GENE ACTION IN MICE.

Part I. The Anatomy and Growth of the Bony Part of the Skeleton of the House Mouse.

Part II. Milk Yield in Mice and the Selection for Maternal Performance.

by

Nigel Bateman, B.Sc.

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Institute of Animal Genetics
University of Edinburgh.

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CONTENTS.

THE ANATOMY AND GROWTH OF THE BONY PART OF THE SKELETON OF THE HOUSE MOUSE.

INTRODUCTION.

This account is of two-fold interest. It is concerned chiefly with the description of the processes which result in the growth of the bony parts of the mouse skeleton as far as they are apparent from a comparison of the skeletons from special strains of mice. The study entails a knowledge of the anatomy of the skeleton and the opportunity has been seized to describe the mouse skeleton - a task that has not previously been undertaken. Hence the second topic of interest.

While the present study of bone growth was conceived as merely confirming the works of many earlier workers, it has three novel features. In the first place, special strains of mice are used instead of the classical madder, anthroquinone stain, or metallic mark techniques, to reveal the process of growth of the skeleton. These special strains segregate for animals which suffer from retarded growth and from a general failure of bone remodelling. Secondly, the investigation is necessarily of very young animals, in place of the more youthful ones, as the segregant animals, essential for this study, rarely live beyond weaning age; and some additional contributions to the problem of bone growth are derived from this limitation. Lastly, a departure has been made from the usual descriptive terms. Whereas previous authors refer to "sites of accretion" and to "sites of erosion" the present author has rejected these static terms for a system

which describes bone movements since, it is felt, that the whole process of bone growth thereby becomes much more comprehensible.

In bone growth we are concerned with two complementary processes: one, accretion, is the depositing of new bone on the surface of the older bone; the other is erosion. The terms accretion and erosion are often referred to as apposition or deposition, and as absorption or resorption. Erosion is not an indispensible feature of bone growth, though it is associated with the growth of almost every bone. Some authors have confined their studies to one or other of these components of bone growth, or have treated them in separate parts of their writings; but the present author considers them to be intimately related. As the description of growth in terms of the multifarious sites of accretion and erosion is confusing, the attempt has been made here to describe growth in terms of bone movements, themselves resulting from characteristic dispositions of the sites of accretion and erosion. This not only reduces the number of entities used in the description of growth in each bone; but creates a saner approach to the problem of growth to the extent that it succeeds in conjuring 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. The description of the composition of these growth systems or patterns is left to a later section of the Introduction.

The discoveries by Grüneberg (1935 and 1948) of two very similar anomalous conditions in the skeleton of the House Mouse have given unique material admirably suited for a confirmatory study of bone growth providing that bone growth is a surface phenomenon only. However, bone growth may be interstitial. Since the reliability of the present technique rests entirely on the fact that bone growth is entirely superficial, it is advisable to digress for the examination of the alternative theories of the interstitial versus superficial nature of bone growth.

In interstitial growth of bone, new bone is produced within the substance of the old. Interstitial growth is essentially an animal method of growth, and it is in this way that the bones' cartilaginous and membranous precursors grow. On the other hand, by superficial growth of bone is meant the deposition of new bone on the surface, not only on the external surface but also on the internal surface of bones which have a hollow structure. Superficial growth implies the existence of a "cambium" or "meristeum" - a concept which doubtless was distasteful to some osteologists because of its botanical associations. However, meristematic growth is also found in the animal kingdom as "meristems" are to be found in the Malpighian layer of the skin and in the germinal epithelium of the ovary, and recently (1948) a spectacular case of a meristem in animal development has been reported by Saunders in the chick in which the mesodermal parts of the limbs and limb-girdles are determined

in serial, proximo-distal order by delineation from an apical epidermal cap of the limb bud. There is, therefore, no a priori reason for rejecting the hypothesis of superficial bone accretion.

Having dealt with the philosophical aspects of the problem we will now turn to the scientific evidence. The earliest factual statements in favour of growth by superficial accretion were made by Stephen Hales in 1727. He showed that metallic marks embedded near the ends of long bones did not get further apart as the bones grew. Had growth been interstitial the marks would have been further separated by the flow of bone substance around them. In this way, Hales conclusively demonstrated that growth was localised terminally.

The next advance in our knowledge of bone growth was made in 1835 by John Hunter. He made use of the fact that a diet of madder gives a red colour to the bone laid down during the period of madder feeding. Thus he was able to show that surface accretion occurred alike to long and flat bones. The findings of Hales and Hunter were confirmed, and their work developed by Flourens (1840) and Kölliker (1873) and others.

Harris (1933) decided that the theory of superficial growth had not been sufficiently accepted and devoted a large section of his book to its proof. He presented additional evidence from the histological picture of the conjugation (epiphyseal) cartilages of normal and rachitic long bones,

concluding that growth in length of the diaphyses of long bones is brought about by the ossification of the intercellular matrix of those parts of the conjugation cartilage immediately adjacent to the bone. He also gave radiographs of the skeletons of children who had suffered severe illnesses and whose growth had been severely arrested. Dense layers of bone were formed with each successive set-back. These "lines of arrested growth" as Harris called them, always first appeared at the bones' surface. He identified the conjugation cartilages as the meristematic layer for the terminal growth of long bones.

The controversy was reviewed again, in 1934, by Brash who further contributed a treatise on the growth of pig and human skull bones.

Lastly, Lacroix (1942-3 and 1946b) in a series of embryological experiments involving the transplantation of pieces of conjugation cartilages and periosteum proved that both are centres of superficial bone production.

Erosion occurs as the corollary to surface accretion since it is impossible for most bones to maintain their complex shapes merely by differential rates of accretion. The description of the sites of erosion was the subject of Kölliker's classic work of 1873. He plotted these sites in the calf and dog in particular, by cutting thick tangential sections, 5 mm. square, of softened bones, which he then examined for Howship's foveolae - rough depressions made on bone surfaces by active osteoclasts. Sites of erosion were found to be a feature of almost every bone. The fact that surface erosion is so

widespread suggests that surface deposition is also widespread.

The last evidence to be presented for the surface nature of bone growth comes from a consideration of the skeletal anomalies whose detailed investigation forms the bulk of this account. It suffices here to state that they can be explained quite simply on the assumption that surface accretion (just as general development) is retarded, and that no erosion takes place. On the other hand, anyone believing in interstitial growth would find it extraordinarily difficult to explain the skeletal anomalies.

Compared with the case for superficial growth, that for interstitial growth is inadequate. No experimental evidence was available for it until 1929 when Kornew claimed to have demonstrated it. He surrounded with metallic rings the metaphyses of rabbits' ulnae and fibulae (i.e., at each end of the bone on the highly concave part near the conjugation cartilages). The rings moved further 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 and Mussleman (1940) made unilateral bone grafts between exposed cancellous surfaces of the vertebral centra of month-old kid-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. The authors, who had just demonstrated that goat vertebrae grow at equal rates at their two ends by ossification of the conjugation cartilages, nevertheless concluded that the grafts had grown interstitially. But Lacroix (1946a) has suggested that the grafts underwent a series of profound histological changes involving decalcification followed by redeposition of the bone, this time, at new surfaces.

Lastly, as late as 1946, failure of melanic exostoses (bony protuberances from the sides of the diaphyses) to become more distant from the growth (conjugation) cartilages was used by Leveuf in evidence of interstitial growth. 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. For either remodelling or cessation of growth at the diseased end could account for the situation in spite of superficial growth.

It is essential that a clear distinction must be made between interstitial <u>accretion</u> and <u>erosion</u> (erroneously believed to be directly involved in bone growth) and interstitial changes of <u>deposition</u> and <u>decalcification</u>. Both the latter are regular, but only accessory features of normal bone growth, being necessary components of the histological changes from spiculate (endochondral) and from dense (periosteal) bone to

the canalated 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 also occurs in pathological conditions - rickets, osteomalacia and syphilis. It results from osteolytic decalcification by the cells contained within the spicules (Lacroix, 1942-3).

In conclusion: superficial accretion and erosion of bone are firmly established as general phenomena, whereas there is, at most, only tentative evidence for rare instances of interstitial growth. In this paper the possibility of interstitial growth will now be ignored.

MATERIALS.

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, resembling Albers-Schonberg's disease (osteopetrosis) in man (Albers-Schonberg 1907) was first discovered in the mouse by Grüneberg (Grüneberg 1935, 1936, 1937 and 1938b where a full account of its genetics may be found). It is a distinctive feature of grey mice which regularly die around weaning. Grüneberg called such mutant mice "grey-lethals" (symbol gl/gl). He 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. In their degree of ossification (of neural arches, transverse processes, sternal ribs, sternebrae, hyoid, pelvis and conjugation cartilages) they resemble at three weeks normal mice only 10 days old. Failure of erosion is inferred:-

(1) from the small size of intra-osseus foramina (which can be enlarged only by erosion),

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

(3) from the persistence of the spongiosa, and

(4) 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 of erosion expected from Kölliker's work.

Furthermore, one would expect erosional anomalies in consequence of the gigantic grey-lethal osteoclasts remarked by Barnicot (1947). As a result of transplanting grey-lethal and normal bones into grey-lethal and normal hosts Barnicot (1941) has concluded that the gene probably does not act directly on the osteoclasts but rather on tissue or body fluids - for grey-lethal bone is eroded in normal hosts and normal bone fails to be properly eroded in grey-lethal hosts. A more sensitive experiment would be to transplant pieces of conjugation cartilage under the renal capsule (following Lacroix' example in other studies, 1942-3) as this would rule out the possibility of the activity of the transplants'

osteoclasts being confused with that of the hosts'. Besides causing this extensive pathological syndrome and the grey coat colour, the gene responsible for these effects irregularly produces kinked vertebrae near the tip of the tail.

An almost identical condition of the skeleton was discovered again by Grüneberg (1948) in mutant mice originally bred by Hertwig (Hertwig 1942). It is impossible always to distinguish these skeletons from those of grey-lethals, though they are generally less retarded and erosional failure is apparently not always so complete. Skeletally, they resemble at three weeks normal mice of the same stock only 14 days old, while 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, unpublished). Hertwig calls her mice "microphthalmics" (symbol mi/mi) since, as well as the skeletal anomalies (which passed unnoticed by her) the mice have rudimentary eyes, a condition due to <u>coloboma retinae</u>. Besides these characteristics, 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 recognised 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.

Heterozygous action of the microphthalmic gene in the skeleton.

Heterozygous microphthalmic and homozygous normal skeletons (from the same stock) do not appear to differ in size, and it seems unlikely that the gene "mi" should manifest itself by affecting erosion in the heterozygous skeleton. Nevertheless, as the gene has so many other heterozygous expressions, it seems worth while to put the matter to more rigid test. The Foramen obturatum is characteristically extremely malformed in the homozygous microphthalmic mouse and is therefore ideally suited to the study of heterozygous actions of the gene on the growth processes of the skeleton.

The right foramina obturata of ten heterozygotes and nine normals were outlined by camera lucida and simultaneously enlarged so that their long axes were of the same standard length. The outlines for all the normals were superimposed, and an "average outline" drawn from them. The outlines of the heterozygotes were similarly used and the "average outlines" for each group were then superimposed on each other - Fig. 48. Any tendency towards the failure of erosion in the heterozygotes should be obvious from this comparison. (Since heterozygotes are, on the average, the same size as homozygotes, differences in shape due to differences in size between the two strains should cancel each other out.) It is noted, however, that only slight differences between the "average outlines" are visible, and such as there are are not likely to arise from a tendency for the normal processes in growth to fail in the heterozygotes.

Perhaps a better test for heterozygous action

would be to compare the average areas of normal and heterozygous foramina enlarged, as before, to the same length. The areas, measured in arbitrary units, with a planimeter, are tabulated below for the individual mice. It appears that the area of

FAMILY	A	B	C	D	F
NORMALS	1.86	-	1.95 2.06	1.93 1.77	1.54 1.65
HETEROZYGOTES	1.88	1.74 1.56	1.97	1.77 1.82	1.71

FAMILY	G	Mean
NORMALS	1.85 1.90	1.83 $\pm$ 0.05
HETEROZYGOTES	1.95 1.69 1.91	1.80 $\pm$ 0.04

TABLE 1. The areas of the obturator foramina of normal and heterozygous microphthalmics after enlargement of the long axis to a standard length. The units are arbitrary. There is no significant difference between the strains.

the Obturator foramen (when enlarged to a standard length) is no different in heterozygotes than in normals and there is no real evidence that the gene for microphthalmia expresses itself heterozygously on the accretional and erosional processes in the growth of the skeleton. This is especially surprising since the gene has very marked effects when heterozygous on the eye and coat colours and on the tail, causing them to be respectively lighter, spotted and occasionally kinked - effects which may be regarded as intermediate between the normal and mutant conditions. Only in its effect on the process of skeletal growth is the gene ineffective when heterozygous.

This remarkable independence of the pleiotropic effects of the gene is further indicated by the author's establishment of families which are characterised by different expressions of the gene in the heterozygotes. For example, in one line the heterozygotes have large head spots and light eyes, while in another they have only a few white hairs on the head but even lighter eyes. In other words, the gene can manifest well in one direction without necessarily manifesting well in another. In view of this independence of the several actions of the gene, it is unlikely that a study of the biochemical causes of one facet of the gene's action would throw light on the cause of another. It is the author's impression that an investigation leading to the ultimate action of the gene would be an extremely exacting test of Grüneberg's hypothesis (1938)[a] of the unity of gene action and of the absence of true pleiotropy. The study of the pleiotropy of the gene is being undertaken by Professor Hertwig.

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 few bones, indeed that no bone, in the mutant animals escapes their 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 the anomalies. For these reasons the two stocks of mice appeared to Dr. Grüneberg as admirably

suited for the study of accretion and erosion in bone whilst the mutant mice themselves appeared to him deserving of full examination in their own right. It was in response to Dr. Grüneberg's direct request that the present author undertook this work.

The method of recognising the sites of accretion and erosion necessarily entailed comparing camera lucida drawings of normal and mutant bones. The drawings of the normal bones have been extensively labelled to serve as a description of the mouse skeleton, see Plates 1-29. Some precedent has been set by Greene(1935) in her "Anatomy of the Rat" but her account of the skeleton is inadequate for many anatomical purposes.

CONCERNING THE METHOD OF INVESTIGATION.

The method of investigation of the manner of bone growth in the present study is entirely new. In this novelty resides the special value of the study in confirmation of those of previous investigations, and out of the difference between this and earlier methods arises the possibility that this work is complementary to previous ones; of course, there are also grave possibilities of the reverse.

The earliest studies of bone growth involved boring holes in long bones and fixing metallic marks. While able to prove that growth was not interstitial (but not excluding interstitial changes at the histological level - such as occur normally in the growth of bone) they yielded little information as to

the pattern of growth.

The technique of madder feeding, evolved in the eighteenth century, was capable of supplying details both on the sites and the rates of accretion throughout the skeleton. The method has been used by Hunter (1837) and Flourens (1840) but their works are almost inaccessible. Brash (1934) and Payton (1932, 1933) are perhaps the latest workers in this field, but together they do not describe the growth of a complete skeleton, and they treat erosion only by inference from the extent of accretion.

The madder technique is limited to those animals which will eat madder. However a parallel technique of universal application has been developed by Schour and Hoffman (1938) who have injected small quantities of a stain, related to madder, into animals of varying ages. The stain is immediate in its effect on bone, and is also lasting - being visible within three hours and lasting for at least four months after the last injection. Schour and Hoffman used alizarin red S but there are other anthroquinone stains of different colours which are also readily absorbed by bone. It is thus quite feasible to make a simultaneous triple study of the sites of erosion, indifferent areas and accretion by studying the skeleton after three intravenous injections of different coloured stains. Further, such material would lend itself to a detailed study of the <u>rates</u> of accretion and erosion (erosion being manifest by the appearance of earlier stained surfaces). Unhappily, Schour and Hoffman used their material only for the

study of tooth growth, and no one has used such an amended technique. The present work was almost completed when this new technique was conceived.

Kölliker (1873) had already attempted the happy combination of a simultaneous study of accretion and erosion in bone growth, 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's plots of the sites of erosion depended on his recognition of Howship's foveolae or of osteoclasts themselves. But his work is no more able to give direct information on the rates of erosion than those of Brash, Payton, Flourens or Hunter, for it seems that osteoclasts can vary in their activity, and that mere numbers are no guide to the intensity of erosion. Thus, Barnicot (1947) has observed the migration of massed osteoclasts across the inside of the Parietale of the mouse although there is unlikely to be any change in the sites of erosion over the period he studied (from birth to three weeks); Kölliker himself has observed osteoclasts before the formation of bone; and Kawata (1924) and Ruth (1937) report ostensibly comparable amounts of erosion of the pelvic symphysis in the pregnant guinea-pig, although they record very different numbers of osteoclasts. Kölliker also figures bone surfaces (his Figs. 3, 6 and 9) for which it is difficult to imagine osteoclasts more closely packed together, and others (his Fig. 5) which although pot-marked all over with Howship's foveolae, have hardly any osteoclasts.

Harris's interpretation of the "lines of arrested growth" (1933) is yet another source for the study of bone accretion. These lines, which are visible in radiographs, are zones of dense bone formed particularly in long bones through a slowing up in the proliferation of the conjugation cartilages during illness, starvation, etc. The amount of bone capping the lines of arrested growth gives a measure of the rate of growth at that site. Harris figures a radiograph showing much of the skeleton of a 36-week-old human foetus with several lines of arrested growth resulting from congenital syphilis. Harris has also estimated the rates of growth at the ends of long bones from the number of cells in the ranks of the conjugation cartilages. Both of Harris's methods, though limited to the growth of cartilage bones, can be applied to the study of growth in any mammal.

Over the preceding methods (with the exception of the suggested development of Schour and Hoffman's technique) the grey-lethal-microphthalmia technique used here has but one major advantage - that it supplies direct information on the rates of erosion. Over many of the above methods it has the additional advantage of being applicable to almost every bone in the skeleton, though it is of course restricted to the mouse skeleton.

Of its handicaps, the greatest is the inability of the investigator to come to any conclusion as to the growth process of a bone without making a comparison between two bones,

or what is worse still, a comparison between two drawings of the bones. Hence error, perhaps creeping, perhaps flooding, is introduced into the interpretation of the differences between normal and mutant bones owing to the confusion of normal variation with differences arising out of gene segregations. While the method has an advantage over Kölliker's in that it displays the sum total of erosion over a long period and can never depict only temporary erosion at the moment of death, even more confusion is likely to arise through the mollification of erosional anomalies due to the site subsequently becoming one of accretion, or to the aggravation of erosional anomalies arising out of an early phase of growth with those derived from a later phase with a different pattern of growth (see pp.118-121). Such situations may well attain in the majority of long bones. In regard to the animals to which it may be applied, this technique has even more severe limitations than the madder technique; for comparable conditions are unknown elsewhere except sporadically in man. Albers-Schönberg (1907) gave the first account of the condition and it is known as Albers-Schönberg's disease or as osteopetrosis; Elliot Smith and Wood Jones (1910) have described the condition in archaeological material from Nubia; Suk (1929) described affected femora; Lightwood and Williams (1939) discovered it

in a two-year-old child. Ingalls and Grossberg (1932) described a "unique" pair of human femora whose anomalies, however, strictly parallelled those of grey-lethals and microphthalmics. They failed to see the significance of the anomaly in regard to bone growth and did not mention whether any part of the skeleton besides the Femur and Tibia was affected.

It is seen that the method used here for revealing the manner in which bones grow has advantages over previous methods since it supplies simultaneous information on both components of growth - both on accretion and on erosion. On the other hand, the method is laborious, a little uncertain and inaccurate, not always adequate to supply the full details of the manner of a bone's growth, and is already far surpassed by another (suggested by the author, but as yet unpractised).

Wait till you s
the snags of th
method!

AGE FOR PREPARING SKELETONS.

What is the optimum age for killing the mutant mice for their skeletons ? Since the formation of ossification centres is not complete until some time after weaning (e.g., tympanohyals do not begin to ossify till 28 days after birth, Johnson 1933), the earliest age allowing complete description of the sites of erosion and accretion is about five weeks. Moreover, the older the animals, the more pronounced will be the differences between normal and mutant mice, thus making analysis more easy, certain and subtle. However, because few

of the mutants live longer than 21 days, and a number of their skeletons were required, it was felt that 21 days was the optimum age at which skeletons could be prepared. Unfortunately this age limitation meant that a few bones not fully formed at this age could not be studied.

Fourteen grey-lethal and 23 microphthalmic skeletons were prepared. In addition, each mutant animal had at least one normal litter mate prepared as control comparison. All the mice were kindly supplied by Dr. Grüneberg.

TECHNIQUES.

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 minutes boiling. There was little danger of disarticulating the epiphyses and compound bones except when the 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 KOH 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's, 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 (Fig. 1). This consisted of a wax square made in three depth sizes, filled to overflowing, placed between a glass plate and a microscopic slide. Distortion was thereby reduced to a minimum. 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.

DRAWINGS.

Bones of three-week-old mice are extremely variable in opacity and are therefore quite unsuitable as photographic subjects. Therefore they had to be drawn. Outlines were made accurately and to scale by camera lucida attachment to a binocular microscope. Magnification in the originals was about 9½ diameters but has been reduced in reproduction to about 6 times. Shading was added without the use of the camera lucida. Assistance in the shading of cleared preparations was obtained by comparison with papaine preparations of comparable bones in

KEY TO FIGURES 2–5

Outline of the bone of the younger mouse

Outline of the bone of the older mouse

Newly formed bone deposited by accretion

Parts of the older bone which are eroded

New bone subsequently eroded

Bone substance common to the older and younger bones.

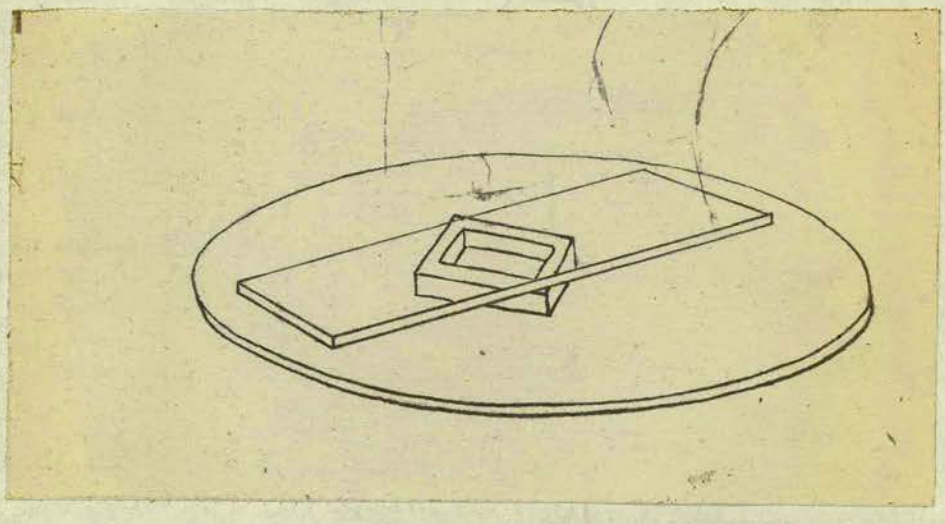

Fig. 1. The glycerol bath in which small bones from alizarin preparations are placed for examination and drawing.

which their form is more easily appreciated.

Lighting was always from the left, from above and to the front.

The order of drawings is always: top; normal (from microphthalmia stock) middle: microphthalmia; and bottom: grey-lethal; or left to right in the same order (+/+, mi/mi, gl/gl).

Diagrams are free-hand interpretations of the original drawings or of superimposed tracings of them.

NOMENCLATURE.

The author has followed the example of Ellenberger and Baum (1926) in using their latinised veterinary nomenclature. This is closely related to the fuller medical terminology (the Basle Nomina Anatomica, or BNA (Jameson 1916) agreed upon at an international convention in Basle in 1895, and is adapted to four-footed animals. The author has found it, however, occasionally necessary to use BNA terms and even to invent new ones, where example has been lacking in Ellenberger and Baum.

He has rejected the current British medical custom of "anglicising" the anatomical nomenclature (see the British revision of the BNA adopted in Birmingham in 1933) (Cunningham) on account of the weird and ungainly hybrid terms which result, and which are intolerably excruciating to any but medical and non-classical scholars.

EIGHT THEORETICAL MOVEMENTS IN BONE DEVELOPMENT.

To appreciate the real character and significance

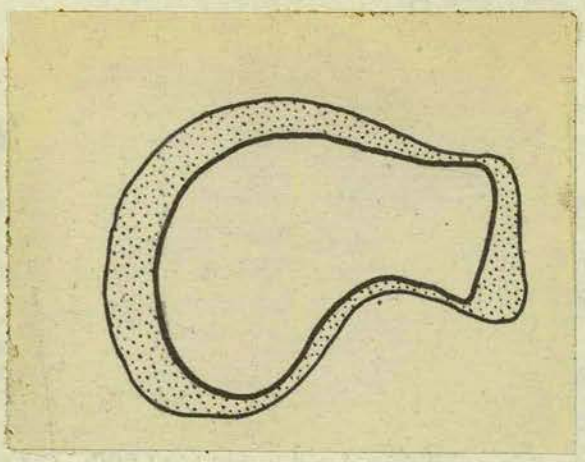

Fig. 2a. An example of external accretion - the Caput humeri.

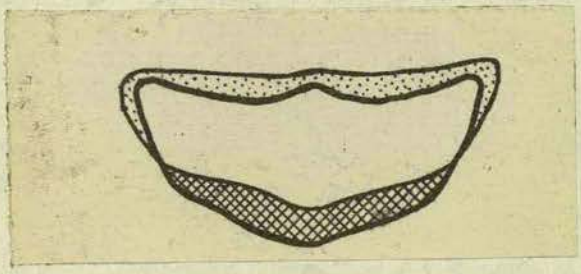

Fig. 2b. External erosion in the Interparietale.

of the processes of accretion and erosion, they must be realised as two complementary aspects of one and the same movement in bone growth. Hence it is important to discuss some theoretical inter-relations of the sites of accretion and erosion as they apply to growth movements. Each of the eight theoretical patterns to be described has actually been observed in practice. In order that an example of each pattern may now be given, a later section of this paper is anticipated. The patterns will be referred to many times in subsequent sections of the paper, and, so that each may be remembered easily, each will be given a descriptive name.

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:-

1. External accretion. 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 (Fig. 2a).

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

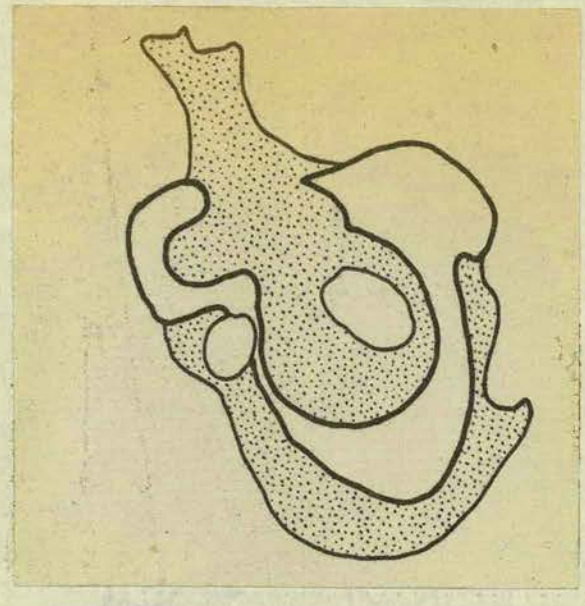

Fig. 3a. Internal accretion (and external accretion) in the ossification of the cartilaginous Perioticum.

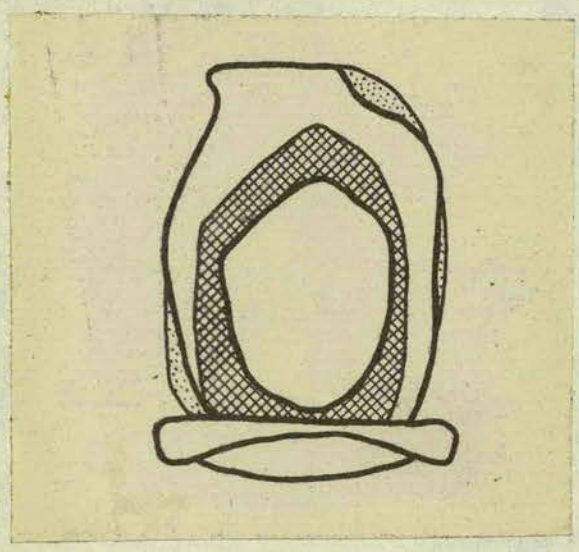

Fig. 3b. Internal erosion of the Stapes for the enlargement of the Foramen stapedis.

3. Internal accretion (Fig. 3a) 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 vestibulae of the Perioticum is an example of it, having at birth three times its final diameter (the Perioticum grows also by external accretion).

Lastly 4. Internal erosion. Intraosseous foramina are enlarged by internal erosion. Fig. 3b 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.

5. Unilateral growth. Unilateral growth is the combination of the first and second patterns such that accretion is restricted to one side of the bone with erosion of the opposite side, that is of the oldest part of the bone (Fig. 4a).

In this way the Manubrium mallei is bent away from the Processus anterior. In this case, unilateral growth is inferred from the difference between normal and mutant mice in the angle between the two processes. For 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.

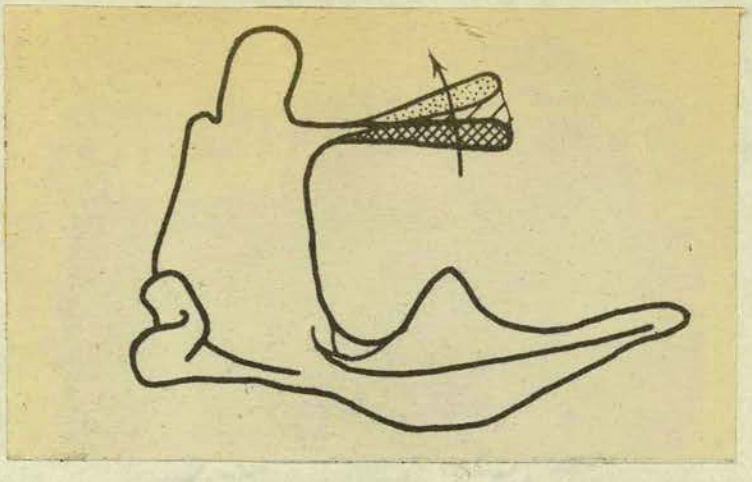

Fig. 4a. Unilateral growth of the Manubrium mallei.

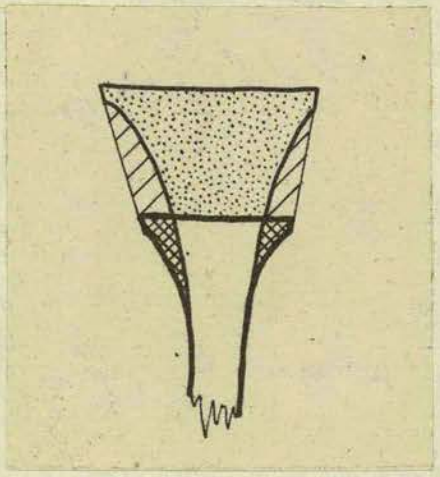

Fig. 4b. Diaphyseal growth at the end of a typical long bone.

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 (Plate 1).

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.

6. 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 (Fig. 4b). The pattern is typical of the 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.

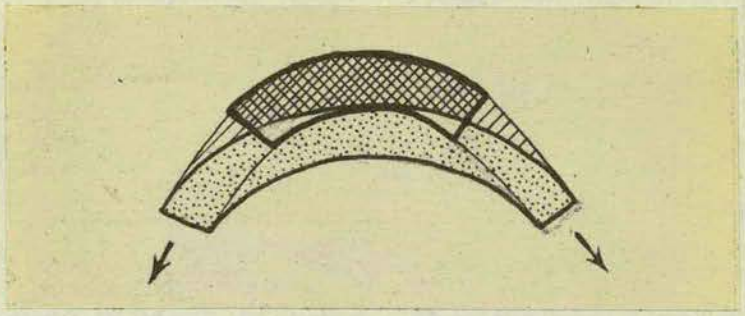

Fig. 5a. Centripetal growth - an ultradiagrammatic representation of an Arcus vertebrae.

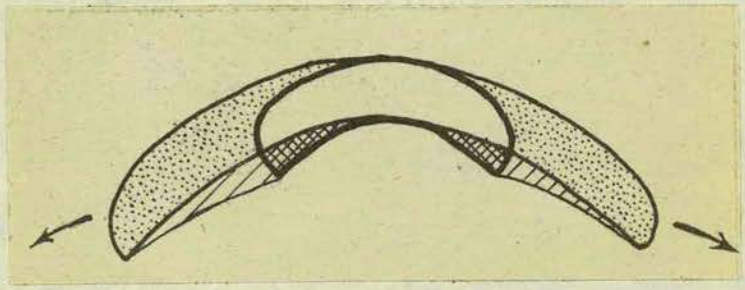

Fig. 5b. Centrifugal growth - an ultra-diagrammatic representation of the Parietale.

7. Centripetal growth. In many Arci 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 (Fig. 5a). (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 Fig. 5a represents a diametrical section across the bone.).

8. 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 (Fig. 5b). 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. 64) 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 "diaphysed 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.

We can now proceed to the analysis of skeletal growth by reference to the anomalies in the mice.

2. GROWTH OF THE TIBIA AND FIBULA (OSSA CRURIS) - A PRACTICAL EXAMPLE OF THE METHOD OF ANALYSIS.

This section studies in detail the condition of the Tibia and Fibula to show how in a particular instance differences in shape between normal and mutant bones are related to normal growth processes. This is followed (Section 3) by an account of the growth of each individual bone in the skeleton of the mouse (grouping metameric bones, like vertebrae, ribs, phalanges etc., together so long as they are sufficiently alike). The reader who is not so intrigued by the growth of individual bones may like to turn now to the Discussion, p.114, where more general problems of bone growth are discussed. Adequate references will be found there to the third section of this Thesis but, if these are to be properly understood, it is advisable that the present section is studied.

Although the theoretical patterns of growth just described are of considerable help in recognising anomalies and in interpreting them, it would be unwise to limit ourselves by reference to them alone. It is essential to make use of two simple principles: that in regions where accretion occurs the retarded, mutant bones will be thinner than normal; and that where erosion normally occurs the mutants will be thicker. An additional aid to analysis is the coarse pitting of the surfaces of mutant bones at sites corresponding to those of erosion in normal mice (Plates 26 and 27). These pits appear to be due to the very local and partial success of the mutant, giant osteoclasts

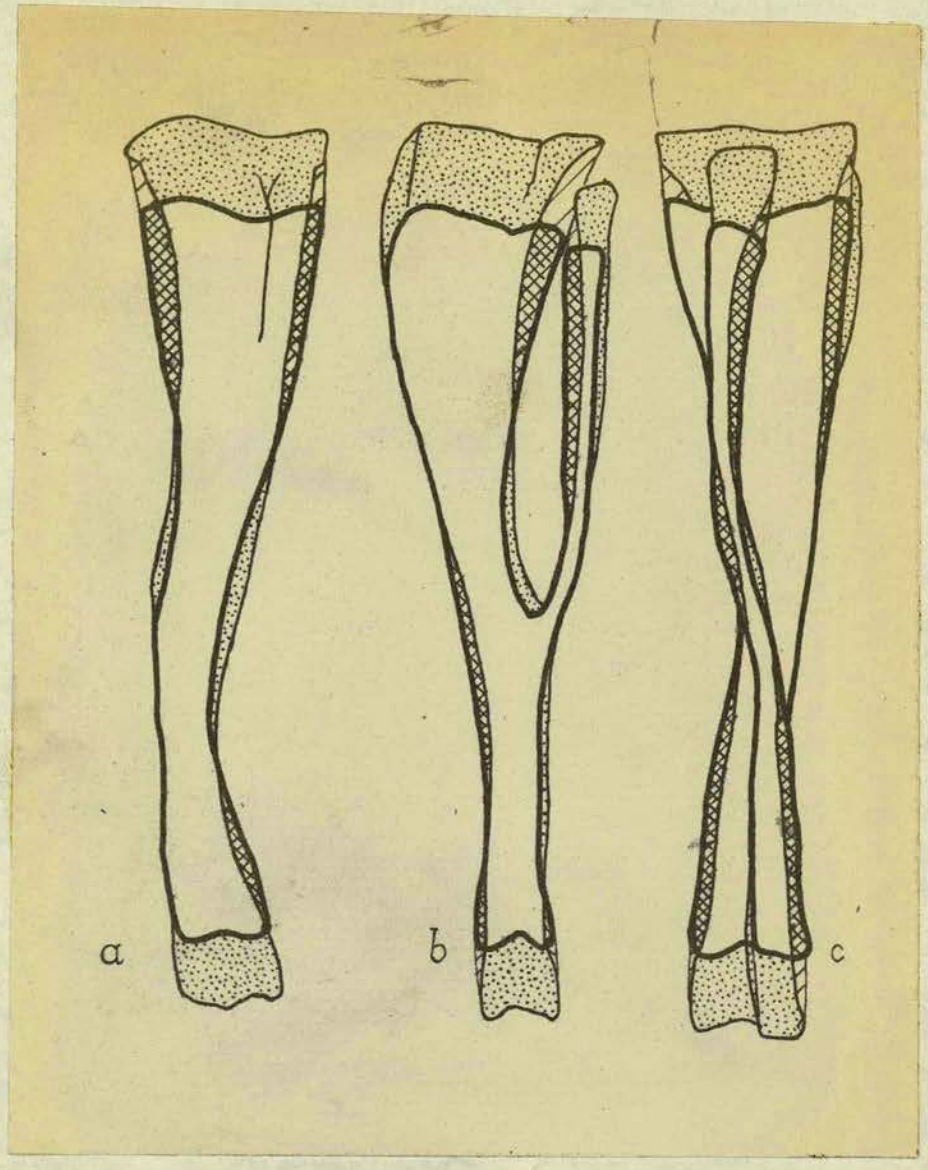

Fig. 6. Interpretation of the differences between the outlines of normal and mutant Ossa cruris (Tibia and Fibula). Heavy line - the mutant bone, lighter line - the normal bone. Stippled areas show the extent of accretional failure in the mutant; cross-hatched areas the extent of erosional failure; hatched areas are where bone should have been formed by the mutant and subsequently eroded. Only those processes visible in optical section are shown. (a) anterior; (b) medial; and (c) posterior aspect.

(Barnicot 1947) in resorbing bone, and no doubt correspond to Howship's foveolae (Kölliker 1873 and Textbooks of Anatomy). In normal mice the osteoclasts leave a much smoother surface even where erosion is extremely rapid (Plate 27). This characeristic pitting of mutant bones may occur without much thickening, and is therefore useful in confirming erosion where differences in shape are too slight to be significant.

Comparison of mutant with normal Tibia and Fibula leads to the following conclusions (see also Fig. 6).

1. That the mutant bones are short. General retardation completely explains this shortness, since the heterogonic relationship between skeletal measurements seems to be constant for mutants and for normals from both stocks (Fig. 7).

The difference in size between mutant and normal bones makes it impossible to ascertain the deviations from normality of the mutants, merely by superimposing the outlines of the bones one on the other. Moreover, if the drawings were scaled to the same size for comparison, the changes in proportion accompanying growth could cause considerable analytical inexactitude. Thus it is in some cases feasible only to make unaided comparisons by eye. Usually, however, as in this case, the consideration of the anomalies in erosion (below) enables the comparisons to be put on a more stringent basis.

2. It is clear that the proximal third and distal quarter of the mutant diaphyses are relatively thick (except for the very ends - Lacroix's "ossification rings"). They are also

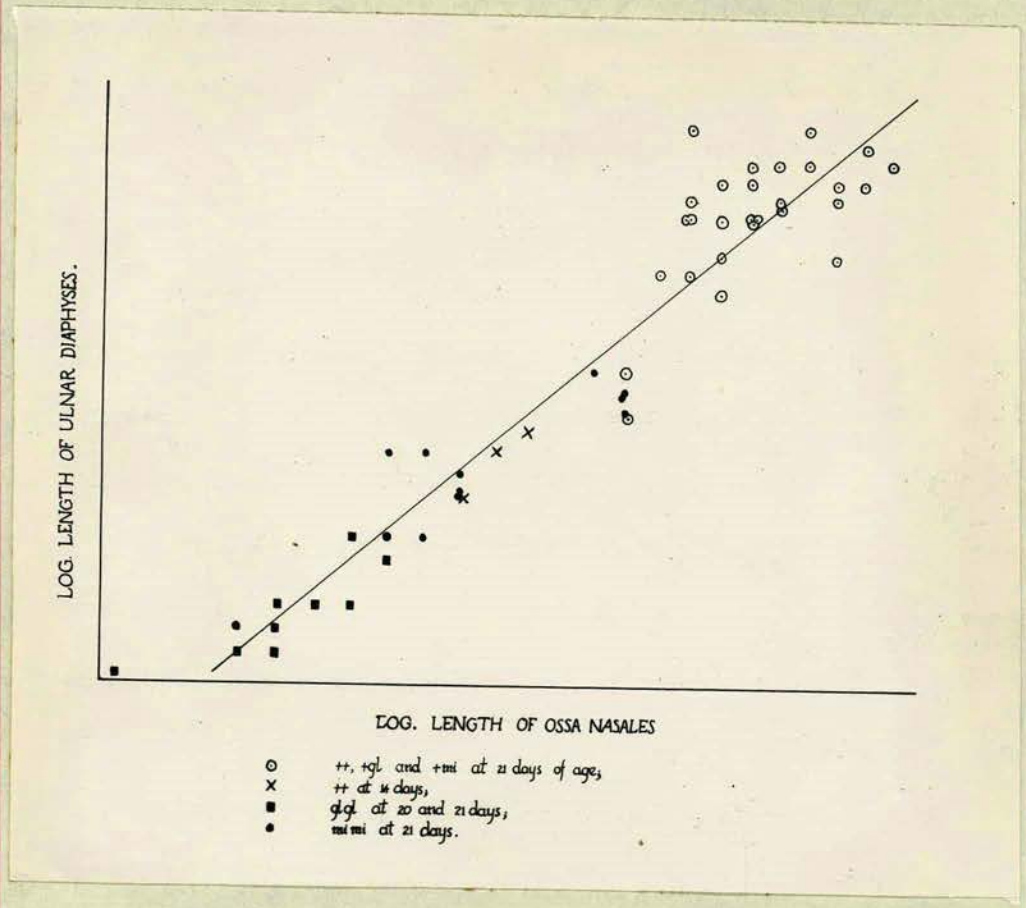

Fig. 7. The allometric relation between the length of the snout (length of Ossa nasales) and the length of an "unrelated" body measurement (length of Corpus ossis ulnae) for normal and mutant mice of 20 and 21 days of age, and for three 14-day-old normal mice.

heavily pitted by osteoclasts, especially on their lateral, medial and posterior surfaces. Both facts indicate that these regions are normally sites for erosion. This being so, it is apparent that the increasing girth of the clumsy ends of the mutant diaphyses record successive stages in the increasing girth of the growth (conjugation) cartilages (vide Fig. 4). If, then, the sides of the mutant bones are projected in the drawings until they are the same distance apart as in the drawings of the normal bones of the same age, it becomes possible to superimpose the drawings of the two bones fairly accurately, and to estimate the growth processes more precisely.

It is not always as easy as it has been in this case to determine the lines of reference to be used in the superimposition of the drawings. Final decision, as to appropriate reference lines, rests with the rigorous test to which all analyses are subject - that there is conformity in the conclusions as to the bone's pattern of growth, whichever mutant is used in the analysis. There is sufficient variation in the size and proportions of the individual bones for the results common to both analyses to reflect the common pattern of growth of the bones. Figures 6a, b and c are constructed in this way.

These Figures show that between 14 days(developmental age of 3-week-old microphthalmic mice) and 21 days (actual age of the normal mice) and also between 10 and 21 days (10 days being the developmental age of grey-lethals actually 3-weeks-old) growth proximally is a little faster than growth distally.

(Ratio of 5:4). The same conclusion is obtained for the earlier period of growth - i.e., from the first appearance of the erosional anomaly, onwards. This is based on the extent of the failure of diaphyseal erosion proximally and distally. This conclusion is in agreement with Harris's observations (1933) on the human child (see his Figure 138); but Payton (1932) states that in the pig's Tibia growth proximally is twice as rapid as distally.

The Figs. 6 reveal some further points of interest, namely:-

3. That the middle region of the shaft is strengthened by external accretion limited to the lateral, medial and posterior surfaces.

4. On the anterior surface of this same region there is some erosion which, in combination with accretion at the opposite, posterior wall, results in secondary unilateral migration of this segment in an antero-posterior direction.

5. The maintenance of the Fossa anterior for the insertion of the flexor tendons of the foot requires the "unilateral" pattern of growth in reverse. Its distal wall is continually being eroded, while it is continually being filled in by proximal accretion.

6. Minor changes occur to the Tibia in relation to the Fibula. At birth, these two bones are independent structures. At 10 days (cf. grey-lethal) fusion is complete distally but the Fibula still retains its identity throughout its entire length. But, by 21 days, the third quarter is almost entirely incorporated in the Tibia, and along the line of fusion, between 10 and 21 days

the Tibia undergoes posterior and lateral accretion to build it up to the same level as the Fibula.

With regard to the Fibula, Figs. 6 show that:-

7. The rates of terminal growth are about equal, as in the Tibia. The pattern of growth is diaphyseal, but unilateral remodelling movements are superimposed on it. These obliterate the erosional component of the diaphyseal pattern on the posterior surface proximally and on the postero-medial edge distally.

8. Near its middle the Fibula changes over from the medial side of the Tibia (proximally) to the lateral side (distally). In young animals (cf. the mutants) the intermediate zone is more transverse but the zone becomes attentuated as the bone gets longer and relatively narrower. In other words, this part of the Fibula, in spite of being rigidly fused with the Tibia, rotates. This change is accomplished by medial erosion above the point of fusion and lateral erosion below it - both being compensated by accretion to the opposite sides (i.e., the rotation results from unilateral growth in opposite directions).

Immediately below the point of fusion the tendency for medial accretion is confounded by diaphyseal erosion but appears paramount further distally.

9. As is seen in the medial view of the mutants the Fibula has no curvature derived from its terminal growth. The bowing out of its proximal half is a subsequent process involving unilateral anterior erosion (enhancing diaphyseal erosion in this region) and unilateral posterior accretion (overwhelming it).

Near the junction with the Tibia the direction of the movement is reversed.

10. The middle regions of the mutant Ossa cruris are very noticeably much more normal than the extremities, both as regards shape and surface texture. These regions correspond to that part of the Fibula diagnosed as juvenile (vide point 8 above). Hence it is clear that up to an early stage of the bone's development its growth is quite normal. The same conclusion is reached when the mutants' clumsy metaphyses are compared with those of Fig. 4b. For the latter was constructed on the assumption that the young bone grew normally at first, failure of erosion being considered only for the latest period of growth. In general, it appears that growth is normal in the mutants until shortly after calcification of the cartilaginous or membranous precursor.

11. In the epiphyses of the Tibia and Fibula there are no differences between mutants and normals indicating failure of erosion in the mutants. The mutant epiphyses are, however, retarded. Epiphyseal growth is therefore by external accretion alone.

12. The intermediacy of microphthalmic between normal and grey-lethal bones is noted here but not considered until the Discussion (p.125).

Having illustrated the foregoing example at some length it is now convenient to describe the growth of the rest of the skeleton in logical sequence.

3. GROWTH OF THE REST OF THE MOUSE SKELETON.

THE SKULL.

Although the skull is a compound bone and must be reduced to its component parts for analysis, the significance of the growth changes in the individual bones can be fully realised only by reference to their place in skull growth as a whole. Therefore, before proceeding to a study of each bone, it will be useful to describe the general trends in skull growth. These trends are revealed by comparing the skulls from the three types of mice depicted in Plates 1-5. Concerning ourselves for the moment only with the retarding action of the mutant genes it becomes apparent that the chief trends accompanying growth are:-

(a) in dorsal view. 1. The enlargement of the whole skull, particularly in the long axis, to a maximum degree anteriorly, and minimally posteriorly.

2. The forward and sideways shift of the Arcus zygomaticus.

(b) in lateral view. 1. The raising of the roof of the skull particularly at the anterior end.

2. The upward migration of the Arcus zygomaticus particularly at the anterior end.

(c) in ventral view. 1. A general elongation of the skull, relatively more rapid behind (in the Pars basilaris of the Occipitale and Sphenoidale aborale) than in front (in the Palatina, Maxillae and Incisiva).

The plane of the Foramen magnum becomes increasingly

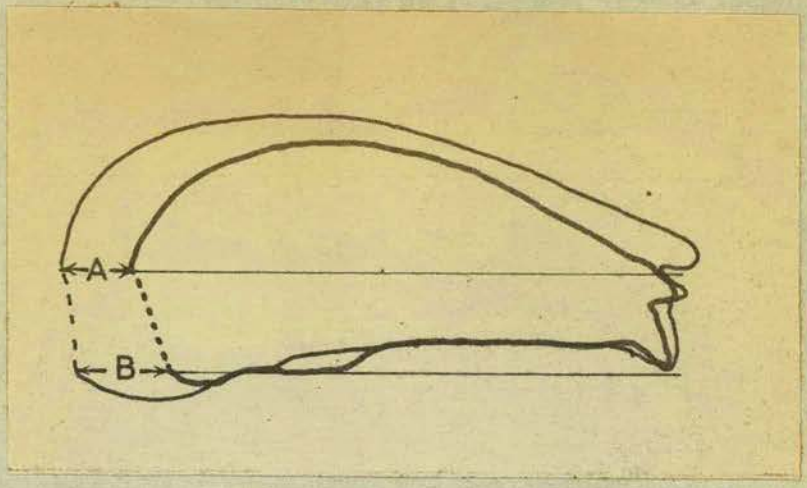

Fig. 8. Diagram to show how differential rates of growth in the roof and floor of the normal skull may bring about the more vertical position of the Foramen magnum.

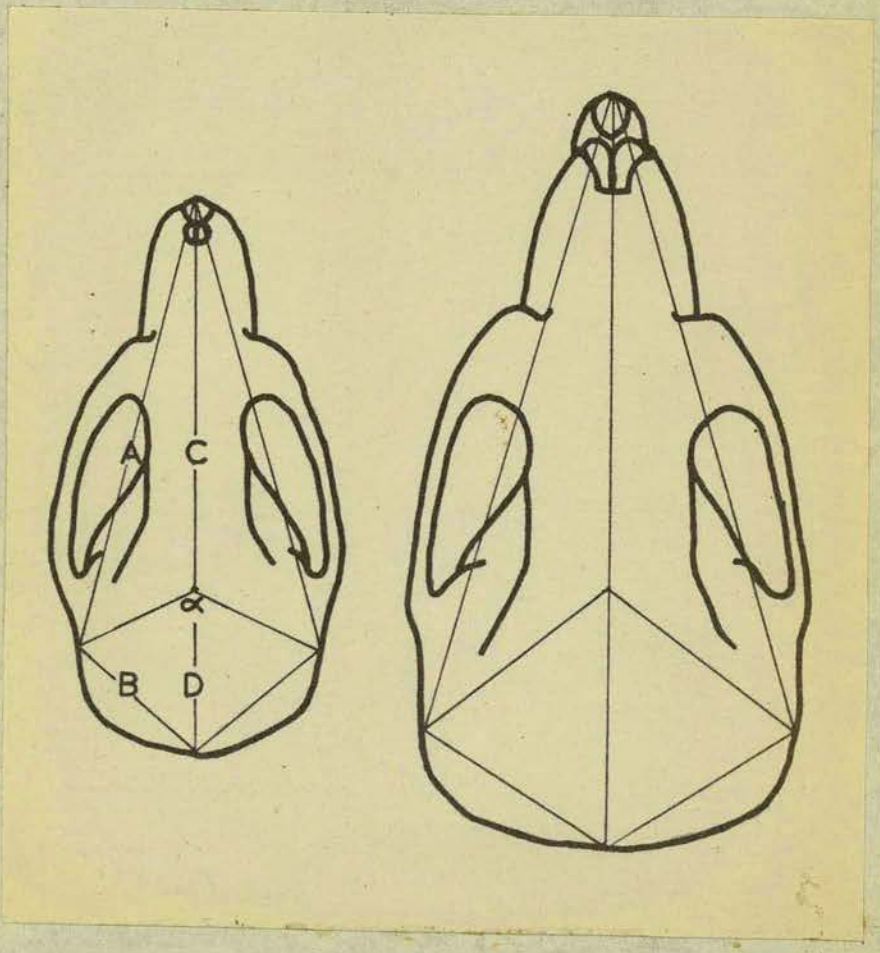

Fig. 9. Rotation of the Bullae tympanicae (i.e. reduction of the angle α) is caused by differential rates of growth fore and aft both laterally and medially (i.e. of A/B compared with C/D).

transverse to the long axis of the skull because the rate of elongation of the floor is greater than that of the roof (at least when no account is taken of the curvature of the roof). Fig. 8. The auditory capsules are caused to rotate, so that the angle between them becomes more acute, by the differences between the sides and the mid-line of the floor of the skull in their rates of growth fore and aft (Fig. 9).

2. The lowering of the palate in the mid-longitudinal region so that it becomes more on a level with the Limbus alveolaris.

3. The baring of the teeth by erosion of the sockets of the teeth from within.

And lastly, 4. The broadening of the anterior part of the palate to accommodate the backwardly growing incisor roots.

It is now convenient to turn to the growth processes of the individual bones of the skull and so to the consideration of the remaining bones of the skeleton.

It may be remarked here that almost no two bones of the head (unless paired, left, right) grow in a similar manner. Each grows in an individual way, and when at all complicated in structure, by a most complicated system of interacting patterns (vide the account of the growth of the Incisûra sphénoidale given in the Introduction, p.26). For many bones, for example the Maxilla (see p.60) the concept of a "punctum fixum" is invalidated, unless perhaps it is regarded as lying somewhere in the space between its numerous processes and laminae. The

The Squama occipitis (C, p.37), Sphenoidale orale (E, p.39) and Lacrimale (Q, p.55) give some idea of the varying extent to which bones of the skull are dependent on their neighbours for normal growth. On the other hand the similarity of the allometry (Fig. 7) between the length of the nasal bones (as a measure of the length of the snout) and the length of the ulnar diaphysis (as a measure of an "independent" body measurement) for normal and mutant bones demonstrates the independence of the growth of the Nasale of certain extrinsic factors, i.e., tooth pressure (de Beer 1940). Lastly, the Occipitale (A, B and C, pp.36 &37) and Tympanicum (G, p.43) may be quoted as showing fundamental age changes in the growth patterns of the bone.

A. OCCIPITALE: Pars basilaris (Plates 3 and 4).

It seems likely that the Pars basilaris grows by external accretion at all borders, particularly at the cartilaginous sutures (synchondroses) with the Pars laterales and with the Sphenoidale aborale. Erosion occurs only (and then only to a slight extent) on the inferior surface on either side of the mid-line just in front of the posterior margin where it serves to model part of the Tuberculum pharyngeum.

B. OCCIPITALE: Pars lateralis (Plates 3 and 4, 5 and 10).

Growth of the horizontal ramus of the Pars lateralis results from external accretion at the synchondroses with the Pars basilaris and from external accretion along the margin of the Foramen magnum. The Canalis hypoglossi keeps pace with the synchondrosis by the reverse of the "unilateral" pattern of

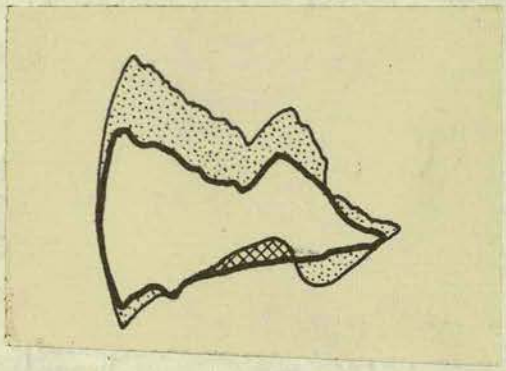

Fig. 10. Growth of the Squama ossis occipitis (Supraoccipital). An interpretation of the differences between normal and mutant bones. Shading as for Fig. 6.

growth. (i.e., internal erosion of its leading margin, with internal accretion to the trailing edge.) The material is unsuited for further analysis.

C. OCCIPITALE: Squama occipitis (Plates 1, 2 and 5; Fig. 10).

The Squama occipitis grows considerably in height and its most anterior parts diverge further, the one from the other, though without increasing much in length (antero-posterior dimension). Both changes extensively involve "centrifugal growth" and, in consequence, almost the whole cerebral surface of the mutant bone is heavily pitted by osteoclasts. With fusion of the Squama and Partes laterales at about 17 days, accretion ceases at the intervening synchondroses so that practically all the increase in height is due to "centrifugal growth" involving the upper margin only.

These growth movements of the Squama are normally co-ordinated with compensating growth changes in the adjacent bones. In the mutants, however, this co-ordination is upset and as a result of the strains exerted on it by the backwardly growing Squamosum the part of the Squama occipitis next to it becomes corrugated; failure to erode the posterior corners of the Interparietale results in this bone overriding the Squama occipitis.

With the fusion of all the Partes laterales, basilaris, and Squama around 20 days their growth patterns must be radically altered. Unfortunately this material is too young to yield any information on the new patterns.

D. SPHENOIDALE ABORALE: (Plates 3, 4, 5 and 6; Figs. 11a, b).

The Sphenoidale aborale consists of five parts; a basal portion (Corpus), two wings (Alae temporales) directed laterally upwards and forwards and the Pterygoidea which support the soft palate.

The main changes accompanying this bone's growth are:

1. Enlargement of the Corpus,
2. Enlargement of the For. orbitorotundum,
3. The outward movement of the base of the Alae so that they become more vertical, and
4. Widening of the soft palate.

It is probable that the Corpus grows by about equal rates at its two ends in the diaphyseal pattern, erosion being strictly limited. Primary growth of the Ala temporales is by "external accretion" to its lateral and posterior borders. The Foramen ovale and Canalis alaris migrate outwards and backwards by internal erosion, enlarging very considerably as they do so. Complete closure of the Foramen ovale of the grey-lethal drawn in Plate 5 resulted from internal accretion to the anterior border of the Foramen. The roots of the Alae temporales and Procc. pterygoidea are carried outwards by medial erosion. Erosion continues with diminishing intensity up the cerebral surface of the wing so that, with corresponding accretion to the outside, the lower part of the wing migrates laterally. In this way the wing secondarily takes up a more vertical position, occupying less of the floor, and more of the sides of the cranium.

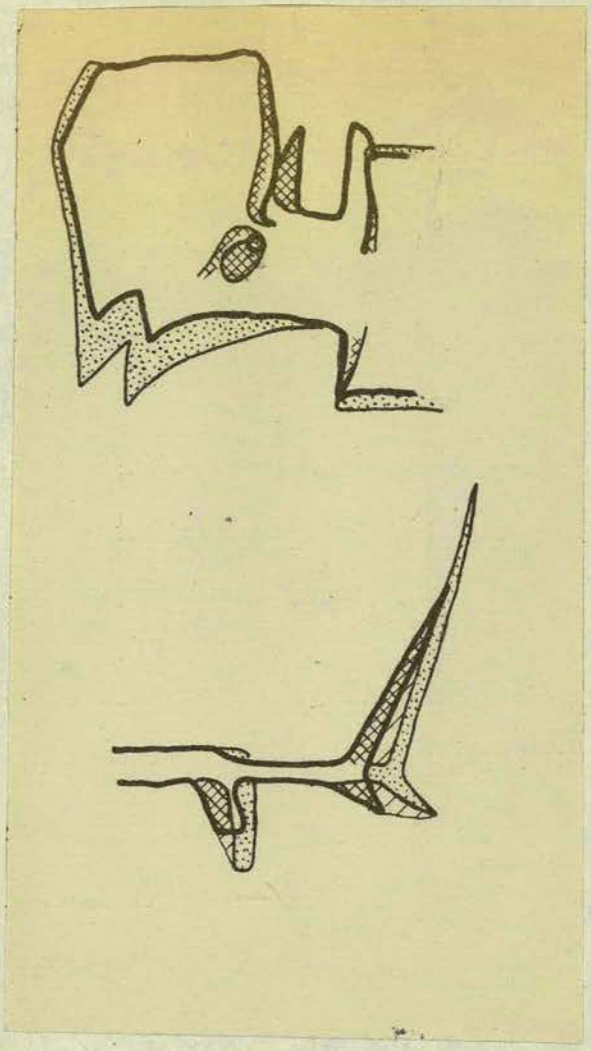

Fig. 11. Growth of the Sphenoidale aborale; above - cerebral aspect, below - vertical section.

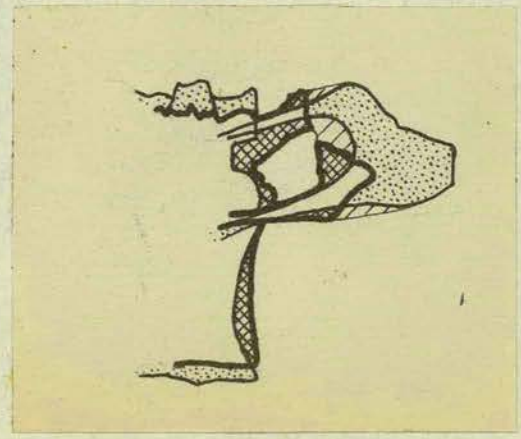

Fig. 12. Growth of the Sphenoidale orale; cerebral aspect.

The broadening of the soft palate results from the divergent growth of the Ossa pterygoidea along both their posterior and ventral margins, and from the "unilateral" growth of their roots.

A remarkable feature of the mutant Alae temporales is seen in the method of attempted erosion of their cerebral surfaces. The osteoclasts appear to be eating their way into the bone in a direction opposite to that of the primary growth of the wings (Plate 6) and towards the zone of greatest need for erosion (Fig. 11b). This suggests that it may be a general rule that the osteoclasts do not merely follow in the wake of accretion, but move up the gradient of erosional need.

In adult mice the Corpus of the Sphenoid thickens enormously to contain sinuses, and the Canalis pterygoideus is developed. They are absent in 3-week-old mice.

E. SPHENOIDALE ORALE (Plates 3, 4, 5 and 10; Fig. 12).

During its early phases of development the Sphenoidale orale undergoes quite remarkable changes of form which it will be well to consider before passing to the processes involved. The following changes occur and are reflected in the differences between the normal and mutant bones at three weeks.

As the Sphenoidale orale increases in length it moves forward in relation to the rest of the skull. In consequence, (1) the Corpus which, in early stages of development curves round the circumference of the anterior, lower quadrant

of the hinder, globular part of the cranium, flattens out into the floor of the fore-part of the cranium.

Similarly, (2), the wings, Alae orbitales, which earlier occupy the front wall of the globular, hinder part of the cranium (the position occupied by the mutant bones) move round to the lateral wall of the narrow, fore-part of the cranium. This movement has two components:-

(i) a change in the relative position of the anterior and posterior rami of the Alae - from the earlier position in which the anterior ramus is placed above the posterior ramus, to the subsequent position of their being more on the same level.

(ii) a steepening of the angle between the Alae of the left and right sides.

and lastly, (3) As a result of 2(i) the Foramen opticum comes to occupy a more horizontal, less vertical plane; and has to be considerably enlarged in order that the Nervus opticus may continue to pass freely out of the skull.

The growth processes involved in these changes are:-

1. "External erosion" of the cerebral surface of the fore-part of the body.

2(i) "Unilateral growth" both downwards and forwards of both rami of the Alae orbitales especially of the anterior ramus.

(ii) "Unilateral" upward growth of both Alae; the rate of migration increasing from their medial to their lateral margins, and

3. "Internal erosion" of the anterior, lateral and medial walls of the Foramen opticum. The latter process erodes the sides of the Corpus which consequently becomes narrower.[1]

The processes so far considered are not directly concerned in growth as they result from the radical change in the site of the bone in relation to the gross anatomy of the cranium. Other processes lead more directly to a bone of larger size, as follows:-

1. The Corpus is lengthened by "diaphyseal growth", erosion of the sides of the back half of the Corpus serving to enlarge the For. orbitorotundum.

2. Growth of the Alae is more complicated. In early stages of development each Ala consists of two distinct rami, one anterior, the other posterior. Each grows out laterally by "diaphyseal growth". The Foramen opticum is enlarged by the considerably enhanced erosion of the sides of the rami bordering on it. At about the third day of normal, postnatal development, the rami fuse laterally and growth of the Alae is thenceforward by "external accretion" and "internal erosion". These processes are continued right down the roots of the Alae so that the roots separate further.

---

1. Owing to the precocious development of the eye-ball the For. opticum is originally large enough to take a N. opticus of almost adult size, and failure of erosion of the borders of the Foramen cannot be regarded as a cause of the rudimentary eye of microphthalmic mice.

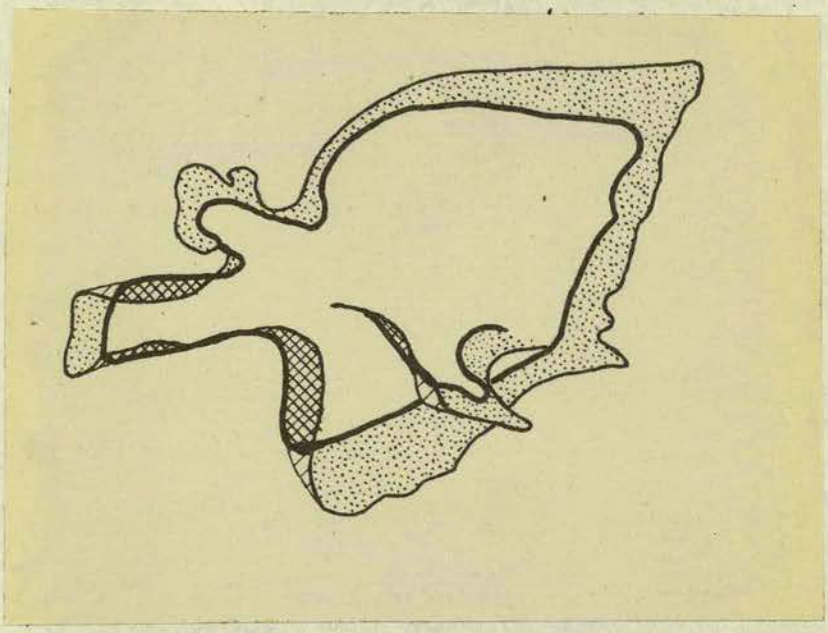

Fig. 13. Growth of the Squamosum; lateral aspect.

A most unexpected feature of the mutants is that they frequently have open optic foramina (Plate 10) and are in this respect comparable to normal mice which are only two days old. This condition may be forced on the Sphenoidale orale by the failure to enlarge the Incisura sphenoidalis of the Frontale. It would seem that the normality of the growth in one bone is dependent on the normality of the growth of the bones intimately surrounding it.

F. Squamosum (Plates 1, 2, 3, 4, 5 and 8; Fig. 13).

Indications that accretion occurs at all the sutural margins of the Squamosum is obtained from the mutants by:-

1. The occurrence of deficient bone at the frontal and parietal sutures (probably partially due to failure to remodel the underlying bones) and also in the Processus caudalis.

2. By the crumpling of the Proc. caudalis (such crumpling - see Plate 5 mi/mi and gl/gl and Plate 8 mi/mi only - can surely occur only in soft, membranous bone). and

3. By failure of "diaphyseal erosion" of the Proc. postglenoidalis.

Erosion of the cerebral surface (Plate 8, gl/gl especially) is probably chiefly associated with the extension of the sutural overlaps with the Parietale, Frontale and Alae temporales and also with the "diaphyseal growth" of both the Proc. postglenoidalis and the Proc. caudalis. The absence of erosional anomalies on the upper and lower surfaces of the

root of the Proc. zygomaticus is cause for regarding it as a fixed point in the bone's growth. Figure 13 is constructed from superimposed drawings with all the above facts in mind.

This diagram shows three further features of interest. The first is the relatively rapid growth of the inferior border which, together with the growth of the superior border of the Alae temporalis, lifts the Squamosum bodily up the side of the cranium. This movement incidentally raises the posterior root of the Arcus zygomaticus and, by bringing it into a more horizontal position, causes it to stand out further from the side of the skull. The latter change is assisted by "unilateral growth" of the Proc. zygomaticus by lateral accretion and medial erosion.

Secondly, that the Proc. zygomaticus grows in length by "external accretion", directed slightly away from the side of the skull, at its suture with the Zygomaticum. External accretion also occurs at the anterior border of the Fossa mandibularis.

Lastly, the Fissura squamotympanica (Green's 1935; Postglenoid foramen) is enlarged by external erosion. This is the only major site of erosion around the margins of the Squamosum.

G. TYMPANICUM (Plates 3, 4, 5 and 6; Fig. 14)

The Annulus tympanicus, which supports the Membrana tympani is the only ossified part of the Tympanicum in the newborn mouse. On the 7th day ossification spreads uniformly

medially, but laterally only from the posterior angle. Three days later it spreads laterally from the anterior corner also. The two lateral ossifications meet on the fourteenth day - the stage attained by the microphthalmic bone drawn in Plate 6. Thenceforward constriction of the mouth of the meatus (Porus acusticus externus) proceeds chiefly from the anterior margin by "internal accretion". When this temporary phase of rapid ossification is over the Meatus is then enlarged by "internal erosion". It is just another sign of the mutants' retardation, therefore, that the horizontal diameter of their Pori is larger than in normal mice of the same age. The roughness of the inner edges of the grey-lethal Tympanicum drawn in plate 5 is due to infiltration of the blastemic membrane by bone spicules; and not to attempts at erosion.

On the other hand, even at the stage of development under consideration, the Porus does increase in "height". This is effected by lowering the floor of the Bulla tympanica. "Internal erosion" and "external accretion", particularly active ventrally, posteriorly and ventro-medially, cause the Bulla to bulge in these directions, and there are corresponding signs of osteoclastic action in the mutants. The Tuba auditiva ossea (Eustachii) grows medially by "external accretion".

Pitting of the bone is elsewhere atypical of osteoclast action, but is characteristic of newly formed auditory bone prior to its receiving a covering of periosteal bone.

The rotation of the Bulla, mentioned in the general account of the skull's growth, does not result from the growth

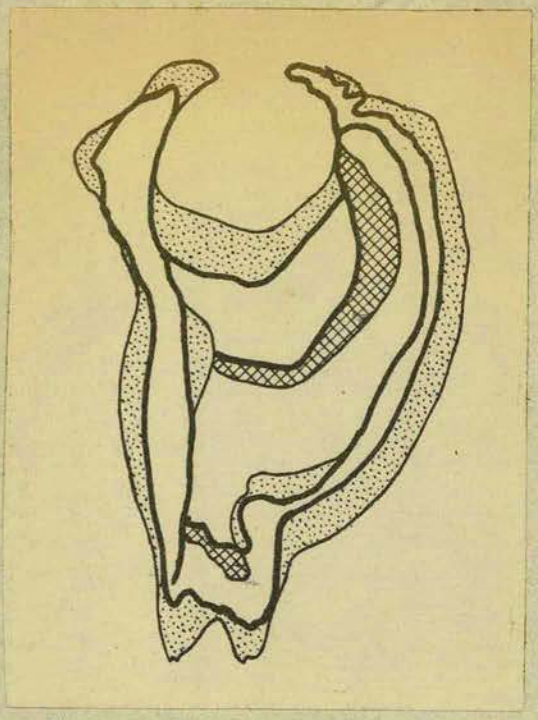

Fig. 14. Growth of the Tympanicum; superior aspect.

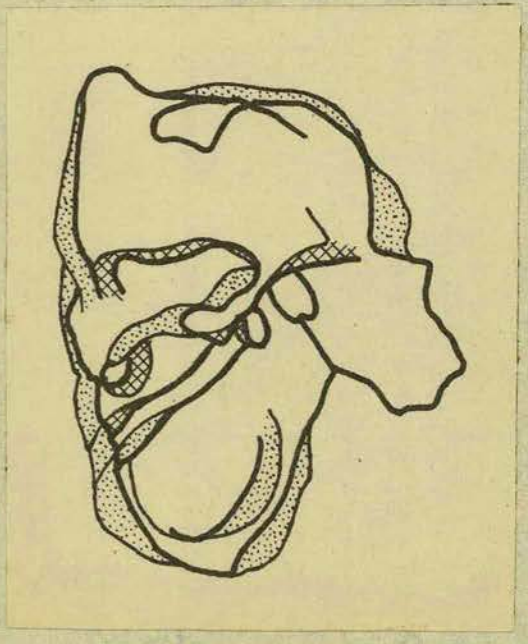

Fig. 15. Growth of the Perioticum; inferior aspect.

processes described above; but is imposed on it by changes of form in the surrounding bones (Sphenoidale aborale and Pars basilaris ossis occipitis).

H. PERIOTICUM: (Plates 3, 4, 5 and 6; Fig. 15).

Like the Tympanicum, very little of the Perioticum is ossified at birth. It is preformed in cartilage but bone rapidly replaces the cartilage between birth and 10 days, although even at 10 days the Proc. stylomastoideus is still incomplete (cf. grey-lethal, Plate 6). In consequence of such late ossification, there are few of those erosional anomalies in the mutants which are helpful for the superimposition of the drawings for analysis. Nevertheless, some information can be gathered on the bone's growth. In the mutants there are signs of intense attempts to erode the lateral wall of the Fossa parafloccularis from within; and, especially on that part of the bone covered by the Squama occipitis, a lack of accretion without. "Unilateral growth", in a lateral direction, of the lateral wall of the Pars mastoidea is thereby indicated. Osteoclast action is, in fact, visible on the whole cerebral surface of the mutant Fossa parafloccularis. That directed medially suggests that the cochleal portion of the bone also migrates by "unilateral growth" - but in a medial direction. In the more developed microphthalmic there is a considerable lack of erosion along the upper side of the Processus mastoideus indicative of ventro-posterior "unilateral growth" for this process. In grey-lethals the Processus stylomastoideus is very incomplete, and the cochleal

region underdeveloped. Figure 15, constructed from the drawings with these observations in mind, shows that "external accretion" in fact occurs at all borders of the Perioticum. The Tegmen tympani, eroded from its dorsal surface, must undergo "unilateral" accretion ventrally. The Fenestrae cochleae and vestibuli are formed in bone by the flow of bone around them, and are subsequently filled in by "internal accretion". (These changes were observed in an age series of alizarin clearances.) However, in the depicted grey-lethal and to a less extent the microphthalmic, these Fenestrae are smaller than normal so, after the completion of initial ossification, in which "internal accretion" plays a part, the Fenestrae are then enlarged by "internal erosion". The shape of the "Fenestra flocculi" (N.B.) even in normal mice, is so variable - varying from a small circle via an egg-shape to a distinct comma - that no information can be gathered on its growth from the comparison of drawings. Its shape, but not necessarily its size, is determined by the extent of deficient initial ossification. At the stage of development reached by 21 days ossification of this region of the grey-lethal bone is only just completed.

I. MALLEUS (Plates 5 and 7; Fig. 16).

Ossification of the Malleus, complete 7 days after birth, is centered between the Proc. longus and the Capitulum. It is therefore in this region that failure of erosion in the mutants is most noticeable. Growth of the Malleus is characterised by a number of local torsions of one part of the

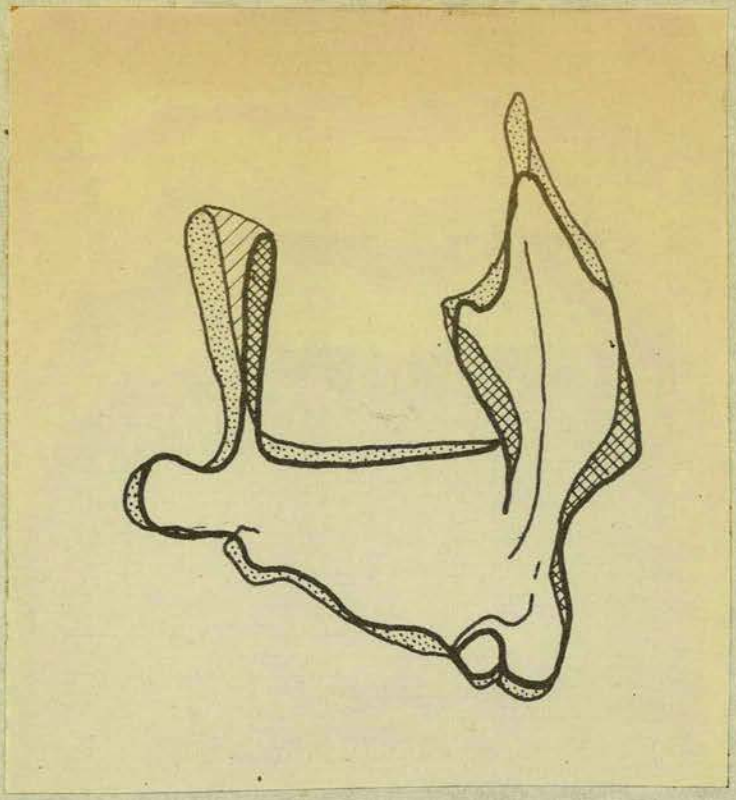

Fig. 16. Growth of the Malleus; medial aspect.

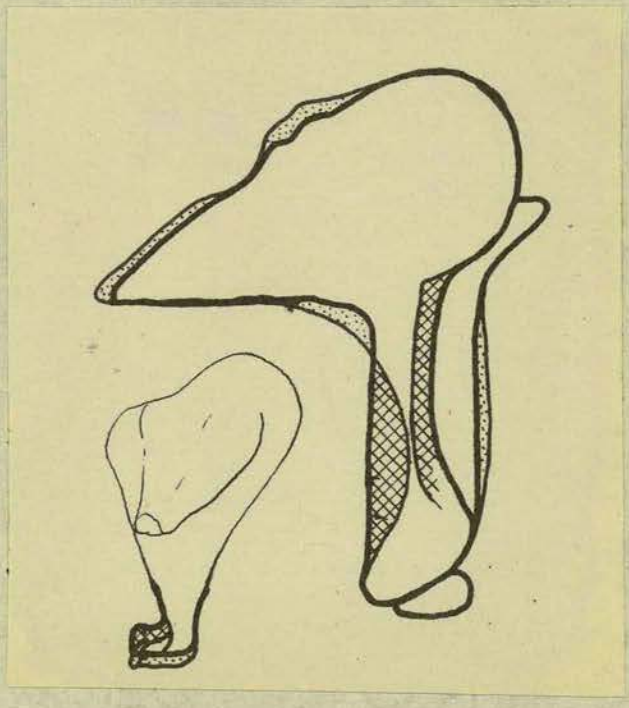

Fig. 17. Growth of the Incus; lateral aspect. Inset, growth of the Processus lenticularis; posterior aspect.

bone relative to another. For example, the Manubrium rotates away from the Proc. anterior and also rotates laterally; the ventro-posterior part of the Collum rotates laterally relative to the dorsal part of the Collum. Each involves "unilateral growth". A fourth torsion results from medial accretion to the Capitulum (posteriorly) and from medial erosion of the root of the Proc. longus. "Diaphyseal erosion" occurs on the anterior borders of the Capitulum and on the posterior margins of the Proc. longus. The root of the Proc. longus is very heavily eroded because it becomes incorporated into the antero-dorsal region of the Collum - to which it is set at right angles. "Diaphyseal erosion" also follows in the wake of accretion to the ventral surface of the Proc. muscularis and in the wake of accretion along the anterior and posterior edges of the Collum. The Proc. longus is rigidly fused with the Tympanicum by 21 days.

J. INCUS (Plate 7; Fig. 17)

The Crus longum of the Incus grows ventrally by "diaphyseal growth". The component of "diaphyseal erosion" coincides with a mild, anteriorly directed "unilateral migration" of the middle region of the Crus. The Lenticulare, and the small Processus lenticularis supporting it grow downwards by "unilateral growth" thus maintaining their position at right angles to the tip of the Crus longum. The Crus breve grows obliquely downwards and posteriorly by "external accretion" occurring at both its upper and lower surfaces. The crest on its lateral side is remodelled, and is bent into an S-shape by

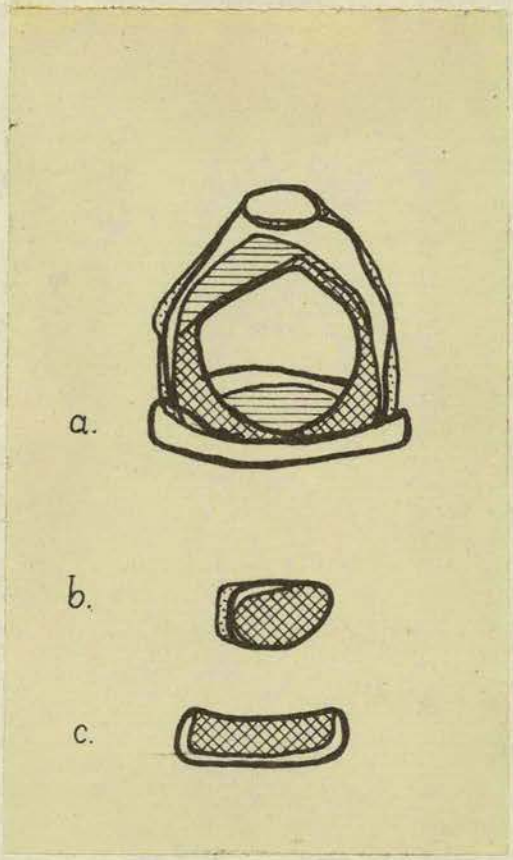

Fig. 18. Growth of the Stapes. (a) medial aspect. (b) cross-section of the Crus anterium. (c) cross-section of the Basis. (Horizontal shading indicates deep erosion of the surface seen in optical view.)

"unilateral" movements - downwards near the site of primary growth, and upwards distal to it. Some "external accretion" takes place on the upper surface of the Corpus incudis though this part probably grows chiefly by the incorporation of the roots of the Crura longum and breve.

K. STAPES: (Plate 7; Fig. 18).

The Stapes grows by "external accretion" occurring at more or less equal rates around its periphery. Failure of "internal erosion" is the cause of the mutant Stapes having a solid Basis and pillar-like Crura; in normal animals, these are very delicate structures and crescentic in section.

The oddity of the mutant bone's shape is inadequately explained by the abnormality of these processes alone. To a very large degree it is owed to the manner of the progress of ossification. Ossification spreads from the Basis, up the Crura anterium and posterium, to the Capitulum. If, for the moment, only the lower half of the bone is considered, it can be seen that there will be a grading in the extent of the erosional anomalies from the point of ossification (where most time will have elapsed for failure of erosion to express itself) to the region where least time has elapsed since its formation in bone. That is: from the Basis to the point of transection of the Crura by the horizontal diameter across the Foramen stapedis (N.B.). However, above this diameter, the Crura again becomes progressively abnormal. For, as ossification spreads, there is here an ever increasing tendency for the bony

part of each Crus to expand and unite in the formation of the Capitulum of the Stapes. Hence, the upper halves of the Crura demonstrate a condition similar to that where growth is "diaphyseal".

It is of interest that the grey-lethal Stapes drawn in Plate 7 is less abnormal than the microphthalmic one. This point is treated in the Discussion (p.124).

L. INTERPARIETALE: (Plates 1, 2, 3, 4 and 5).

"Centrifugal growth" brings about the elongation of the Interparietale in the transverse axis of the skull. "External accretion" at the anterior margin is suggested by the deficiency of bone at this site in some mutants. However, in spite of accretion, the Interparietale actually gets shorter, so that the posterior margin must be the site of "external erosion". Failure of proper remodelling of the mutant bone causes it to override the Squama occipitis at its ventro-posterior corners (Plate 5).

Quite the most remarkable feature of the growth of this bone is the inversion of the usual relationship between the rates of accretion and erosion at its opposite margins. The Interparietale may be larger in the long axis of the skull at eight days than it is in the adult. In this bone, therefore, "external erosion" proceeds more rapidly than "external accretion". Table 2 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

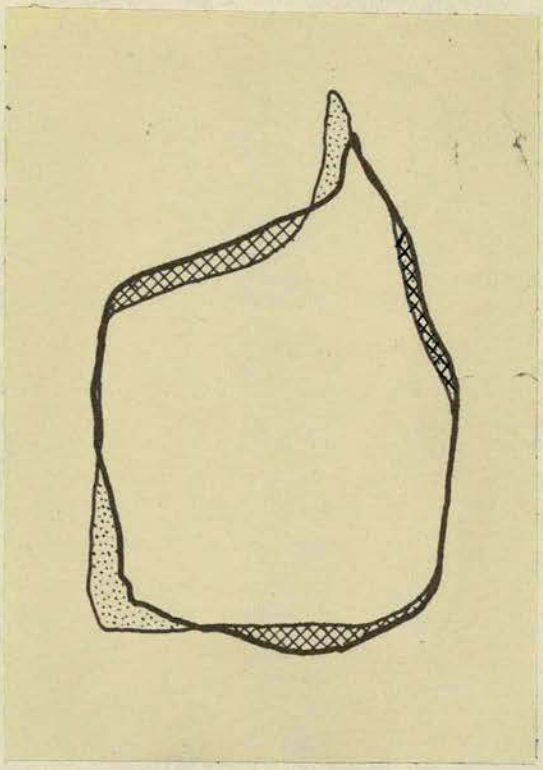

Fig. 19. Growth of the Parietale; internal or external aspect.

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~~ than by the excess growth of the skull.

TABLE 2.

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

M. PARIETALE: (Plates 1, 2, 5 and 9; Fig. 19).

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 Proc. frontalis (N.B.).

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

The thickening of the mutant bones near the periphery (cf. Fig. 5b) is not so considerable as might be expected. The situation (which results from the failure of accretion) recalls the inhibition of accretion in the Manubrium mallei. But if something similar were to occur in the mutant parietals they would be subjected to severe stresses by the more normally growing bones surrounding them. That such stresses occur in the mutant skulls is suggested by the frequency with which their skulls burst in the parietal region during preparation of the skeletons. Incidentally, the consequent buckling of the Ossa parietalia, particularly when isolated, warns us of attaching too much significance to discrepancies in the outlines of normal and mutant bones.

N. FRONTALE (Plates 1, 2, 5 and 9; Fig. 20).

The Pars nasofrontalis of the Os frontale (i.e., its horizontal lamina in the roof of the skull) grows anteriorly and posteriorly in the "centrifugal pattern". Osteoclasts are particularly active anteriorly on the cerebral surface, where they assist in raising the Proc. zygomaticus of the Frontale and so in lifting the anterior root of the Arcus zygomaticus itself. The Proc. zygomaticus is the site of the highest rate

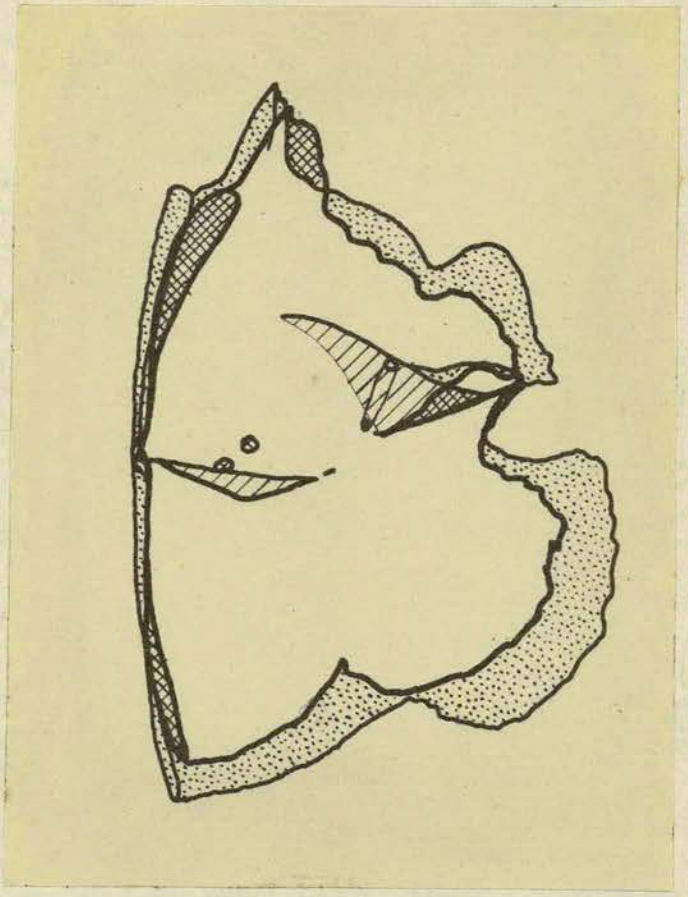

Fig. 20. Growth of the Frontale; internal aspect.

of forward growth. As this growth is directed not only forwards but also laterally, the front part of the Arcus zygomaticus is thereby carried well away from the side of the skull. The lateral wall of the Proc. zygomaticus is remodelled by "diaphyseal erosion". This smooths the curve of the anterior part of the orbit. More posteriorly, the ~~radius of~~ curvature of orbit is again reduced by lateral accretion to the Arcus superciliaris. This also results in a slight broadening of the Frontale. Along the Sutura coronalis of the bone the rate of backward accretion increases towards the mid-line. The Arcus superciliaris is formed at the junction of the horizontal (frontal) and vertical (temporal and orbital) parts of the bone. It is raised and sharpened by accretion to the outer (dorsal) surface, and there is corresponding erosion of the cerebral surface. Both are part of the "centrifugal" growth pattern of the frontal portion. The Sutura saggitalis is not involved in medial growth (though there is some dorsal - "centrifugal" - accretion).

The Crista ethmoidalis (N.B.), on the cerebral surface of the Pars orbitotemporalis is the most characteristic feature of this part of the bone in the mutants where it shows that its peripheral growth is obliquely forwards and downwards. Precise superimposition and analysis of the drawings is allowed by this fact and by the occurrence of "diaphyseal growth" of the zygomatic process and "centrifugal erosion" of the frontal portion.

The Pars orbitotemporalis is seen in Fig. 20 to grow

at all its free borders - anteriorly, ventrally and posteriorly. The Incisura sphenoidalis is enlarged partially by the regression of ventral accretion away from its borders, and partially by "internal erosion" of its anterior margin. The anterior wall of the lower end of the Crista ethmoidalis is scooped out by erosion, and the upper "tail" of the crest is carried forwards by "unilateral" growth. The For. ethmoidale moves obliquely downwards and forwards, and the "Foramen frontale" (N.B.) anteriorly by the "reversed unilateral pattern" (i.e., erosion of their leading edges and accretion to their trailing edges). In the mutants, accretion in these foramina is partially responsible for the animals' death by causing their complete closure and constricting the Nervus ethmoidalis anterior, and the vein supplying a transverse blood sinus. The Fossae frontales are moulded by local accretion and erosion processes following centrifugal accretion along the anterior margin.

O. INTERFRONTALE[1] (Plates 1, 2 and 10; Fig. 21).

[1] The Interfrontale is always present in mice from the grey-lethal and microphthalmia stocks, but it is not an unfailing characteristic of all mice. According to Keeler (1933) it is probably inherited as a single recessive. His data is however very inadequate.

The Interfrontal bone has two parts: the anterior Caput (N.B.) which is stout articulating below with the Ethmoidale, and the Pars caudalis (N.B.) which is dorsoventrally flattened. In grey-lethals and microphthalmics the Interfrontale is bent dorso-ventrally, a relic condition of that in very young mice in which the cranium bulges high above the ethmoidal region of the skull. In such mice the Pars caudalis lies in the

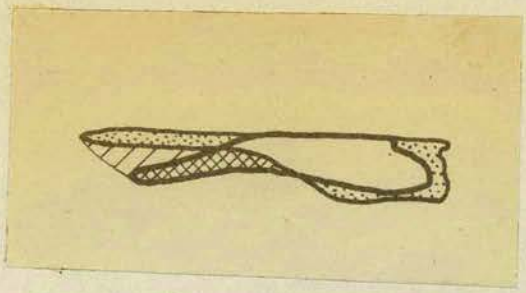

Fig. 21. Growth of the Interfrontale; lateral aspect.

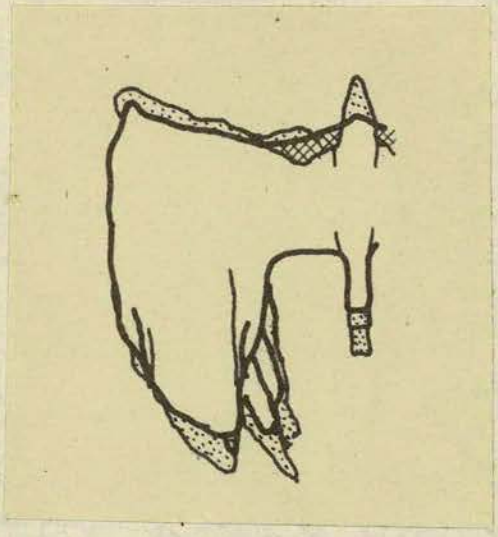

Fig. 22. Growth of the Ethmoidale; dorsal aspect.

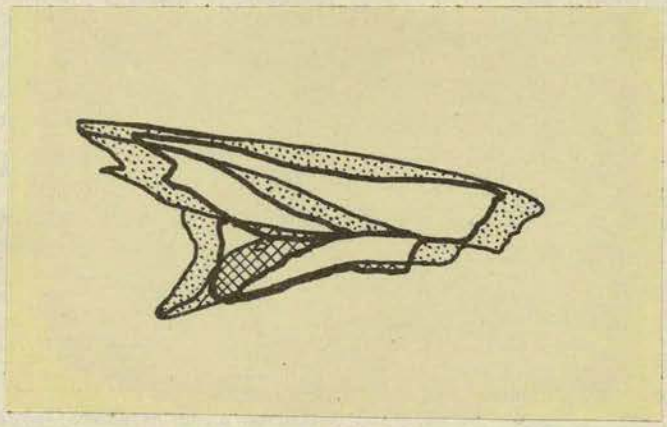

Fig. 23. Growth of the Nasale and Concha dorsalis; lateral aspect.

roof of the cranium, while the Caput slopes down towards the nasal region. In normal mice at three weeks the curves of the cranial and nasal regions of the skull are continuous and the outer surface of the Interfrontale is therefore much flatter. Because the inferior surface of the Caput is eroded from behind, it is concluded that the bone's growth in length results from "diaphyseal accretion" at the front end, though there may be some posterior accretion also. There is some further accretion on the sides and ventral surfaces of the Caput, just behind the anterior growing region. Erosion of the under side of the Proc. caudalis is indicative of the "unilateral migration" which corrects the early flexion of the bone.

The shape of the Interfrontale is so individually variable as seen from above that no further information can be gathered as to its methods of growth.

P. ETHMOIDALE: (Plate 10; Fig. 22).

The Ethmoidale is one of the most variable bones in the mouse skeleton and there is a danger of attaching too much significance to variations between the drawings of the normal and mutant bones. Nevertheless the following conclusions appear significant.

The bone is ossified in three parts: one for the Lamina perpendicularis; the others, one for each Labyrinthum. It is not altogether surprising therefore, that the stalk of the mutant labyrinthus (especially in grey-lethals) displays

failure of "diaphyseal erosion" - on the posterior edge as seen from above, which is indicative of earlier medial growth. Since fusion of the three parts occurs early (at least before 10 days - cf. grey-lethals) it is very apparent that all further growth of the Labyrinthi must result from "external accretion" and "internal erosion". The Cellulae ethmoidales have been opened by fragmentation when the skull was disarticulated.

The bone is not amenable to further analysis.

Q. LACRIMALE: (Plates 1, 2, 5 and 8).

The mutant Lacrimale is jammed against the orbital plates of the frontal and maxillary bones, and is constricted inside the unenlarged Canalis infraorbitalis. In this situation the Squama is prevented from growing even at the usual subnormal rates for mutants and is inflicted with a largely extrinsic distortion.

Only two aspects of its intrinsic growth are clear - that the Caput (N.B.) grows upwards in the "diaphyseal pattern" and that "diaphyseal erosion" of the ventral and anterior walls of the Collum (N.B.) is intense.

R. NASALE: (Plates 1, 2, 5 and 8; Fig. 23).

Growth in length of the Nasale results partly from accretion anteriorly and, by analogy with the Concha dorsalis (see below) with which it fuses around 9 days, from posterior accretion also. The middle of the medial edge is raised by "unilateral" processes so that the contour of the anterior margin rises steadily up to the posterior margin through the

medial edge. Laterally, "unilateral" processes carry forward the vertical face for sutural articulation with the Incisivum so that it maintains its place relative to the growing tip of the bone. They also raise the lateral part of the bone almost to the same level as the medial edge. As a result, the Nasale becomes less like a quartered cylinder (the shape determined by its terminal growth anteriorly) and more like a horizontal sheet; and, by occupying less of the side of the skull becomes specialised as a roofing bone.

S. CONCHA DORSALIS: (Plate 8; Fig. 23).

The Concha dorsalis grows in length, posteriorly, by "diaphyseal growth". Though there is no direct evidence of growth anteriorly, some is to be expected from analogy with the Nasale (with which it ankyloses at about 9 days). "Diaphyseal erosion" on the lateral side is extremely vigorous, for the solid, transverse, posterior block is thereby transfigured into the dainty, longitudinal, anterior lamella. A formmen which perforates the lamella near its posterior end is carried backward by the "reversed unilateral pattern" of growth.

T. VOMER: (Plate 8; Fig. 24)

The mouse Vomer consists of a "Basis" (N.B.) which is produced above into two large Alae vomeris. The Basis articulates below with the Proc. palatinus of the Maxillae. The Alae vomeris consist of two regions: The Alae septales (N.B.) are anterior, and the Alae ethmoidales (N.B.) posterior. The Alae septales are vertical lamellae lying immediately above the

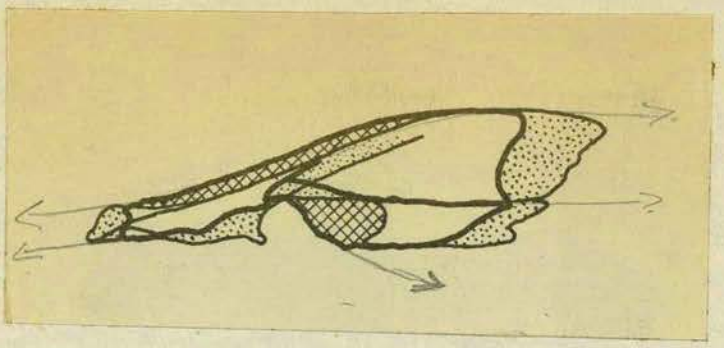

Fig. 24. Growth of the Vomer; lateral aspect.

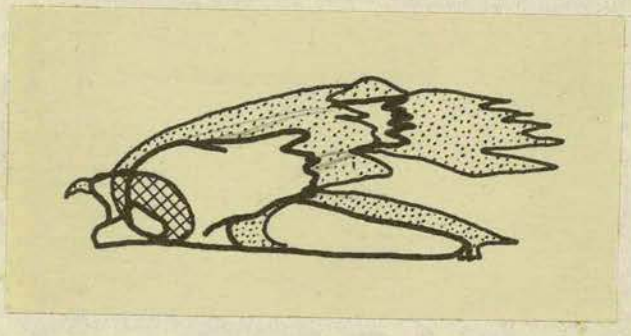

Fig. 25. Growth of the Incisivum; inferior aspect.

Basis and carrying between them the cartilaginous Septum nasi. The Alae ethmoidales are horizontal and lie at either side but distinct from the S. nasi. They articulate above with the Labyrinthi of the Ethmoidale. The ragged appearance of the Alae ethmoidales is due to their fragmentation on removal from the Labyrinthi.

The Basis and the Alae septales grow "unilaterally" downwards and forwards. Erosion from behind is intense. The upper edges of the Basis are eroded so as to convert it into the lower part of the Alae. Because the labyrinthi do not separate to any great extent, the angle between the elongating Alae ethmoidales becomes progressively smaller, by particularly active accretion on their antero-medial side. Posterior growth of the Alae ethmoidales is therefore of the "diaphyseal pattern" with strong "unilateral" tendencies, increasing anteriorly, in ventral and lateral directions. Thus there is actually medial erosion and lateral accretion at the roots of the Alae ethmoidales whereby the Basis and Alae septales are converted into them.

U. INCISIVUM (PREMAXILIA): (Plates 1, 2, 3, 4 and 5; Fig. 25).

The Os incisivum lengthens by "external accretion" which is restricted almost entirely to its posterior margins. The importance of this posterior site of growth is seen from the character of the anterior opening of the Canalis infraorbitalis in the mutants. This opening has borders on the Incisivum and on the Processus zygomaticus of the Maxilla. In the mutants, these two parts of the periphery of the opening

are widely separated while normally, the premaxillary margin of the foramen is carried backwards by "diaphyseal erosion". Independent evidence of posterior growth on the palatal surface can be inferred from the continuation forward in the mutants of the lateral margins of the Fissurae palatinae as crests of bone. These crests probably mark earlier limits of the Fissurae which, it appears, are being filled in by accretion at their anterior borders. Dorsally, at the suture with the Frontale, this backward growth is directed obliquely upwards; while ventrally growth at the suture with the palatal region of the maxillary bone, is directed slightly downwards.

Thus the Incisivum increases in height, posteriorly, simultaneously with its increase in length. However, increasing height is not restricted only to the posterior margin of the bone but, especially anteriorly, the whole dorsal edge is also a very important site of upward growth. Thereby the Incisivum not only compensates for the removal of the Nasale from the side of the skull but also heightens the skull in this region. There is a similar, though a much less significant site of accretion, on the ventral surface, just behind the incisor teeth.

Growth directed anteriorly is negligible, as is suggested by the approximately normal distance in the mutants between the Foramen incisivum and the Spina nasalis anterior.

There is no medial growth, for this would necessitate "diaphyseal erosion" of the anterior margins of the Fissurae palatinae for which there is no evidence. Thus all increase in breadth of the bone results from "external accretion" to the lateral wall. Just in front of the zone of "diaphyseal erosion" (for carrying back the anterior margin of the Canalis infraorbitalis) the lateral wall of the Incisivum grows outwards. This results in the anterior margin of the anterior opening of the Canalis infraorbitalis becoming visible from above.

Figure 25 was constructed on the assumption that no medial growth occurs, and that the Processus frontalis grows backwards with "diaphyseal erosion" of its lateral margin.)

Eruption of the incisor tooth.

In most mutant mice the Dens incisivus is confined to the anterior half of the Os incisivum. Normally, however, the tooth extends right back, through the whole length of the incisive bone, and is deeply cupped by a special part of the Maxilla. The extension of the tooth root through the posterior half of the lateral wall of the Incisivum requires extremely intense "internal erosion" of the posterior wall of the Alveolus for the tooth. The formation of the Alveolus inside the lateral wall of the Incisivum is cause for accretion both to the outside and to the inside surfaces of this wall. While extending in length, the Alveolus also increases considerably in diameter, so as to accommodate the ever-enlarging tooth. This process is reflected anteriorly by "internal erosion"

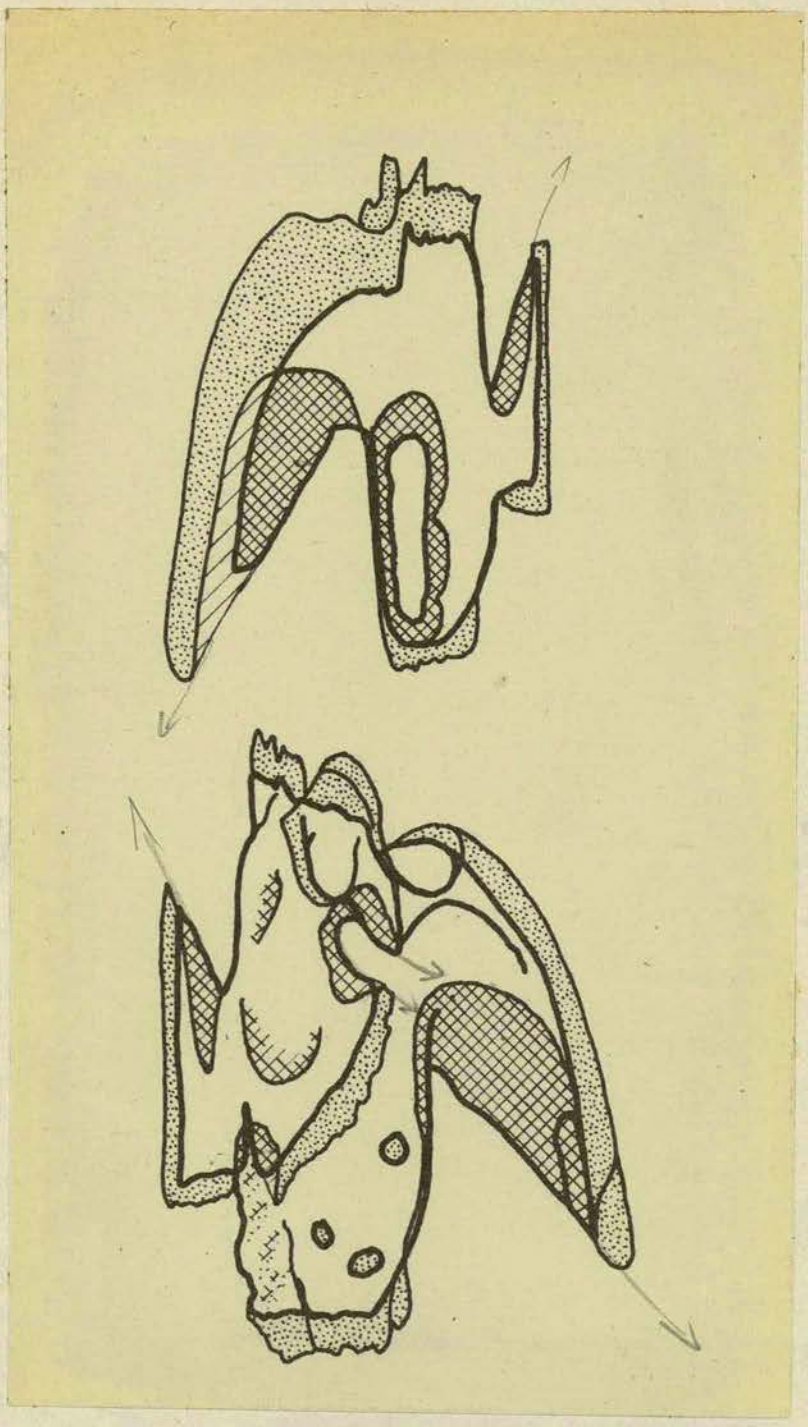

Fig. 26. Growth of the Maxilla; above - inferior aspect, below - superior aspect.

chiefly of the lateral and posterior margins of the mouth of the Alveolus. It is the failure of this erosion which prevents the eruption of the incisor teeth in the mutants.

V. MAXILIA: (Plates 1, 2, 3, 4, 5 and 11; Fig. 26).

A prominent feature of growth in the Maxilla is the "unilateral" growth of the root of the Proc. zygomaticus, simultaneously outwards, upwards and forwards. Right at the posterior tip, the Processus continues obliquely backwards by "external accretion", and it is natural that this newly formed bone requires no immediate remodelling. Thus the activity of the components of the "unilateral" movement increases anteriorly. Growth at the posterior tip of the process is directed backwards, strongly laterally and slightly upwards.

Projecting downwards from the ventro-antero-lateral border of the Proc. zygomaticus (Crista facialis) is an obliquely vertical flange of bone forming the lateral wall of the Canalis infraorbitalis, and merging into the Corpus of the Maxilla just in front of the Dentes molares. Erosion of the ventral border of the Proc. zygomaticus transforms it into the upper region of this flange. The flange grows outwards by "unilateral growth" - erosion of its medial side serving to broaden the C. infraorbitalis. In harmony with the Proc. zygomaticus, its growth anteriorly is also directed upwards and outwards.

The Proc. frontalis, which bridges the C. infraorbitalis between the Proc. zygomaticus and the Lamina infraorbitalis, grows directly upwards, "unilaterally". The "unilateral erosion"

of its ventral border serves to heighten the C. infraorbitalis.

The C. infraorbitalis is not enlarged ventrally or medially.

The Lamina infraorbitalis, separated from the L. orbitalis by the Incisura lacrimalis, grows rapidly upwards and forwards. The L. orbitalis grows equally rapidly upwards and backwards. The Inc. lacrimalis is enlarged mostly by "diaphyseal erosion" of the posterior border of the L. infraorbitalis, though "diaphyseal erosion" of the anterior border of the L. orbitalis also contributes to its development. The basal portion of these Laminae are moved sideways by "unilateral growth" which allows the expansion of the Fossae maxillares (N.B.).

Attached to the medial side of the L. infraorbitalis there is normally a deep, horizontal, crested, delicate cup, the Alveolus incisivus superior, which houses the tip of the root of the incisor tooth. In the mutants, however, it is represented by an enormous, crested, horizontal sheet. Normally, this cup develops by "external accretion" on its dorsal, ventral and medial surfaces, and anterior edges, together with strong internal erosion from in front. Its extremely subnormal development in the mutants is possibly a result of the absence of induction from the incisor root, which, in these animals, is usually trapped in the fore part of the Os incisivum.

The Proc. palatinus of the Maxilla grows at equal rates anteriorly and posteriorly in the "centripetal pattern".

There is also a little medial accretion. "Centripetal erosion" of the convex, upper surface of the Proc. palatinus takes the form of "diaphyseal erosion"; for there is very intense erosion of the antero-medial border of the Processus, directed particularly posteriorly. This serves to enlarge the Fissura palatina by erosion of its posterior border, and to broaden it by erosion of its postero-medial border. The lateral borders of the Fissurae palatinae are not eroded. There is some "centripetal accretion" to the ventral, concave surface of the Processus. This site of accretion spreads over the palatal surface on to the body of the maxilla and so lowers and levels the roof of the palate.

The Fossae maxillares in the floor of the nasal cavity are modelled by erosion of the floor of the cavity (compensated by the just mentioned palatal accretion) and by erosion of the bases of the Ll. orbitalis and infraorbitalis (the erosion here being part of the "unilateral" growth of these laminae).

The notch for the For. palatinum majus grows by erosion of the medial border of the palatal process and by dorso-lateral erosion of the base of the L. orbitalis. "Internal accretion" at its anterior end completes the processes involved in its backward migration.

The troughs and crests on the "rough" for articulation with the Os Palatinum are probably formed by alternating zones of accretion and erosion which migrate backwards in harmony with the "centripetal accretion" of the posterior end. The failure

of erosion of the mutant Palatinum opposite the sites of accretion in this region of the Maxilla may cause the mal-development of the crests in the "rough" of the mutant Maxilla.

Eruption of the molar teeth.

Eruption of the Dentes molares involves gross changes in the Limbus alveolaris of the Os maxillaris. First, the foramina above the tooth roots are closed by "internal accretion" - thereby completing the initial ossification of the maxillary bone. In the young bone, as in grey-lethals and most microphthalmics, all the margins of the alveoli, with the exception of the posterior one, are turned inwards so as partially to cover the molar teeth. The walls of the alveoli must therefore be eroded before the first and second molars can begin to erupt; whereas the third molar in mutants may occasionally manage imperfectly to erupt without any evidence of erosion[1]. The all-round erosion of the internal walls of the alveolus causes its enlargement both in length and breadth. Erosion of the posterior wall is associated with accretion to the posterior border of the alveolar region of the bone.

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1. Very occasionally the molar teeth of microphthalmics erupt sufficiently to break through the gums. Once again, however, (cf. section on the incisor teeth in the Discussion) there is little evidence that there has been successful erosion, but only an indication that development of the teeth has been sufficiently precocious for them to break through the fragile inturned portions of the alveolar borders shortly after their initial ossification. The maxillary bone of the microphthalmic skull drawn in plates 1 - 5 presents this picture - of broken walls of the alveolus - though none of the teeth has got so far as to perforate the gums.

With the passage of the bulky crowns from the body of the Maxilla, the uppermost part of the alveolar region becomes narrowed down and reduced in height and the alveoli become shallower. This results from dorsal, especially dorso-lateral and dorso-medial erosion accompanied by "internal accretion" in the roof of the alveoli. This "unilateral" lowering of the alveoli continues into old age so that in old mice the teeth are to be seen rooted in a very shallow and broad bed which encroaches on the palate to a quite considerable extent.

W. PALATINUM: (Plates 3, 4 and 11; Fig. 27).

Erosion of the anterior border of the lower end of the Foramen palatinum majus suggests that the Pars horizontalis of the Palatinum grows anteriorly by "diaphyseal growth". Similarly, erosion of the antero-lateral border of the Lamina pterygopalatina (N.B.) indicates "diaphyseal growth" of this plate in a posterior direction. These inferences are corroborated by the existence (normally) of "diaphyseal erosion" of the lateral side of the Proc. orbitalis, anteriorly, and of the dorsal margin of the Proc. sphenopalatinus, posteriorly. Moreover, in ventral view, the Pars horizontalis, which then appears at a higher level than the Lamina pterygopalatina, spreads posteriorly over it and the articulation for the anterior end of the Pterygoideum, and extends along the medial border of the Lamina pterygopalatina. The articulation for the anterior end

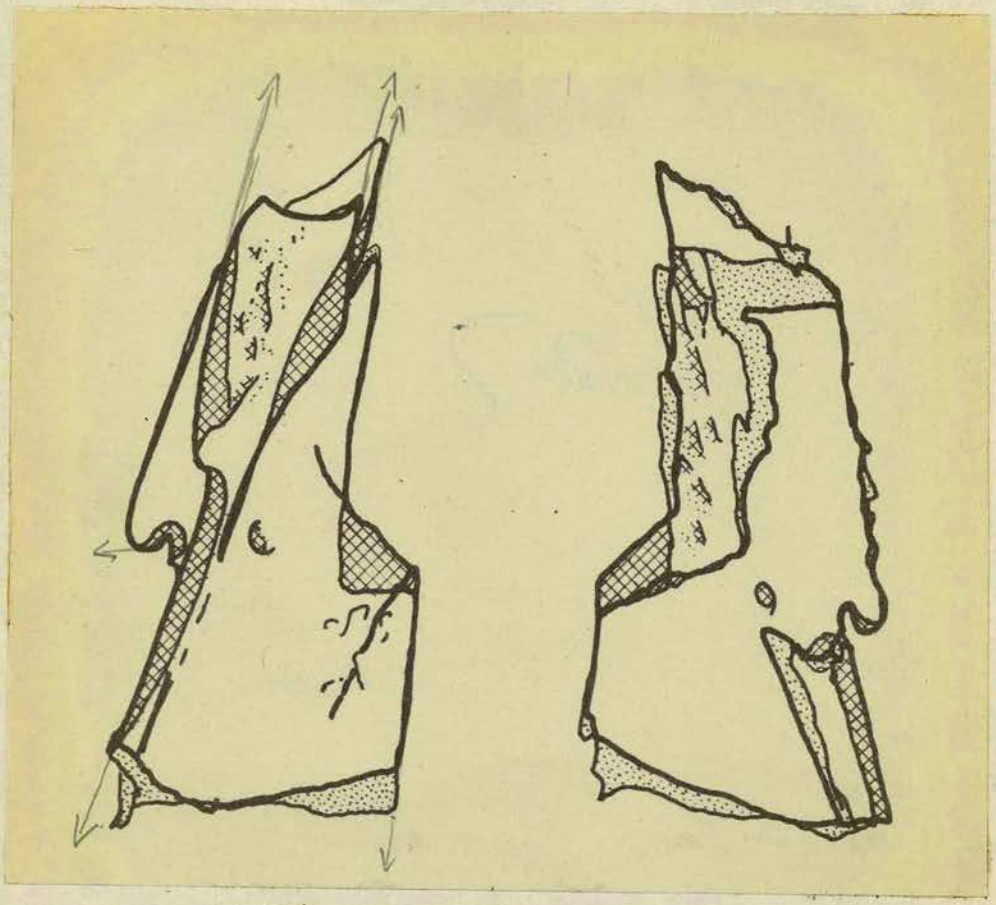

Fig. 27. Growth of the Palatinum; left - superior aspect, right - inferior aspect.

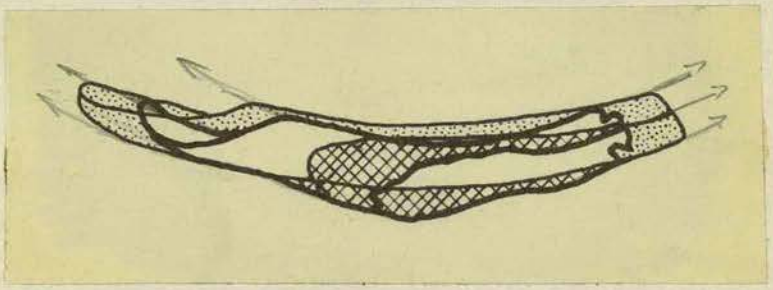

Fig. 28. Growth of the Zygomaticum; lateral aspect.

of the Pterygoideum grows posteriorly while the medial border of the Lamina pterygopalatina migrates medially. This latter process brings the medial articulation for the Pterygoideum into a more vertical position. The palatal surface of the Pars horizontalis subsequently becomes pitted, probably by a combination of highly localised erosion and accretion processes.

Growth in breadth occurs chiefly by accretion to the lateral side of the Pars horizontalis (which ventrally tends to spread laterally over the articular surface for the alveolar region of the Maxilla). Almost the whole length of the Pars perpendicularis (i.e., from immediately behind the anterior zone of diaphyseal erosion to the end of the Proc. sphenopalatinus) is subject to a lateral movement by "unilateral growth".

There is evidence of very slight medial growth. For example, the mutants consistently show a clumsiness of the posterior Spina nasalis aboralis, which could be rectified by a little erosion of its lateral border; and there are signs that the mutants lack "diaphyseal erosion" which extends the whole length of the nasal surface of the Pars horizontalis, just lateral to its medial edge.

The dimensions of the Meatus nasalis are enlarged by:-

1. "Unilateral erosion" of the medial side of the Pars perpendicularis.

2. "Unilateral erosion" of the roof of the Meatus (the corresponding component of accretion heightens the

Proc. orbitalis).

3. "Diaphyseal accretion" to the medial edge of the Pars horizontalis.

and 4. "Diaphyseal erosion" of the nasal surface of the Pars horizontalis.

It is lengthened by accretion to the anterior borders of the Pars horizontalis and Proc. orbitalis. Probably there is no backward growth of the Meatus nasalis.

The Foramen palatinum minus in the mutants is either obliterated or is represented only by a pin prick. It should be enlarged by "internal erosion" with "reversed unilateral growth" conducting it laterally.

The crests and depressions in the region of articulation with the Alae temporales of the Sphenoidale aborale are carried postero-laterally by a series of "unilateral" movements.

In the mutants, the articular surface for the alveolar region of the Maxilla is maldeveloped partly owing to the palatine bone's intrinsic failure in erosion, and also because failure of erosion in the Maxilla inhibits accretion in corresponding parts of the Palatinum. Normally, accretion and erosion processes alternate, the one with the other, and shift anteriorly.

X. ZYGOMATICUM: (Plates 1-5; Fig. 28).

The Zygomaticum, as seen in lateral aspect, grows

"centripetally". At the two ends, peripheral growth takes the form of the "diaphyseal pattern"; for anteriorly, the ventral border of that part of the Proc. maxillaris which projects above the articular surface for the Maxilla is eroded just behind the growing region; and posteriorly, the medial side of the Processus temporalis is very heavily eroded - so heavily, indeed, that there are very marked signs of attempts at it in the mutants. The Corpus of the Zygomaticum (which, at least in lateral view, can be regarded as having posterior and anterior borders which limit the sutural overlaps of the Squamosum and Maxilla) migrates backwards in the "unilateral" pattern. The erosion of its anterior border serves to enlarge the lateral articular surface for the maxillary bone.

The whole length of the dorsal edge of the Zygomaticum is a site of accretion. This is a component of the ventro-dorsal "unilateral" movement which is the other component of "centripetal growth". The corresponding component of erosion, however, is restricted to the upper limit of the lateral articular surface for the Maxilla, and to the inferior border of the Proc. maxillaris. Posteriorly, the erosional trend is obliterated by the extension of the peripheral accretion right round the end and on to the ventral edge.

The bone broadens by external accretion to the lateral surface. There is no sign of any significant accretion to the medial surface.

The mutant bones display a strange medial bending

of the tip of the Proc. maxillaris, of such magnitude, that extreme signs of attempted erosion in the mutants would be expected to accompany it if it were due to intrinsic failure of normal growth processes. Actually, there is no sign of erosion, either on the medial or on the lateral surface. It must therefore be concluded that this feature of the mutant bones is unrelated to failure of intrinsic growth processes, but is probably imposed on the bone by extrinsic factors such as abnormal curvature of the orbital surface of the Proc. zygomaticus of the Maxilla, with which the tip of the Zygomaticum is in intimate contact.

THE MANDIBULA (Plates 12 and 13; Fig. 29).

In spite of its quite complex shape erosion enters very little into the growth processes of the Mandibula. Apart from the removal of the walls of the Alveoli as a prerequisite of the eruption of the teeth (a process which anyhow, is of only short duration and of no significance in the growth of the bone as a whole) there is evidence of erosion along only one small section of those borders which can be seen in side view. Even at this site - the more anterior part of the Margo interalveolaris - erosion has only minor effects, since the mutant bones are not grossly abnormal on this score. It can be concluded, therefore, that the remaining regions of the outlines of the side view are either "indifferent areas" (Kolliker 1873) or are sites of "external accretion". Quite accurate estimates of the rates of accretion along these margins can be made if the outline

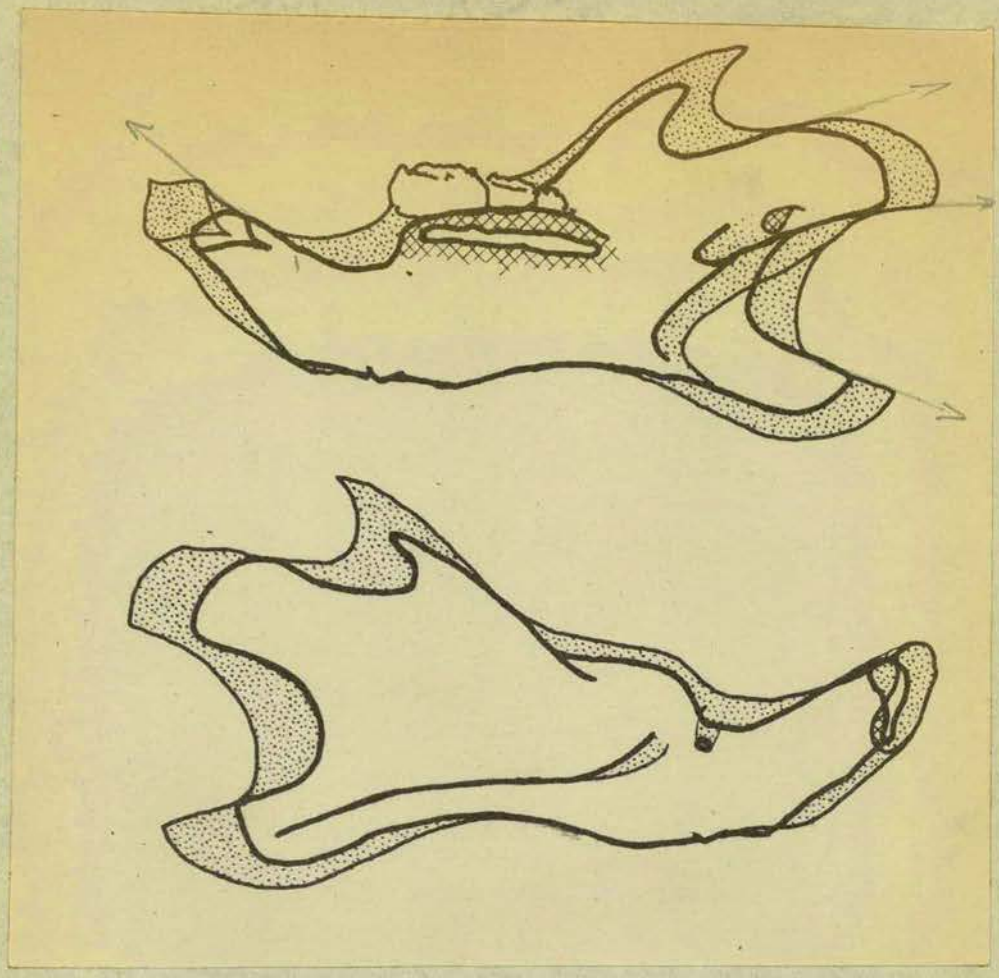

Fig. 29. Growth of the Mandibula; above - medial aspect, below - lateral aspect.

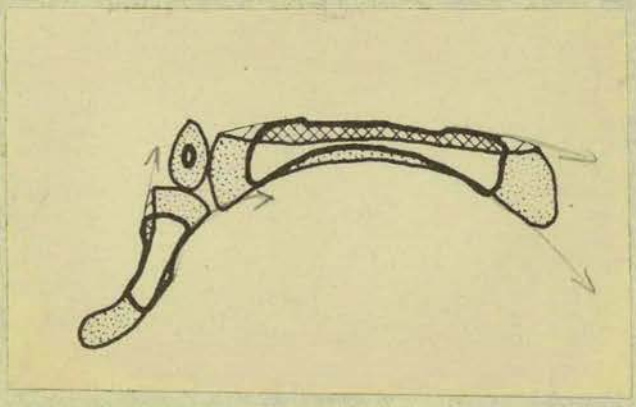

Fig. 30. Growth of the Hyoideum; inferior or superior aspect.

drawings of normal and mutant bones are correctly superimposed. Three decisive factors for this superimposition are the primary directions of growth indicated in the mutants for the Margo interalveolaris, for the inferior border of the Proc. condyloideus and for the superior border of the Proc. angularis. The directions of growth are obtained by the projection of these radii outwards. It can then be arranged, as in Figure 29, that corresponding parts of the normal outline fall on them.

Figure 29 establishes that the anterior part of the Margo interalveolaris is the only site of regular erosion of those borders which can be seen in side view; and demonstrates that almost all the rest, including the alveolar regions, are active sites of accretion. Generally, the rate of growth backwards is faster than that forwards; and, except perhaps posteriorly, growth upwards is more important than growth downwards. But now to detail.

The Corpus of the Mandibula grows obliquely upwards and forwards (there is no ventral growth) by "external accretion" to its antero-dorsal and antero-ventral surfaces. As already mentioned, there is some slight "diaphyseal erosion" along its dorso-posterior border, although the effects of this are normally soon nullified by upwardly directed accretion along the rest of the Margo interalveolaris. This same trend in upward growth, carried on to the lateral side of the jaw, raises the For. mentale. Originally (compare with microphthalmia in Plate 13) the For. mentale opens laterally on to the side of the Mandibula. But

by the concentration of accretion on the lateral side of the jaw around the lower borders of the foramen it is turned round so as to open upwards. (The For. mentale of both grey-lethals and microphthalmics is occasionally grossly enlarged (see Plate 13, g1). In these cases the soft root of the Dens incisivus, being unable to grow backwards because of the failure to absorb the posterior wall of its socket, has found a way into the Canalis mandibularis and so out of the For. mentale. Here it forms a misshapen, calcified lump (Grüneberg 1935). The borders of the foramen apparently grow around it so forming the crater.) The walls of the Alveolus incisivus inferior are eroded from within, although the edges of the opening are sites of accretion for its forward and upward growth.

After the Dentes molares are allowed to erupt by the removal of the walls of the molar crypts, the process is reversed, and the whole dorsal surface of the Pars molaris becomes a site of rapid accretion. This remarkable feature, of accretion to the alveolar margins accompanied by "internal accretion" in the bottoms of the crypts, has been observed both by Kölliker (1873) and by Brash (1934) in the ox and pig respectively. The molar teeth, with their sockets, slowly slide obliquely forwards and laterally; the posterior part of the lateral alveolar border becomes continuous with the anterior edge of the Proc. coronoideus; and the anterior part projects beyond the general level of the side of the jaw.

Much of the lateral surface of the jaw undergoes accretion. The Crista masseterica moves forward with the Pars alveolaris by accretion to its more anterior slopes.

The upward migration of the molar teeth leaves room in the base of the mandible for the backward extension of tip of the root of the Dens incisivus to its adult location in the base of the Proc. angularis. Earlier in its development, however, it is confined, as it is in the mutants, to that part of the jaw in front of the For. mentale. The tunnelling for the socket of the Dens incisivus is accompanied by ventral accretion in front of the Proc. angularis and by a marked swelling showing on the lateral and medial surfaces of the bone.

The Proc. coronoides grows upwards and backwards by "external accretion" to its posterior and superior (which is also its anterior) borders. Its more basal parts migrate medially by a "unilateral" movement.

The backward growth of the Proc. condyloideus is in the "diaphyseal pattern". The medial side and the upper half of the lateral side of the Collum are heavily eroded. The dorsal edge of the Collum is a site for upward growth; the ventro-posterior edge, a site for ventro-posterior growth. On the medial side the latter continues over the base of the Proc. angularis which thereby becomes incorporated into the base of the Proc. condyloideus. Just anterior to the zone of "diaphyseal erosion" on the medial side, accretion is again especially active; it is directed obliquely backwards and

carries the Foramen mandibulare back with it.

The Proc. angularis grows strongly backwards and downwards. There seems to be no secondary migration of the continuation of the Crista masseterica on its lateral surface.

THE HYOIDEUM: (Plate 10; Fig. 30).

Throughout the period up to the age of three weeks the Hyoideum is in an early stage of development, for the Cornua majus and minus have not passed beyond the phase of rapid ossification of the predmodelled cartilages. Nevertheless, the mutants' deformities do reflect some more permanent features of the bones' growth.

Thus it is very apparent that the extremities of the Basis of the Hyoideum grow in the "centripetal pattern" in a slight ventrodorsal curve and in a stronger antero-posterior curve. The antero-posterior curvature of the bone is flattened by that "unilateral" movement which is a component of the "centripetal" pattern of growth.

In the mutants the distal end of the Cornu majus is tipped by a quite considerable length of cartilage. However, in three-week-old normal mice this cartilage is absent. The conclusion is drawn that distal growth by ossification of this cartilage is quite rapid between the formation of the ossification centre and three weeks. But there is also proximal growth, for a site of "diaphyseal erosion" of the lateral border of this end of the bone is indicated in the mutants. Similar anomalies distally and on the medial surface show that at

this end, too, growth is in the "diaphyseal pattern".

At least up to the third week of normal development the Cornu minus grows only by "external accretion". Ossification has not yet begun by the 10th day of normal development as is shown by the grey-lethal bone drawn in Plate 10.

Summary

It is seen that the bones of the head each presents an individual and complicated pattern of growth.

THE VERTEBRAL COLUMN: (Plates 14-18; Figs. 31-33).

With regard to the elucidation of their individual growth processes, the vertebrae are disappointing material; for during the first three weeks of post-natal life their growth processes are radically changed. The change is necessitated by the transition from three-piece to unit construction. As a result, each vertebra presents a compound and confused picture of two entirely different and successive patterns of growth. However, the vertebrae have one redeeming feature - that they are constructed on the same general plan. With only three exceptions, each has originally two lateral Arcus vertebrae and a single median Centrum or Corpus. The Atlas has no Centrum, but an Arcus ventralis instead; the Epistropheus (Axis) has two centra (the more anterior, the Dens, being the ortogenetic centrum of the Atlas); and lastly, the more posterior caudal vertebrae have no Arcus and consist of centra only. Because of the repetitive nature of the plan of construction of the vertebrae, it seems likely that their

growth processes, too, are fundamentally the same. It is thus likely, and perhaps legitimate, to form a complete picture of the growth of a "generalised vertebra" from snippets of information about the growth of particular vertebrae. It is expected that some vertebrae are affected preponderantly by the earlier phase of growth; and that others are affected more by the later one; while yet others are affected almost equally by the two phases. It is not surprising, therefore, to find that the conclusions drawn from individual vertebrae are sometimes contradictory or equivocal. But the combined information, gathered throughout the vertebral column, can be reasonably sorted out so as to reveal the two successive patterns. The account is limited only to those vertebrae drawn, which were chosen merely to show the extent of variation in vertebral form, and not an account of the amount of information each could yield on vertebral growth.

The early phase of growth.

The Arcus vertebrae.

In the early period of growth the Arcus vertebrae has two sutures: one, dorsally, with its fellow of the opposite side; the other, ventrally, with the centrum. Both are synchondroses and are very wide in the new-born mouse, the centres of ossification being separated by broad zones of cartilage. As is to be expected, the extremities of the Arcus are active sites of growth whereby the circumference of the vertebra is increased. In the Atlas, Epistropheus, sixth

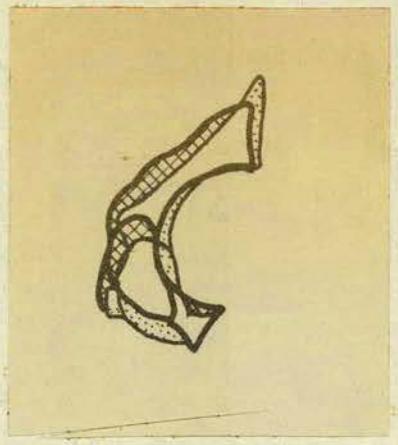

Fig. 31. Centripetal growth in the Arcus vertebrae of the Epistropheus.

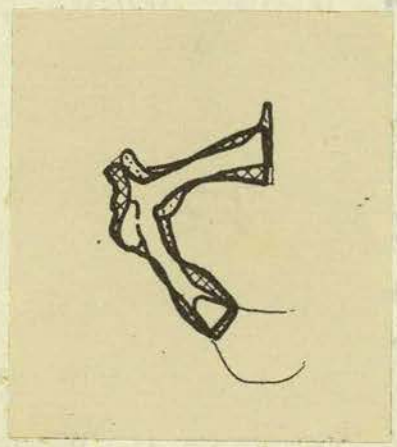

Fig. 32. Centripetal growth inthe Arcus of the seventh Vert. thoracalis. Note its diaphyseal growth at the interneural and neuro-central sutures.

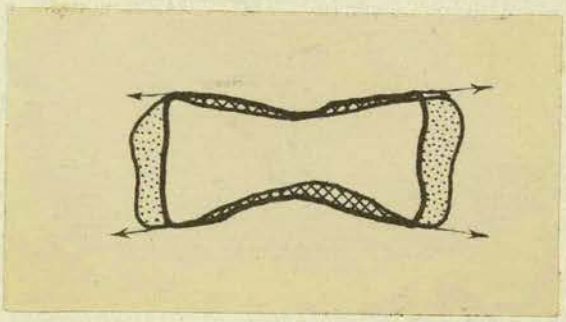

Fig. 33. Growth of the Corpus vertebrae.

cervical, and second and seventh thoracic vertebrae growth at the interneural suture (as is seen from the clumsiness of this extremity in the corresponding mutants) is "diaphyseal". There is similar evidence of "diaphyseal growth" at the neuro-central suture of the second, and especially of the seventh, thoracic vertebrae. The direction of primary growth at these sutures varies considerably between vertebrae. For example, in the seventh thoracic vertebra the direction of growth for the dorsal site is almost horizontal, while its ventral growth is directed 45° downwards. Precisely the opposite situation attains in the Epistropheus; while the second thoracic vertebra supplies the intermediate condition. In spite of these variations, it is general that growth at these sutures contributes more to the breadth than to the height of the vertebrae. This tendency towards excessive breadth is corrected by a "unilateral" movement of the more lateral parts of the Arci towards the centre of the Foramen vertebrale. In other words, growth of the Arci vertebrae is at this stage "centripetal".

For some obscure reason, the "unilateral" component of the "centripetal" pattern fails entirely in the mutants (just as, it may be remembered, it failed in the Manubrium mallei). As a result, in spite of the smaller size to be expected from their retardation, the mutant vertebrae may be even broader than the normal vertebrae, and the girth of their Arci across the horizontal diameter too narrow so that their For. vertebrales are far too wide. Thus positive proof of

"internal accretion" in normal vertebrae is to be found in the Atlas, Epistropheus, seventh thoracic and lumbar vertebrae. In all of these, in spite of their retardation, the For. vertebrale is actually broader in the mutants than it is in the normal mouse. However, owing to the excessive narrowness of the mutants' Arcus vertebrae, not all these vertebrae, but only the Epistropheus (Fig. 31), the second and the seventh thoracic (Fig. 32) conclusively demonstrate the existence of the corresponding process of "external erosion". The consideration of the dispositions of the Foramina transversaria for the Atlas, Epistropheus and third cervical vertebrae confirms the "centripetal" pattern of growth for these vertebrae; for in these, the foramina are more widely separated in the mutants than normally. It is to be concluded that in normal vertebrae the Forr. transversaria move medially by the "reversed unilateral" pattern of growth.

The Procc. spinosi ossify late. That of the second thoracic vertebra[1] 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 Arcus are still separated by cartilage. The latter develops by a 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.

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1. This spine is absent in the grey-lethal mouse figures in Plate 15. This anomaly is not necessarily due to retardation for Grüneberg (1950) has noticed that this spine is absent in about half the animals in the stock which segregates for the grey-lethal gene and which supplied the mice for the present study.

No direct information on the growth of the Procc. articulares is available from the present material, but in the first phase of their growth they are no doubt subjected to the same "unilateral" movements to which their regions of the Arcus are subjected. There is no evidence that they undergo any dorso-ventral (or ventro-dorsal) movement.

In cervical and thoracic vertebrae the Proc. transversus like the Arcus of which it is part, has two phases of growth. During the first phase, "centripetal erosion" of the Arcus around the base of the Proc. transversus converts bone from the Arcus into the base of the Proc. transversus. At the same time, the tip of the Processus (in conformity with the rest of the Arcus) is eroded. Thus the measurements across the Procc. transversi of the mutants' Atlas, Epistropheus, third and sixth cervical, and second and seventh thoracic vertebrae are very nearly equal to, and in some cases exceed, those measurements for the normal vertebrae. This feature is more significant when allowance is made for the retardation of growth in the mutants.

Now to the examination of the growth of the Arcus vertebrae in "length", i.e., growth in the antero-posterior axis. In this axis the mutant vertebrae are everywhere thinner than normally. This is indicative either of anterior accretion, or posterior accretion, or, of course, of both. There is in fact, evidence of both. Because the processes effecting growth are almost certainly independent of the partite or unit construction

of the vertebrae, there is no reason to suspect that accretion at both anterior and posterior edges does not occur simultaneously in the same vertebra. It is unfortunate, as far as the analysis of growth at these edges is concerned, that the vertebrae are more-or-less ring-shaped. It means that quite slight differences in the angle from which the vertebrae are viewed may themselves cause quite large differences in the outlines of the drawings. Such an uncontrollable possibility utterly invalidates the normal procedure for disclosing the growth processes - of superimposing outline drawings. The observations on growth anteriorly and posteriorly have therefore to be restricted to very limited sections of the circumference of the bone which can supply information independently of superimposition.

Anterior accretion occurs in the region of the Foramen vertebrale laterale of the Atlas. In microphthalmics (see side view) this Foramen is too distant from the anterior edge (owing to the failure of "reversed unilateral migration" of the foramen anteriorly). In grey-lethals, on the other hand, it is too near. This apparently contradictory difference between the two mutants does not, however, affect the conclusion. For in seven day-old normal mice the Foramen is still open anteriorly. It is closed by the flow of bone, by anterior accretion, around it. (That this in fact takes place, and not the opposite - that the foramen sinks into the Arcus vertebrae by eroding it - is proved by the existence of the foramen in the mutants in spite of erosional failure.) Thereafter the Foramen migrates forwards by "reversed unilateral

growth" as more bone is added to the anterior edge of the Arcus.

In a similar way, the position of the Foramen transversarium of the Epistropheus indicates that there is posterior accretion in this region. Just as was the case for the For. vertebrale laterale of the Atlas, the For. transversarium of the Epistropheus is formed in bone by the bone flowing around it. The Atlas, too, provides evidence of posterior accretion. The For. alare inferiore of the grey-lethal Atlas is a mere pin-prick far too near the anterior edge, and far too far from the posterior edge. Unlike the For. transversarium of the Epistropheus and the For. alare superiore, it is obviously formed early in the ossification of the neural arch and is enlarged by "internal erosion" while migrating backwards by the "reversed unilateral process". In this region, anterior accretion must be less important.

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 Arci vertebrae differ widely in this respect.

The Corpus.

While there is no indication of erosion in anterior or posterior views of the Corpora (centra), it is apparent that erosion does sometimes enter into the processes which cause its growth in length. Indeed, growth in length of the most posterior caudal vertebrae, which consist of centra only, and the centra of the second, third, and fourth sacral vertebrae is accomplished in the "diaphyseal" manner. These centra are

peculiar in that their sides and bottoms are normally highly concave. The form of all the other vertebrae is much more rectangular because the zone of "diaphyseal erosion" in the former is replaced by "external accretion" around the circumference of the bone. As far as can be estimated from the appearance of the mutant caudal vertebrae, the rate of accretion is about equal at the two ends (Fig. 33). This feature is probably general.

The Arci haemales (chevron bones)

The ragged appearance inside the V of the mutant Arci 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 A. 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 A. haemalis, each into three more-or-less independently growing pieces - the two Arcus vertebrae and the single Centrum. At varying times between one day (for the caudal vertebrae) and 14 days (for the cervical vertebrae) fusion occurs between these pieces. At this juncture growth is by no means completed and profound changes in the patterns of growth are induced. In theory it is to be expected that the "centripetal" pattern of growth of the Arcus gives way to a combination of "external accretion" and "internal erosion"; that

the direction of the "unilateral" movements of the Forr. transversaria is changed from medial to lateral; while the centrum, or the Procc. articulares, or both become subject to vertical "unilateral" migrations away from one another. Evidence of these later processes is supplied by the vertebrae themselves.

"Internal erosion" is shown by the anterior vaudal vertebrae. It is located on the more dorsal borders of the For. vertebrale and appears as a ragged edge in the mutants. Again, in the Atlas, "internal erosion" of the more dorsal section of the Arcus dorsalis (this site extends well beyond the zone affected by the "diaphyseal" component of the earlier "centripetal" growth pattern" is revealed by a trail of bony fragments lying in the For. vertebrale of the normal vertebra . (Because the preparations of the vertebrae were made as alizarin clearances from which no flesh is removed and in which only calcified tissues stain, fragments of bone removed by the osteoclasts, but not yet digested by them, can appear as stained particles located near the zone of erosion.)

On the other hand there is in no case any sign of "internal erosion" of the neural surface of the Corpus. The expected increase in the distance between the Procc. articularis and the Corpus should therefore result from an upward "unilateral movement of the former.

With the fusion of the Arcus vertebrae the Procc. spinosi embark on the second phase of their growth. The spine of the Epistropheus no longer grows in the "diaphyseal" pattern

but merely by "external accretion" to its tip. It is not until this second phase of growth that the spine of the second thoracic vertebra forms - by a local excess of upwardly directed "external accretion" in the mid-dorsal region of the fused Arcus vertebrae.

It was noted when describing the first growth phase of the vertebrae that the distance between the tips of the Procc. transversi in the mutants often approached, and in some cases even excelled, that measurement in normal mice. However, this is not always the case, even in those mutant vertebrae where, fusion of the Arcus having only just taken place, the anomalies due to failure of "centripetal growth" are maximum. In these instances (the lumbar vertebra is the best example, the Atlas a possibility) methods of growth belonging to the second phase for the Proc. transversus may overlap with those of the first phase for the rest of the vertebra. "Second-phase" growth of the Proc. transversus is "diaphyseal". The distance between the tips of the Processes in the mutant third cervical (grey-lethal only) lumbar, sacral and caudal vertebrae is far too little. "Diaphyseal erosion" can be seen just behind the tip of the Proc. transversus of the normal sixth cervical vertebra. In the sacral vertebrae it is the anterior, posterior and ventral surfaces which are eroded; while in the caudal vertebrae "diaphyseal erosion" occurs on the anterior, dorsal and ventral surfaces of the process and is accompanied by extensive accretion to the posterior edge.

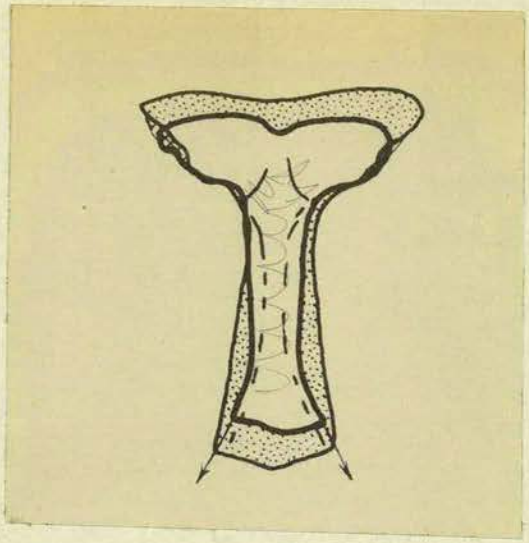

Fig. 34. Growth of the Manubrium sterni; inferior aspect.

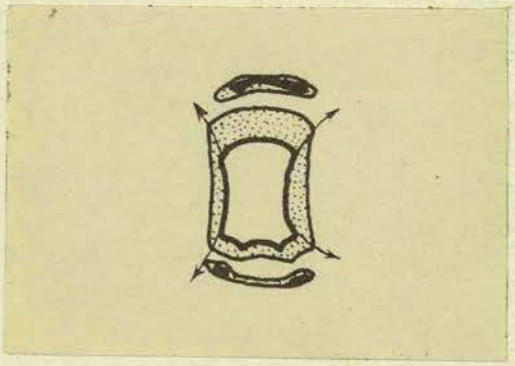

Fig. 35. Growth of the Sternebra tertia.

THE STERNUM: (Plate 19).

The Sternum has three regions, together composed of seven segments. 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.

THE MANUBRIUM STERNI: (Fig. 34).

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.

Two distinct differences can be seen between the mutants drawn in Plate 19. The first, the deep Incisura semilunaris of the microphthalmic bone, is irrelevant to the analysis of growth since it is a feature of normal variation in the stock. The second difference, the absence of anomalies in the Capitulum of the grey-lethal Manubrium, has a bearing on growth and is related to the procedure of ossification. In the new-born mouse the anterior part of the Corpus is the only part of the Manubrium represented by bone, the rest being

cartilaginous. The bone appears as a parallel sided cylinder from which ossification steadily infiltrates the posterior cartilage. But not until the eighth day after birth does the ossification enter the cartilage of the Capitulum: by ten days, ossification of the cartilages is complete. But it is to the tenth day of development that grey-lethals are retarded. Thus the Capitulum of the grey-lethal Menubrium is at that stage of development at which the rapid ossification of its perfect cartilaginous model has only recently been completed. It is for this reason that there are here no erosional anomalies in grey-lethals.

THE SECOND TO FIFTH STERNBRAE: (Fig. 35).

These small sternebrae frequently show an anomaly in the mutants similar to that observed in some of the mutant vertebral centra. In both situations accretion around the waist of the bone fails almost entirely although accretion at each end of the bone (as is usual for accretion in the mutants) is only moderately retarded. Thus, while the width of the waists of the mutant sternebrae at 21 days is only slightly larger than the diameter of this region in new-born mice, the diameter of their ends is considerably greater. Hence the curves of the sides of the mutant bones are practically identical with those curves traced by the growth of the conjugation cartilages. It is apparent from the shape of these curves that growth in the second to fifth sternebrae probably proceeds equally at each end. Erosion appears to play no part in their growth.

Paired centres of ossification are found in each epiphysis around the eighth day of post-natal development. Thus, for the moment at least, epiphyseal growth is by "external accretion" alone.

THE PROCESSUS XIPHOIDEUS.

The anterior, ossified, end of the xiphoid process is much broader than the bony parts of any other sternebra at birth, though later they all come to equal it in breadth. The posterior part of the xiphoid process, even in the three-week-old mouse, is cartilaginous. Unless erosion occurs anteriorly, and there is no evidence of it, the Proc. xiphoidea must grow chiefly at its posterior end. Up to the sixth or seventh day, this is on an ever decreasing surface; but, subsequently, the posterior extremity widens, and the "diaphyseal erosion" pattern on the dorsal surface becomes apparent. (Other difference between the outlines of the Procc. drawn in Plate 19 are due to differences in the angles from which they are viewed.)

THE COSTAE (RIBS): (Plate 19).

The mouse normally has twelve pairs of ribs but occasionally there is a thirteenth. The first twelve pairs are very regular in their form, but the thirteenth, when it occurs, is highly variable in length and may be only unilaterally represented. Thus Plate 19, in which Ribs 1, 7 and 12 are drawn, shows the extent of regular variation in the form of the ribs. The seventh is perhaps the most typical of all, and it

Variation betwe
12 - 14 pairs,
with 13 pairs m
common.

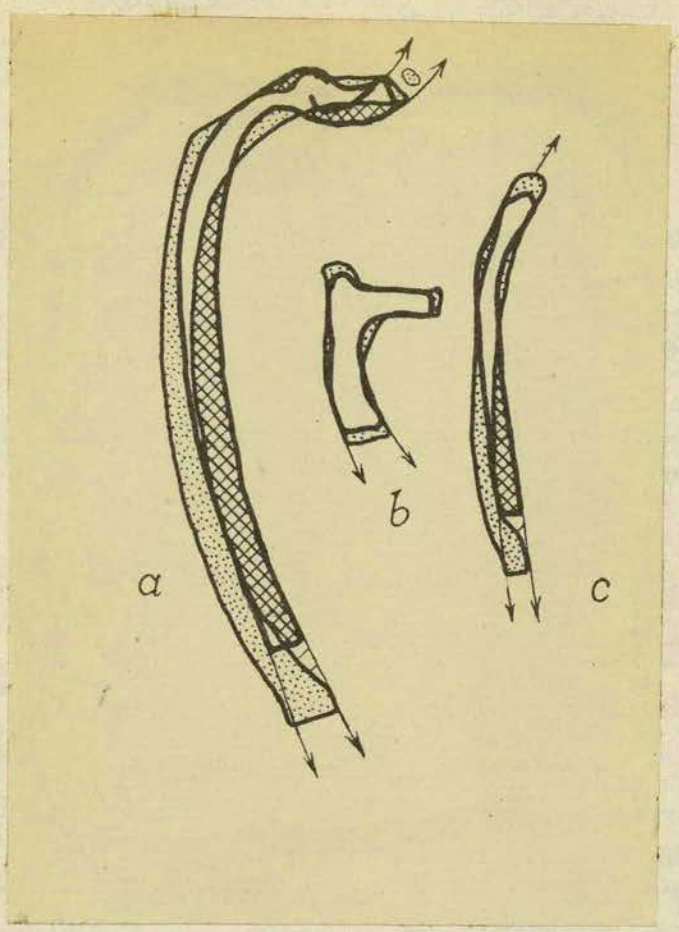

Fig. 36. Growth of the Costae; anterior aspects of (a) the seventh, (b) the first, and (c) the twelfth.

is for this reason that its growth will be described first, while the descriptions of the growth of the first and twelfth ribs are included only to demonstrate the range of variation in the ribs' growth processes. Each rib consists of two parts: an Os costale, with a Cartilago costalis at its sternal end. Ribs 1-7 are Costae Verae since their cartilages articulate with the Sternum; ribs 8-10 are Costae spuriae - their cartilaginous parts do not reach the Sternum but each touches the one in front; and ribs 11, 12 and 13 are Costae fluctuantes as they lie freely in the body wall and articulate only with the Columna vertebralis.

THE SEVENTH RIB: (Fig. 36a).

If it is assumed that growth occurs at each end of the seventh rib (as the presence of the cartilaginous junctions and the orientation of the trabeculae encourage one to believe) then it is obvious from the distortion of the mutant bones that normally each end is extensively eroded from the medial side. This being so, the absence of any considerable erosion in the mutants necessitates that the outlines of their medial surfaces trace the directions of terminal growth. A second point of considerable interest is that the region in which the mutants have attempted erosion is about seven times longer distally than it is proximally. It is on these data that Figure 36a is constructed. It shows that the original curvature of the bone, which is determined by the graded rates of terminal growth between the lateral and medial surfaces, 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.

THE FIRST RIB: (Fig. 36b).

The form of the first rib differs so much from that of the seventh that it is not surprising to find considerable differences in their growth processes, too. To outward appearances, erosion seems to play an entirely insignificant role in the growth of the first rib. Nevertheless, the accretional components of the three "unilateral" movements of the seventh rib do remain. It seems likely from the exceeding curtailment of its Corpus that the relative terminal growth rates are not identical with those of the seventh rib; but no reliable estimate of the new growth rates can be made.

THE TWELFTH RIB: (Plate 36c).

The twelfth rib appears to grow in a strikingly similar manner to the seventh, in spite of its very different shape. Since the first and twelfth ribs represent the extreme range of regular variation in rib shape, it is safe to conclude that the growth processes of the seventh rib are fairly typical of those of the others.

The twelfth was the one rib which, on account of the

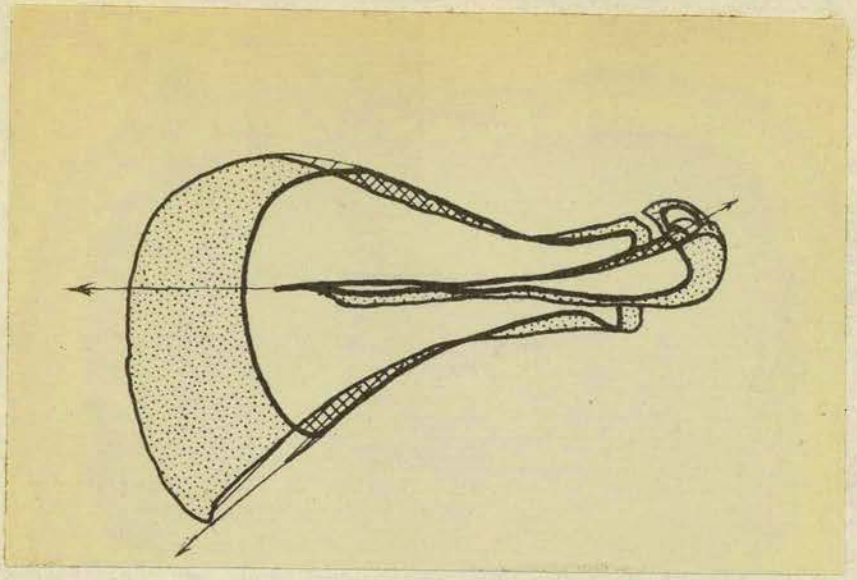

Fig. 37. Growth of the Scapula; lateral aspect.

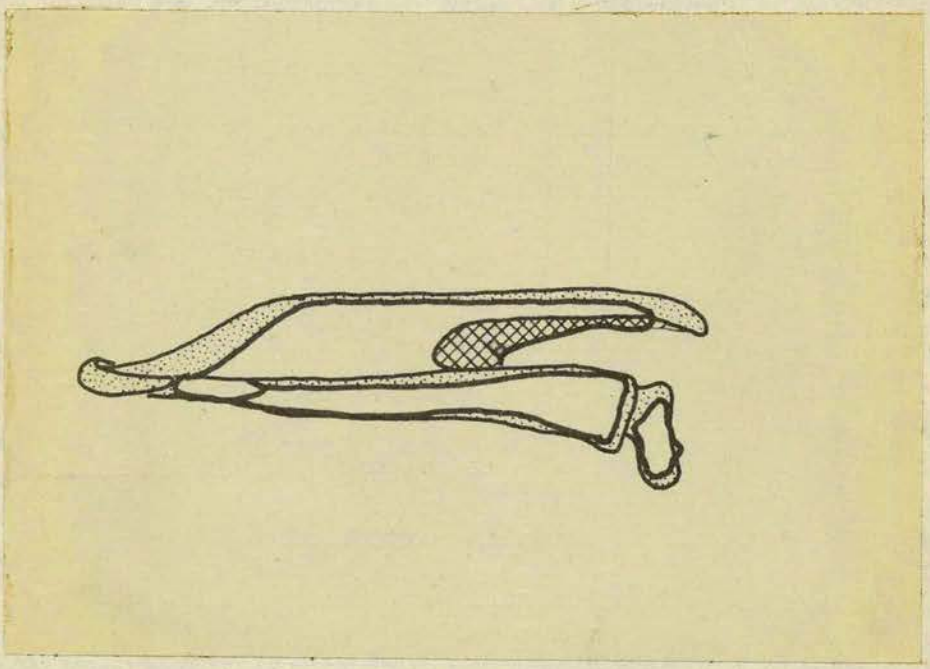

Fig. 38. Growth of the Scapula; ventro-posterior aspect.

slightness of its curvature, could be held immovably in the glycerol bath to be drawn from the medial aspect. This view shows that the middle of the Corpus is expanded antero-posteriorly presumably by accretion to both edges. It is a feature possibly characteristic of the other ribs, too.

THE SHOULDER GIRDLE.

SCAPULA: (Plates 20 and 21; Figs. 37 and 38).

Three features of the Scapula enable an accurate analysis of its growth processes to be made by the superimposition of the outlines of the lateral aspects of normal and mutant bones. The first of these is the steep cliff produced just below the Margo vertebralis by intense erosion of the Facies lateralis. Being associated with signs of erosion, though less intense erosion, of the Facies medialis, it is proof that the Margo vertebralis is a very important site for growth in length and that this growth is "diaphyseal". Therefore, the Margines cervicales and axillares of the mutants respectively trace successive positions of the Anguli cervicales and thoracales. Conversely, it is possible to predict future positions of these angles by projecting these margins vertebrally. Unfortunately, only the Margo axillaris is sufficiently simple in form for it to be so projected. The second feature is the possibility of projecting the base of the mutant Spina scapulae in a similar way. The clumsiness of the cervical margin of the mutant Acromion, which indicates a failure of local "diaphyseal erosion", is the last feature. Like the other two, its

prolongation predicts future positions of the tip of the Acromion. Armed with these three lines which forecast the positions of anatomical features of the bone after further development, the outlines of the normal and mutant bones may be superimposed as in Fig. 37 and the study of the normal growth processes can be made in detail.

The figure proves that there is very little growth in the region of the Cavitas glenoidalis and that the Margo vertebralis is the main site for growth in length and breadth. As mentioned earlier, growth is here "diaphyseal" in pattern. The rate of growth along this margin increases from the Angulus thoracalis to the A. cervicalis so that the former angle gradually moves forward in relation to the rest of the margin until, in adult mice, it is actually the leading part of the bone. The Fossae supra- and infra-spinata are extended and hollowed out by "diaphyseal erosion" of the Facies lateralis. Erosion of the Fossa supraspinata extends to the Margo cervicalis, which is therefore thin. On the other hand, erosion of the Fossa infraspinata does not extend as far as the Margo axillaris, which is therefore quite thick. In fact, the M. axillaris is heightened by accretion to both its lateral and medial edges in the region of the Collum. "Diaphyseal erosion" of the Facies medialis is quite slight.

The comparison between microphthalmic and normal Scapulae shows that there may also be "diaphyseal erosion" of the more vertebral regions of the M. cervicalis and axillaris (although

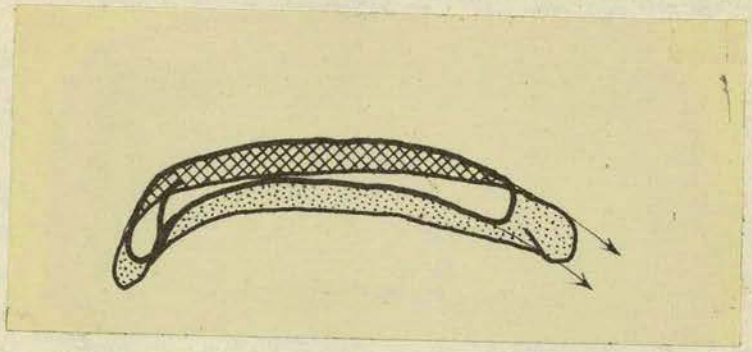

Fig. 39. Growth of the Clavicula; posterior aspect.

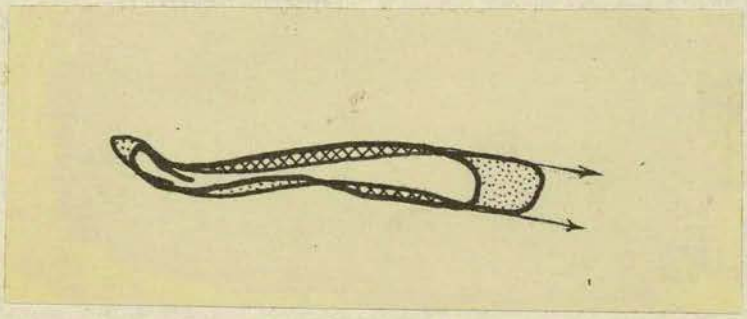

Fig. 40. Growth of the Clavicula; dorsal aspect.

nearer towards the Cavitas glenoidalis there is accretion to these margins). On the other hand, when the grey-lethal bone is compared with the normal there is no evidence of erosion of either of these margins, while accretion to them extends much further towards the vertebral margin. Where before there has, as in the Manubrium sterni and Atlas been encountered such a fundamental difference in the results of analysis depending on the mutant used for comparison with the normal mouse, it has been associated with age changes in the early growth of the bone. Hence it is highly probable that this remarkable situation in the Scapula is significant of some change in the allometric properties of the bone occurring between the ages of 10 and 14 days which are the developmental ages of grey-lethals and microphthalmics respectively. It is suggested that during this 4-day period the bone becomes relatively broader at the vertebral end so that "diaphyseal erosion" of the axillary and cervical margins is required during its subsequent growth in length. This hypothesis is corroborated by observations made on the bony parts of the Scapulae of newborn, 12-day-old and three-week-old mice. The average ratios of the length to breadth in these three groups were respectively 1.33, 1.54 and 1.45. (They were based on 9, 13 and 11 bones respectively.) It is seen that a temporary change in the proportions of the Scapula has occurred in the 12-day-old mouse. The reduction of this ratio to that in the 3-week-old mouse necessitates "diaphyseal erosion".

The Spina scapulae migrates in a vertebral direction by the "unilateral" growth process along the blade. The

erosional component not only enlarges the Incisura spinoglenoidalis by removing the more medial parts of the edge of the Spina scapulae; but, by leaving the more lateral parts, transforms them into the root of the Acromion. By posterior accretion to its free, lateral, edge, it curves over towards the Margo axillaris and is heightened.

The Acromion, which is less precociously developed than the Collum, 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 undergoes "diaphyseal erosion", for a site of accretion, continuous with that on the free border of the Spina scapulae, extends along its axillary border. At its vertebral end the Acromion is formed out of the Spina scapulae by the "unilateral erosion" of the latter. The Acromion is heightened by "unilateral growth" - the erosional component of which also serves to enlarge the Incisura spinoglenoidalis. The epiphysis of the Acromion does not ossify till some time after weaning.

CLAVICULA: (Plate 20; Figs. 39 and 40).

The Clavicula of the mouse consists of a long body and a short ramus, which meet at the "Angulus claviculae" (N.B.). The body, or Corpus claviculae, articulates with the Manubrium sterni; the ramus, or "Extremitas acromialis" (N.B.) articulates with the Acromion; and represents the "acromial end" and "conoid tubercle" of the human bone.

Because the Extremitas acromialis is actually longer

in the retarded mutants than it is in normal mice, "unilateral growth" involving its tip and the Angulus claviculae is indicated. If the A. claviculae were the site of "unilateral accretion", then the Corpus would be excessively thick in this region. As this is not so, it is concluded that the "Extremitas acromialis" grows by "unilateral accretion" to its tip. This is one of the two observations used in the construction of the analytical figures. The other observation is that the Corpus grows at its sternal end in the "diaphyseal pattern". The direction of growth at the sternal end is therefore recorded in the mutants. Figure 39 is constructed on this combined information[1]. Figure 40 is constructed so that the rate of growth at the sternal is the same as that discovered in Fig. 39.

The analysis in Figs. 39 and 40 shows that the Corpus ~~sternalis~~ tends to be quite strongly curved (as it is in the mutants) as a result of the "diaphyseal accretion" at the Sternal extremity. However, the original curvature of the bone is normally considerably reduced by a "unilateral" movement in a dorso-posterior direction. This "unilateral" movement, which extends throughout the Corpus , , is identified with that

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1. The conclusions as to the normal growth pattern are unaffected by the remarkable degree to which "diaphyseal erosion" has succeeded in the grey-lethal Clavicula drawn in Plate 20 (that is when the grey-lethal drawing is used for comparing with the normal bone).

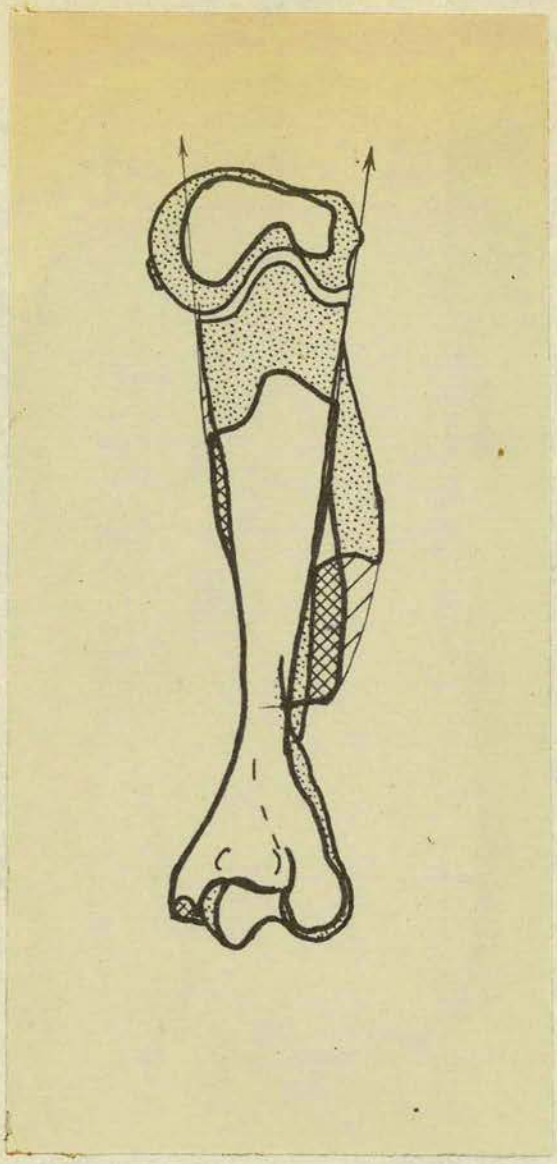

Fig. 41. Growth of the Humerus; posterior aspect.

resulting in the growth of the Extremitas acromialis.

HUMERUS: (Plate 22; Fig. 41).

The diaphyses of the mutant Ossa humeri betray a gross failure of "diaphyseal erosion" at the proximal end, and an equally noticeable failure of "unilateral erosion" of the Crista humeri. On the other hand, there is extraordinarily little sign of erosional failure at the distal end, although, if growth here were of any consequence, failure of "diaphyseal erosion" would again be a prominent feature of the mutants. Fig. 41 is, in fact, based on the assumption that very little length is contributed by distal growth.

The following information can be drawn from the figure. Practically all increase in length of the Humerus results from "diaphyseal growth" at the proximal end of the diaphysis. At this end erosion is minimal along the base of the Crista humeri and on the Collum. The Collum and the Crista tuberculi minoris are thrown into prominence by erosion on either side of the Collum. The Crista humeri and Tuberositas deltoidea move proximally by "unilateral" growth while increasing their height by accretion to their antero-lateral margin. The Corpus humeri appears to migrate antero-laterally, but only on account of intense accretion to its anterolateral surface, for there is no erosion on the opposite side. The Crista epicondyli lateralis and its proximal continuation, the Margo lateralis, become increasingly prominent by accretion to their anterolateral edges; while the former is thrown into still greater prominence by a

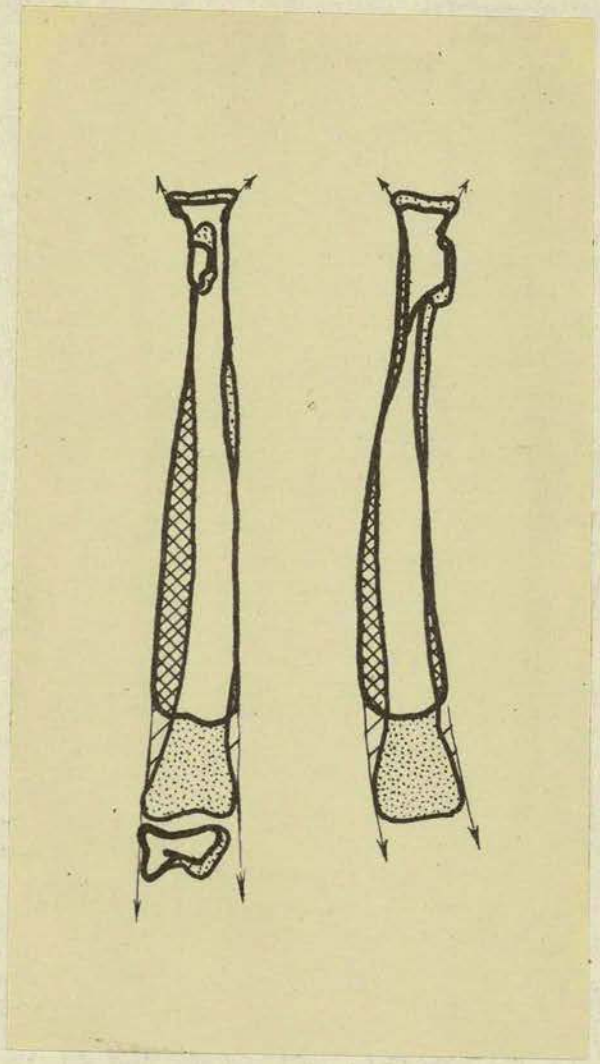

Fig. 42. Growth of the Radius; on the left - ventral aspect, and on the right - medial aspect.

small amount of "diaphyseal erosion" of the anterior surface of the root of the Epicondylus lateralis. The E. medialis grows in a medial direction with "diaphyseal erosion" of its distal border.

Neither the Caput nor the Trochlea humeri has any anomaly in the mutants which is indicative of erosional failure, and it appears that both the proximal and distal epiphyses grow by "external accretion" alone. In consequence the proximal epiphysis of the mutants closely resembles normal, 10-14 day-old epiphyses. Whereas the proximal epiphysis is a separate entity in the growth of the bone for long after weaning, the distal one fuses with the diaphysis at about 7 days.

OSSA ANTIBRACHII.

RADIUS: (Plate 21; Figure 42).

The mutant Ossa radii display at both proximal and distal ends that failure of "diaphyseal erosion" which, in the now familiar way, is so helpful in the precise analysis of the bone's growth processes. The following conclusions can be drawn from the superimposition of the outline drawings.

The rate of growth is far greater distally than proximally - indeed, about eight times as much. The Tuberositas radii, situated near the proximal end, appears as the "fixed point" in the growing bone, for it has sites of accretion on both its proximal and distal slopes. The Collum, at the proximal end, is eroded on all sides, but at the distal end "diaphyseal erosion" is very much limited. It is particularly intense on the dorso-medial surface and radically alters the primary curvature

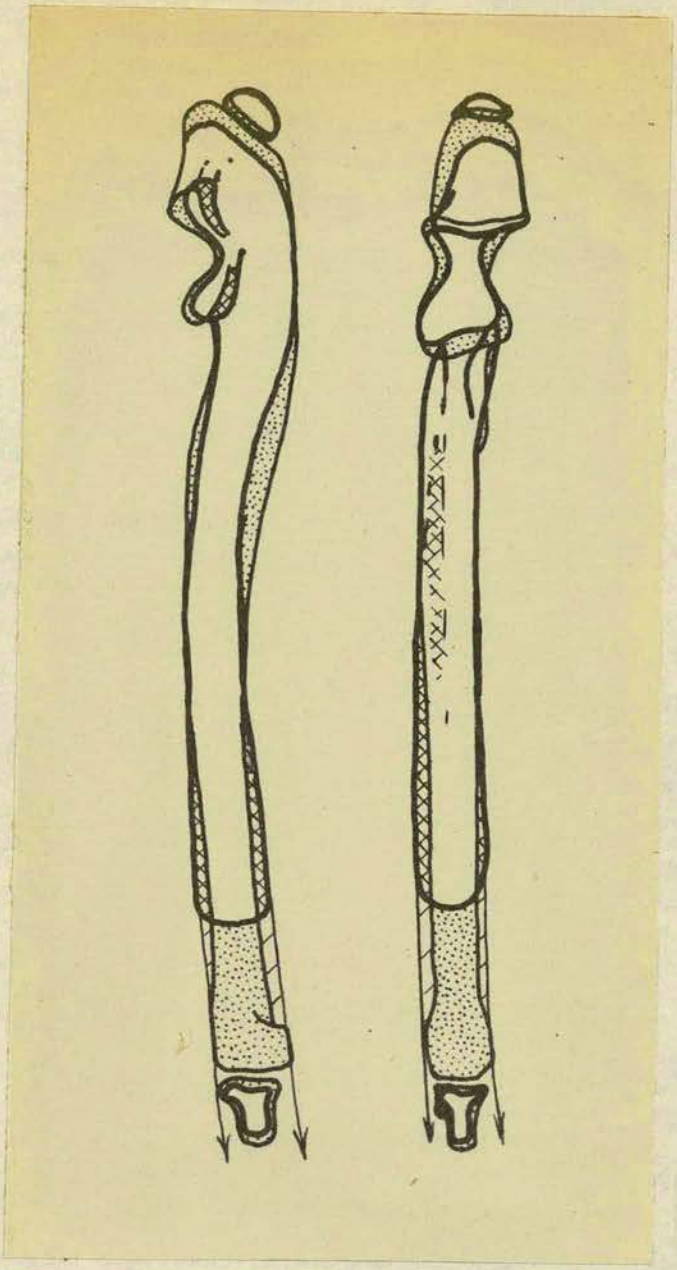

Fig. 43. Growth of the Ulna; left - medial view, right - dorsal view.

of the bone. Just distal to the Tuberositas radii the Corpus migrates ventrolaterally through the "unilateral" opposition of accretion on the one side with "diaphyseal erosion" of the other. The distal epiphysis suffers no erosion; the proximal epiphysis fuses with the Collum at about 14 days.

ULNA: (Plate 22; Fig. 43).

While the primary direction of growth of the ulnar diaphysis is clearly indicated by its clumsiness in the mutants, the situation at the olecranon end of the bone is by no means as clear. However, the shortness of the mutant Proc. olecrani indicates that there is growth in length at this end; and the presence of an epiphysis (the Olecranon) suggests that this growth is by accretion in the conjugation cartilage. But it is still impossible to estimate the relative rate of growth at this end because the "diaphyseal pattern" is not involved. Luckily, the position of the fixed point in the bone's growth can be diagnosed independently, without a knowledge of the relative rates of growth at the two ends. (The "fixed point" is that part of the bone which maintains its relative position throughout the bone's development.) The narrow waist of the mutant Incisura semilunaris is evidence that this is the earliest formed part of the Incisura. The more normal size of its lips - the Procc. anconeus and coronoideus - appear to be the sites for its primary growth. As the Incisura semilunaris is not otherwise distorted in the mutants, it can be concluded that its lips grow upwards and obliquely away from one another, while the "waist" is at least very close to the fixed point. Now there is sufficient

information for the construction of the analytical diagram (Fig. 43).

It transpires that, as with the Radius, though not to the same relative extent, it is the distal end which is the major site for growth in length: the rate of growth at the proximal end is only about 1/6th of that distally. Distal growth is in the "diaphyseal pattern", but (in surprising contrast to the Radius with which it is so closely associated) erosion is strongest on the ventro-lateral side, and not (as in the Radius) from the dorso-medial side. The dorso-ventral flexion of the bone which results from the "diaphyseal" growth of its distal end, is quite strong, but it is modified in part by a minor "unilateral" movement just proximal to the zone of distal "diaphyseal erosion", and partly by ventral and some dorsal accretion between this "unilateral" zone and the Proc. coronoideus. As part of the upward growth of the Incisura semilunaris its medial margins (which are overturned) are "unilaterally" eroded, and the Proc. anconeus is "diaphyseally" eroded from the medial side.

The whole of the medial side of the Ulna becomes highly sculptured in the course of its development. Although it is not possible from the present material to determine whether a particular feature on this surface depends on accretion or erosion for its maintenance, it is probable that the medial surface of the bone presents a mosaic of very local, interacting and migrating sites of accretion and erosion.

A most remarkable feature of the mutant Olecranon is

the larger size it attains as compared with that of the developmentally more advanced normal mouse. While this phenomenon could be due to random variation in the size of the bone, it is well to remark here that this is the first time that there has been even a suspicion of erosion in epiphyseal growth. This observation is in marked contrast to Payton's (1933) who found that the "unilateral pattern" (he, of course, did not call it by this name) was of extraordinarily frequent occurrent in the epiphyses of the pig. He discovered it in all the twelve epiphyses he studied. In many cases, erosion was in excess of the resultant gain in length; in one case as much as 4½ times greater. An explanation of such a fundamental difference between our observations must be found. It is not unlikely that the "unilateral pattern" is characteristic of older epiphyses, such as Payton was examining, whereas the epiphyses in the present study have rarely passed through the initial phase of "external accretion" to their centres of ossification which lie deep in the cartilaginous precursors.

MANUS: (Plate 23).

The drawings of the Manus were made from alizarin clearances of the intact hand. In such preparations it is impossible to orientate all the bones correctly with regard to other members of the comparative series. The comparisons of normal and mutant bones must therefore be made in the three dimensions simultaneously - any attempt in analysis in two dimensions at a time, by the familiar method of superimposing outline drawings, being inadvisable. The method of

simultaneous three-dimensional study necessitates the rejection of the usually precise method of comparing outlines in favour of purely "by eye" comparisons. The author has found that conclusions from provisional "by eye" comparisons, even of quite complicated bones like the Ulna, Costae or Maxilla, were well borne out by later exact methods of analysis. However, although considerable confidence can be placed in the following description of growth in the Manus, it is important to realise that the analysis is essentially rule of thumb.

THE CARPUS.

No mutant carpal bone shows any anomaly which might be attributed to erosional failure, and it is therefore likely that growth, up to the 14-day stage represented by microphthalmics, is solely by "external accretion". It is not surprising that there should be no sign of erosion, for ossification of the cartilaginous carpals does not begin until the 3rd day after birth. The initial phase in the growth of cartilage bones - ossification of the cartilage - does not involve bone remodelling.

This material is unsuited to a more detailed study of the bones' growth.

THE METACARPUS: (Fig. 44).

With the exception of the Metacarpale primum, which more resembles the phalanges in its growth than the other metacarpal bones (see the Discussion, ~~section 2~~, p.127) - the diaphyses of the Metacarpus elongate chiefly by "diaphyseal growth" at their distal ends. "Diaphyseal erosion" extends all

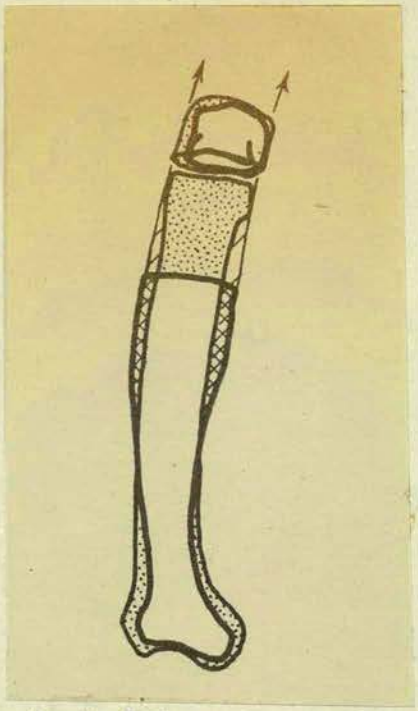

Fig. 44. Growth of a typical Os metacarpale or metatarsale.

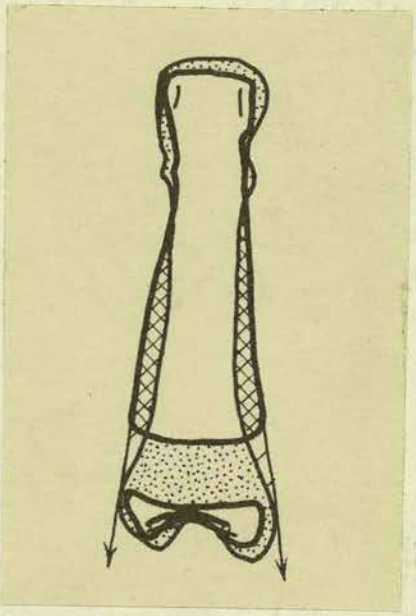

Fig. 45. Growth of a typical Phalanx proximalis, from above.

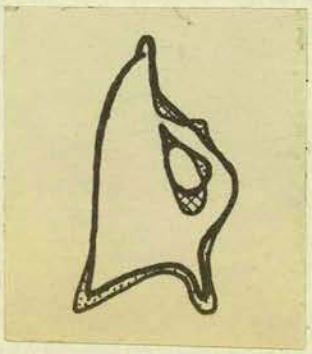

Fig. 46. Growth of a typical Phalanx distalis, from the side.

round the circumference just behind the "ossification ring". "External accretion" at the proximal end enlarges the Basis but contributes little to the bone's length. It also extends well up the sides of the diaphysis where it increases the girth of the bone.

Growth of the Capitulum, which is still separate from the diaphysis at three weeks, seems to be by "external accretion" only.

THE DIGITI MANUS.

Proximal phalanges (Fig. 45). (Under this heading the growth of the Phalanges prima and secunda of the Digiti 2-5 and P. prima only of the first is discussed.)

Osteoclast action, which plays its part in the "diaphyseal erosion" of the Corpus, can be seen at the proximal end of the diaphysis in normal mice, while its failure in the mutants produces their characteristing bulging. Erosion of the plantar surface is very limited. However, the distal ends of the mutant bones, though smaller than normal, are in no way deformed, nor is there any sign of erosion in the normal bone in spite of the fact that any considerable growth at this end would also be in the "diaphyseal pattern". It is concluded that the contribution to length by distal growth is negligible.

It is of interest that the growing end of these Phalanges is opposite to that of the Metacarpales except for the Metacarpale primum which, like the Phalanges, also grows at its proximal end.

The growth process of the exceedingly small Phalanx prima of the first finger is not so clear as that of the others; but since it has a proximal epiphysis, like the proximal phalanges of the other fingers, it is at least probable that its growth pattern is essentially similar.

The bilobed epiphyses at the proximal ends of the proximal phalanges grow by external accretion only. They fuse with their diaphyses at about fifteen days after birth.

The distal phalanx. (Fig. 46). (The Phalanx secunda of the first finger and the Phalanges tertia of the others are considered under this heading.)

The distal phalanx of the mouse is perforated by a transverse foramen (the Foramen transversarium phalangis, N.B.) which is enclosed below by a curved spicule of bone. "Diaphyseal erosion" of the proximal end of the spicule has failed in the mutants. The conclusion, then, is that ossification spreads through the cartilaginous phalanx from its distal end towards it proximal end. The mutant Foramen transversarium phalangis is always very large and may be even larger than in normal bones. The fixation of its distal border in bone, while its yet unossified proximal border is still being remodelled can explain the excessive size of the mutant foramen. This suggestion involves the migration of the Foramen in a proximal direction and suggests that the rate of growth at the proximal end of the phalanx is greater than that at the distal end of the bone.

THE OSSA SESAMOIDEA.

There is no evidence in any sesamoid bone of erosion. Their growth is therefore by "external accretion" alone.

THE PELVIS: (Plate 24; Fig. 47).

The right and left halves of the mouse Pelvis, the Ossa coxae, are readily separated. Only in adult male mice is there ever bony fusion, and then only by a few spicules extending across the Symphysis pelvis (Sym. ossis pubis) (Ruth 1936) and in three-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 mms. 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 three weeks in the acetabular region; the Pubis and Ischium 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 triradiate figure, Y, into a $\curlyvee$-like figure, associated with a slight overlapping of the bones.

(While retaining the veterinary term Tuba coxae for the ventral edge of the Ala ossis ilium the author has changed the positions of the Spina iliaca anterior superior and the Spina iliaca anterior inferior. In the veterinary nomenclature, these two terms, taken directly from the B.N.A., though somewhat inappropriately, mark respectively the cranial and caudal limits of the Tuba coxae. This is intended to be equivalent to the Spina iliaca anterior of man.

The Spina iliaca anterior inferior of man carries the origin of the Musculus rectus femoris and it seems fitting that the Spina iliaca anterior inferior of the mouse should also carry the origin of this muscle. The author has therefore altered its position on to the Corpus ossis ilium. Greene (1935) also uses the term (or rather her translation of it - inferior ventral spine - awful conglomeration of languages!) in this sense for the rat, although very different from current veterinary practice.

In man the Spina iliaca anterior superior marks the caudal limit of the Ala and the caudal limits of the origins of the Musc. tensor fascia latae. Why not in animals also ? Greene (1935) and Ellenberger and Baum (1926) however place it at the cranial limit of the Ala.

Owing to the discrepancies between the present and other authors' definitions of these features, and because of the inappropriateness of taking the terms directly from man, it seems reasonable to translate them into terms 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.

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.)

OS ILIUM.

At the two ends of the Ilium growth is in accordance with the "diaphyseal" pattern but the erosion is restricted to the outer surface (Facies glutea) at the anterior end, and to the inner surface (Facies pelvina) at the posterior end. The

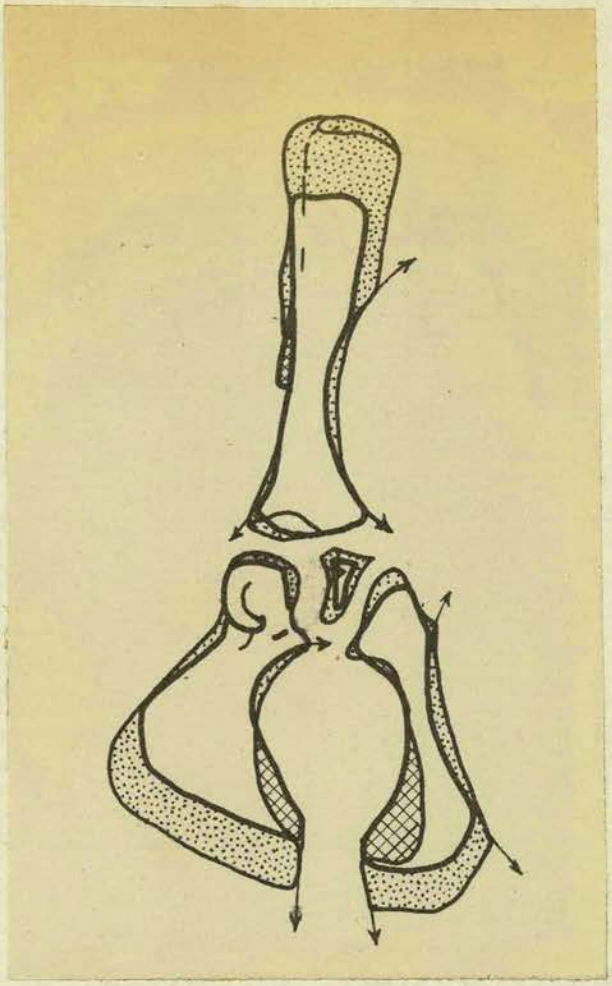

Fig. 47. Growth of the Pelvis (Os coxa); lateral aspect.

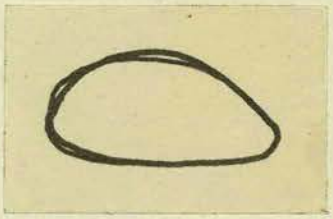

Fig. 48. The difference between the average outlines of the Foramina obturata of ten heterozygous microphthalmics (+/mi) and nine homozygous normals (+/+) enlarged to the same length; +/mi's - heavy line, +/+'s - light line. The differences are not likely to arise from a partial failure of normal growth processes in the heterozygotes.

rate of growth at this end of the bone declines until its cessation with the ossification of the acetabular sutures at three weeks. At this end the extent of growth is a little greater on the inner than on the outer side so that the Ilium finally slightly overlaps the Pubis on the inner surface.

The Tuber sacrale, which is formed partly from the Ala ossis ilium by the "diaphyseal erosion" at the anterior end of the bone, migrates forward by "unilateral growth". On the other hand, neither the Tuber coxae nor its posterior limit (the Spina iliaca ventralis caudalis, N.B.) involves the "unilateral" growth pattern in its forward movement because the need for erosion is obliterated by the coupling of extensive accretion along the whole ventral border of the ilium with the tendency for the site on the Tuber to move forwards.

Despite the greater size of the grey-lethal bone as compared with that of the Microphthalmic figured in Plate 24, it appears on analysis to lack accretion at the acetabular end to a greater degree than does the smaller microphthalmic bone. This situation is clearly connectedwith the difference in the developmental ages of the two mutants, and surely reflects the falling off in the rate of growth at the acetabular suture towards its cessation at 21 days.

OS PUBIS.

Just like the Ilium, the Os pubis grows at very unequal rates at its two ends, the slower growth at the acetabular end ceasing altogether at three weeks. Also like the Ilium, growth

at each end is "diaphyseal" in pattern. "Diaphyseal erosion" is restricted to three sites:- one to each end of the more dorsal border which encloses the Foramen obturatum; the other to the Facies pelvina near the junction of the Rami acetabularis and symphysicus. The R. acetabularis migrates ventrally in the "unilateral pattern" through the association of accretion to its ventral border and ("diaphyseal") erosion of its dorsal border. In a similar way, the R. symphysicus may be regarded as migrating "unilaterally" backwards by the combination of accretion to its posterior border with("diaphyseal") erosion of its anterior, obturator border. Both the Eminentia iliopectinea and the Tuberculum pubicum are points of vigorous ventral growth, so obliquely directed, that they neither of them involve the "unilateral" pattern in their divergent movement in the antero-posterior axis of the bone.

Differences between the Ossa pubes in the two mutants.

The substantial differences in the form of the two pubic bones which are drawn in Plate 24 in no way affect the conclusion as to the method of growth of the normal bone. That the method of analysing differences between apparently arbitrarily superimposed outline drawings of normal and mutant bones in terms of normal growth processes survives marked variations in the form of the bones used in the analysis affords considerable confidence both in the method and in the results of analysis. It implies, moreover, that variations in the form of identical bones occur within the same general pattern and not to differences in the

patterns themselves. They may be due to differences between the different individuals in the relative rates of accretion and erosion at the various sites. For this particular case, it is suggested that the differences between the mutants are due to differences in the time and rate of ossification of the cartilaginous models; that the centre of ossification was formed later in the grey-lethal bone; but that once initiated, it proceeded more rapidly than in the microphthalmic bone. (It may be remembered that a similar suggestion was made to account for the differences between the mutant Stapes.)

OS ISCHII.

The Os ischii, like the other pelvic bones, also grows very little at its acetabular end. Distal to the Acetabulum, growth in length is quite rapid, and the edge of the Ramus symphysicus which borders on the Foramen obturatum is heavily eroded in the "diaphyseal" pattern. On the Facies pelvina of the Ramus there is some further but mild "diaphyseal" erosion.

The very restricted growth at the acetabular end is also "diaphyseal" and there is accretion to both the ilial and pubic sutures; (the first contributing to the length of the Pelvis, the other to its breadth). "Diaphyseal erosion" is seen on the Facies pelvina, while the buttress of the Facies lunata migrates forwards "unilaterally".

Between the sites of "Diaphyseal" growth at the two ends of the bone the Corpus is thickened by "external accretion" both to its dorsal edge (which is free) and to its ventral border

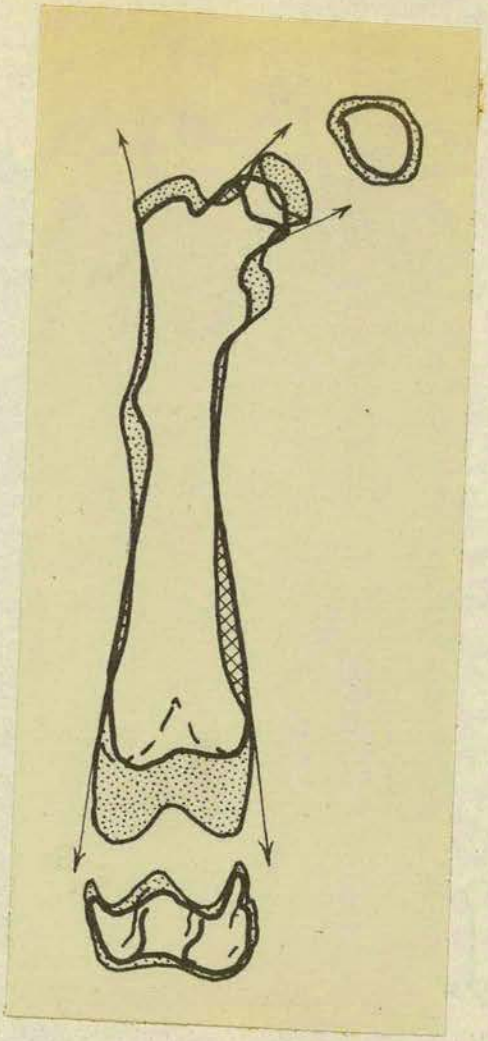

Fig. 49. Growth of the Femur; dorsal aspect.

(which limits the For. obturatum).

OS ACETABULI.

The Os acetabuli is a small slip of bone adjacent to the anterior end of the Pubis and forming the pubic wall of the Acetabulum. Since there are no anomalies in the mutants which certainly indicate failure of erosion, its growth appears to be by "external accretion" alone.

OS FEMORIS: (Plate 25; Fig. 49).

The directions of growth of the Collum femoris and of the distal extremity of the Corpus, which are clearly indicated by the mutants owing to the obvious failure of "diaphyseal erosion" in these places, enable the drawings to be superimposed for the detailed analysis of the bone's growth processes.

It is at once seen, Fig. 49, that the distal end of the shaft is the major site for growth in length and accretion to the proximal end contributes perhaps one-fifth (20%) to the total increment. The distal growth is "diaphyseal" and erosion of the Planum popliteum is exceptionally heavy. The ridges on either side of the Fossa intercondyloidea are eroded so that this surface becomes flattened from side to side. This "diaphyseal erosion" also causes the Planum to become strongly concave from end to end. Erosion of the medial side is not quite so intense, and is almost negligible laterally and dorsally. An erosional cliff seen on the dorsal (extensor) surface of the normal bone marks the proximal limit of the wide "ossification ring" (Lacroix).

The girth of the middle of the shaft is increased by "external accretion" on all sides. The appearance of distal migration of the Trochanter tertius is obtained, without the aid of erosion, by the great discrepancies in the rates of accretion to its distal and proximal edges.

At the proximal end of the bone, growth of the shaft is directed obliquely medially. The Collum femoris (supporting the separate, articular Caput femoris) grows obliquely upwards and medially by the "diaphyseal" pattern; its root becomes incorporated in the Corpus by similarly directed "external accretion" which extends across the Trochanter major, T. minor and the Linea intertrochanterica posterior. No doubt there is also accretion in the floor of the Fossa intertrochanterica.

There is no evidence of erosion in the patterns of growth of any of the four epiphyses.

OSSA TIBIA AND FIBULA: (Plates 26 and 27; Fig. 6).

The growth of these two bones has been extensively described and discussed in the introductory passages - pages 28 - 33 . No further account need by given here.

PATELLA: (Plate27)

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

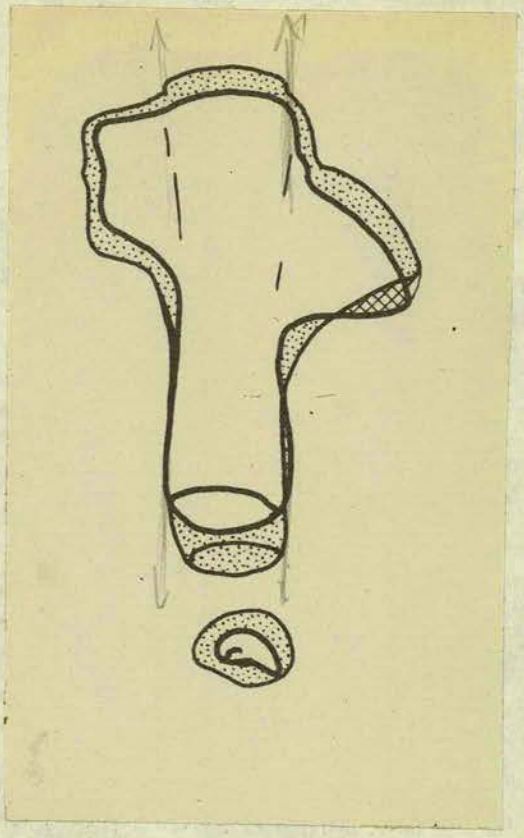

Fig. 50. Growth of the Tarsi fibulare; ventral aspect.

THE PES: (Plates 28 and 29)

(Unlike the domesticated animals and man, the mouse has three in place of two, proximal tarsal bones. Rather than regard the supernumary bone as an unparallelled 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.

With the exception of the Os tarsi fibulare (Calcaneum) the mutant tarsal bones present no malformations which can, with certainty, be attributed to failure of erosion. Their growth is apparently solely by "external accretion".

OS TARSI FIBULARE: (Fig. 50).

At its posterior end the Fibulare grows by the "diaphyseal" pattern, there being in the mutants a mild failure of "diaphyseal erosion" just in front of the Tuber calcanei. It appears that the Sustentaculum tali migrates forwards by "unilateral" growth, while the Proc. trochleatis, which apparently ossifies at a later stage is still in the initial phase of growth; namely, ossification of its cartilage by "external accretion". The girth of the middle of the body also enlarges by "external accretion".

METATARSUS AND DIGITI PEDIS.

The growth patterns of the metatarsal bones, the Phalanges and the Ossa sesamoidea appear precisely similar to those of the Manus and there seems little need for further comment. However, it should be noted that the Phalanx secunda of the fifth toe bears in the microphthalmic mouse which is drawn in Plates 28 and 29, a bony tumour. This has no significance as

regards normal bone growth. (The loose particles of bone seen in the drawings are almost certainly "accidents of preparation" as no similar manifestations have been seen in other alizarin clearances.)

OS EXTRAORDINAIRE.

OS PRIAPIS.

The penis bone grows at its proximal end by "diaphyseal growth".

. . . . . .

COMPARISON OF RESULTS WITH OTHER AUTHORS'.

The account of the growth processes of the individual bones of the skeleton of the mouse, as given here, is in general agreement with the accounts of other workers studying pigs, cattle and man, at least as far as each has elaborated and providing the bones are at all comparable in shape.

With Kölliker's description of the sites of erosion in the calf, there is very little disagreement, although each author has found minor sites of erosion of which the other makes no mention. But there are some more important discrepancies:

1. Kölliker describes additional erosion

(i) of the medial side of the Proc. jugularis of the Pars lateralis ossis occipitale. (The present author's account of the growth of this bone was notably deficient.)

(ii) of the upper surface of the Proc. zygomaticus of the Squama temporalis;

and (iii) of the medial side of the Lacrimale.

2. Profound differences in the form of the bones in the calf and mouse may account for the majority of discrepancies in the descriptions of the growth of the Mandibula, Incisivum and Ulna. In spite of the dissimilar forms of the Mandibulae, there is agreement in regard to the relative rates of growth forward and backward, and in the gradient in ventral growth from before back. In the Incisiva, however, Kölliker found anteriorly directed growth whereas the present author found only backward growth.

3. In connection with describing the process of accretion in the shaft of the Humerus, Kölliker made a profound mistake, although the authors agree concerning the sites of erosion. Kölliker notes that the distance of the insertion of the deltoid muscles from the upper conjugation cartilage increases relative to the distance from the lower epiphyseal line. Stating that this age change can hardly be due to a <u>downward</u> migration of the deltoid muscles, he regards it as proof that there is about equal growth at the two ends of the bone. There is, however, no justification for his premise, for Grüneberg and the present author have both obtained proof of the <u>upward</u> migration of the deltoid muscles (see p. 116 and Fig. 54). If there is a similar muscle migration in the calf, there is clearly indicated very unequal growth at the two ends of the bone, with maximal rate of growth proximally - completely in agreement with the mouse.

In the Ulna there are again discrepancies resulting

from the marked differences of its form between the two animals.

For all the other bones that Kölliker investigated, there is at least a passing similarity of results.

With Brash's account (1934) of the growth of the pig skull there is again very little definite disagreement. Brash found ventrally directed growth of the hard palate (of which movement in the mouse the present author is not aware). Brash also found a faster rate of accretion to the Limbus alveolaris of the Maxilla, than to the general surface of the palate, so that the palate was heightened: on the other hand the mouse palate becomes shallower, the alveolar borders being eroded. There is a possibility that the growth process described for the mouse is characteristic of young animals, while that described for the pig characterises older ones - equally remarkable age changes having been described in the mouse for the vertebrae.

Payton (1932) investigating the relative rates of growth at the two ends of each of the six major long bones of the fore and hind limbs of the pig, observed in every case a quite different relationship to those described here. For the Humerus, Radius, Ulna and Femur the mouse seems to have much more extreme relative rates of growth; for the Tibia and Fibula much more equal rates of growth. These differences are probably species differences.

The rates of growth of the mouse Tibia, Femur and Humerus are, however, quite compatible with those obtained for the human bones by the analysis of Harris's radiographs (1933)

of syphilitic skeletons. The human Scapula and Ilium also grow as in the mouse.

Concerning the long limb bones the present work is unique in its frequent reference to the "unilateral"pattern of growth. Such a method was observed only once by Payton (1932) where it occurred in the pig's Ulna, and Kölliker supplies no evidence of it in the calf. By contrast, the pig's epiphyses regularly grow in the "unilateral pattern" (Payton 1933) although no other author has observed this phenomenon. Gottesleben (1939), Boerema (1942) and Dubreuil (1912) have all commented on the failure of accretion at the diaphyseal surface of the epiphyses, but none of these authors has observed erosion there. Owing to the uniquity, yet generality, of Payton's observation it appears that further, confirmatory investigation is justified. Perhaps Kölliker, not suspecting erosion at this surface, failed to separate the epiphyses from their diaphyses in his examination of the bones. Perhaps the present material cannot hope to confirm Payton's findings, since the epiphyses have hardly developed beyond their initial phase of growth - that of all-round "external accretion" within the cartilaginous epiphyses.

The conclusions of Duhamel (1743), Hunter (1837) and Flourens (1840) who have also written general treatises on bone growth, are not discussed here, as the present author has been unable to obtain their works.

With this account of the discrepancies between the present author's account of bone growth and those of previous workers, the description of the growth of individual bones in the mouse skeleton is concluded. In the following section, more general aspects of bone growth are discussed.

4. DISCUSSION.

No treatise on bone growth would be complete without some discussion as to the degree to which a bone's growth is intrinsically determined. Previous work in this field has given somewhat conflicting results which Murray and Selby (1930) have neatly summarised by concluding that the early development of bone is largely intrinsically determined and that extrinsic factors become of importance only in determining the finer points of later development. This 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 in embryonic tissues (the trajectorial theory) and believed erosion to be a necrotic process.

The work of many experimental embryologists, notably among earlier workers Murray and Huxley (1925), Fell (1928) and especially Fell and Robison (1929) have shown the remarkable degree to which explanted blastemic rudiments of limbs can self-differentiate in tissue cultures, even when stripped of their musculature. Lacroix (1946b and 1947) has demonstrated the power of bone, and even mere fragments of bone, to grow in the most unusual environments. Bone of normal histological appearance and actively growing in the "diaphysial pattern" was formed from pieces of the periosteum or conjugation cartilage (Lacroix) or even from marrow cells (Pfeiffer, 1948) when these were transplanted, for example, under the kidney capsule. Lacroix considers the conjugation cartilage to be the osteogenic

centre because any membrane, for example the kidney capsule, when in contact with it, receives the potency to produce periosteal bone. And an alcoholic extract of the conjugation cartilage (Lacroix, 1947) was capable of inducing an osteoma of the thigh (when injected into the thigh muscles) which showed some of the features of "dyaphysial erosion".

But no bone cultivated in vitro has been allowed to develop beyond embryonic stages, and the possibility remains that the bone must be subject to some extrinsic factors for perfectly normal development. This is clearly indicated by Nicholson's account (1937) of a tumour-like cyst formed in the region of the buttocks of a 16-year old Chinese girl. It contained a daughter cyst and a digitate pad, but the digits were more like those of a hand than a foot - they were so long. The degree of perfection in normal development was graded proximo-distally: the terminal phalanges were lenticulated, but the other phalanges and metacarpals (or metatarsals) were underdeveloped, having none of the tuberosities which characterise these bones. The proximal ends of the metacarpals were thinly ossified and were more or less fused with each other and to two huge, unidentifiable lumps of bone. These in turn articulated with a large triradiate bone having no resemblance to any known bone. This lack of differentiation may be related to the absence of skeletal musculature in the teratoma.

On the other hand, Grüneberg's suggestion in his first account of the anomalies of the grey-lethal mouse (1935) that

the shortness of the snout was attributable to the failure of an adequate stimulus for growth from the maldeveloped incisor tooth is invalidated. Although he modified this statement in proof, de Beer (1940) unfortunately quoted the original suggestion. The present author plotted the length of the Ossa nasales against an unrelated body measurement, (the length of the Corpus ossis ulnae) log by log, for normal and mutant mice at 21 days of age and for three normal 14-day-old mice. The regression line relating the measurements to each other is common to all these groups of mice (fig. 7), whence it appears that the shortness of the snout of the mutant animals cannot be due to any circumstance peculiar to this region of the skull.

While it seems that bone accretion is remarkably free from the effects of factors extrinsic to the bone, this is far from being the case for erosion. Jores (1920) demonstrated the effectiveness of continuous pressure in causing bone erosion by tieing little rubber bags filled with water under pressure or with mercury, over the Procc. spinosi of the thoracic vertebrae in guinea pigs and rabbits.

Jores' work has stimulated interest in the activity of pressure as an erosion-causing agent, but it is not impossible that the release of tension may be equally effective. Thus in sectioned material of grey-lethals, Grüneberg (unpublished) found that the Tuberositas extended distally beyond the insertion of the Deltoideus and Pectoralis muscles, whereas in normal mice the muscles insert at the tip of the Tuberositas.

(The present author has confirmed this anomalous situation in dissections of microphthalmic mice - and one of such dissections with a normal for comparison, is figured in fig. 54). Thus it is not impossible that the migration of these muscles along the Crista humeri may be the normal stimulus (to which the anomalous mice cannot respond) for erosion of the distal border of the Crista and Tuberositas, although the possibility is not excluded that migration of the muscles and the erosion of the bone may not be causally related to each other, and may both be controlled by a third agent.

Loeschke and Weinnoldt (1922) have recorded instances in man in which abnormally high brain pressures produced by hydrocephaly, brain tumours and premature synostosis in the skull have caused unusual amounts of erosion of the inner surface of the skull. Exposure of the diploe and even fenestration resulted in some cases. They also showed that erosion ceases, and may be replaced by accretion when the cause of 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 variation in form, and the findings of Loeschke and Weinnoldt, that the cerebral tables of the skull bones respond to pressure stimuli, is not necessarily true of other parts of the skeleton.

In this connection, Payton's (1933) observations on the growth of epiphyses are especially relevant. He studied the rate of erosion relative to the total increase in size of the twelve epiphyses from the six largest long bones in the limbs

of the pig. Although the pressure on both surfaces of each epiphysis must have been equal, he found very unequal rates of erosion. In every epiphysis the articular surface was accretional, the diaphyseal surface erosional; in one, the amount of erosion was more than four times the total increment in size. Nor was there any correlation between the relative rates of erosion in adjacent epiphyses. It is clear that pressure cannot be the cause of erosion; though it might be the stimulus to which any given bone, or part of a bone, may or may not respond. Thus the emphasis is again laid on the intrinsic properties of the bone.

This conclusion is corroborated by the consideration of the metatarsal bones. The Metatarsale primum differs from the others in that its centre of "diaphyseal growth" is at the proximal instead of the distal end. Yet it functions as a normal metatarsal bone and must be subjected to very similar mechanical factors as the other metatarsals. A similar situation attains in the Manus.

Of special interest in connection with the degree of determination of the growth patterns of bones by extrinsic factors are the changes in growth processes which are associated with increasing age. The pattern of a bone's growth usually changes at least once. For while "external accretion" is the initial process by which all bones become ossified from their cartilaginous or membranous precursors, there are indeed few bones, apart from some carpal, tarsal and sesamoid bones, which never waver from this original pattern. Not long after the

formation of the centre of ossification ("external accretion") most bones embark on their final or, may be, on a transitory pattern of growth. One illustration of a more complex situation is given.

Such a change occurs in the vertebrae, which, as expounded earlier (pp. 73-82), switch from the transitory pattern (of "centripetal" growth for the Arcus vertebrae and of "external accretion" for the Corpus) to a tertiary pattern, consequent upon the fusion of the vertebral segments and compounded of "external accretion" and "internal erosion" involving the Arcus and Corpus as a single unit. Similar radical alterations in the pattern of growth of a bone as a whole must occur whenever two bones fuse - as when epiphyses fuse with the shafts of long bones, when the sacral vertebrae join together and when the bones of the auditory capsule or the pelvis unite.

This plasticity in bone growth is more frequently reflected by the history of one piece of bone. It was indicated by Kölliker (1873), when he stated that "indifferent areas" in the bone of one suckling pig were sometimes areas of accretion or erosion in another pig of only slightly different age. In fact even the general growth pattern of a bone may be reversed.

An example of the <u>reversal</u> of secondary and tertiary patterns of growth is afforded by the Os tympanicum. After the formation of the ossification centre in the Annulus tympanicus, bone spreads medially and laterally through the

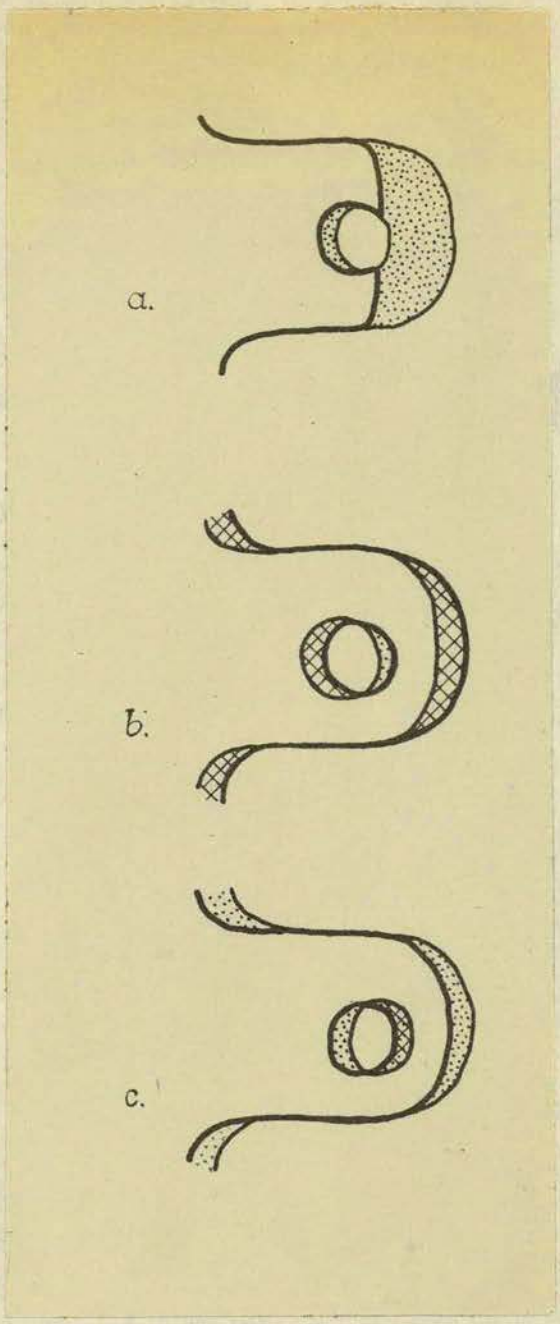

Fig. 51. Plasticity in bone growth; the changing growth patterns of the Proc. transversus.

Bulla ossea. The lateral extension soon enters the wall of the Porus acusticus externus, whose ossification proceeds by "internal accretion". However, this second growth phase is not final, for at about 14 days it is reversed and, like the rest of the Bulla, the Porus undergoes "internal erosion".

The history of the Foramina transversaria of the cervical vertebrae also affords an example of contrasting secondary and tertiary patterns of growth. Figure 51 shows the sequence of events. During the early stages of the "centripetal" growth of the Arcus vertebrae ossification spreads through the cartilaginous Processus transversus and encroaches on the Foramen transversarium. It is at this stage that the bony part of the For. transversarium is being formed by "internal accretion" (fig. 51a). Later, in accord with the inward migration of the lateral section of the Arcus (as an integral part of the "centripetal" pattern of its growth) the For. transversarium also moves inwards, by the "reversed unilateral" pattern of growth (fig. 51b). With the subsequent fusion of the Arcus and Corpus, the growth pattern of the vertebra as a whole is radically altered and the direction of the migration of the For. transversarium is reversed (fig. 51c).

It is noteworthy that a section of a long bone may be subject to no less than four different growth processes during the full course of its development. Consider a disc from the diaphysis of a long bone; let one side of the disc be adjacent to the conjugation cartilage and the whole of the disc be contained within the "ossification ring". At the moment, then,

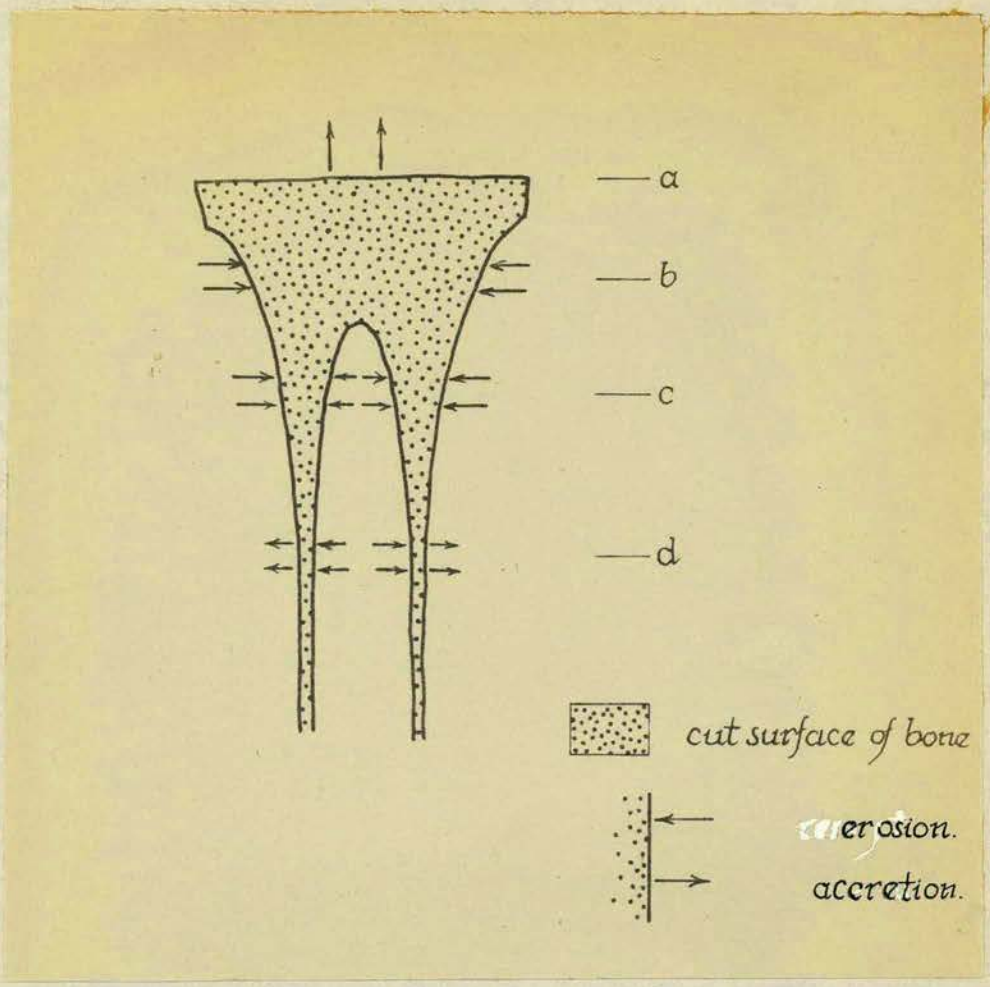

Fig. 52. Plasticity in bone growth: the four successive patterns of growth affecting a piece of a long bone initially at (a).

it is subject to "diaphyseal (external) accretion" by ossification of the matrix of the conjugation cartilage. (First phase).

Later, (through the continued deposition of bone at this surface) the disc comes to occupy a position slightly nearer the middle of the bone, and is then suddenly subject to"diaphyseal erosion" of its circumference. (Second phase).

With continued growth at the conjugation cartilage the disc occupies the position where the marrow cavity is being formed and the bone is attacked not only by "diaphyseal erosion" from without, but by "internal erosion" from within. (Third phase).

In the final phase of its development "diaphyseal erosion" has ceased and has been replaced by "external accretion" so that the middle of the shaft becomes of greater girth. "Internal erosion" enlarging the marrow cavity continues.

These four phases of growth follow one another in time and in space, since bone distal to the epiphysis represents older sections. Thus the sequence can be represented as in figure 52, where the four growth processes occurring at any one time in the growth of a long bone are depicted. It can be seen that in the course of the growth of a long bone all of these four phases are migrating away from the Punctum fixum towards the growing ends of the bone, each subsequent phase tending to encroach on each previous phase. This dynamic picture of the growth of a bone truly reflects the plasticity of its growth.

The examples of plasticity in growth so far given have

been crude, each illustrating a changed pattern of growth. But every bone is a more subtle example of plasticity. It is doubtful whether any bone maintains a consistent allometry, inferring that, even while a bone is growing with a certain pattern, the relative rates of accretion and erosion at the several sites comprising the pattern are constantly changing. Perhaps the most remarkable instance of this kind was seen in the growth of the Scapula (p. 89).

It seems impossible that such radical changes in the patterns and rates of growth occurring both within bones at different ages, and concurrently between adjacent regions of the same bone can be associated with equally fundamental variations in the factors which are presumed to control the bone's growth. It seems preferable, in fact, to hide our ignorance of the agents which effect final control over the complexities of bone development by stating them to be intrinsic. To extrinsic factors such as pressure and tension, are bequeathed those abnormalities in development which enable, for example, a skilled anatomist to recognise the skeleton of a cobbler, or the labile bone to form ball and socket joints on either side of an unsplinted fracture, or the cessation and arrest of growth with severe illnesses.

Little enquiry has yet been made into the nature of the intrinsic control over bone growth and erosion. The greatest advances in this direction have been made by Lacroix (1947), who has been able to identify the centre of control both of accretion and of erosion in long bones with an

alcohol-soluble substance to be found in their conjugation cartilages. It would appear that the action of the conjugation cartilage is to inhibit rather than to encourage erosion as dead bone is absorbed (Hancox, 1947).

Hancox (1949) has raised the question whether trophic gradients can be responsible for the patterns of erosion. Such a situation cannot be general, though it may be true of individual bones (note again the emphasis on the individuality of the bone's intrinsic properties) since in "diaphyseal" and "centripetal" growth it is new bone which is eroded, while in "unilateral" and "centrifugal" growth it is the oldest bone which is eroded.

Grüneberg (1948) expressed the hope that a comparative study of the grey-lethal and microphthalmic skeletons might furnish some information concerning the causes of bone erosion. For it appeared from his preliminary investigation of the two mutants that the microphthalmias frequently showed less failure of erosion than the grey-lethals. Unfortunately, it is not necessary that the more normal appearance of the one mutant compared with the other results from its relative success in bone erosion. For differences in the time and in the rate of ossification in individual bones can both lead to an appearance similar to that associated with partial erosion.

Dr. Grüneberg was affected in his conclusion by two facts. The first was that microphthalmic mice frequently outlive grey-lethals and a mouse reported by Hertwig (1942) has actually been sufficiently normal to breed. The other

was his own observation that the incisor and third molar teeth in particular occasionally protrude beyond the guns although occlusion, of course, is not normal. Let us then consider the question of tooth eruption as our first example of para-erosional phenomena.

second and sometimes first molars.

Fairly normal eruption of the teeth may be the outcome of their precocious development in such a way that they project beyond the borders of the alveolar bone before it becomes heavily ossified. In such a case the alveolar bone would either be modelled around the base of the teeth; or, if a thin bony crypt encasing the teeth were already formed, they could still succeed in breaking through its fragile substance. One certainly gets the impression that the molar crypt, which is figured in the microphthalmic skull of Plate 4, has been broken through rather than eroded.

The above example, while being instructive, is perhaps a very special case and a more likely common cause for a semblance of bone erosion is the delayed ossification of a bone. Supposing a bone is ossified later than usual, that bone will have the appearance of being more normal simply because so much of the process of remodelling, which would otherwise have involved bone erosion, occurred while the "bone" was in a cartilaginous or membraneus condition. It is quite plausible that this situation explains the more normal appearance of the grey-lethal Stapes of Plate 7, of the grey-lethal Vomer of Plate 8, of the grey-lethal Sternebrae

of Plate 19, and of the grey-lethal Pubis of Plate 24. It should be noted that in these three examples it is the grey-lethal bone which appears more normal than the microphthalmic, although it was the latter which was presumed to be the more capable of eroding bone. There may be a second prerequisite for the approach to normality in these cases - that ossification, once initiated, proceeds rapidly.

The rate of ossification is certainly of paramount importance for achieving para-erosion in those mutant bones which grow in the "diaphysëal pattern". Consider the Tibia. It is preformed in cartilage and it grows at its two broad ends at almost equal rates. Shortly after its proliferation, the new cartilage at the ends of the shaft is remodelled, being increasingly narrowed down in the metaphysëal region until it attains the small diameter of the diaphysis proper. Even in mutant animals remodelling of the cartilage is normal. Now if it is supposed that ossification spreads slowly through this perfectly formed cartilaginous model, the bone will be extending through the metaphysëal region over a protracted period - progressing slowly from completely remodelled to the less remodelled regions of the "bone". The microphthalmic Tibia of Plates 26 and 27 conforms to this condition. On the other hand, if the rate of ossification is high, the transition zone would be quickly calcified and the bone would have the more obtuse form of the grey-lethal Tibia of Plates 26 and 27. Differences in the rate of ossification may also explain the more abnormal condition of the grey-lethal Malleus of Plate 7.

Here the centre of ossification lies between the Capitulum and the Proc. longus, and the root of the Proc. longus is ossified quite early. If the progress in ossification is slow (as is suggested in this instance for the grey-lethal bone) the mutant bone would reflect the anterior migration of the Proc. longus, which would appear as an extended lump on the side of the Collum mallei. If the rate of ossification were faster, the root of the Proc. longus would then be more clearly delineated though it would be no less abnormally placed. This condition is seen in the microphthalmic bone.

However untrustworthy may be the suggestions in the individual cases illustrated above, the general implication stands: that factors, other than erosion can produce a semblance of partial erosion, and it is suggested that the time and rate of ossification are the two most important of such factors. Whatever the reason for these para-erosional phenomena, it is clear that the comparative study of these two mutant stocks cannot reveal the causes of bone erosion. It is, however, perhaps possible that a study of osteoporotic skeletons in man might help to reveal the controlling agents in bone erosion.

So far, only the causes of bone growth and remodelling have been considered, and they have been traced, at least as far as <u>normal</u> growth is concerned, almost entirely to unknown intrinsic factors. Only in the skull do natural extrinsic forces, such as brain pressure, appear considerably to affect bone development, but even in the skull, it is not the pattern

of growth which is determined so much as the distribution of highly local excesses in erosion which occur within the framework of the intrinsic growth pattern. Let us now turn to the methods of bone growth and modelling. We have seen that the growth patterns are an intrinsic property of the bone, now we must attempt to find out when these patterns are determined; or, in other words, when a bone is determined, for a bone is defined by its characteristic pattern of growth.

The bones of the limbs are finally determined at an incredibly early stage in embryonic development. The limb bones of the chick are delineated in proximo-distal sequence by the proliferation of an apical epidermal cap in the earliest limb bud (Saunders, 1948). If this cap is removed at progressively later stages of development, so pregressively fewer of the more distal bones are missing. There is slight regulation to the extent that only whole bones are missing, but otherwise this determination is final. If fore- and hind-limb bud caps are interchanged, then chimaeric limbs result. There is no axial regulation. The mesodermal structures are determined almost on the instant that they are proliferated from the epidermal cap, for if parts of the mesoderm are sliced off with the cap, then parts of bones may be missing.

While there is no ontogenetic plasticity in bone determination, Broom (1930) has raised the question to what extent there may be phylogenetic plasticity. He believes the Os carpale primum to represent the Metacarpale primum of

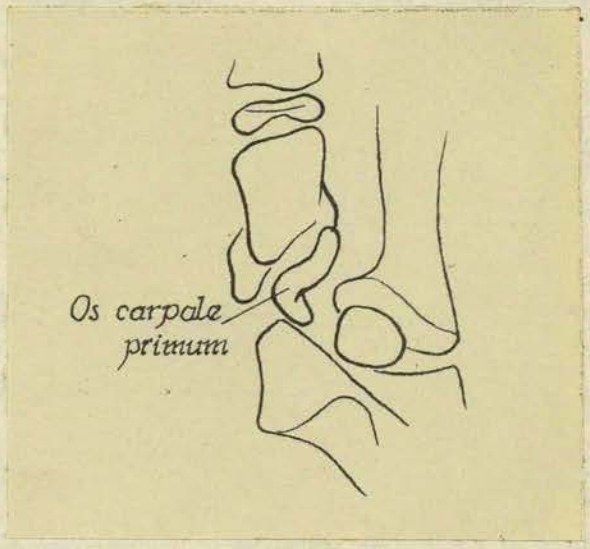

Fig. 53. Free-hand drawing of part of the Manus of a 10-day old mouse to show how the Os carpale primum resembles a typical phalangeal epiphysis of older mice.

therapsid reptiles and the Metacarpale primum of living mammals to be a transformed phalanx. It may be noted that the Metatarsale primum grows in a manner atypical of the other metatarsals but typical of the diaphyses of the phalanges; a similar situation attains in the hands. Moreover, as figure 53 shows, the Os carpale primum in the 10-day old mouse very closely resembles the proximal epiphysis of a typical phalanx. (Contrast with Broom's hypothesis). Any theory which attempts to explain the distribution of the epiphyses in the hands and feet in such a manner presupposes two postulates:- Firstly, 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.

In so far as phylogeny is related to ontogeny, a "primitive" living tetrapod might be expected to supply evidence concerning the first postulate. But it seems characteristic of the living Tetrapoda that the first digit, when present, has at least one element less than the others, and it appears that the postulated changes must have occurred early in the phylogeny of tetrapods. However, evidence from the fossil mammal-like reptiles certainly stresses the ephemerality of these elements. Thus the Pelycosaurs have the full quota of carpal bones (Ossa carpi radiale, intermedium and ulnare, and Ossa carpales 1-5, and two Ossa centralia) (that is, three more than in the mouse) while having, by modern standards, four

supernumerary phalanges. (Their phalangeal formula is 2 3 4 5 3 in place of the mammalian 2 3 3 3 3). The Gorgonopsids lack only the Os carpale 5, but some of the intermediate phalanges are very reduced in size. The Dicynodonts, however, have the typical mammalian phalangeal formula; they, too, lack the Os carpale 5, but possess the mammalian Os carpi accessorium. On this evidence it is perfectly possible for whole segments of the digits and carpus to disappear in the course of evolution without being retained as transformed bones: and conversely, for them to appear without being converted from some already existing bone. This palaeontological evidence of the ephemerality of the bones of the hand suggests that epiphyses may be equally ephemeral. Proof of this is available from the observations in human pathology. Burke (1930), Ogilvie (1931a), Brailsford (1934) and Pryor (1936) have all observed cases of metacarpal bones (sometimes the first, Burke, Ogilvie and Brailsford, sometimes the second, Brailsford and Pryor) with two epiphyses instead of the normal single epiphysis for these bones. Brailsford has also observed phalanges with epiphyses at each end. None of these authors comments on or figures correlated deficiencies in the rest of the skeleton of the hand, and it is as though epiphyses can be created from nothing; so also the carpals. Ogilvie (1931b) records a case of bipartite Os carpi radiale, which, being bilaterally symmetrical, can hardly have been caused by fracture and in the course of the discussion of his paper, another similar case was put on

record. Thus it appears unlikely that the Os carpale primum can represent an earlier metacarpal bone (Broom) or a phalangeal epiphysis (the present author) and it is equally doubtful whether the Metacarpale primum of man or mouse is actually a phalanx merely transformed in function.

The doubt thrown on the theory of the evolutionary transmutability of the elements of the hands and feet is accentuated by the malaccord of the second postulate - that the distribution of the epiphyses is determined by a gradient orientated in the proximo-distal axis of the limb - with the observations of Pryor (1936) in the field of human pathology. Pryor describes the thumbs of two children in one of whom the Metacarpale primum is distally bifurcated, in the other completely separated into preaxial and postaxial segments. In the first case the postaxial process (i.e. that nearest the Metacarpale secundum) has a distal epiphysis (like the Metacarpales 2-5) besides the common proximal epiphysis. The preaxial process, like a typical Metacarpale primum, has no distal epiphysis. In the second case, the postaxial segment of the first metacarpal has no proximal epiphysis (unlike the preaxial segment which is typical of the 1st metacarpal) but the X-ray photograph of neither hand is sufficiently clear to say definitely that it has a distal epiphysis. Nevertheless, it is apparent from Pryor's observations that the distribution of the epiphyses of the metacarpal bones is determined by a gradient in the transverse plane of the hand. Thus the postaxial bifurcation of the thumb comes within the

threshold concentration of the field which determines a distal metacarpal epiphysis. The question arises whether the same graded field determines the presence of a proximal epiphysis. In view of the large number of cases reported of metacarpal bones (first and second) with two epiphyses, the absence of reports of metacarpal bones with no epiphyses appears especially significant. If two separate gradients were concerned, one for the presence of a proximal epiphysis, the other for the presence of a distal one, metacarpal bones with no epiphyses should appear about as commonly as those with two. It is concluded then, that there is only one gradient which determines the presence and absence of both epiphyses. It is clear that a different threshold determines the distal epiphysis to that which determines the proximal one, for it would otherwise be impossible for a metacarpal bone to have both. It is also concluded that the similarity of the Metacarpale and Metatarsale primae to the phalanges both in regard to the position of their epiphyses and to their patterns of growth is not indicative of their common phylogeny.

Bony epiphyses are a fairly recent invention in evolution and make their first general appearance in mammals. Birds and reptiles have the proximal tibial epiphysis (Harris, 1933) and many more reptiles and amphibia have terminal calcified cartilages (Parsons, 1904). There has been much speculation, particularly among medical men, as to their function and significance. Parsons (1903-5) recognised three types of epiphyses - atavistic, pressure and traction. In

the light of the foregoing discussion the former must be regarded with suspicion. Pressure epiphyses occur at the articular ends of long bones; and traction epiphyses are similar to sesamoid bones since they occur where there is an angle in the line of traction of a pulling muscle. Such a classification does not explain the advantage that pressure and traction epiphyses have over mere extensions of the diaphysis: it merely states their location. Traction epiphyses develop independently of muscular tension (Appleton, 1922) and in this respect differ from sesamoid structures. As Broom (1930) states, epiphyses are formed at the more rapidly growing ends of long bones where ossification "is delayed", but they also occur very late in development at the tips of the several processes of the vertebrae, and the slowly growing Corpora vertebrales and Sternebrae have an epiphysis at each end. On the other hand, the Costae have epiphyses at the more slowly growing end. Thus mere delay in terminal ossification is no certain criterion for the development of an epiphysis.

Nicholson (1937) has advanced yet another idea concerning the significance of epiphyses, for which he is indebted to Professor T. B. Johnston - that epiphyses are formed as ossification and remodelling become more extensive and complex with increased growth in length, (as compared with the short limbed reptiles). In this regard it is of interest that, as small and large mammals (e.g. mouse and elephant) have about the same number of epiphyses, Professor Johnston's thesis

seems hardly valid. Nicholson then elaborates - "modelling and remodelling are patently less extensive and profound from two or more centres than from one". This concept is certainly true of the skull but not at all necessarily for the long bones where the epiphyses are anyhow situated outside the zone of greatest "modelling and remodelling".

The present author, however, fails to advance, with conviction, any new theory to account for the evolution of epiphyses. The occurrence of separate growth cartilages for epiphysis and diaphysis (Harris, 1933) may enable the trabeculae to be orientated in a way better suited to withstand the stresses to which each part of the bone is subjected, yet the conjugation cartilages at the distal end of the Femur and at the proximal end of the Humerus are so highly infolded that it is questionable whether these cartilages can better serve this purpose than a single "epiphyseal" growth cartilage alone.

While the role of the epiphysis in bone growth is still unsolved, that of the osteoclast is much clearer. For although in a comprehensive review of research on the osteoclast, Hancox (1949) came to the conclusion that in relation to bone absorption "osteoclasts must still be regarded as enigmatical structures", such indecision in the light of the present contribution is no longer permissible.

In the first place it is no longer possible to regard the osteoclast as unrelated to the process of bone erosion, since the sites of erosion described by the present author are mostly comparable with the sites of the osteoclasts

described by Kölliker (1873) in another species.

It remains to be decided whether the osteoclast is an active participant in bone erosion, or whether it is passive or merely a by-product. The latter has been held by Wilton (1937) and by Häggquist (1934), Retterer (1917) and von Recklingshausen (1910) before him. It is, however, quite impossible that the osteoclast is a by-product of erosion since they occur with almost normal frequency in grey-lethals (Barnicot, 1947) in which there is no bone erosion at all. If any supporting evidence is required it suffices that Kölliker found osteoclasts before ossification centres were formed.

What, then, is the role of the osteoclast in bone erosion? Does it in fact absorb bone, or are we to believe with Jaffe (1933) that it merely removes bone cells? Ruth (1937) observed osteoclasts lining the symphysial tables of the Ossa pubis and ischii of the pregnant guinea-pig, but considered their number too small to cause the extensive erosion which occurs in these regions of the pelvis during the later days of pregnancy. He relegated the osteoclast to the role of removing bony and organic debris produced by other means than erosion. It is strange that Kawata (1924, quoted by Ruth) observed vast swarms of osteoclasts in the same situation. However this may be, Ruth's contension that absorption results from small islets of bone formed around osteocytes in the intrasymphysial ligament is untenable. For how can the pressure exerted by these bones be any greater than that exerted by the ligament itself? Now Hancox (1946)

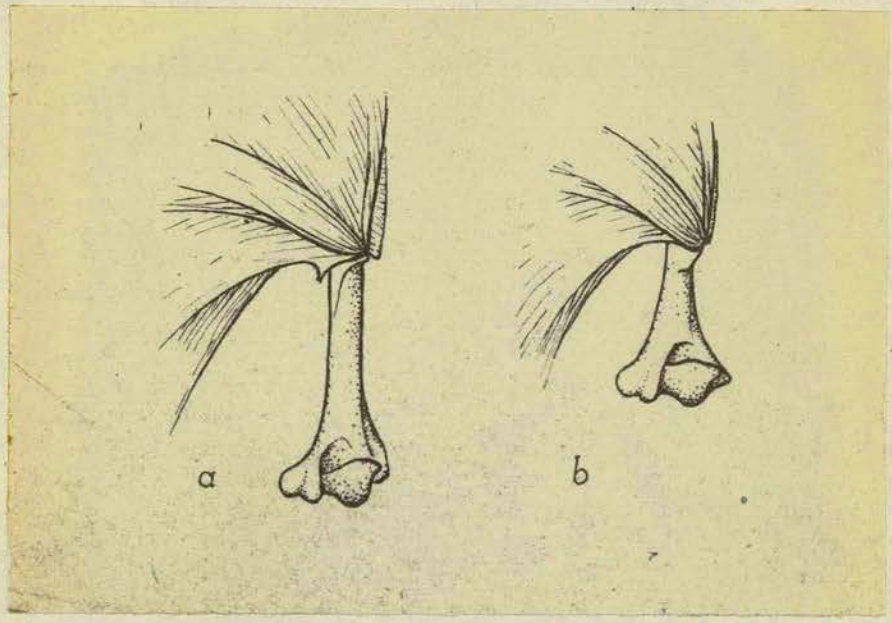

Fig. 54. Dissections of the fore-arm of (a) a normal, and (b) a microphthalmic mouse at 21 days. Note how the deltoid muscles have migrated up the Crista humeri in the mutant. Is this release of muscular tension the normal stimulus for its erosion?

has shown that osteoclasts certainly can digest cell debris since they tunnel through the plasma clots of tissue cultures. But can they absorb bone too? In this regard, Hancox again (1947) has demonstrated that dead, boiled bone (which cannot therefore absorb itself) can be eroded on its transplantation to chick chorio-allantois. This leaves no reasonable alternative to the supposition that the hollows (Howship's foveolae) into which the osteoclasts so neatly nestle, are in fact a product of the activity of the osteoclast. Trailing behind the tip of the Tuberositas deltoidea and on the inside of the mid-dorsal region of the Arcus dorsalis of the Atlas vertebra the author has observed in alizarin transparancies very small particles of bone (see Plate 14). It would be of exceeding interest to discover whether these particles have actually been ingested by osteoclasts. The author feels that the first of these sites would be admirably suited to the micro-cinematographic study of the osteoclast which, as Hancox (1948) suggested, would settle the question of the osteoclast finally, and far more neatly than by the above argument.

The problem of bone growth has been far from fully discussed in the present account, as it is very incompletely stated in terms of the distribution of epiphyses and the role of the osteoclast. There remain problems concerned with the control of the rates and extent of primary ossification - both endochondral and periosteal; - with the control of the distribution and activity of the osteoclast; and with the significance and causes of the substitution of the primary

spiculate and dense deposits of bone by the highly vascular endosteal bone based on the Haversian system and constructed as a breccia. But these fascinating topics for further research are problems beyond the scope of the present account.

5. SUMMARY.

1. Bone growth is shown to be a surface phenomenon. Though interstitial processes do occur, they alter the structure of the bone only at histological levels.

2. Methods of studying bone growth are discussed and two new methods are described. One of these, due to Grüneberg (1948) involving the comparison of normal and pathological skeletal conditions resulting from the segregation of either of two recessive genes, is used in the present study.

3. The growth of the mouse skeleton at an early age is described in terms of patterns of accretion and erosion on normally visible surfaces. Eight patterns which are considered to be fundamental in bone growth are described.

4. The general results of earlier workers in this field are confirmed.

5. The growth pattern of a bone may change several times in the course of the life of a mouse, even to the extent of the growth processes being reversed at several sites. This plasticity in the growth processes evidences the almost total lack of extrinsic factors controlling bone growth. Extrinsic

factors are relegated to the control of abnormal growth.

6. The distribution of epiphyses of the metacarpal bones is shown to be determined by a transversely graded field. The anomalous position of the epiphyses on the Metacarpale primum and Metatarsale primum, and their associated anomalous growth, are not considered evidence of their evolution from phalanges; nor is the Os carpale primum regarded as a modified metacarpal bone or phalangeal epiphysis.

7. The enigma of the role of the epiphysis in bone growth and bone function is discussed without solution.

8. The osteoclast is shown definitely to cause bone erosion.

The anatomy of the normal mouse skeleton at the age of three weeks is depicted in a series of half-tone, camera-lucida drawings in twenty-nine plates. Labelled outline drawings are appended to each plate. Latinised nomenclature is used throughout.

ACKNOWLEDGEMENTS.

The author is deeply indebted to Dr. Hans Grüneberg for the gift of this problem and for the interest he has shown throughout its investigation. He is grateful to Mr E. D. Roberts for tuition in drawing, and wishes to thank Professor C.H. Waddington for permission to continue this work in Edinburgh. Dr. D.S. Falconer kindly advised on the arrangement of the text and photographed the pencil drawings. The author has been in receipt of a Training Grant from the Agricultural Research Council.

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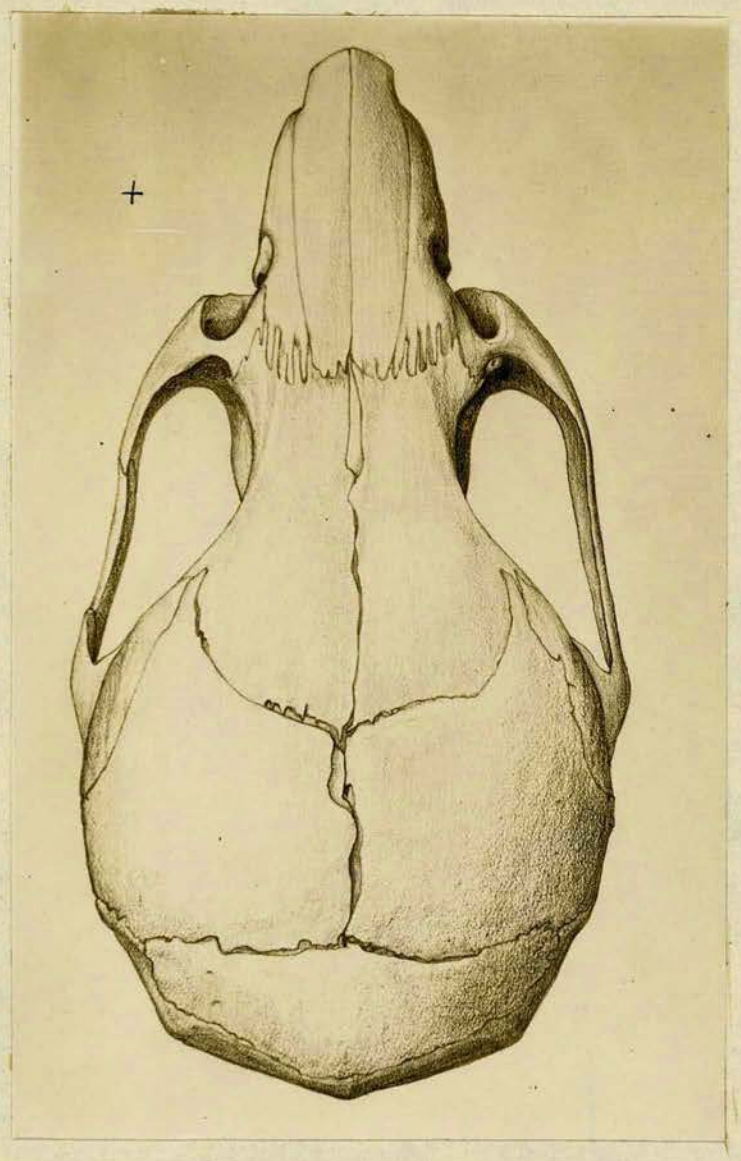
PLATES 1 - 29.

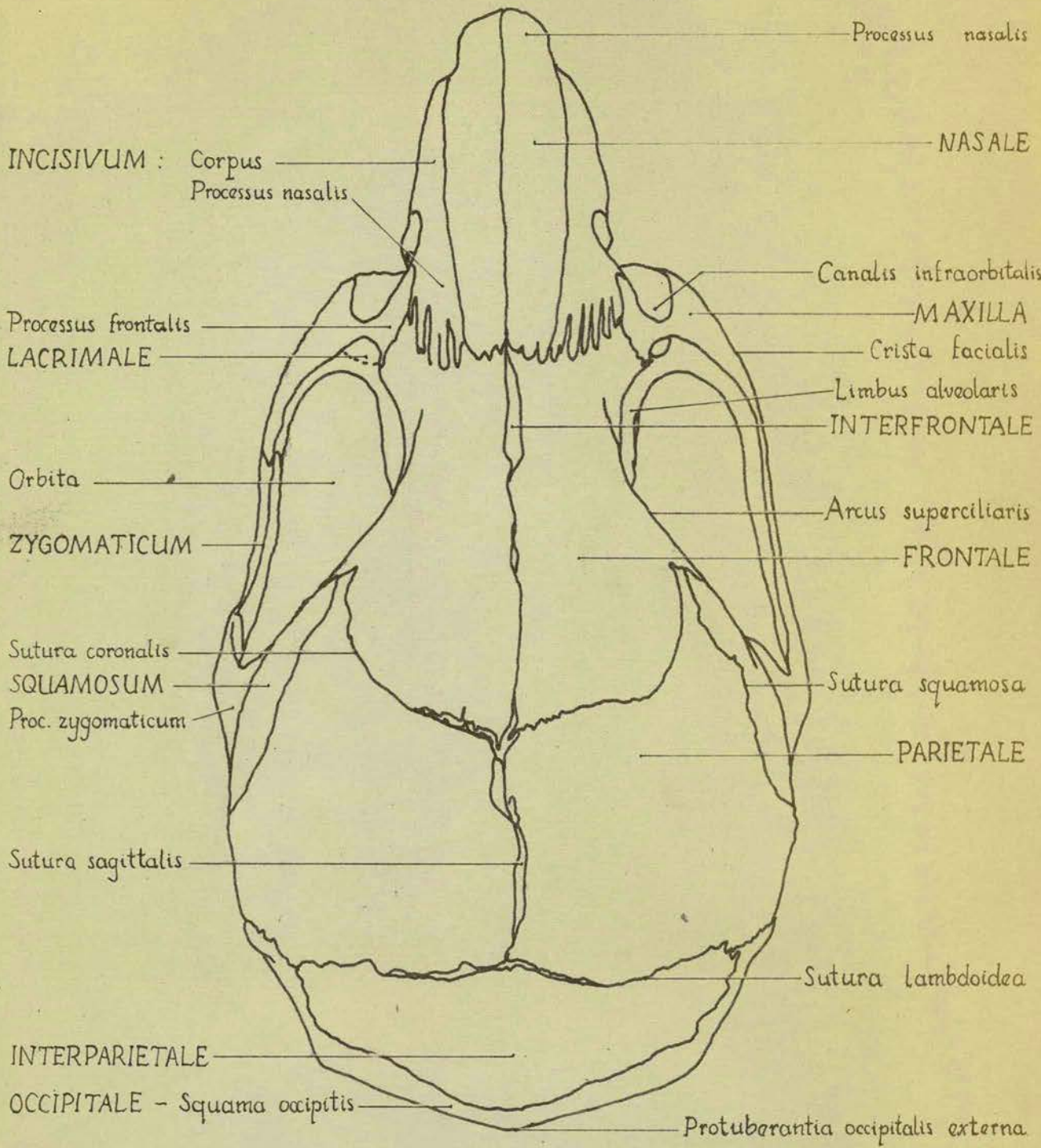
The skeletons
of normal, microphthalmic and grey-lethal
mice,

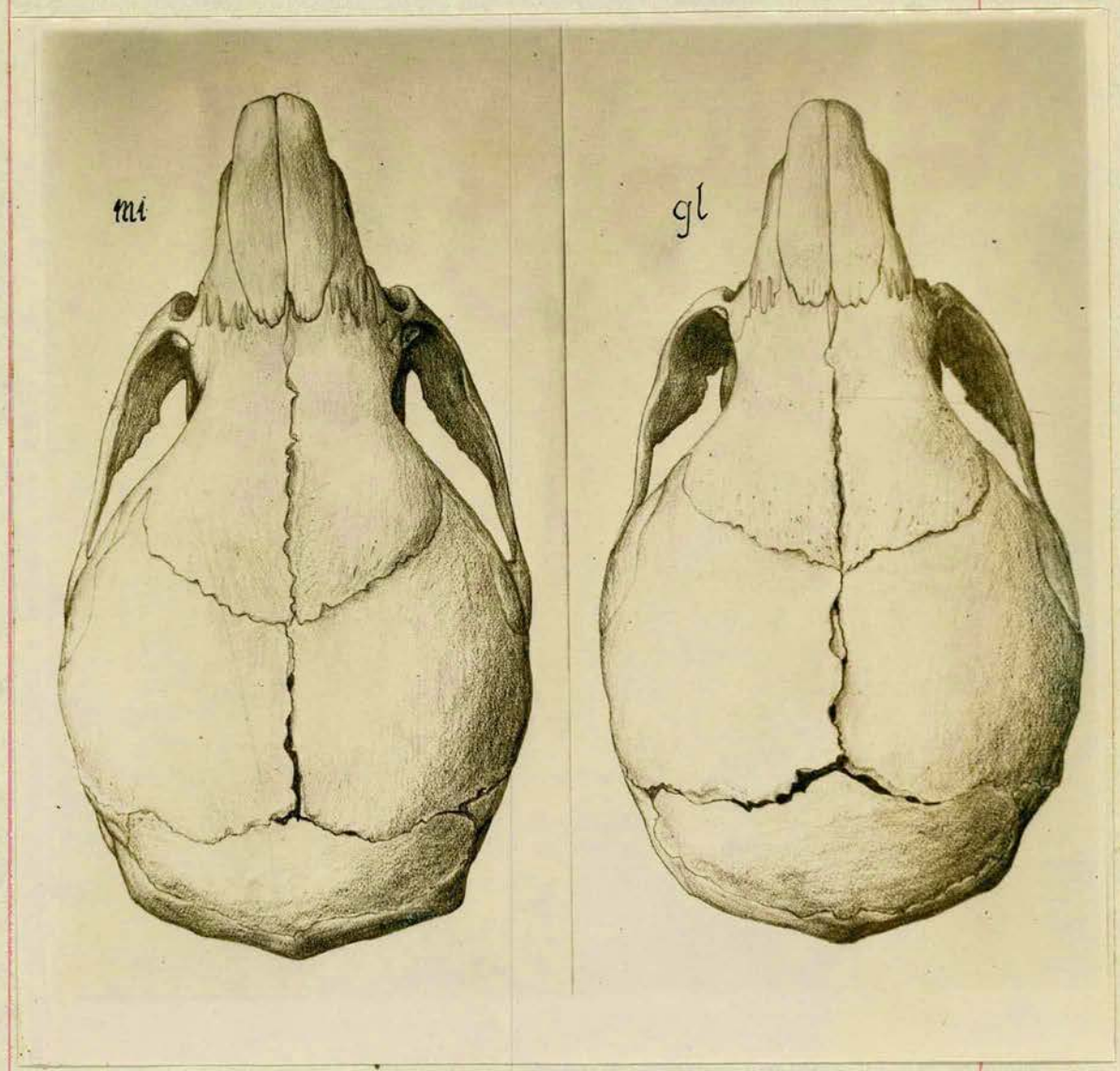
including

The anatomy
of the normal mouse skeleton
at three weeks.



SKULL — dorsal aspect

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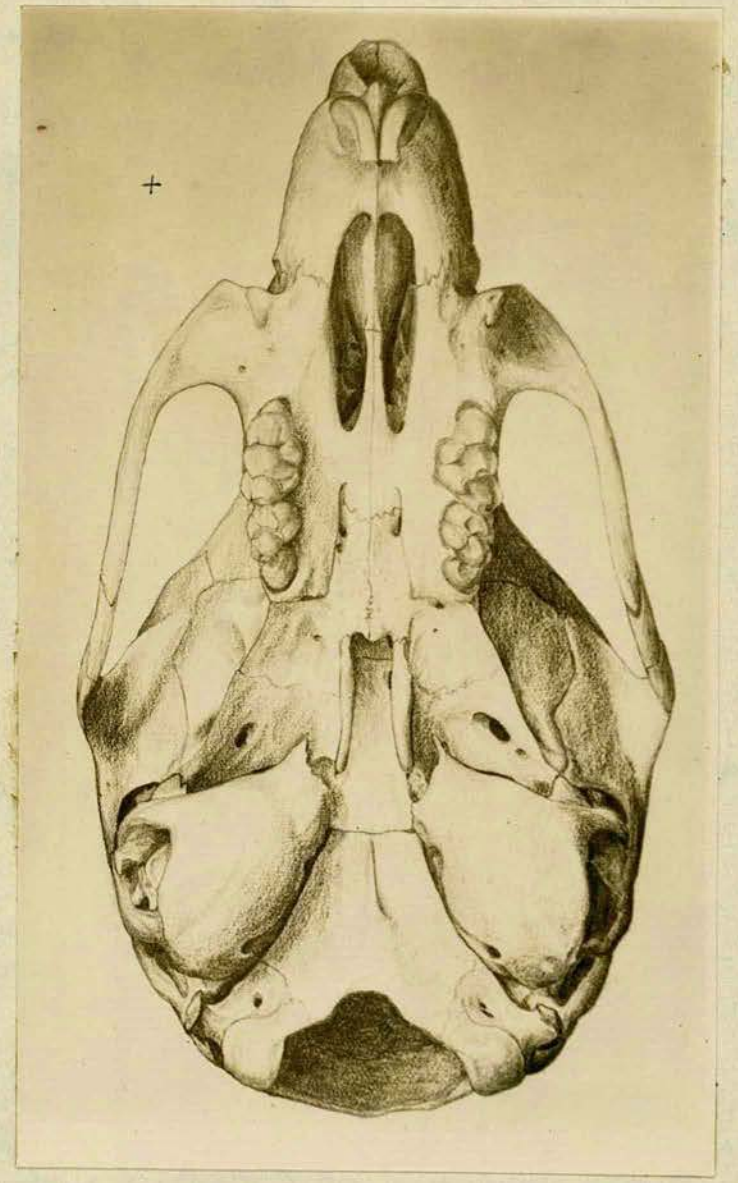
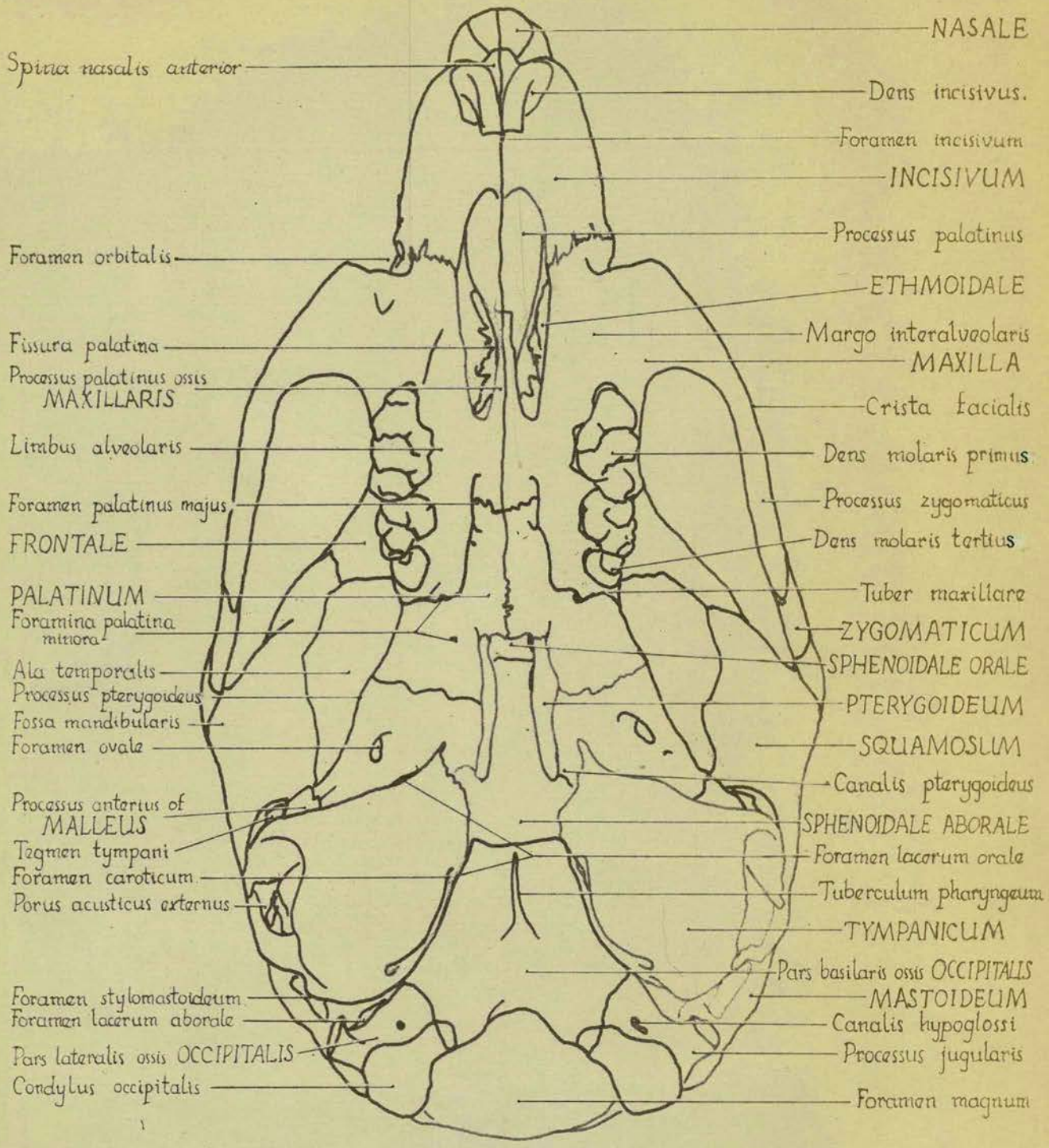
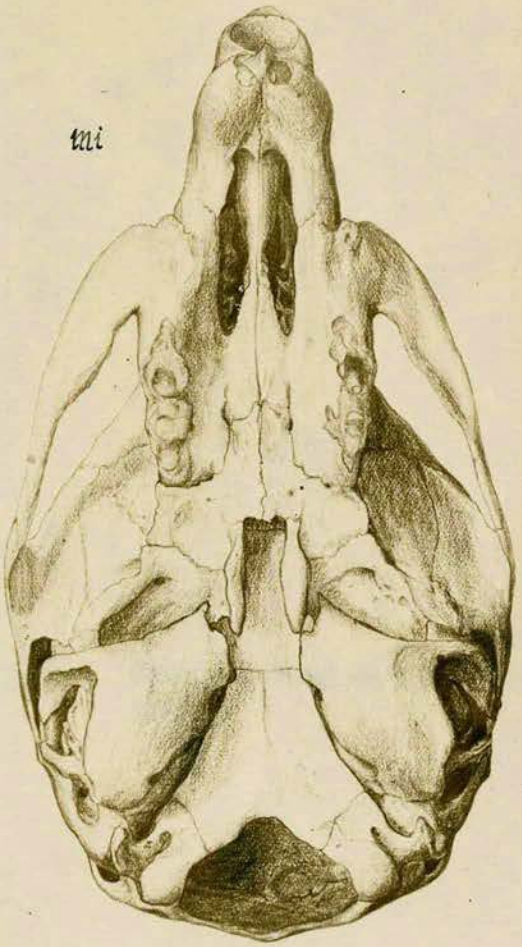


SKULL — inferior aspect

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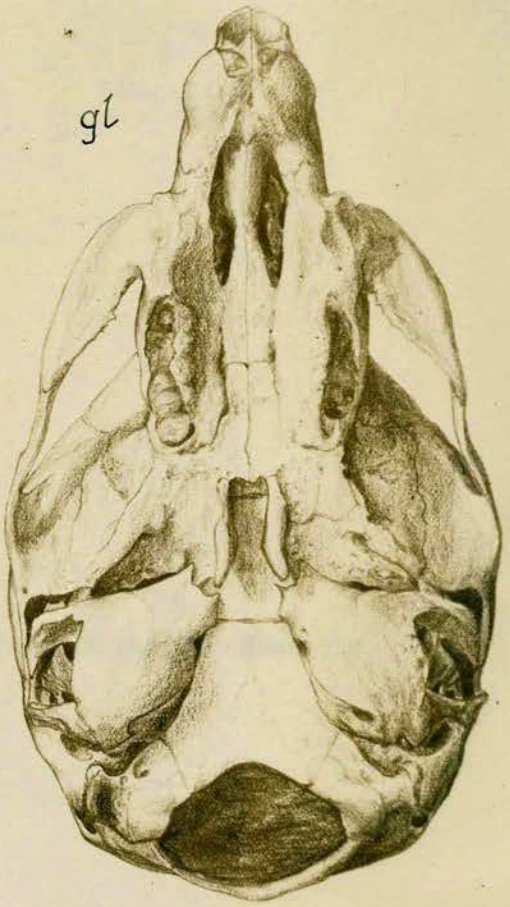
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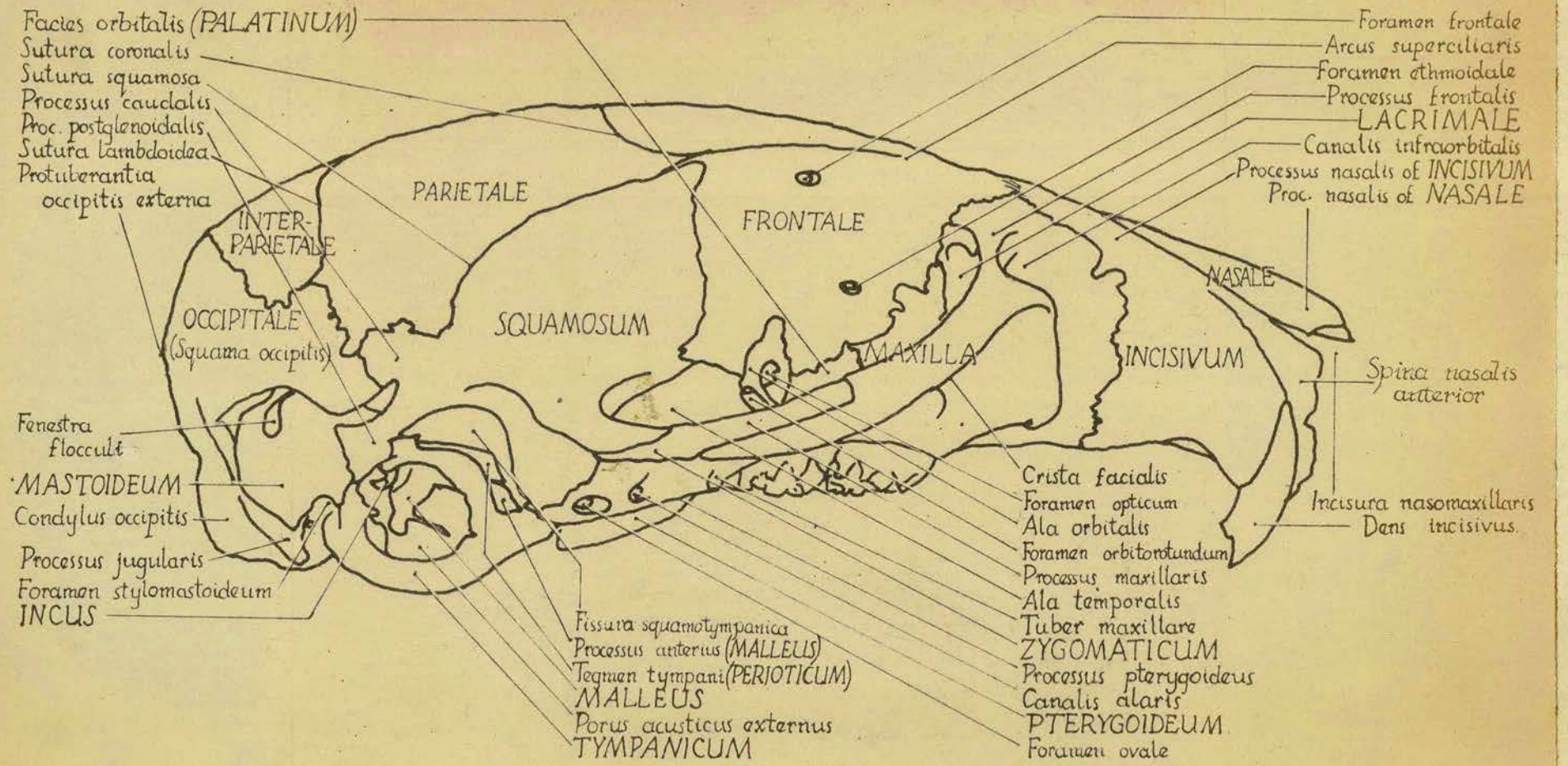
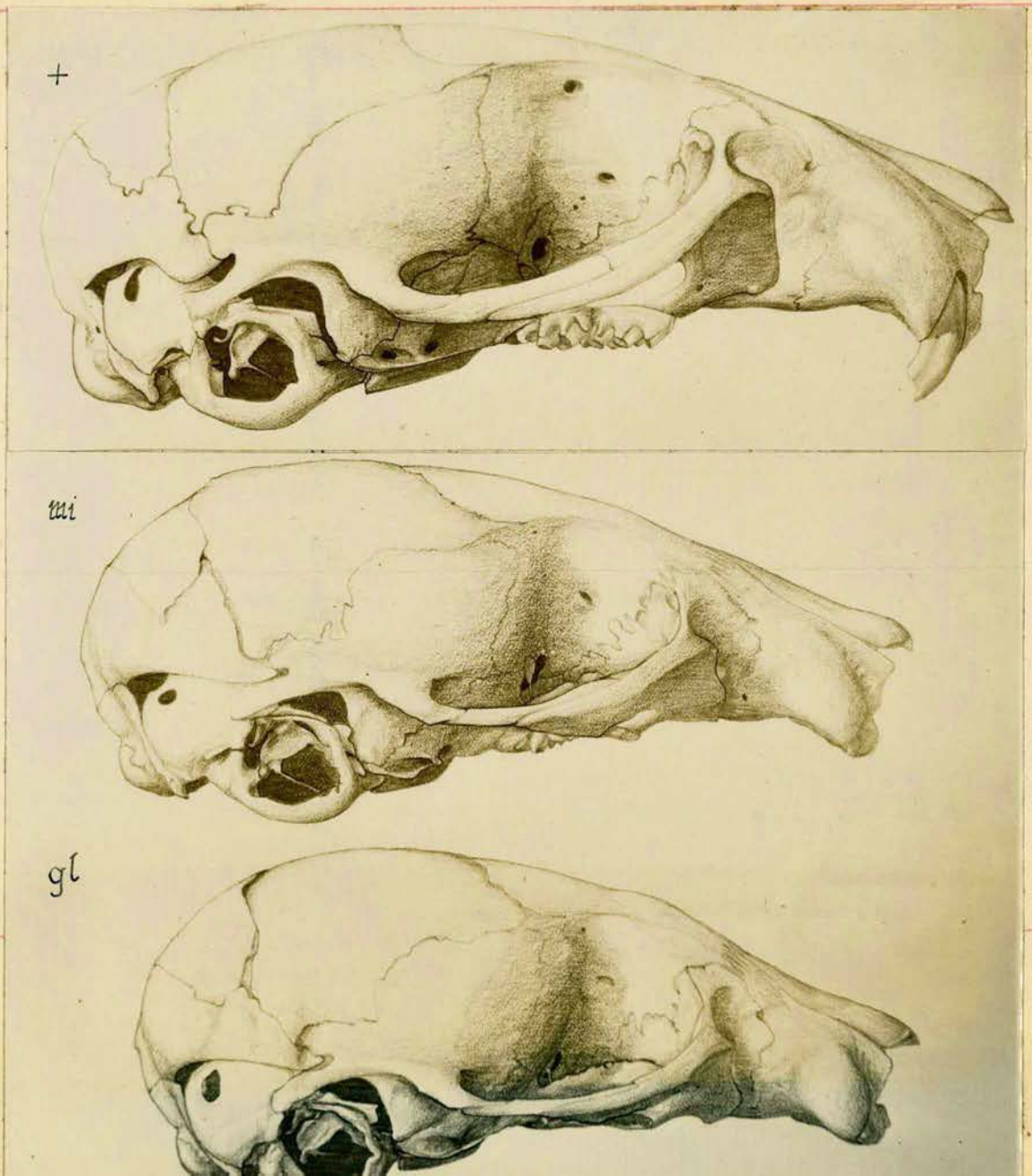
SKULL— lateral aspect.

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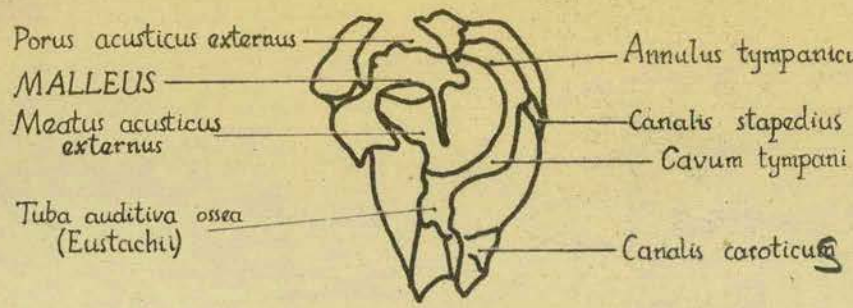
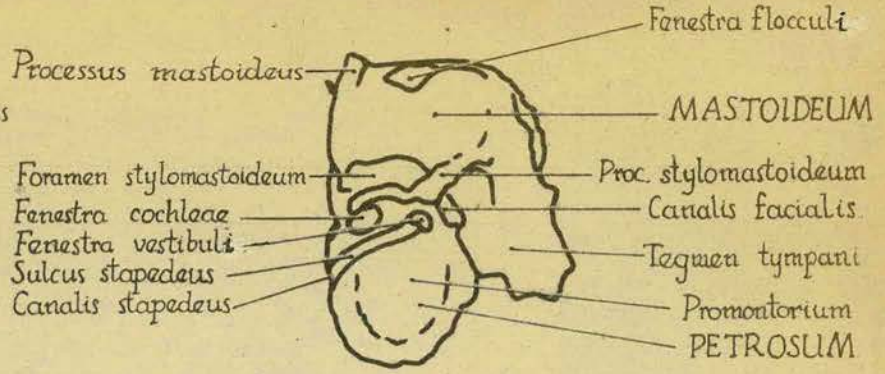
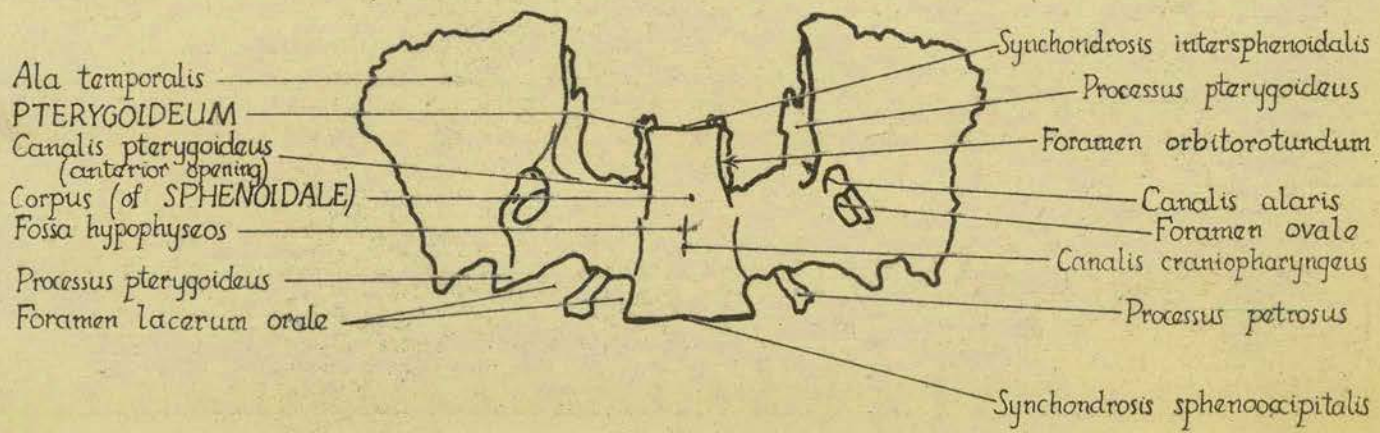
TYMPANICUM — superior aspect

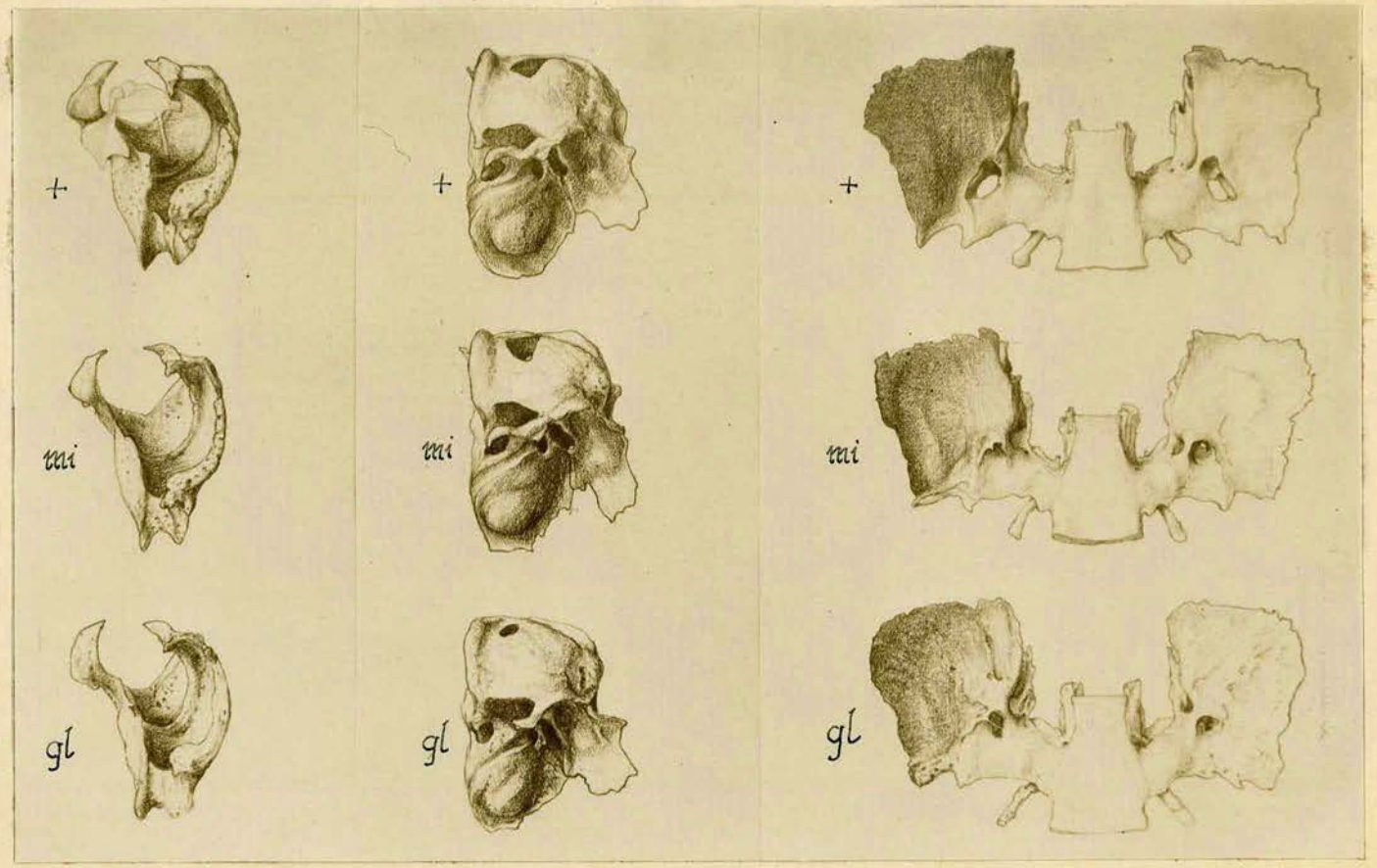


PERIOTICUM — interior aspect.



SPHENOIDALE ABORALE — cerebral surface.

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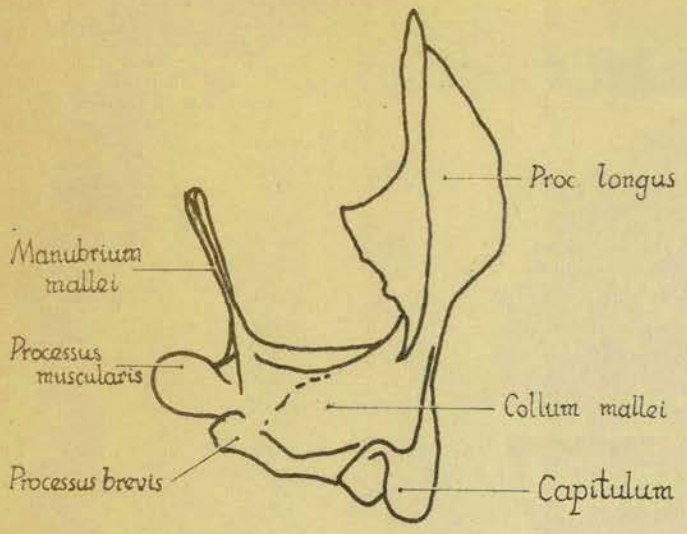
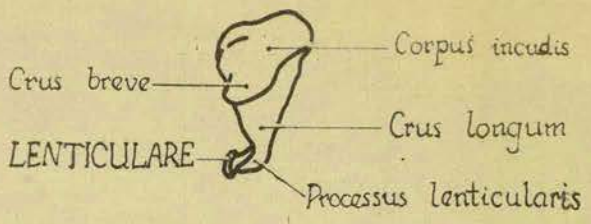
MALLEUS × 30

medial aspect

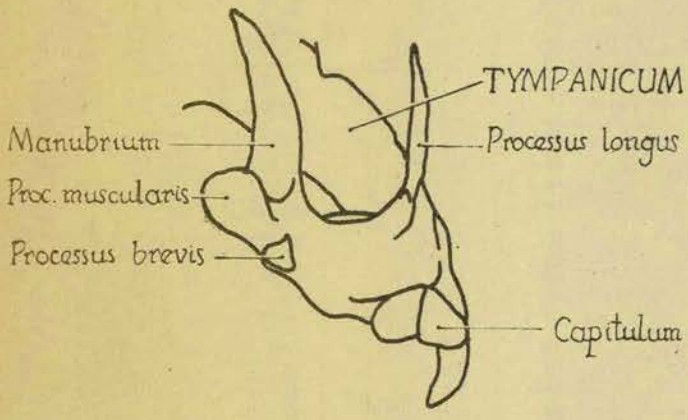


INCUS × 30

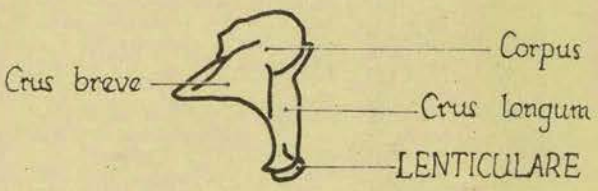
posterior aspect



ventro-medial view

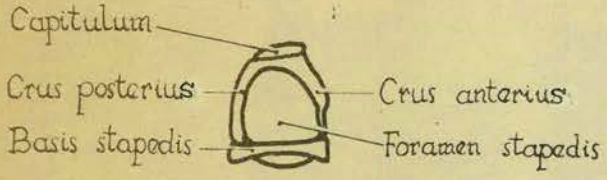


lateral aspect



STAPES × 30

ventro-lateral aspect



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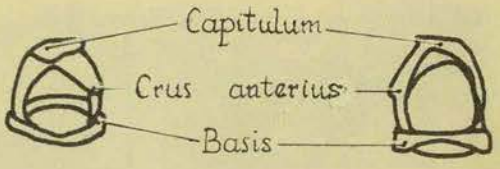
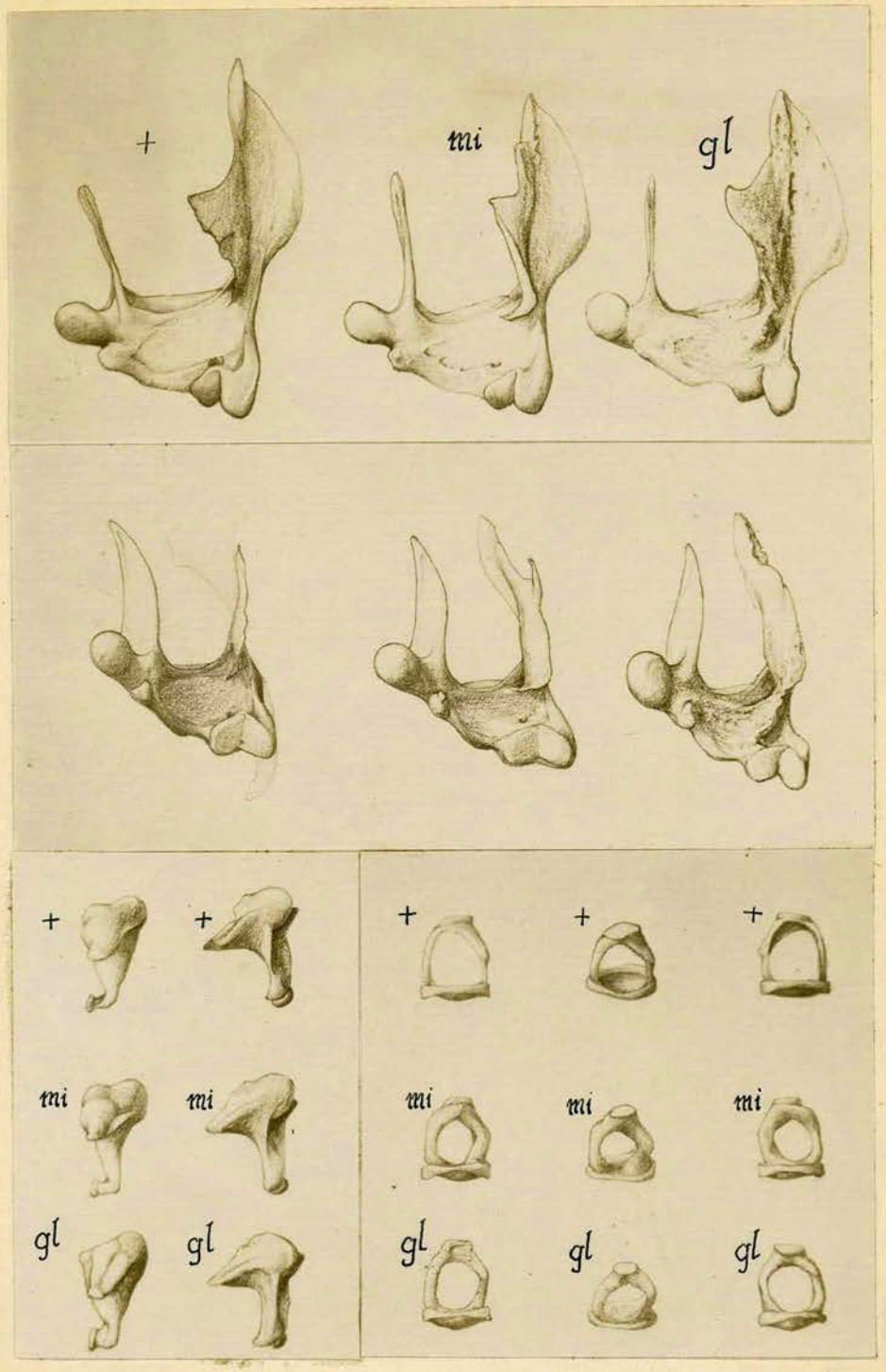


dorso-medial aspect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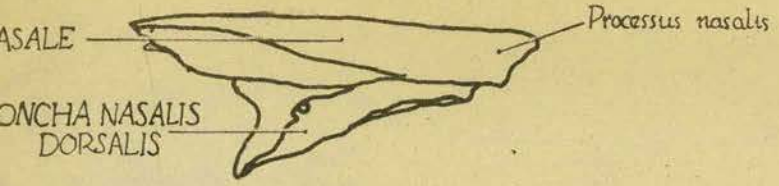
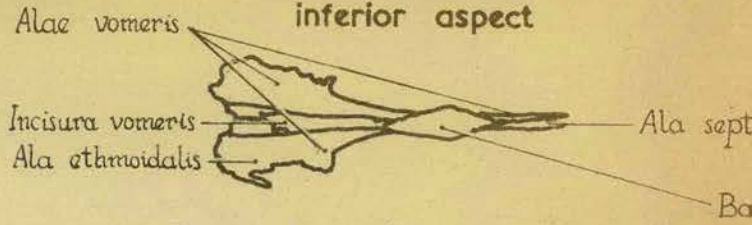
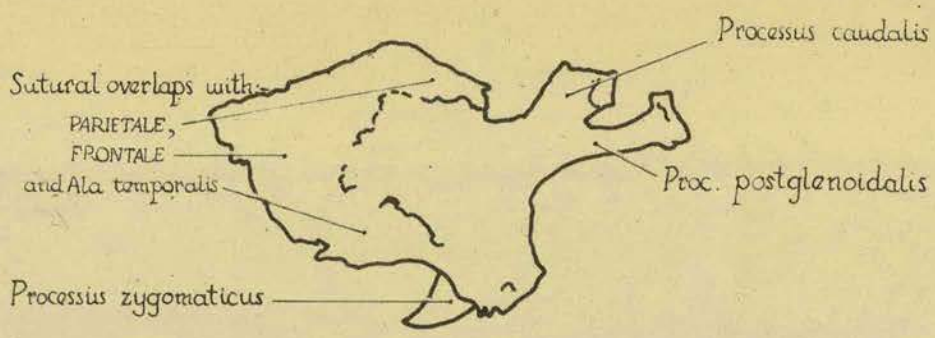
NASALE AND CONCHA DORSALIS
lateral aspect



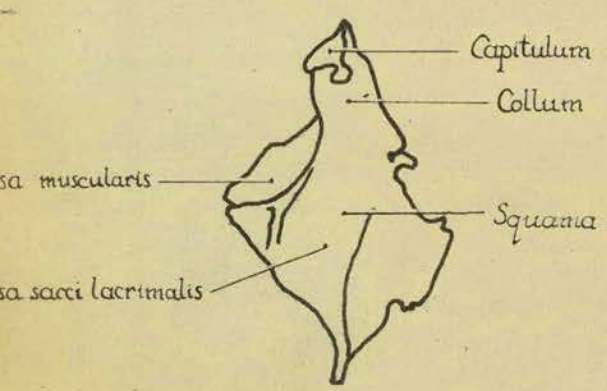
VOMER
inferior aspect



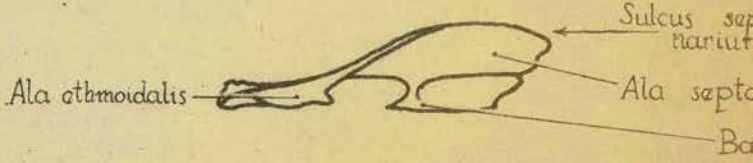
SQUAMOSUM
internal surface

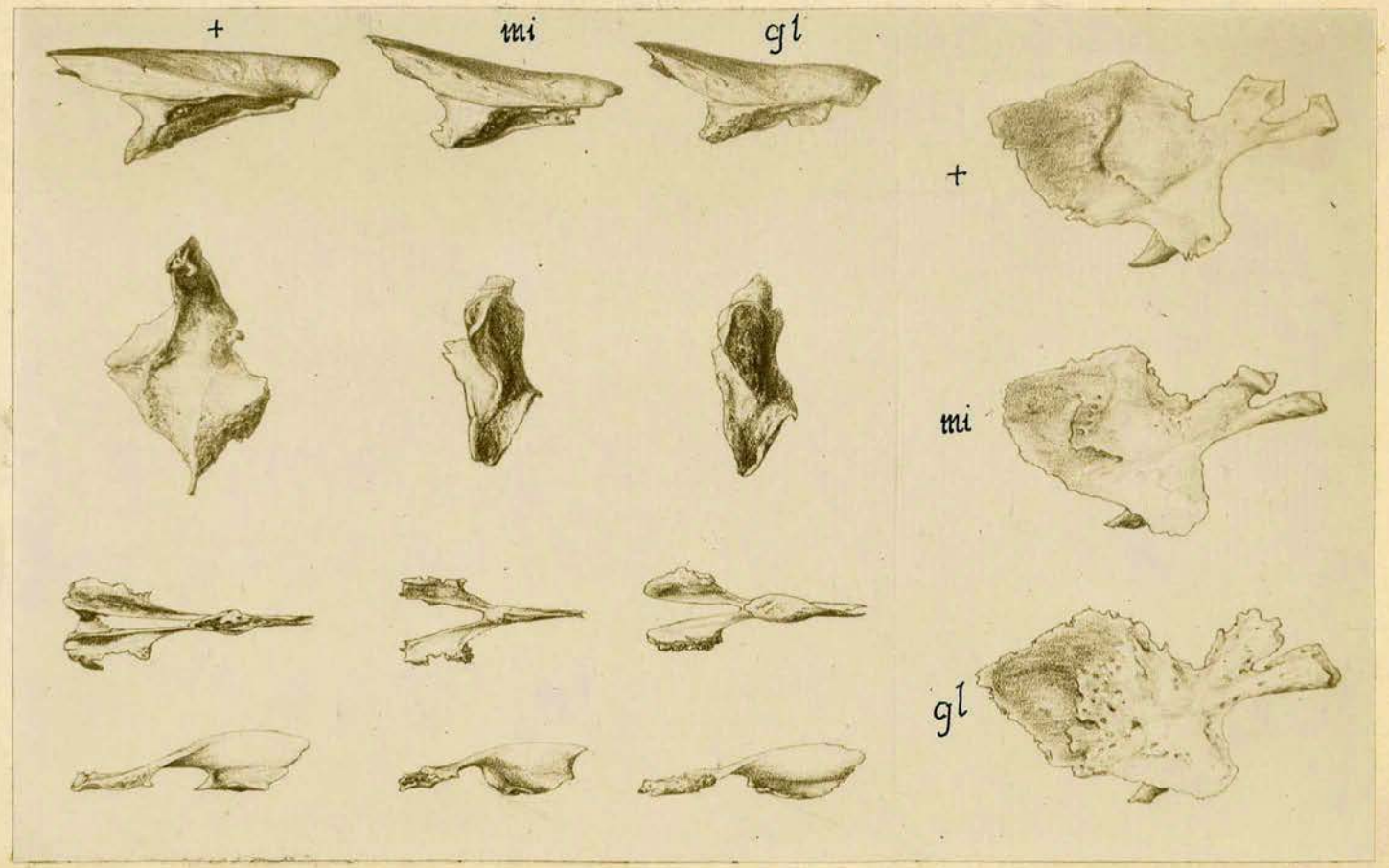


LACRIMALE
facial surface



VOMER
side view

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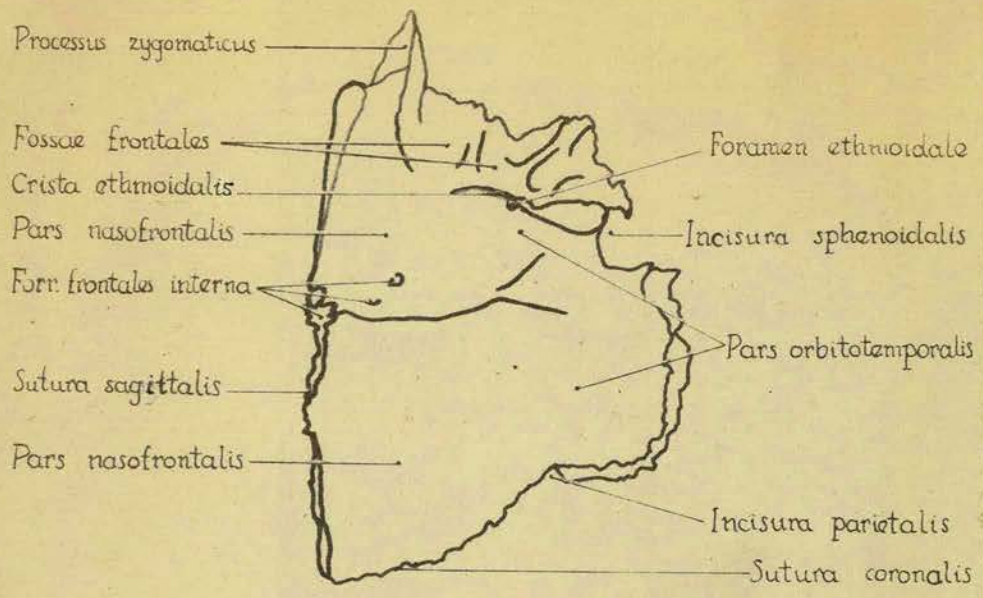
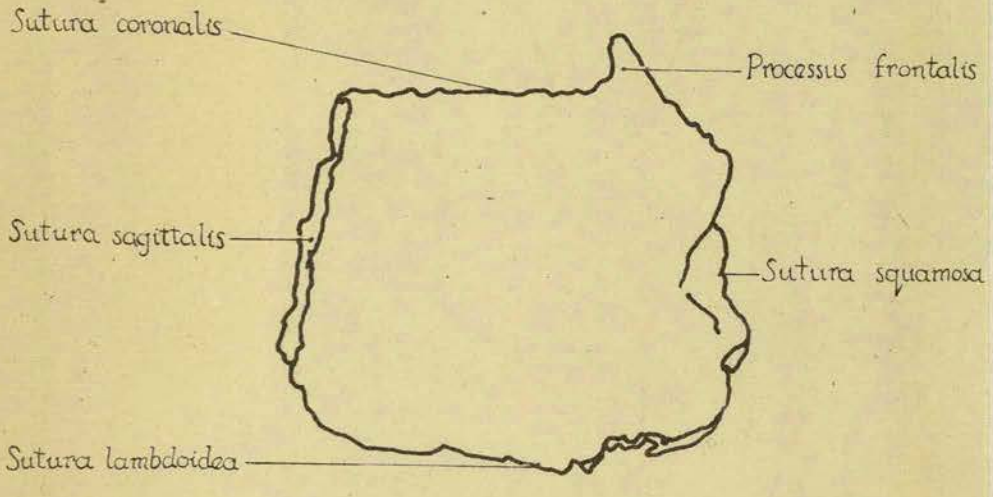
FRONTALE

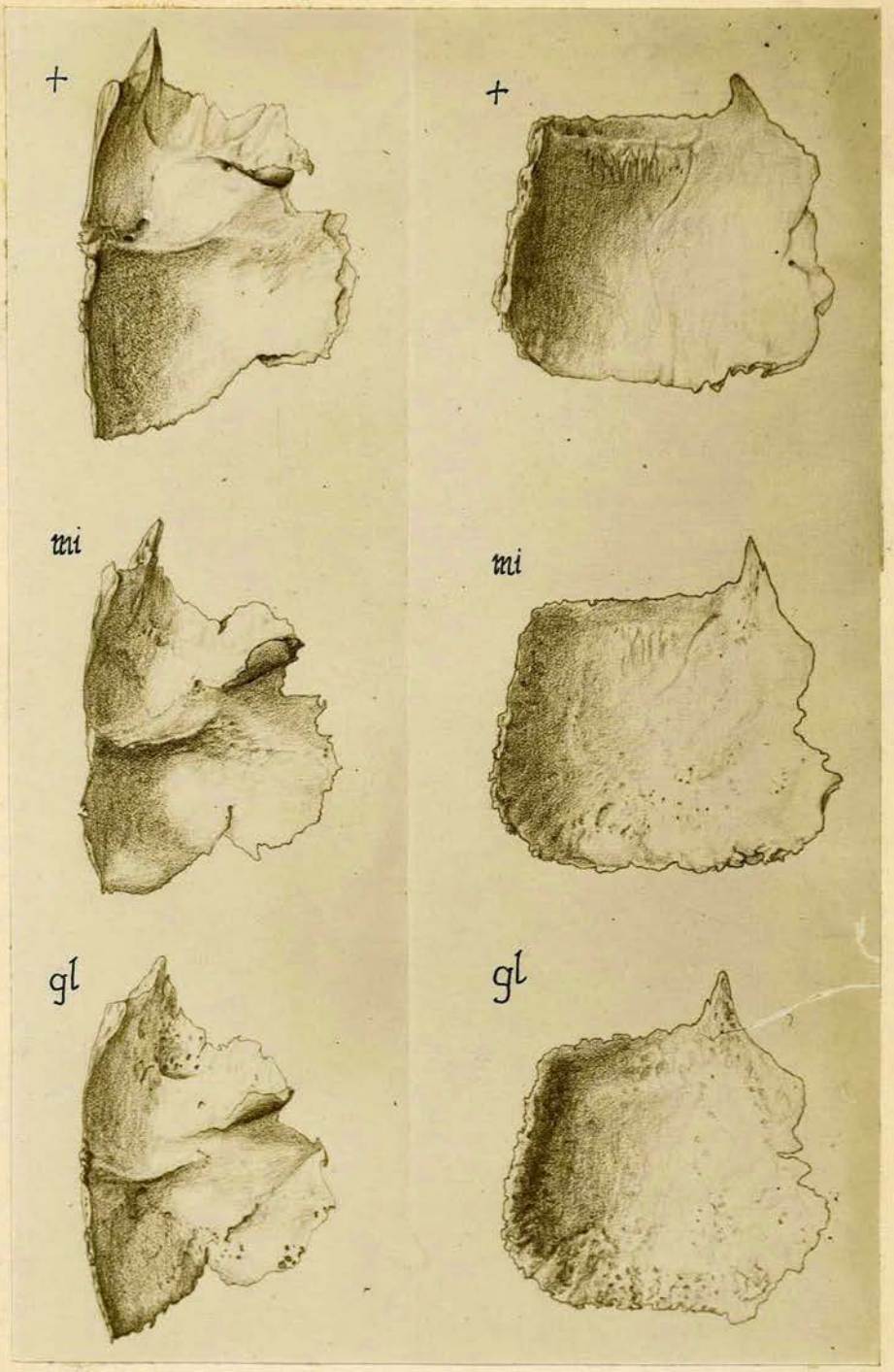
internal surface – medio-ventral aspect



PARIETALE

internal surface

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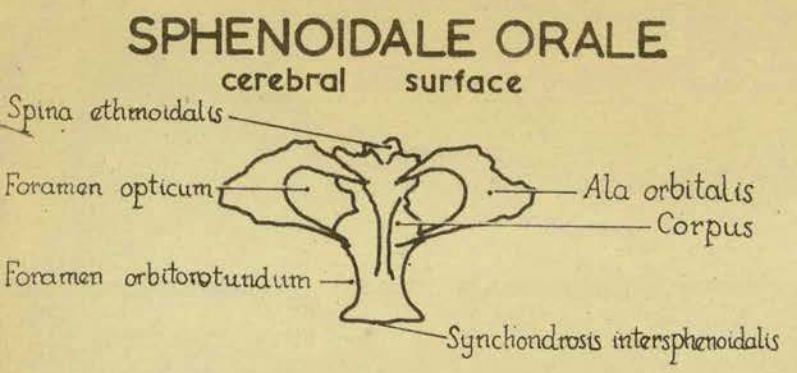
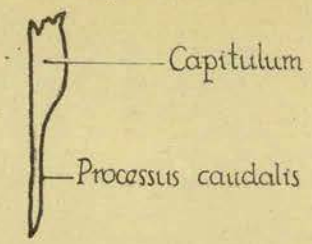
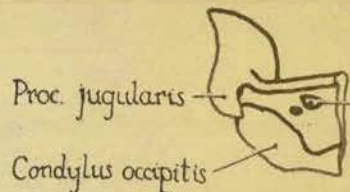
SPHENOIDALE ORALE
cerebral surface



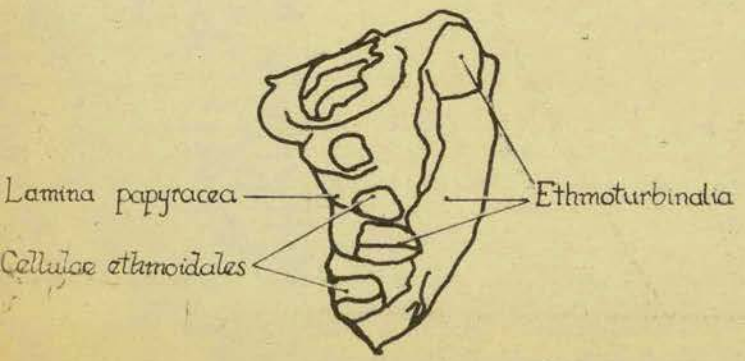
INTERFRONTALE
lateral aspect



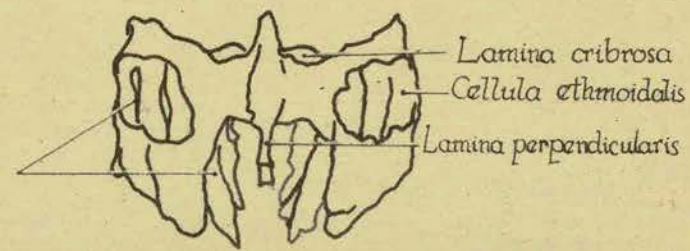
OCCIPITALE – PARS LATERA
naso-ventral aspect



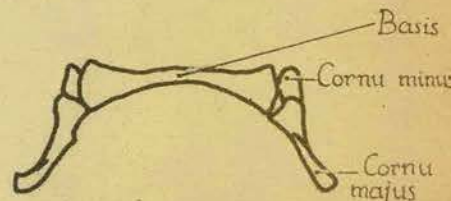
ETHMOIDALE
lateral aspect

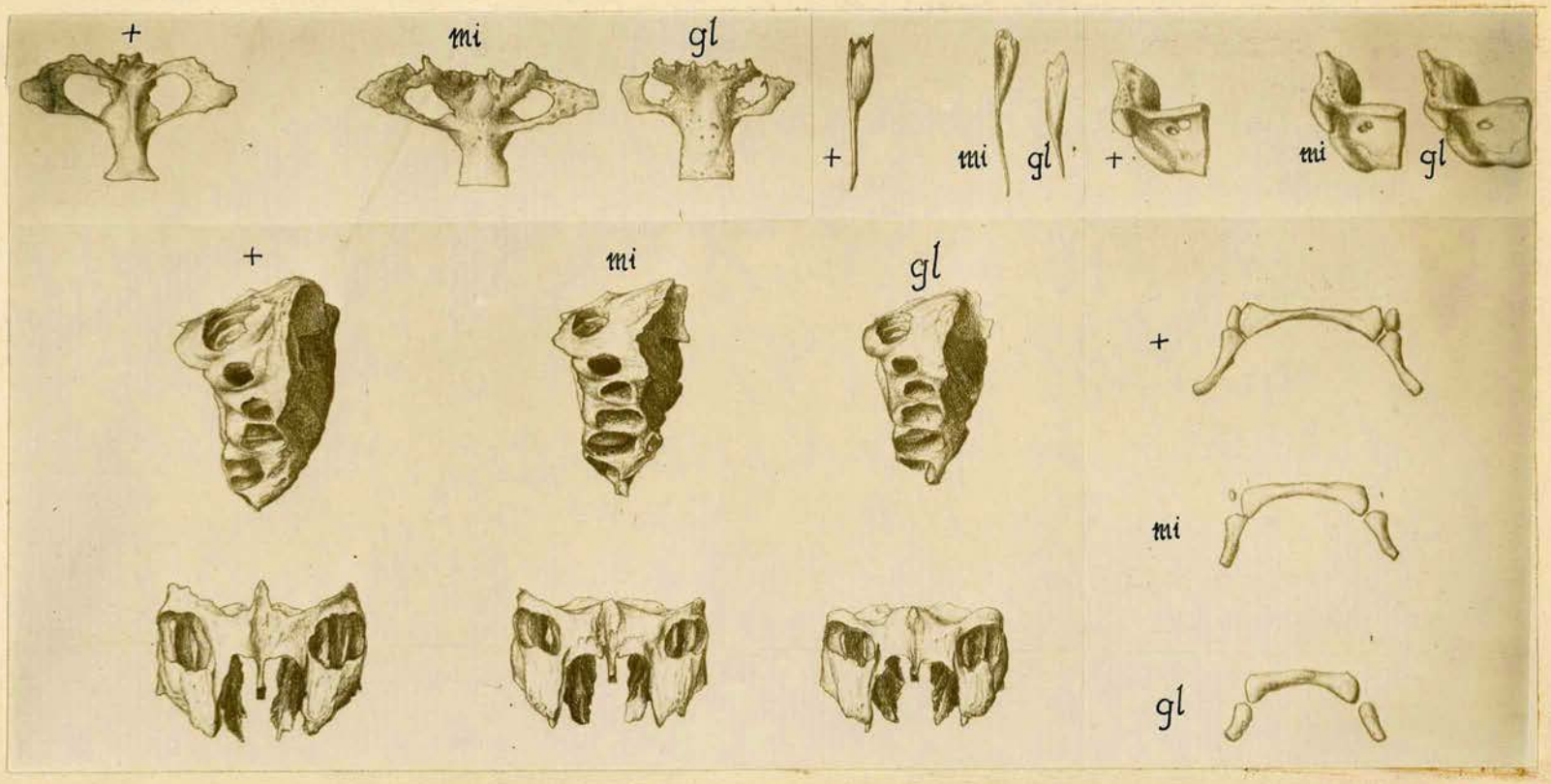


dorsal aspect



HYOIDEUM
inferior aspect

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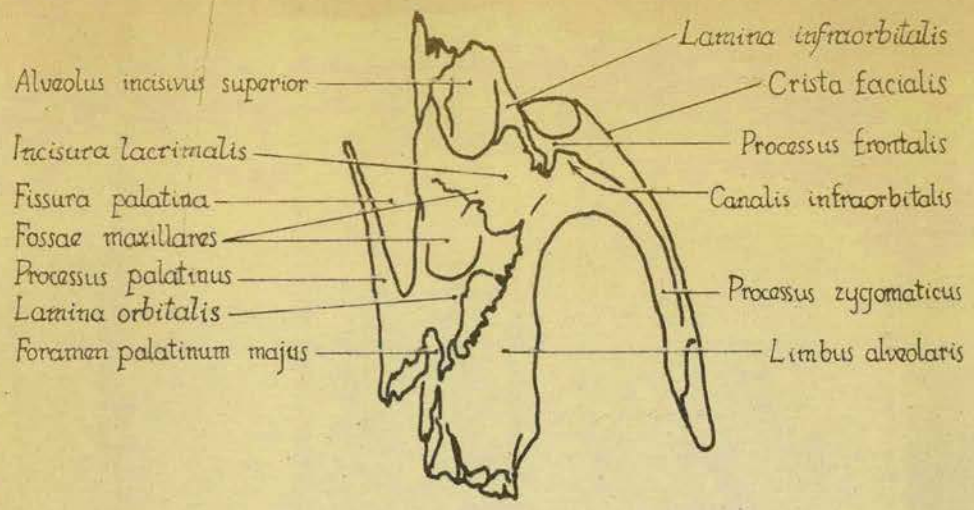
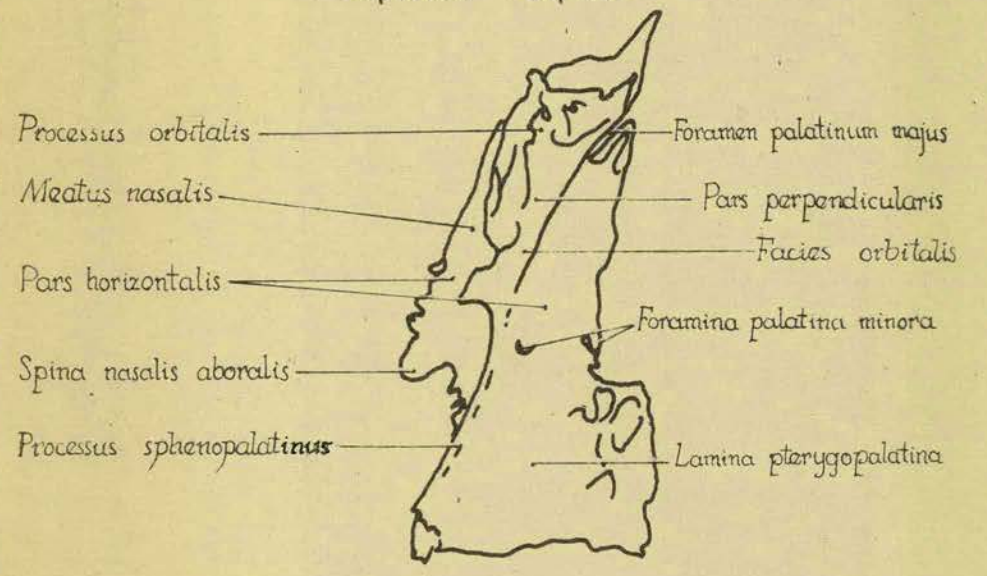
MAXILLA (×6)

superior aspect

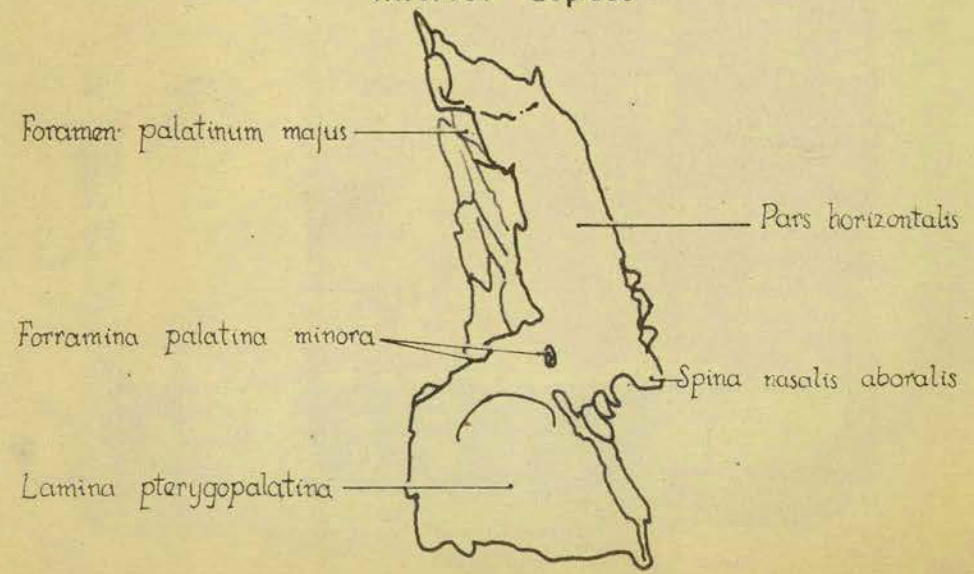


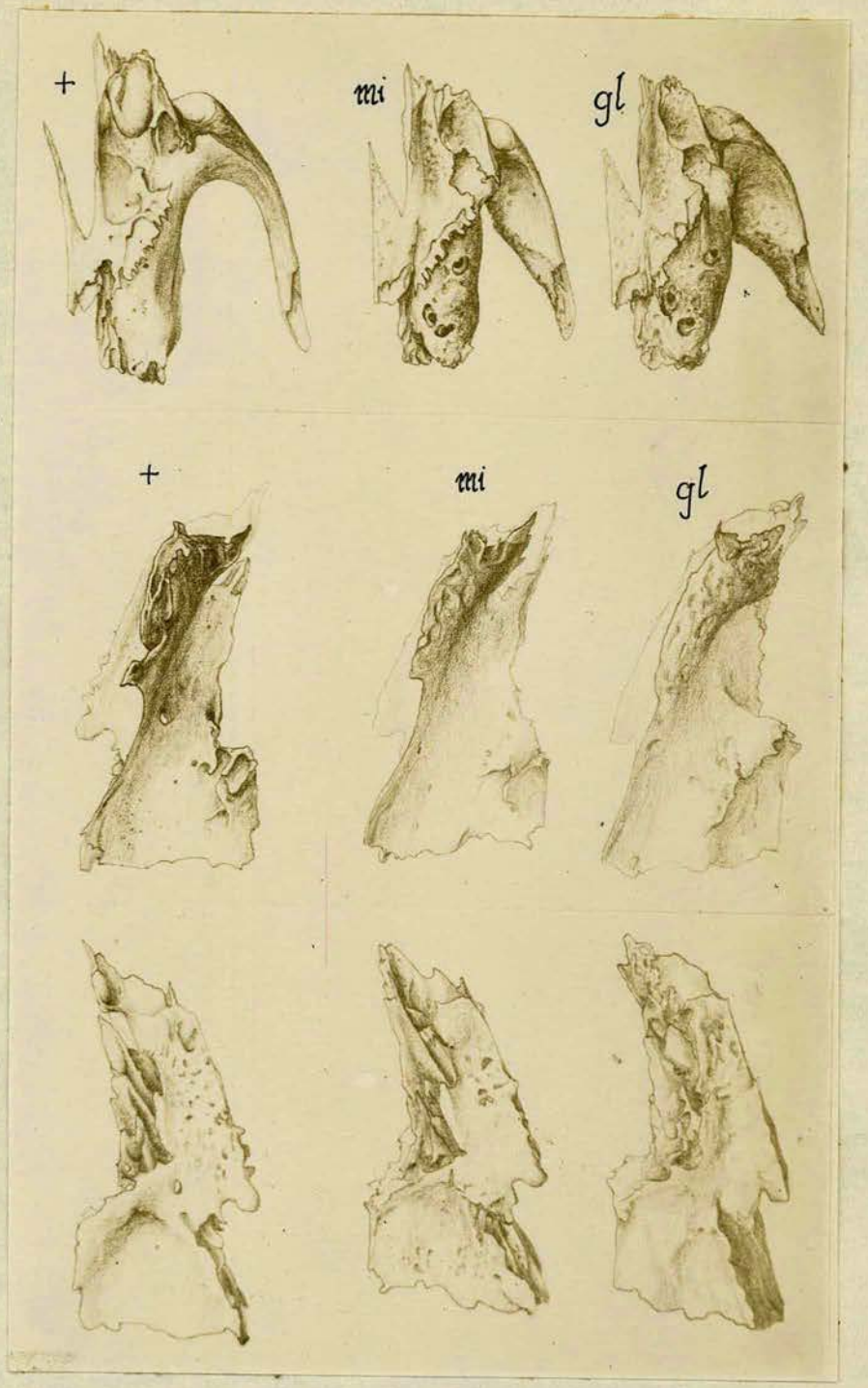
PALATINUM ×18

superior aspect



inferior aspect

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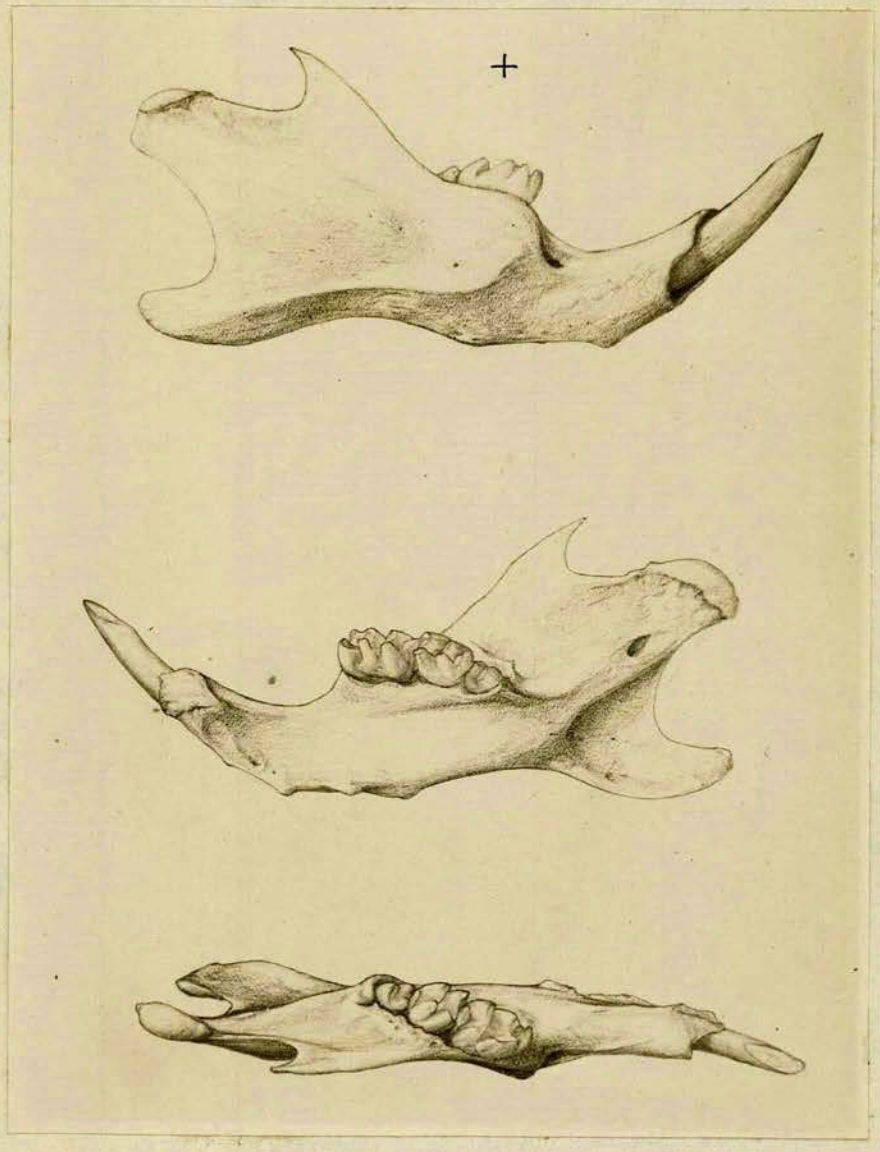
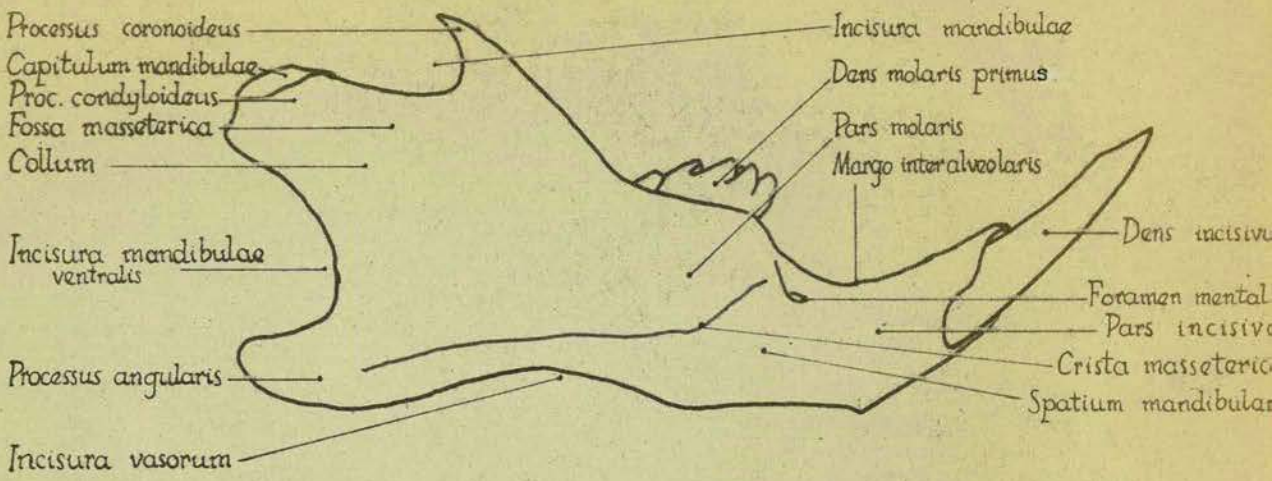
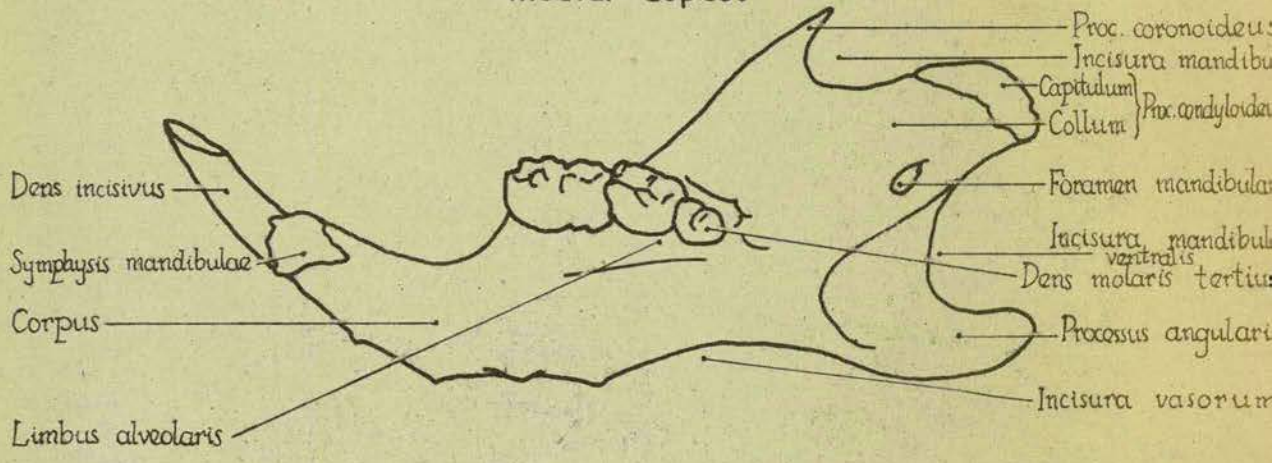


MANDIBULA

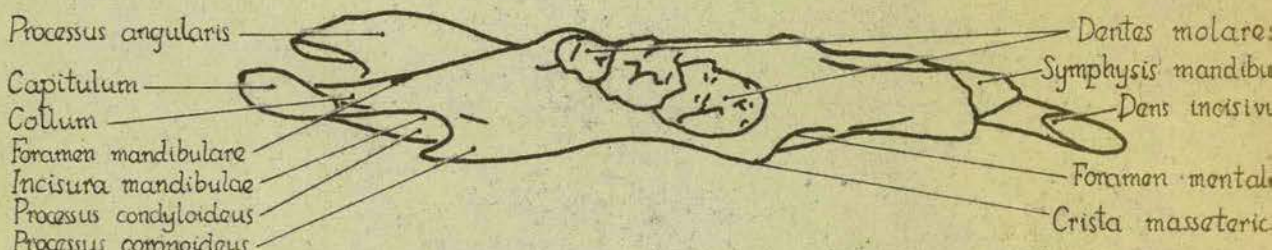
lateral aspect

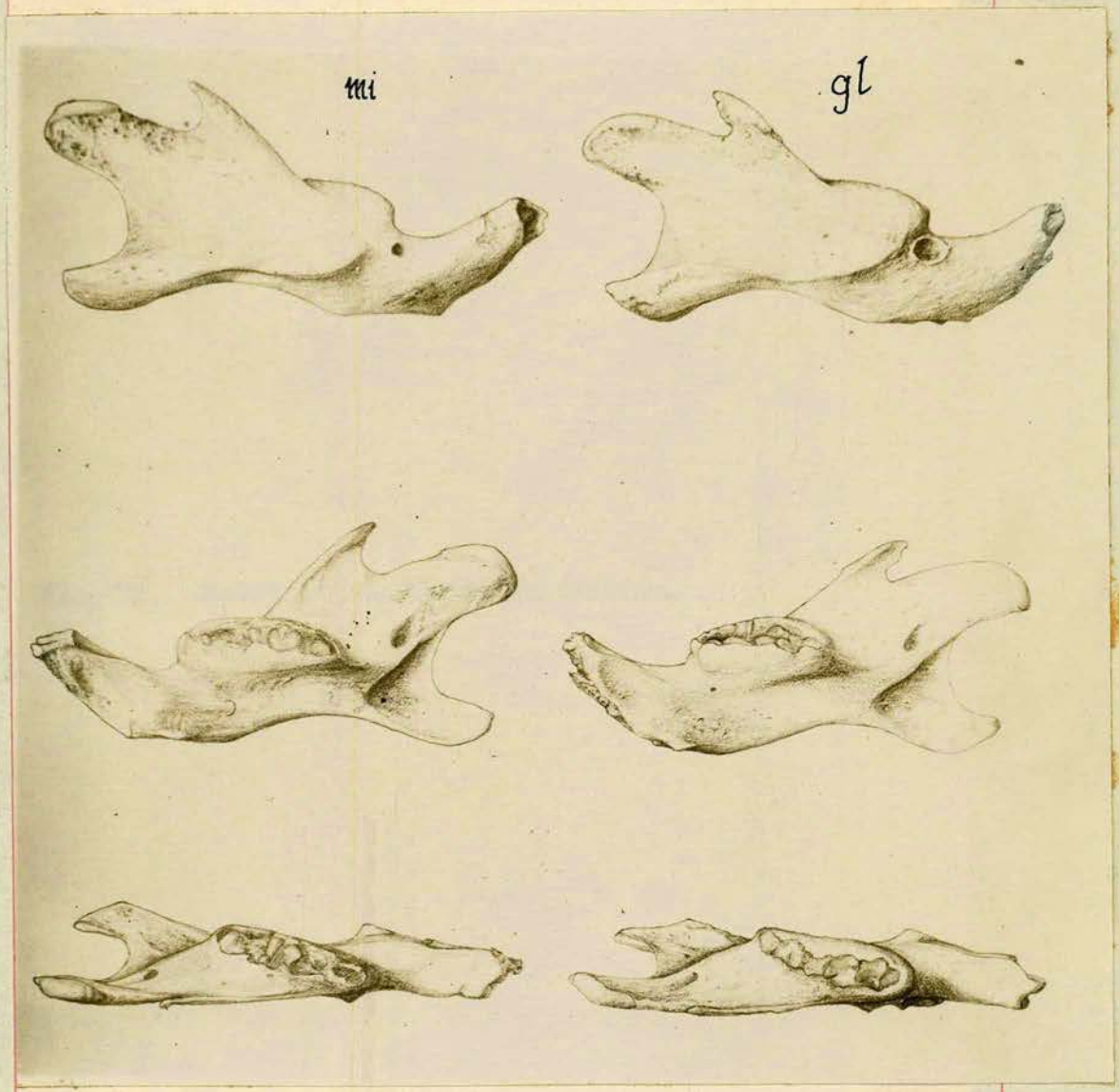


medial aspect



superior aspect

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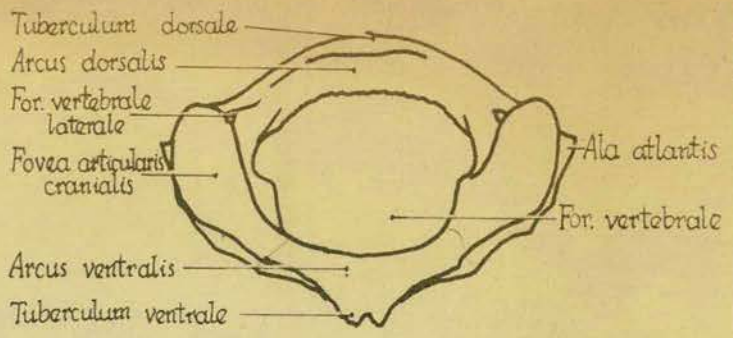
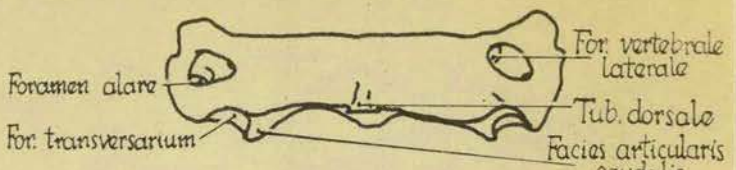
ATLAS

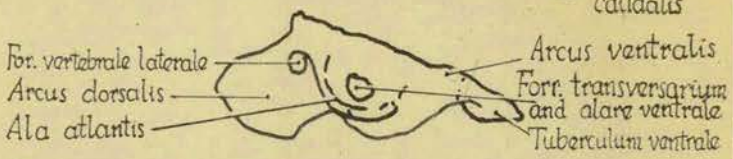
cranial view



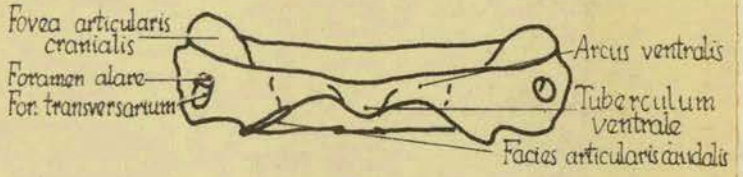
dorsal view



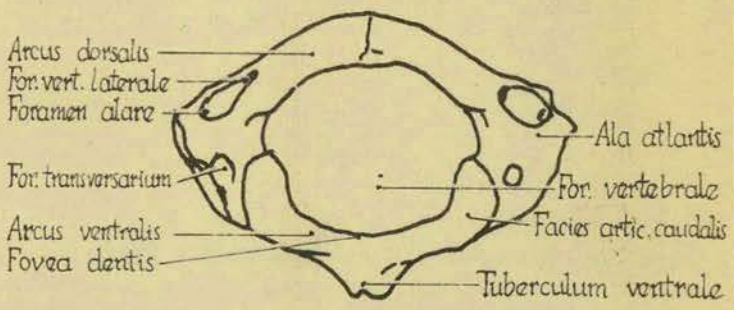
lateral view



ventral view

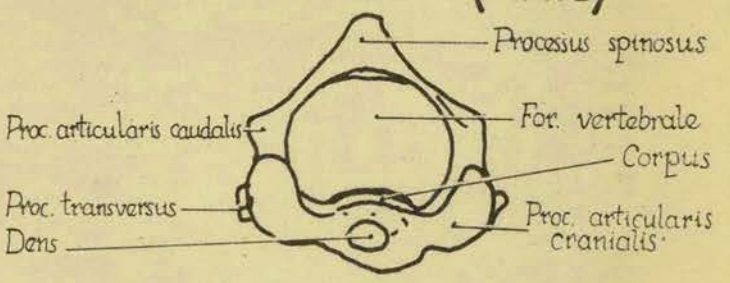


caudal view

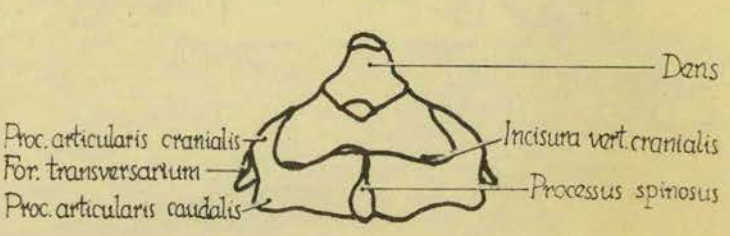


EPISTROPHEUS (AXIS)

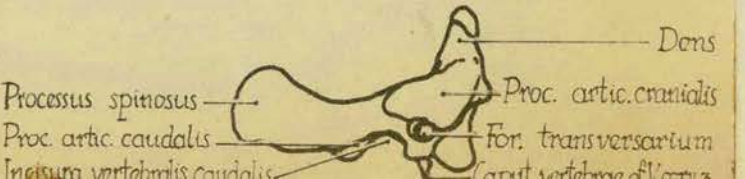
cranial view

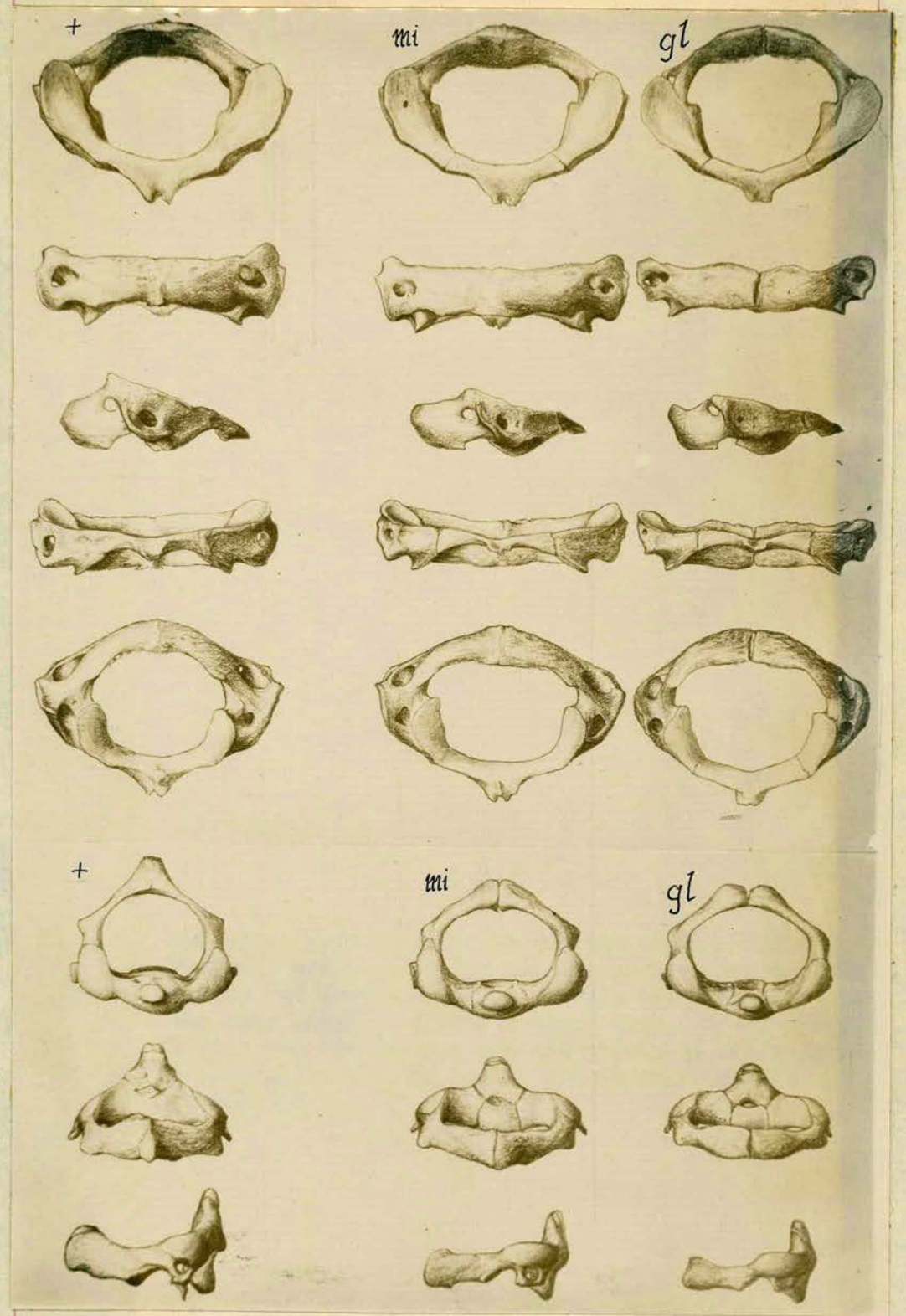


dorsal view



lateral view

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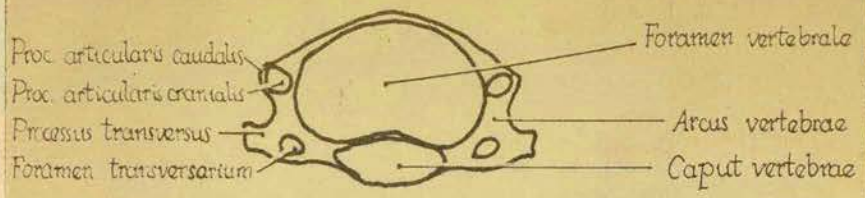
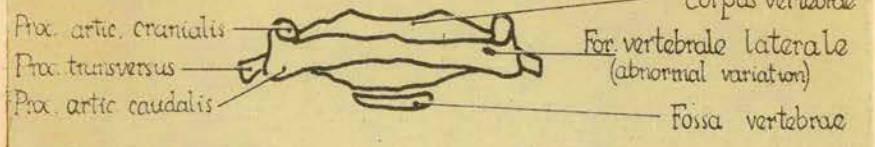
THIRD VERTEBRA CERVICALIS

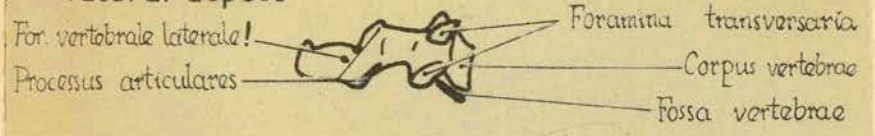
cranial aspect



dorsal aspect

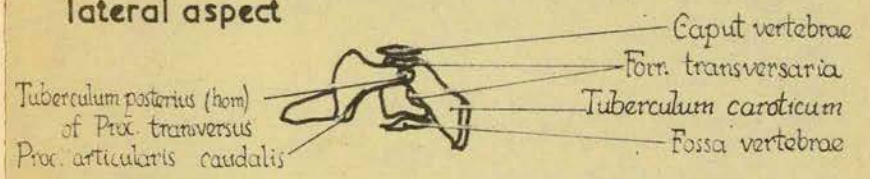


lateral aspect

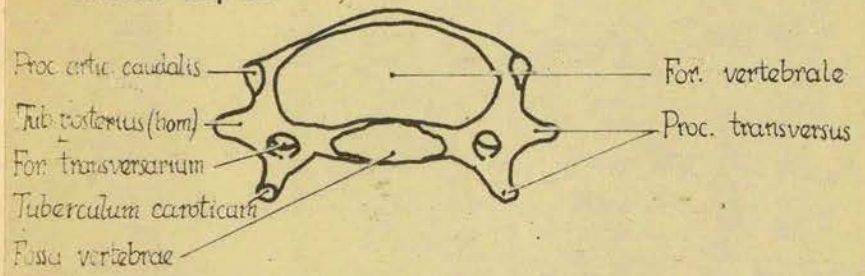


SIXTH VERTEBRA CERVICALIS

lateral aspect

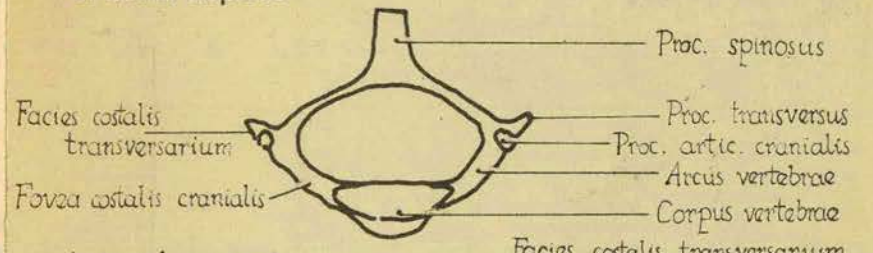


caudal aspect

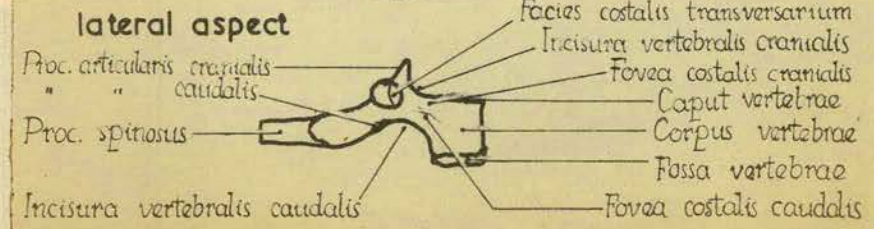


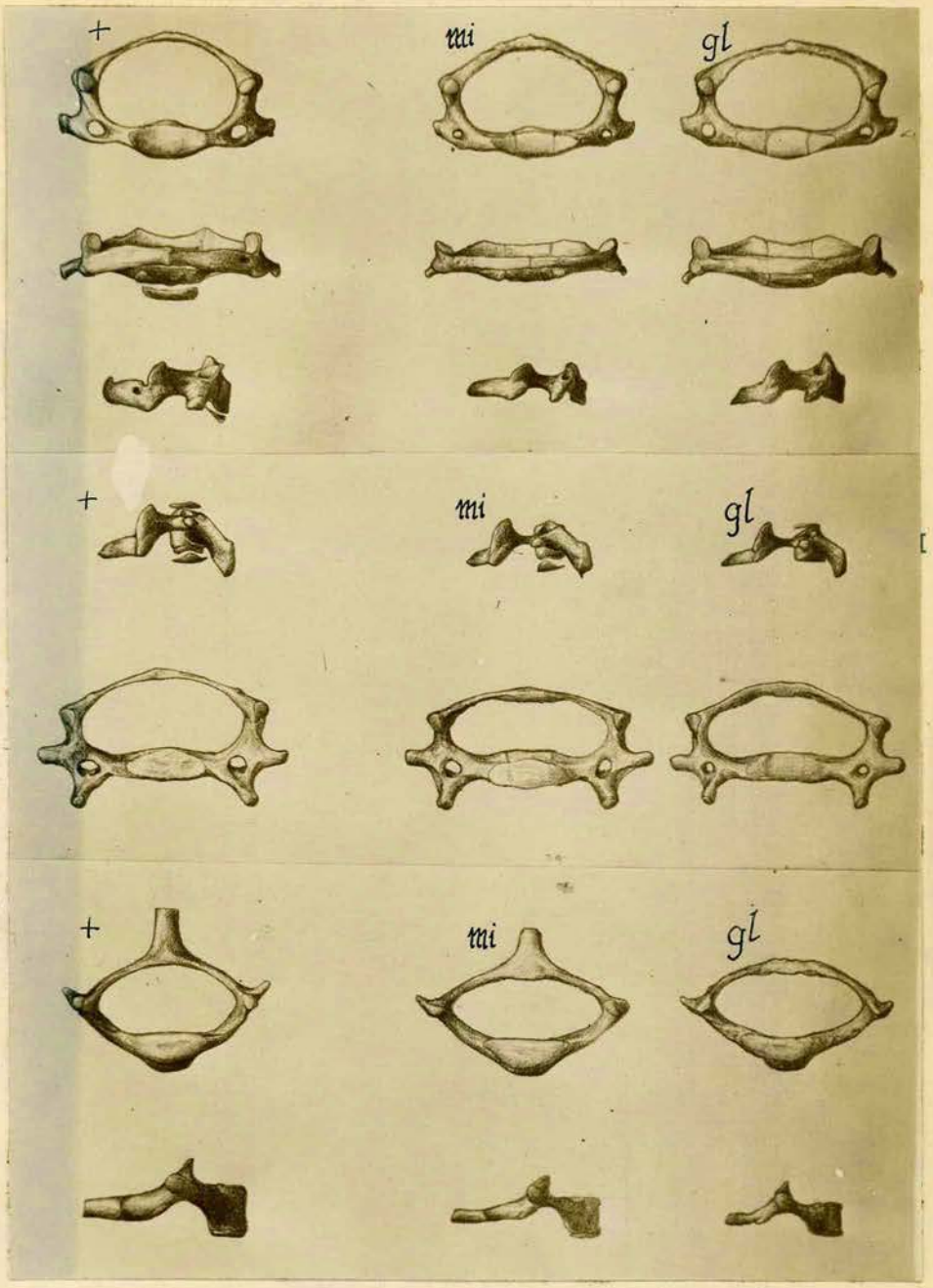
SECOND VERTEBRA THORACALIS

cranial aspect



lateral aspect

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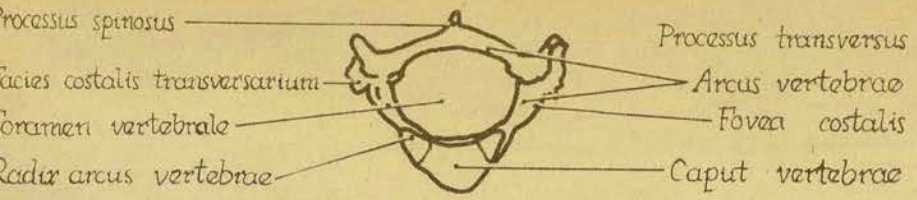
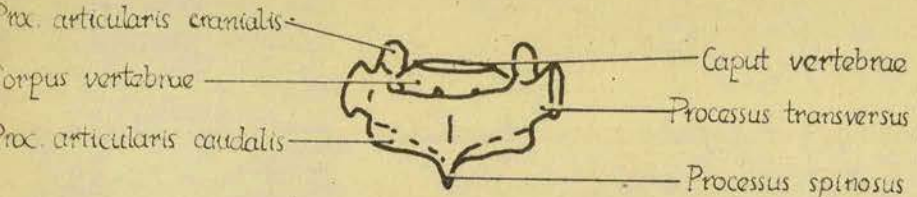
SEVENTH VERTEBRA THORACALIS

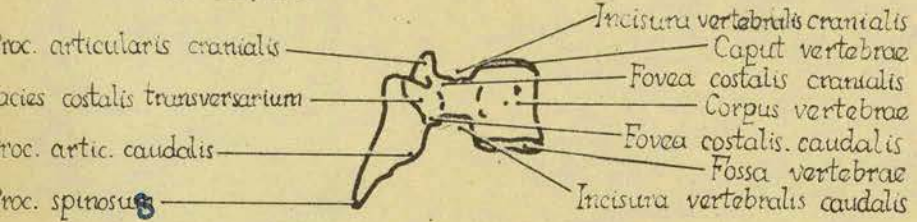
cranial aspect



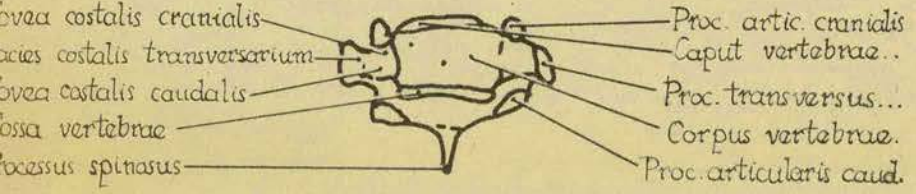
dorsal aspect



lateral aspect

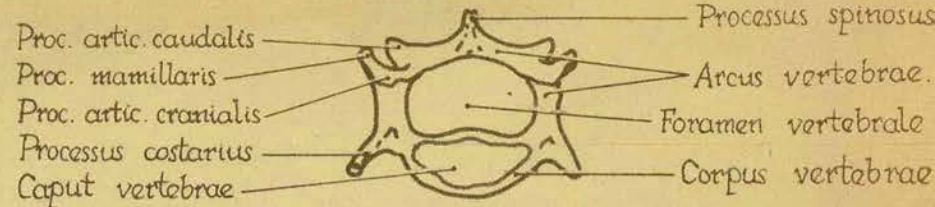


ventral aspect

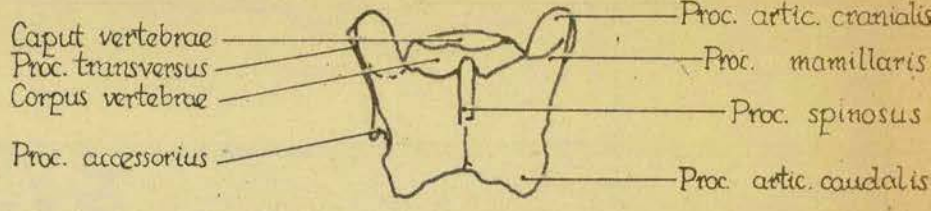


VERTEBRA LUMBALIS – THIRD FROM SACRUM

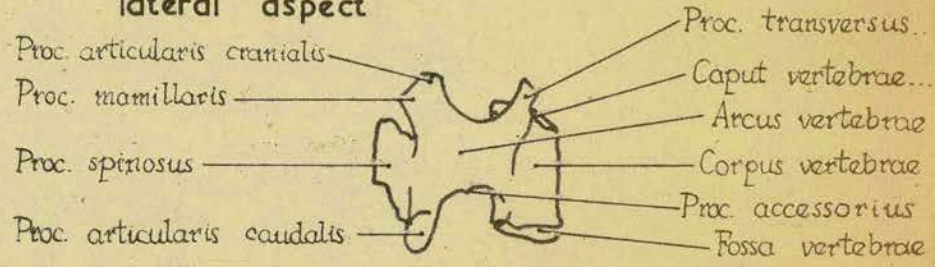
cranial aspect



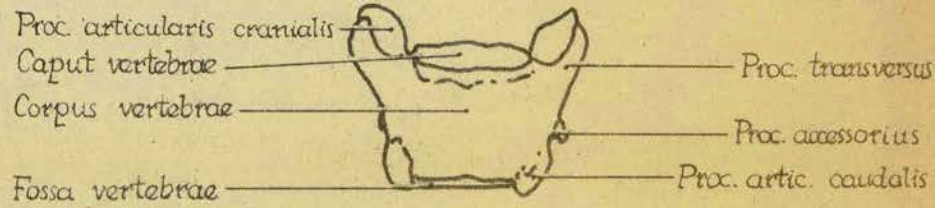
dorsal aspect

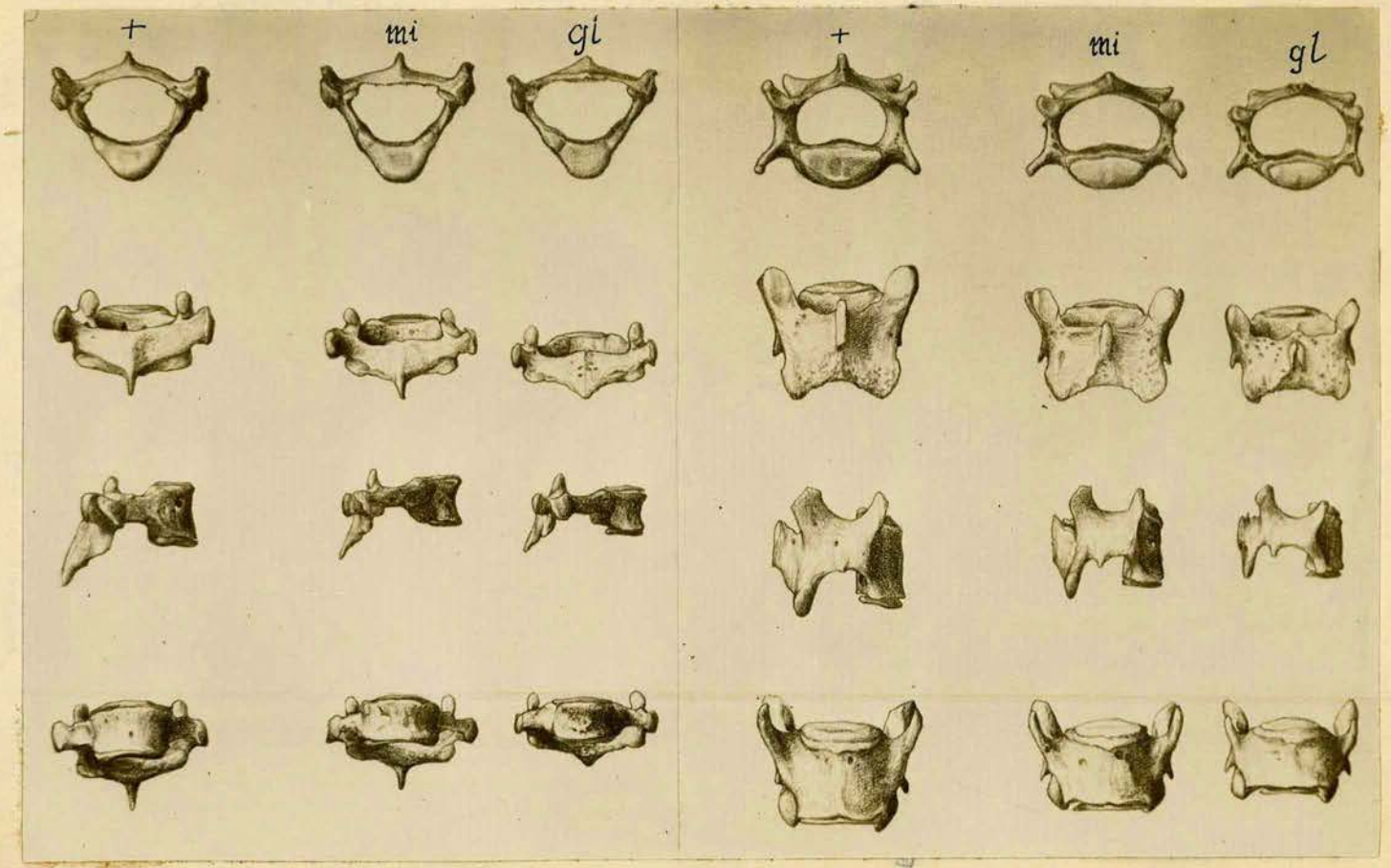


lateral aspect



ventral aspect

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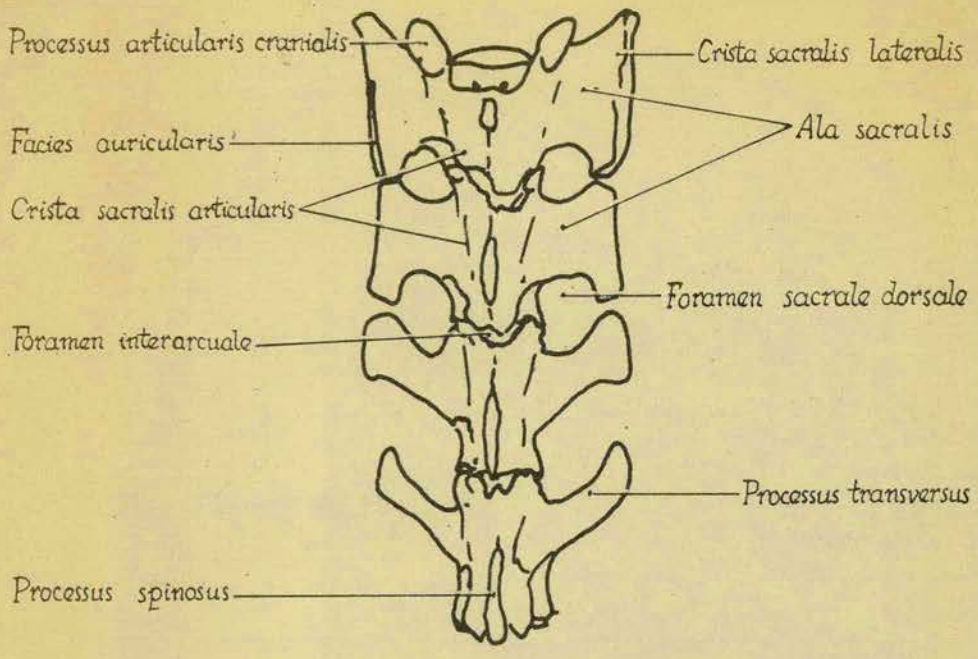
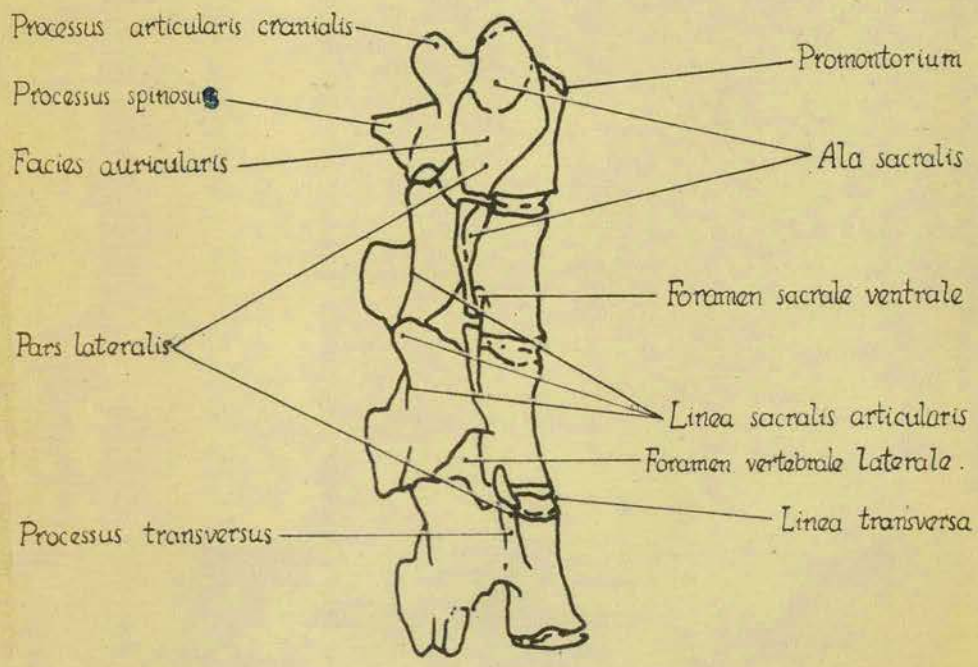
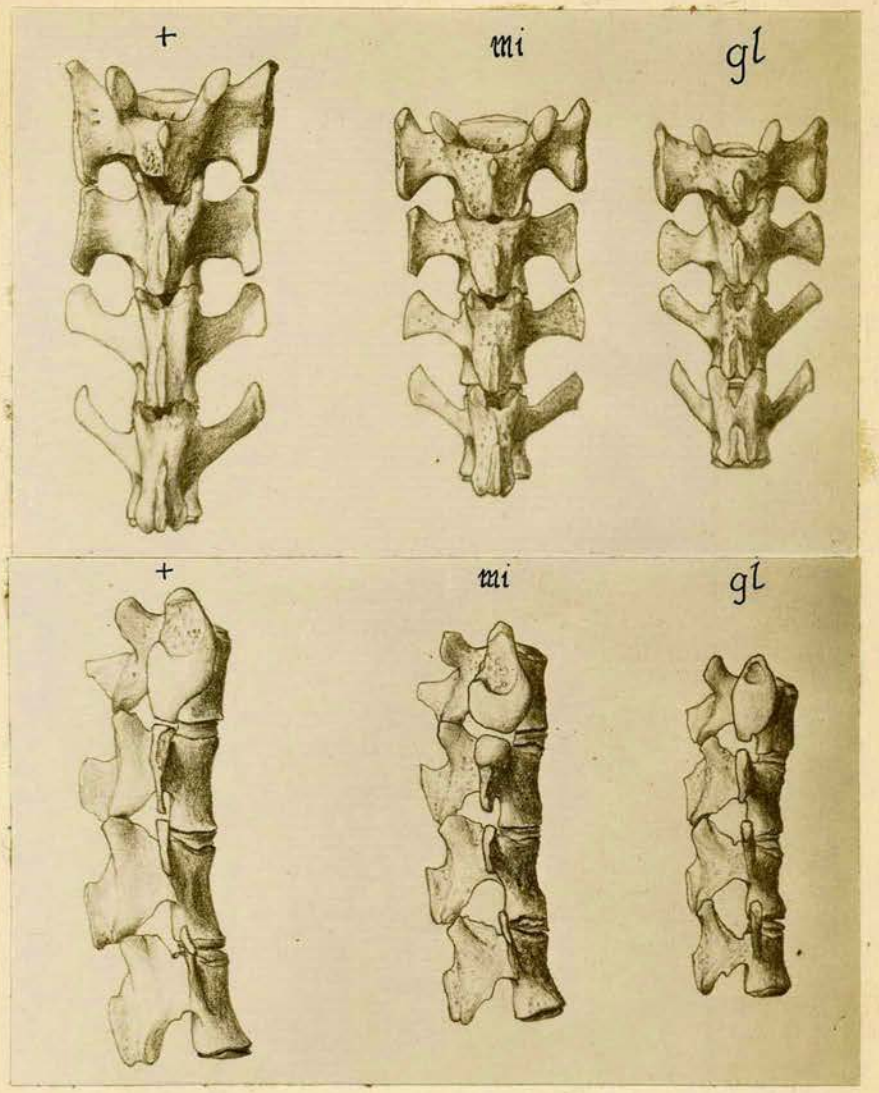
SACRUM

dorsal aspect



lateral aspect

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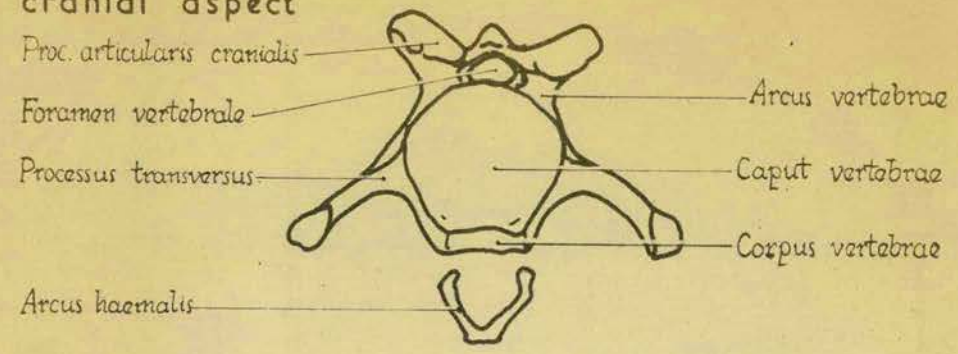
VERTEBRAE COCCYGEAE

THE FOURTH

cranial aspect



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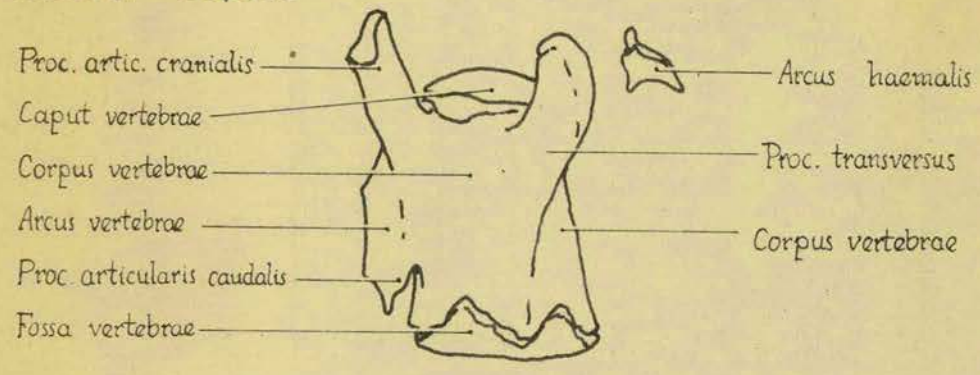
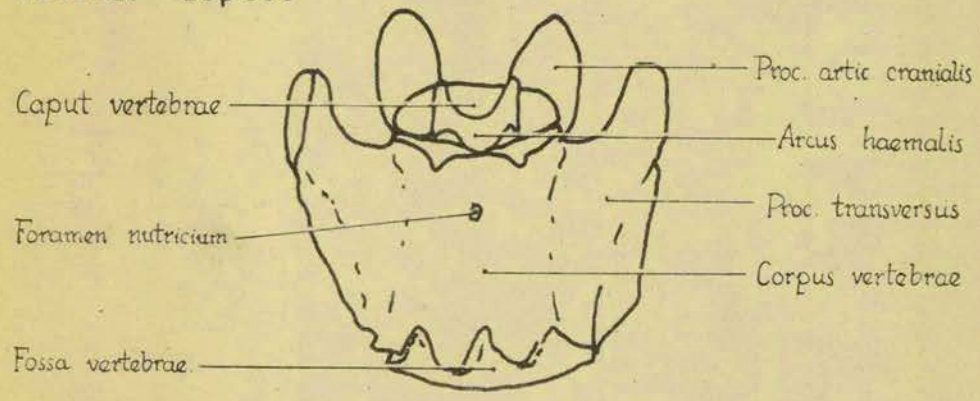
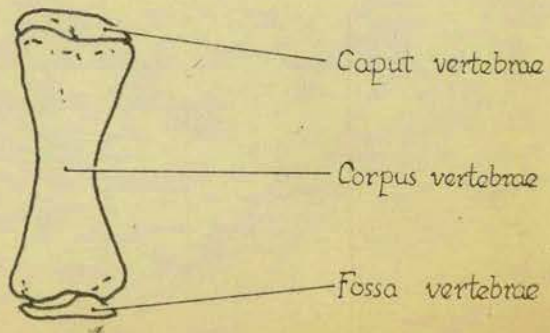


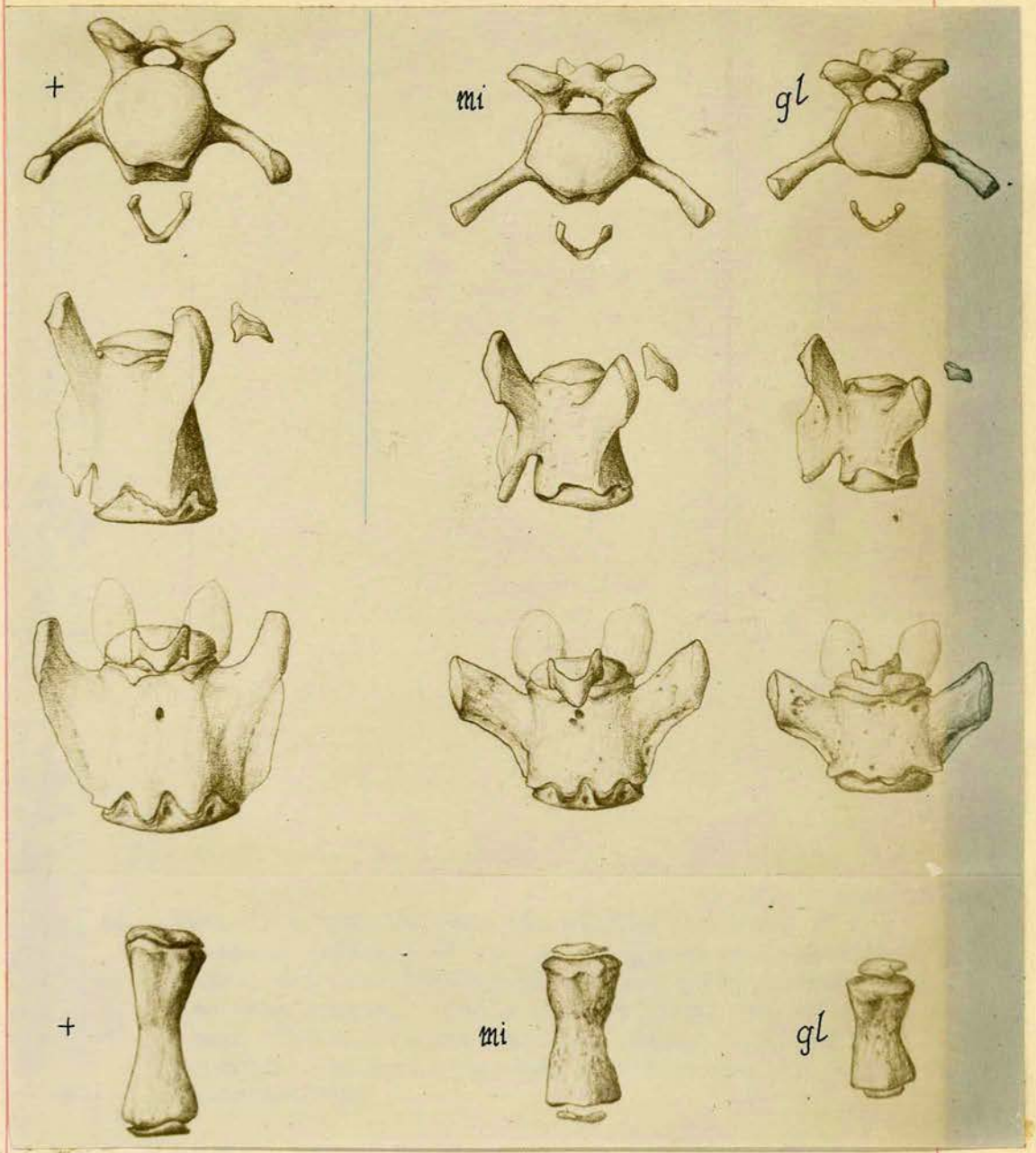
ventral aspect



12TH FROM THE LAST

lateral aspect

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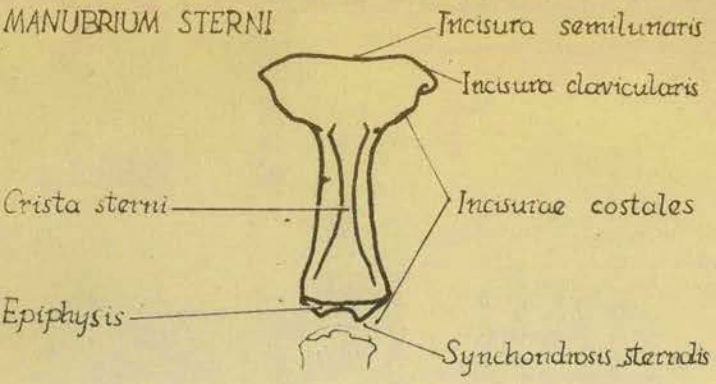
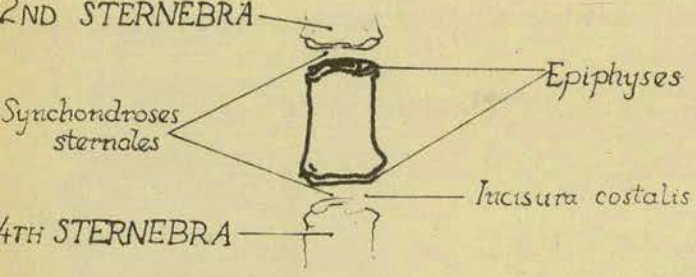
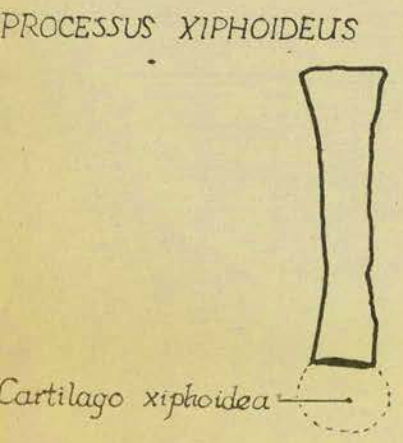
STERNUM

ventral aspect

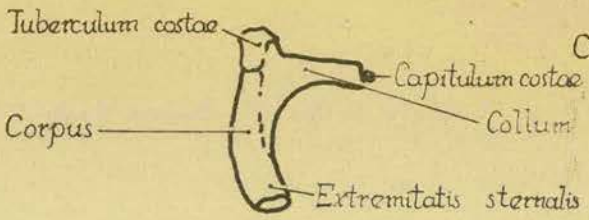






COSTAE (RIBS)

1ST anterior aspect



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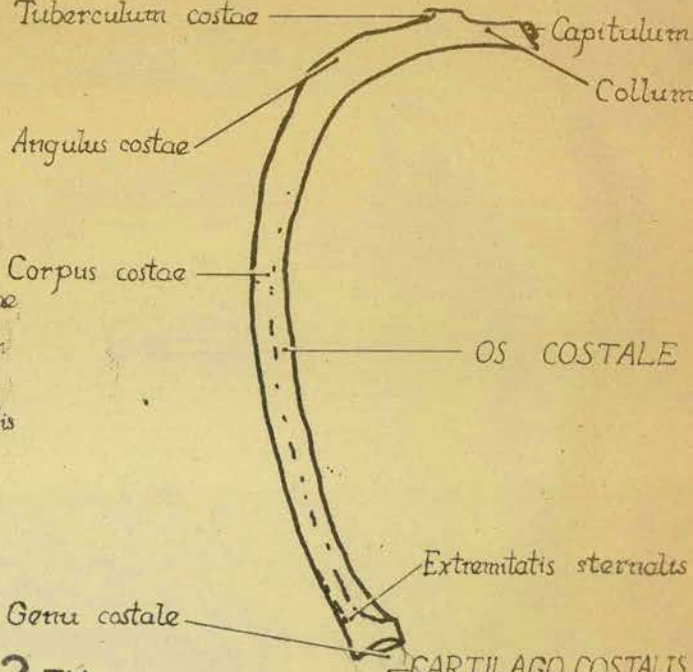
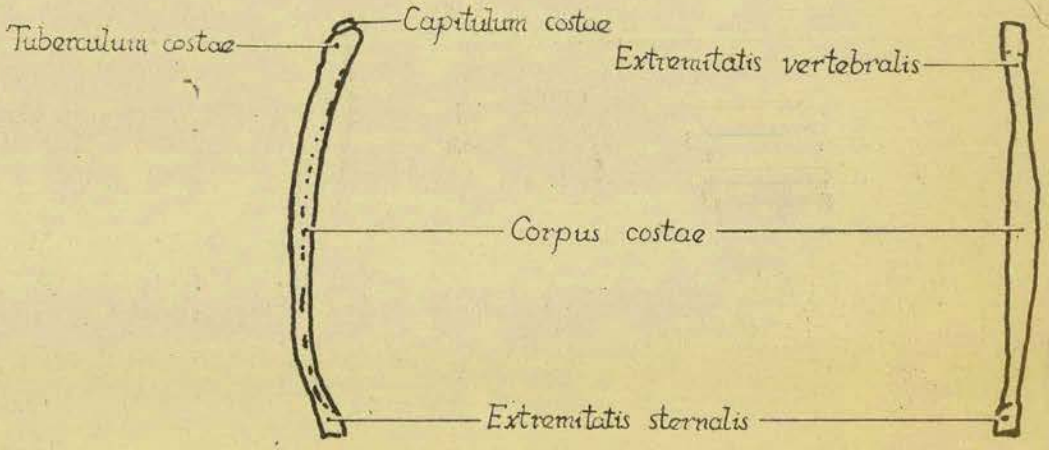
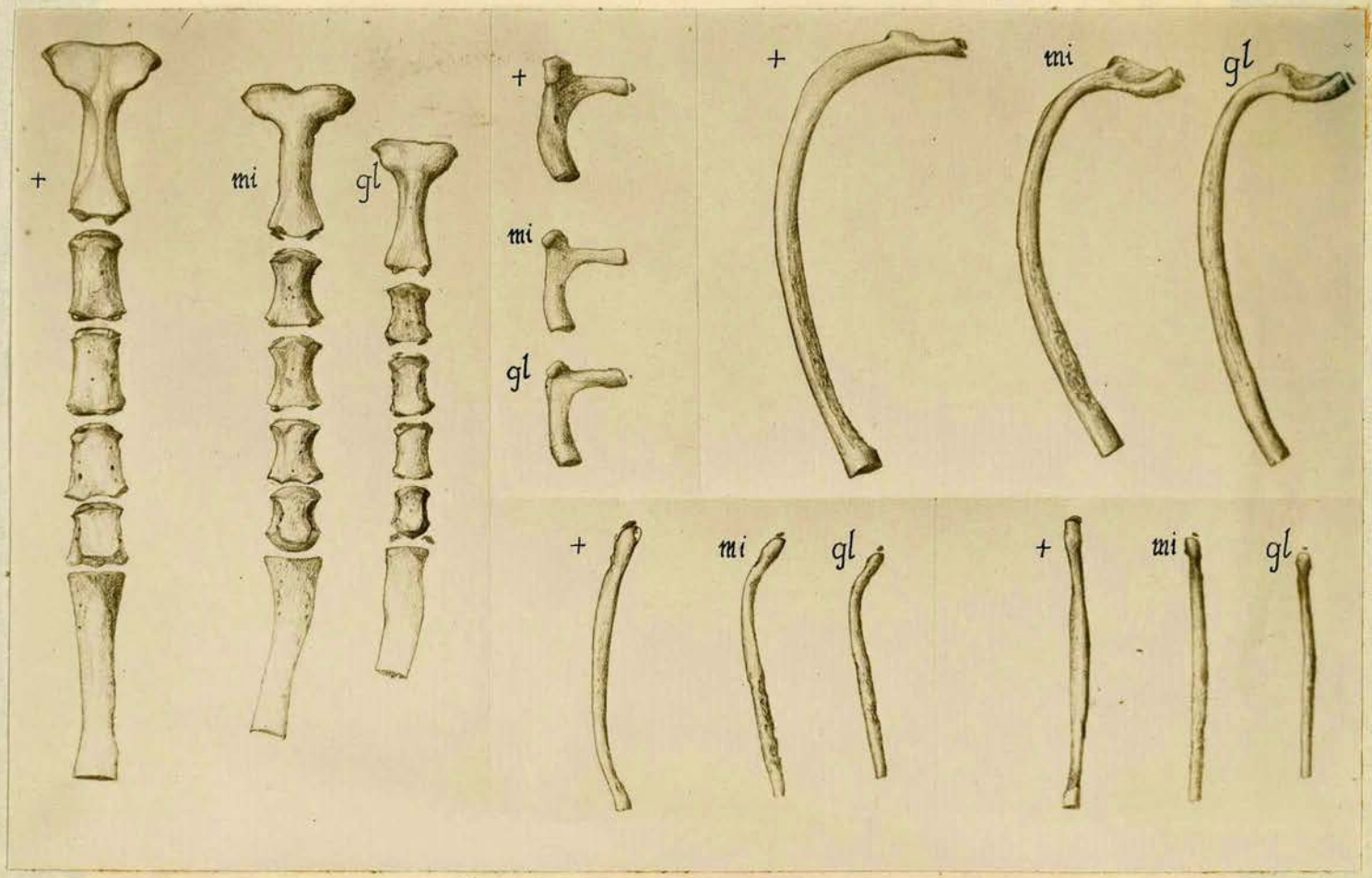


12 TH

anterior aspect

medial aspect

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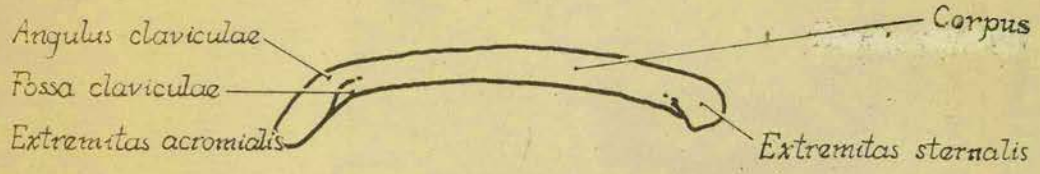
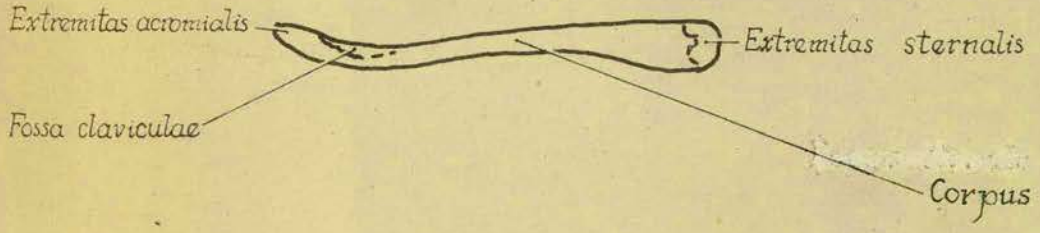
CLAVICULA

posterior aspect

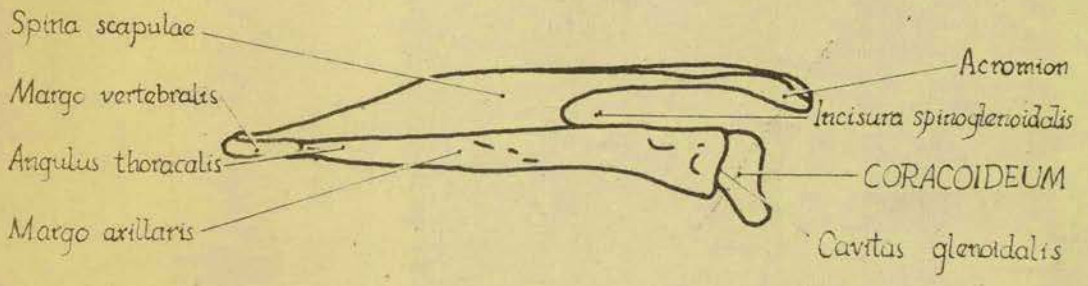


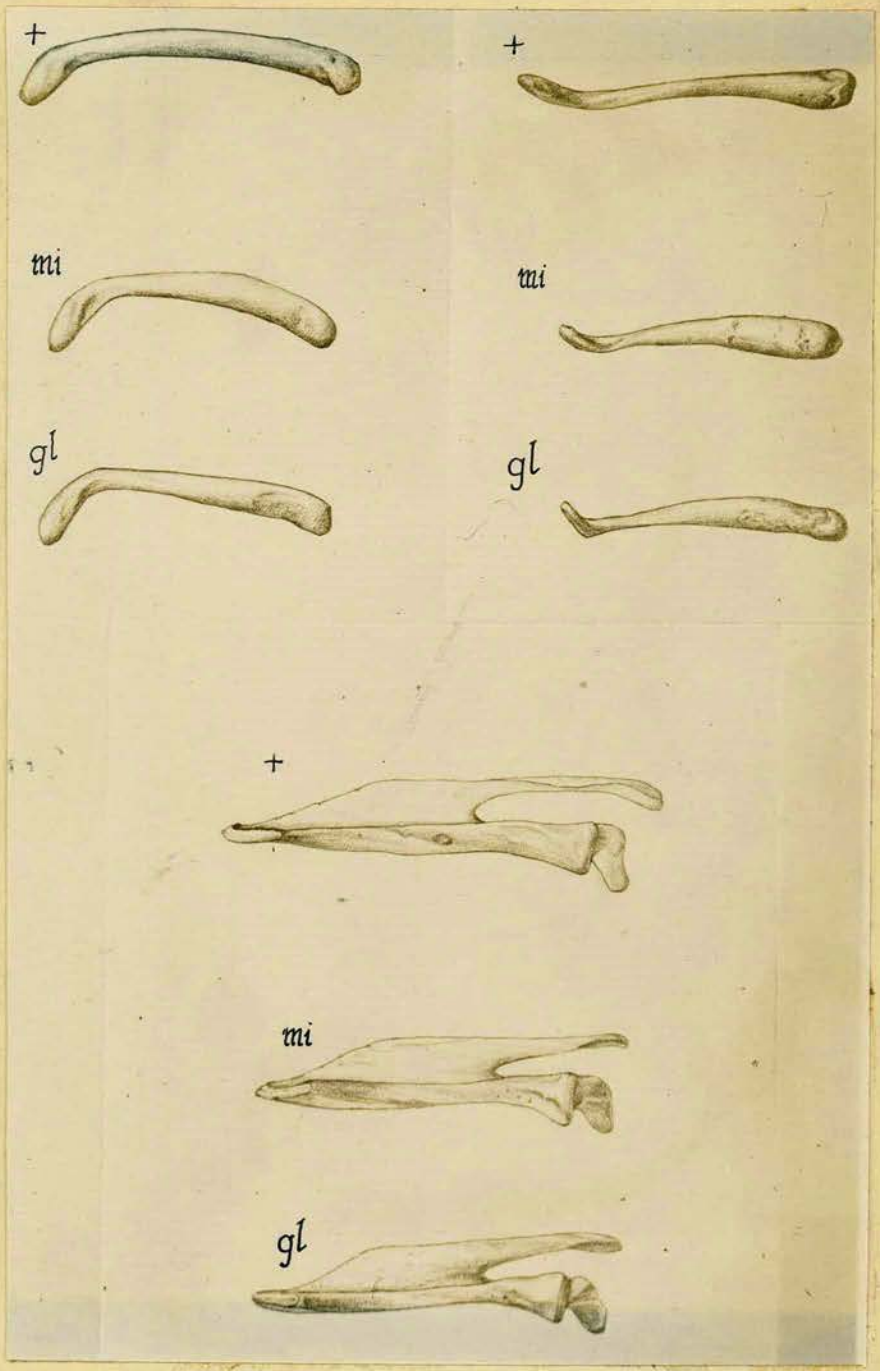
dorsal aspect



SCAPULA

ventro-posterior aspect

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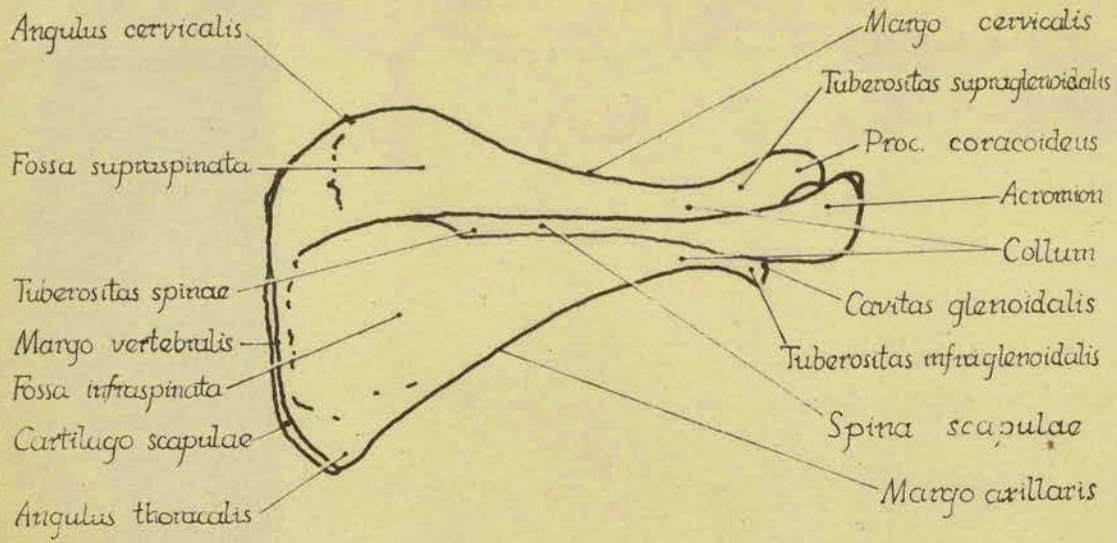
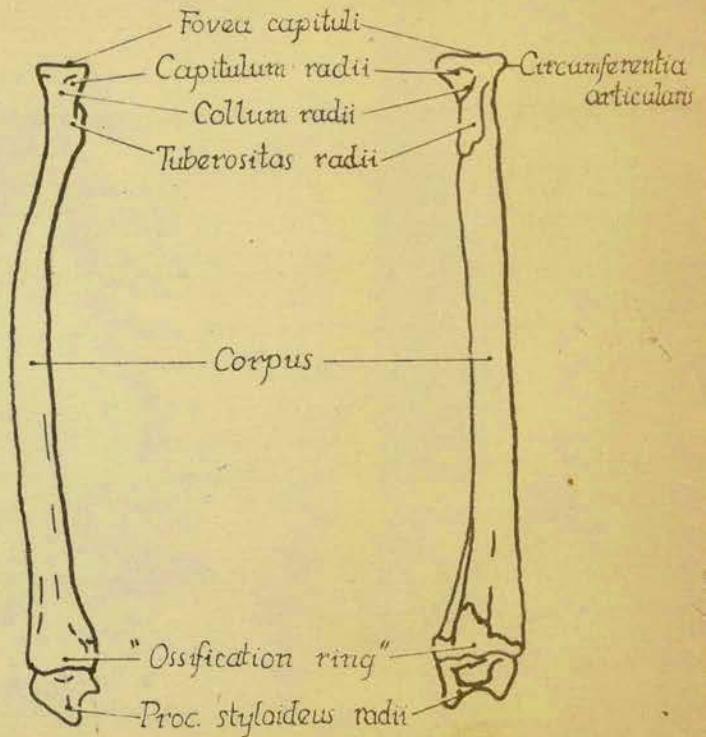
SCAPULA

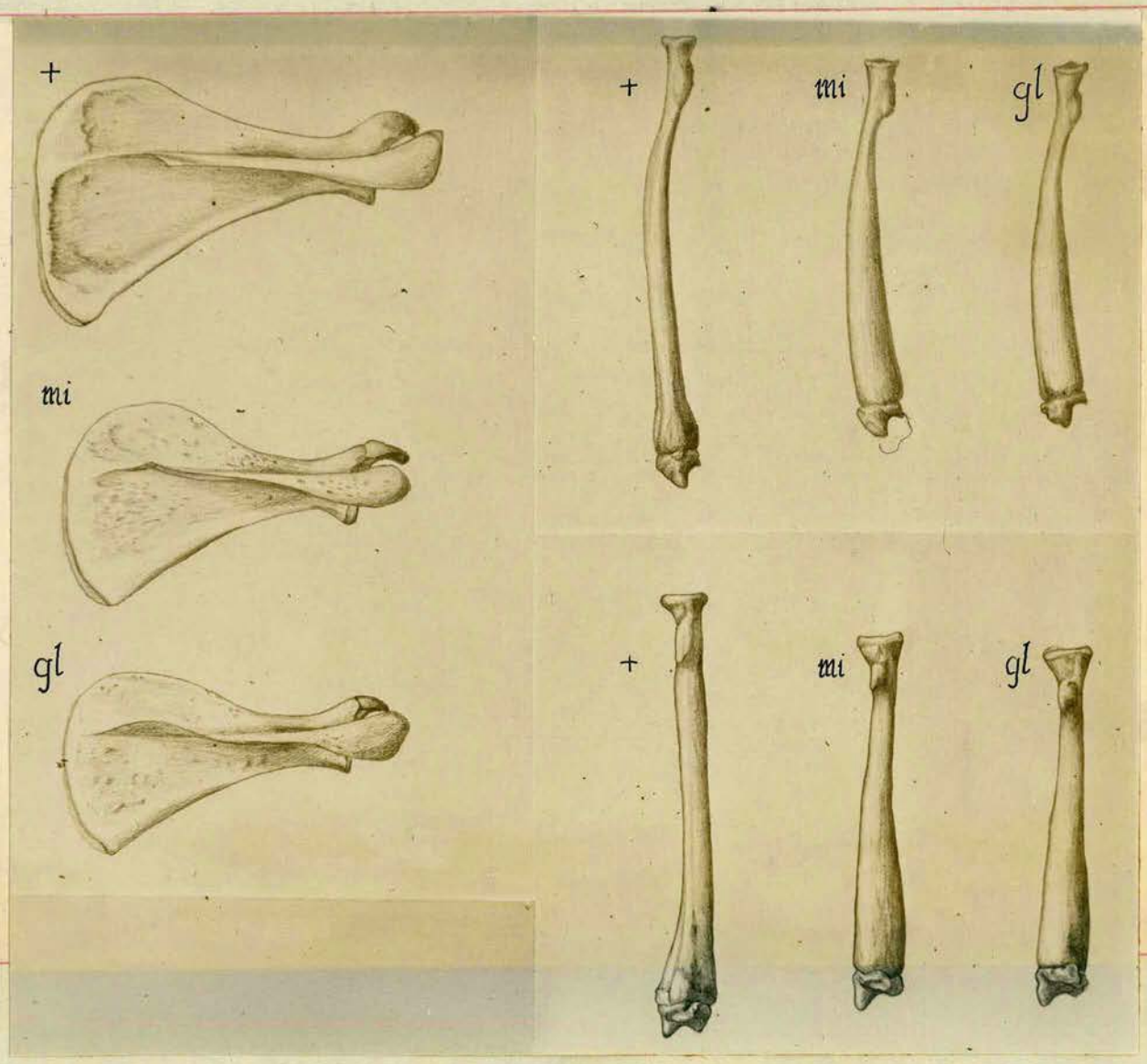
lateral aspect



RADIUS

medial aspect ventral aspect

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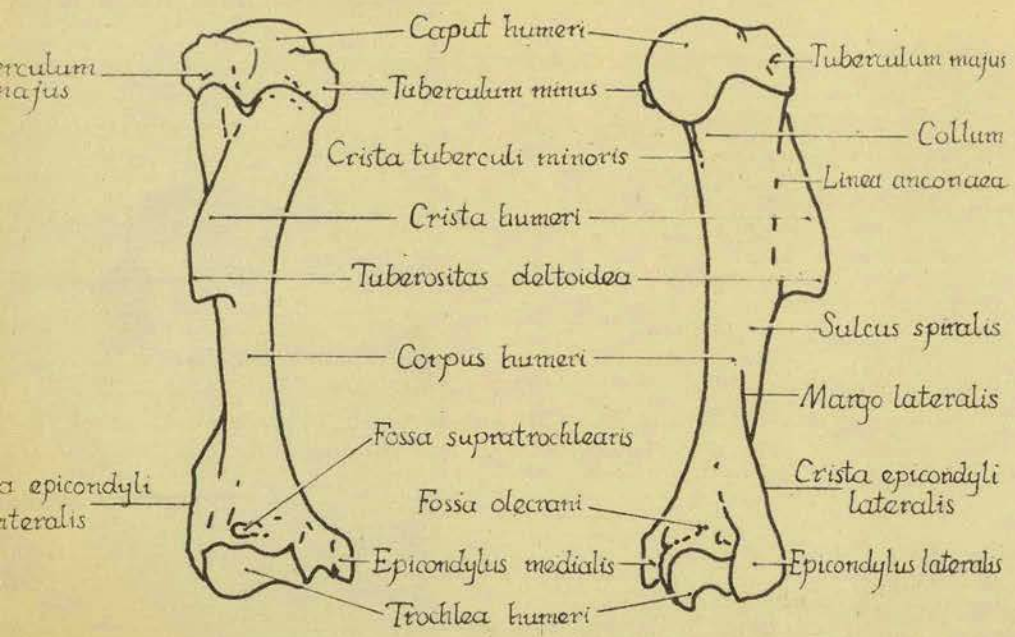
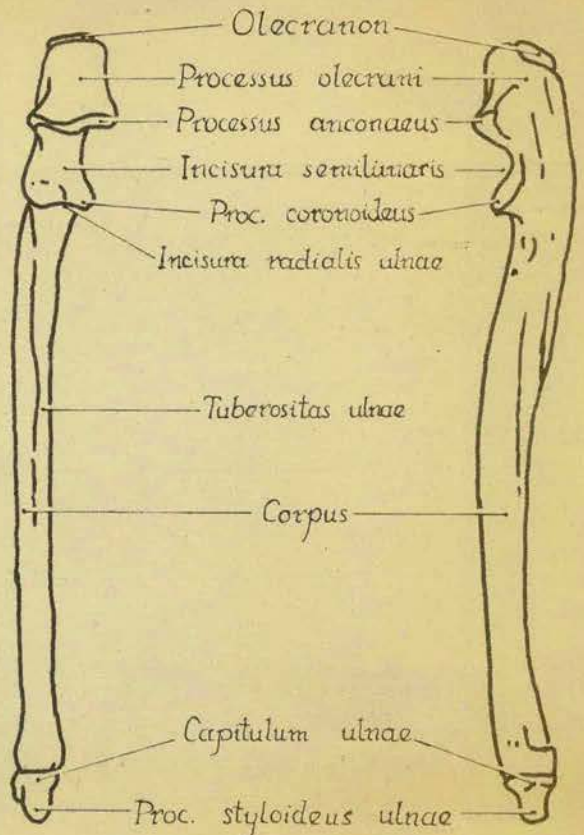
HUMERUS

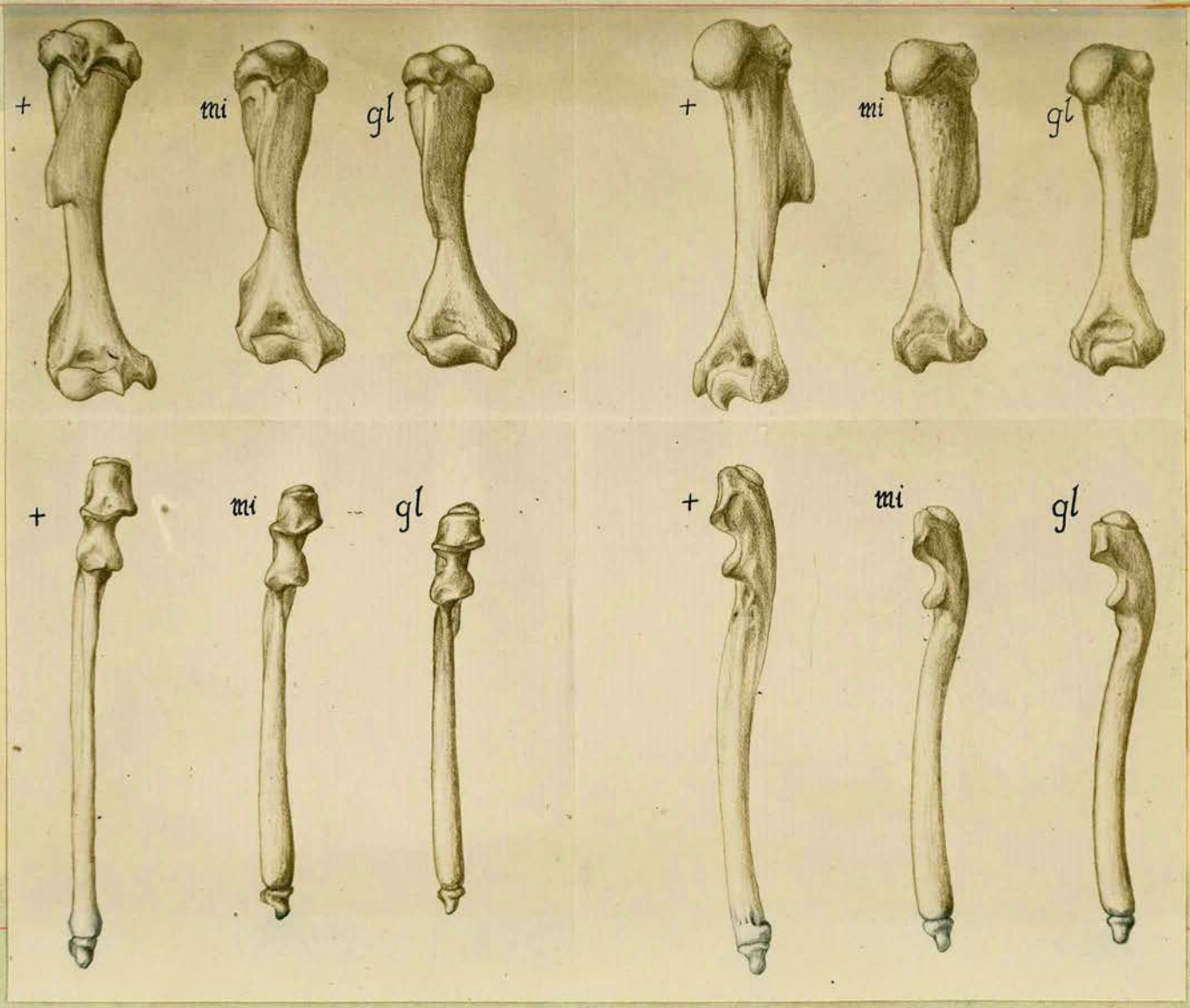
anterior view posterior view



ULNA

dorsal view medial view

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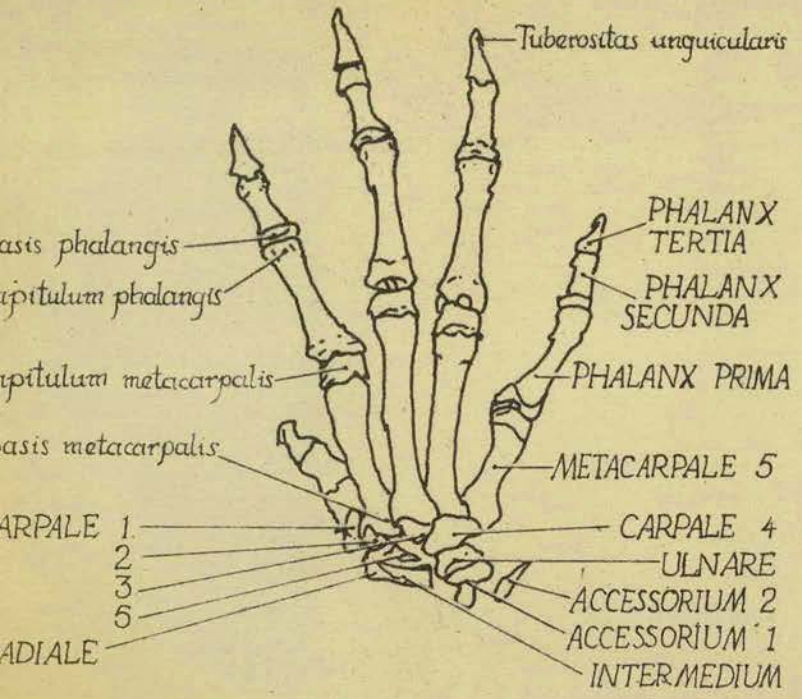
MANUS

dorsal aspect



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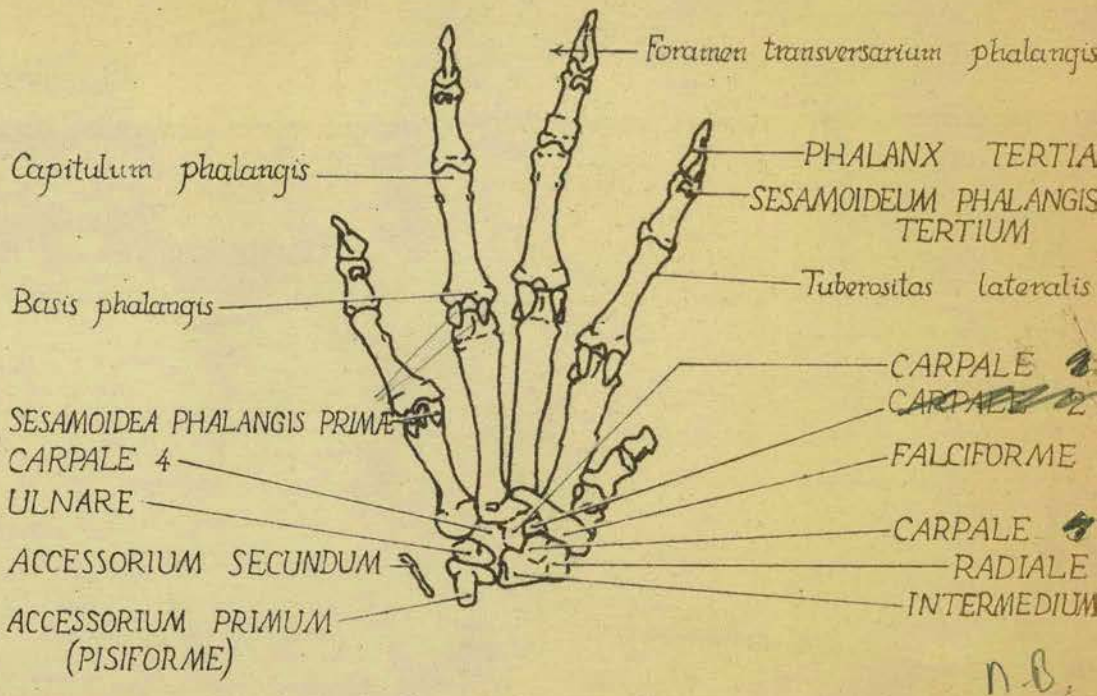

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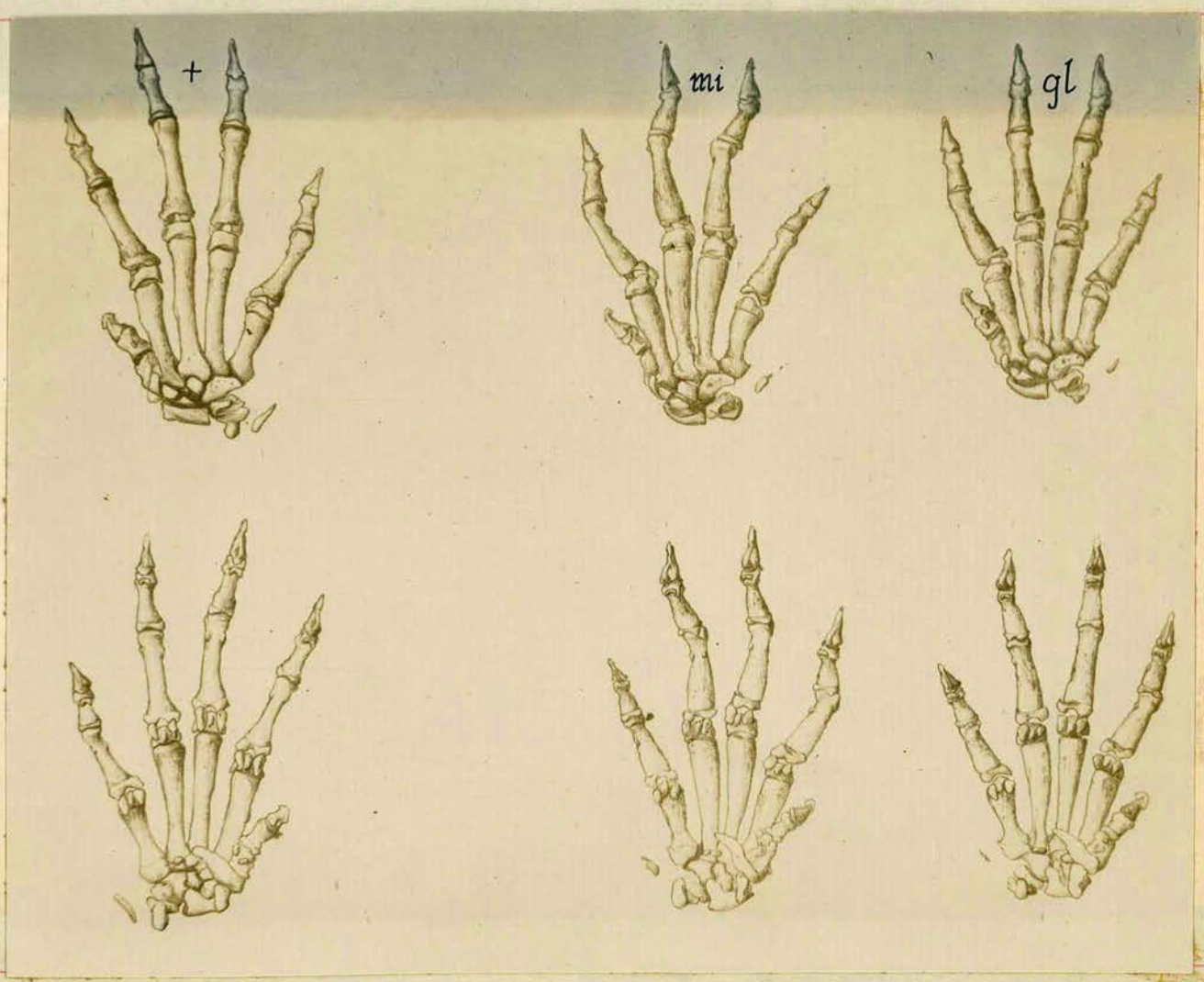
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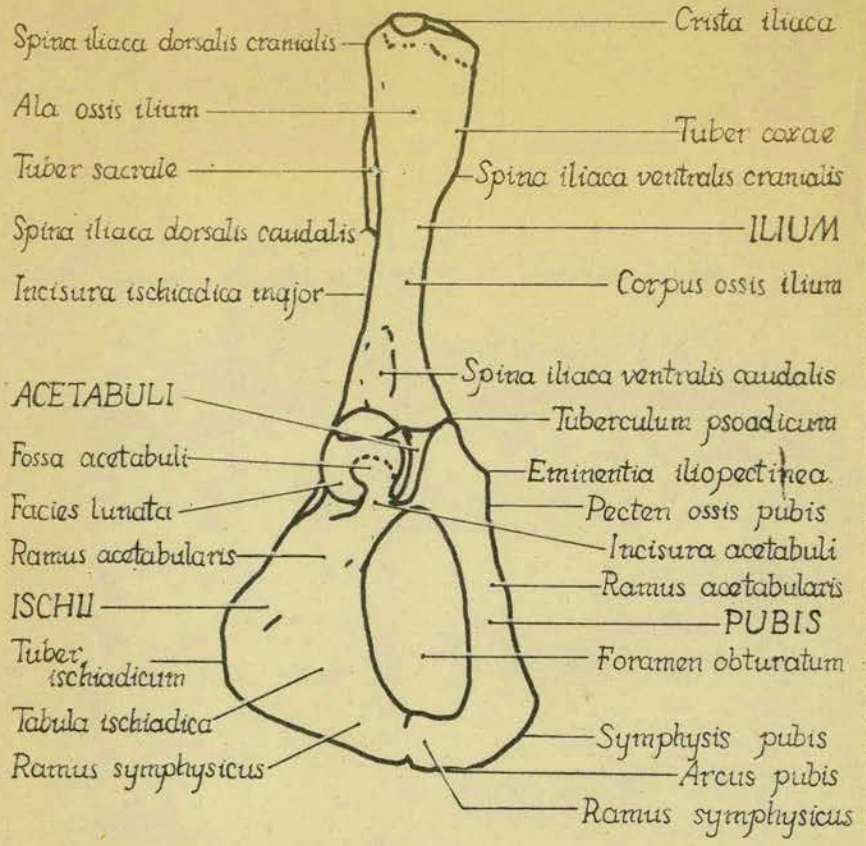
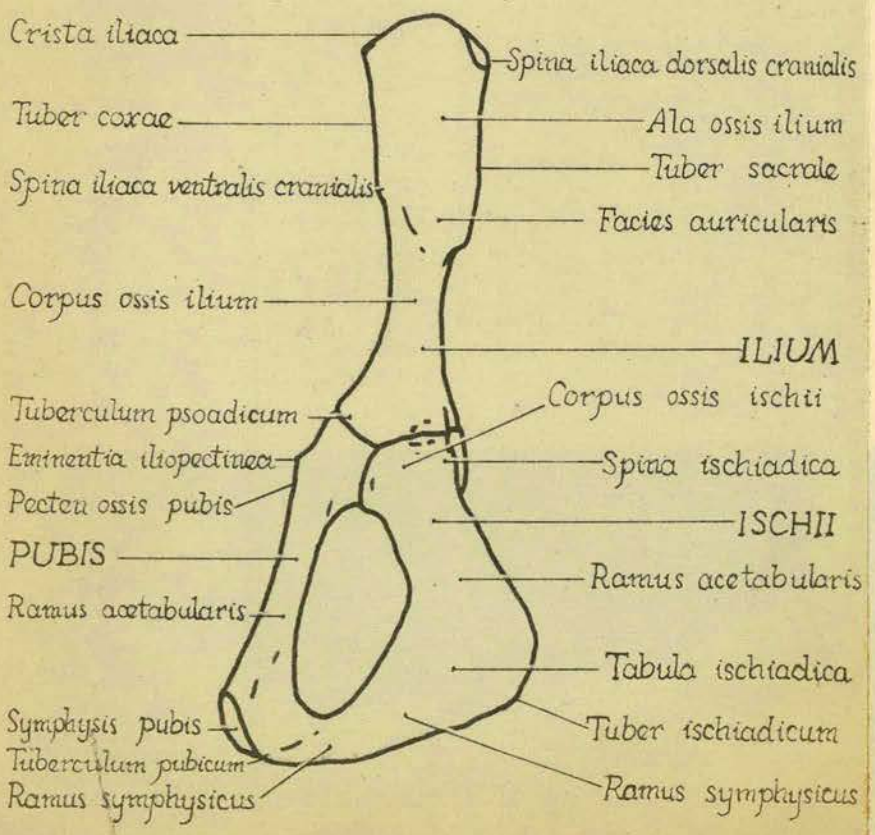
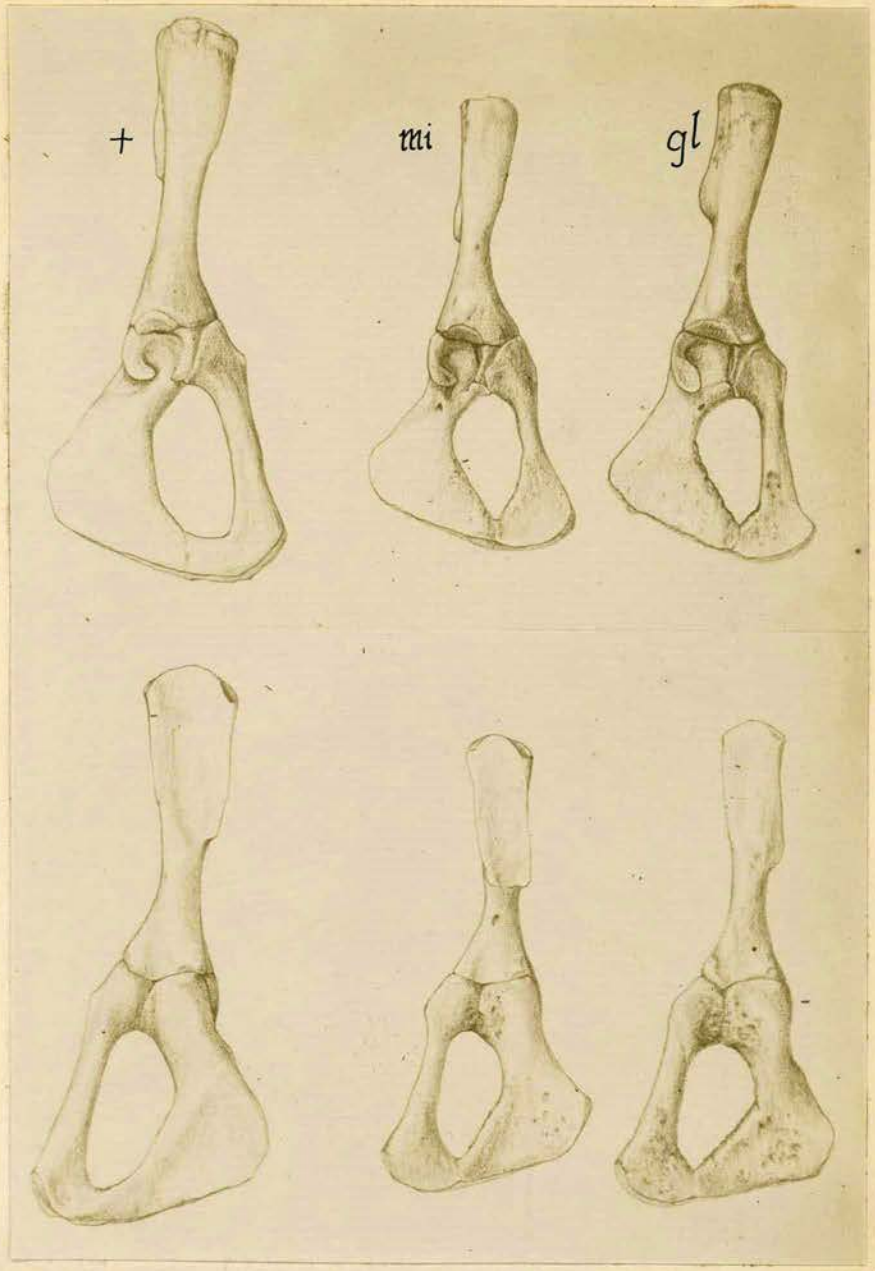
PELVIS

external surface



pelvic surface

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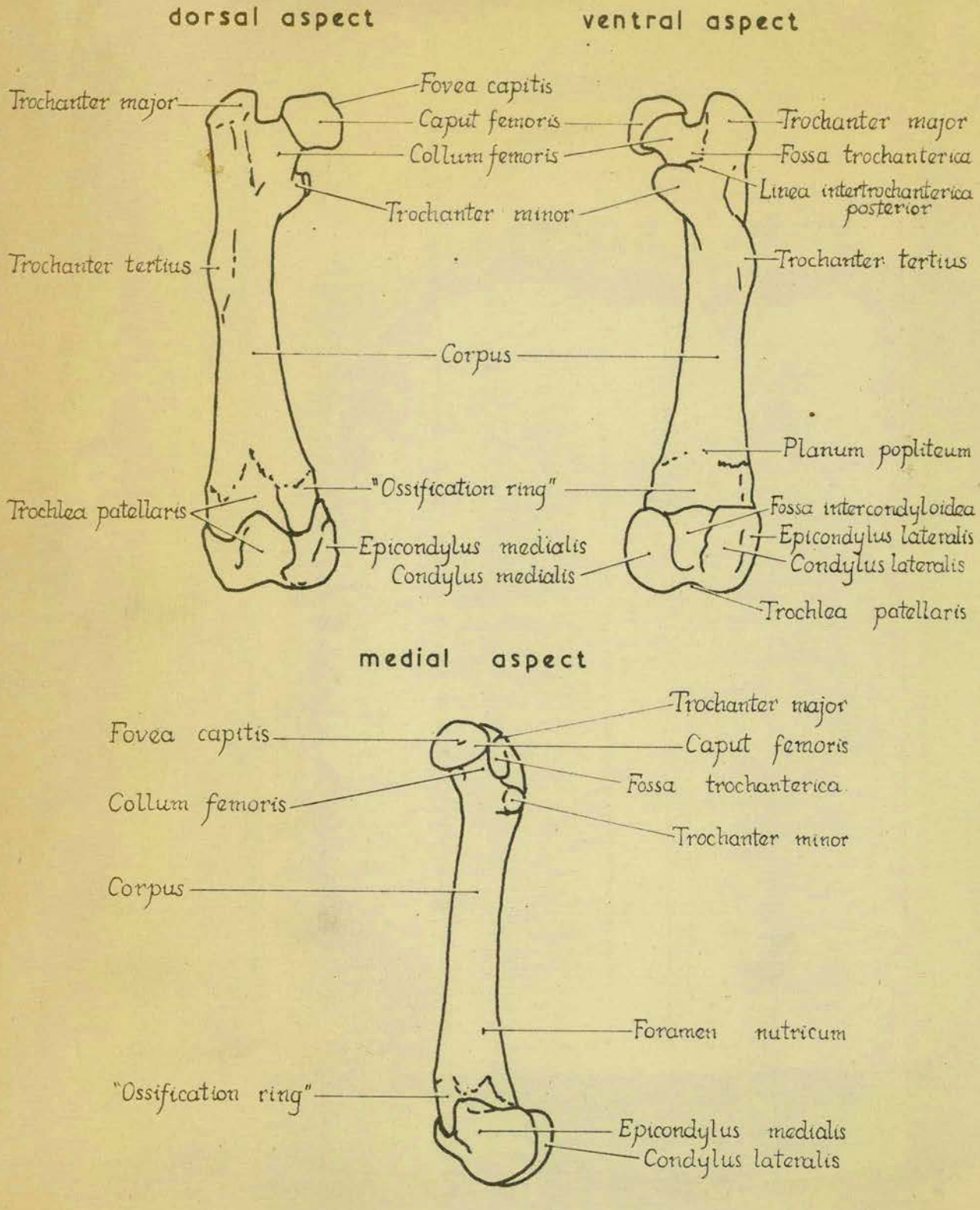
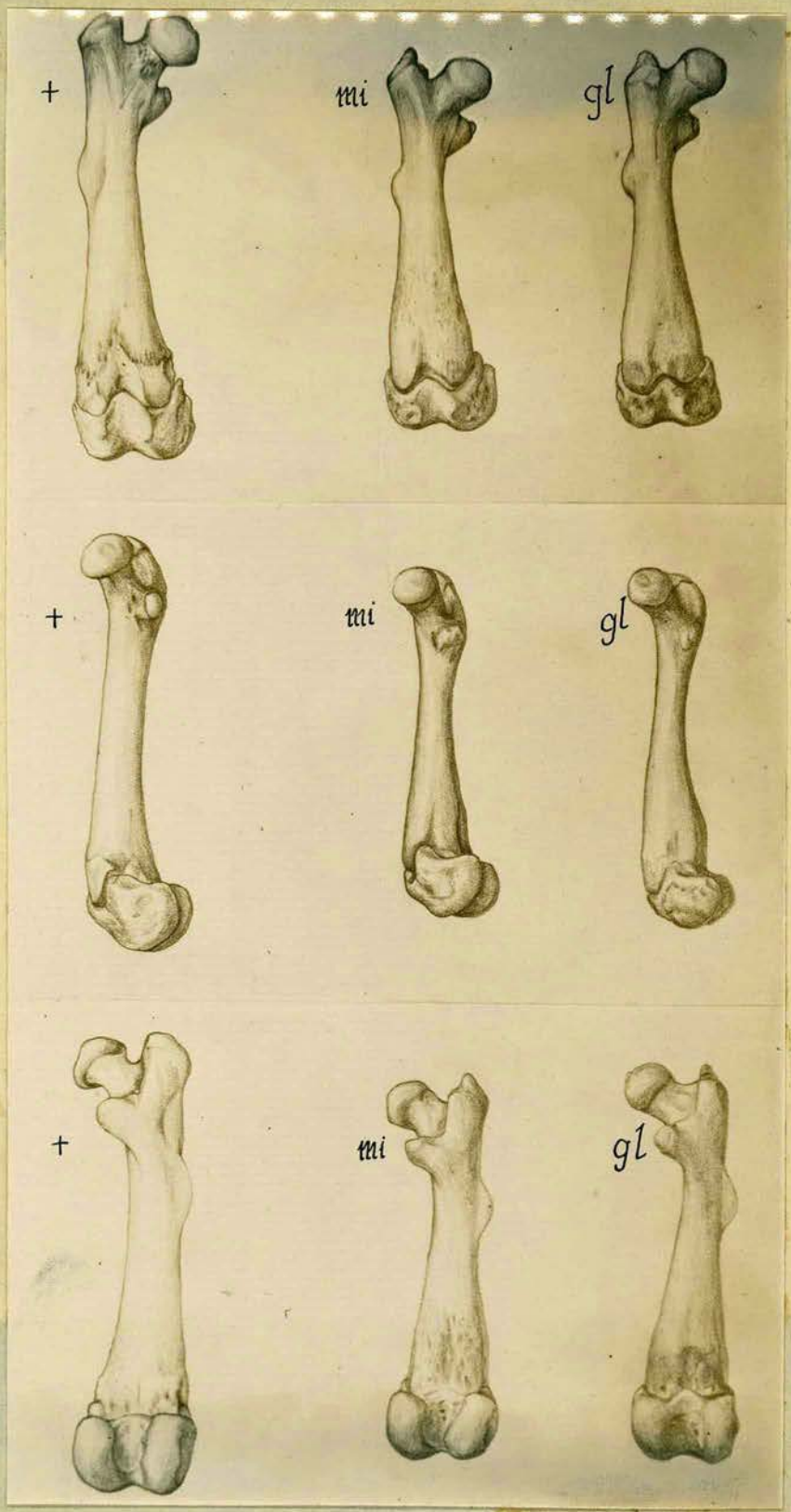
FEMUR


dorsal aspect
ventral aspect
Trochanter major
Fovea capitis
Caput femoris
Collum femoris
Trochanter minor
Trochanter tertius
Corpus
"Ossification ring"
Trochlea patellaris
Epicondylus medialis
Condylus medialis
Trochanter major
Fossa trochanterica
Linea intertrochanterica posterior
Trochanter tertius
Planum popliteum
Fossa intercondyloidea
Epicondylus lateralis
Condylus lateralis
Trochlea patellaris
medial aspect
Fovea capitis
Collum femoris
Corpus
"Ossification ring"
Trochanter major
Caput femoris
Fossa trochanterica
Trochanter minor
Foramen nutricum
Epicondylus medialis
Condylus lateralis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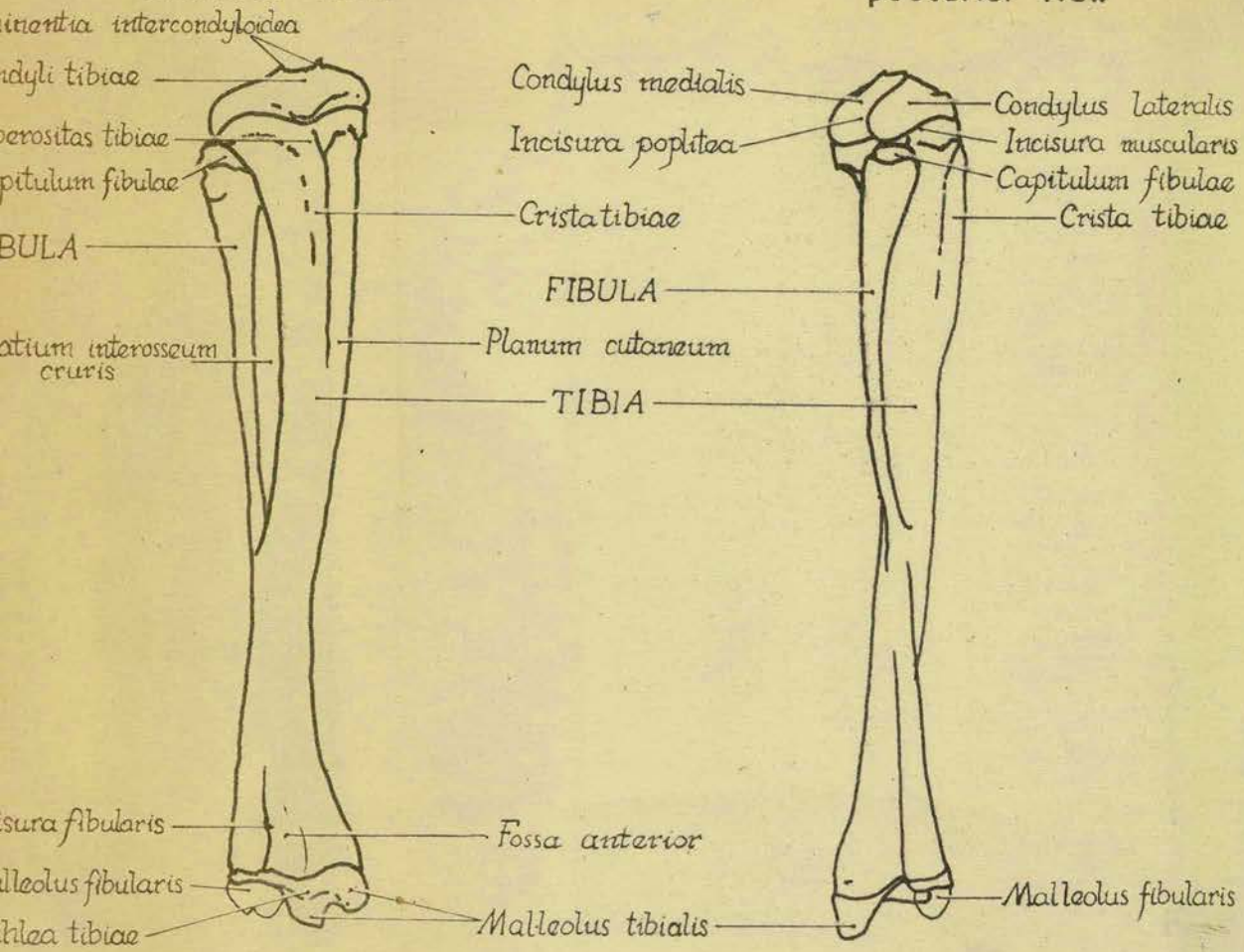
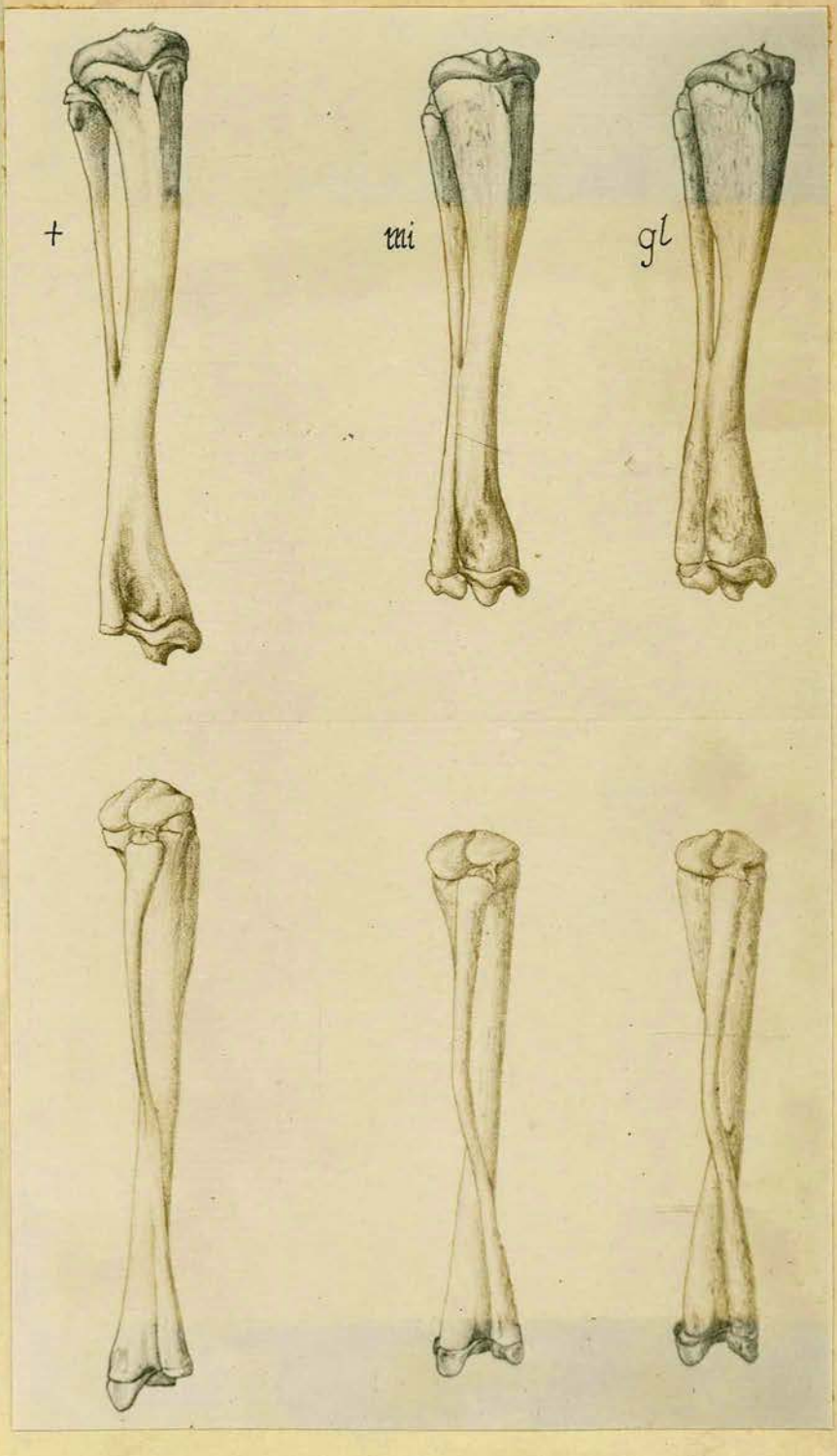
OSSA CRURIS

anterior view

posterior view

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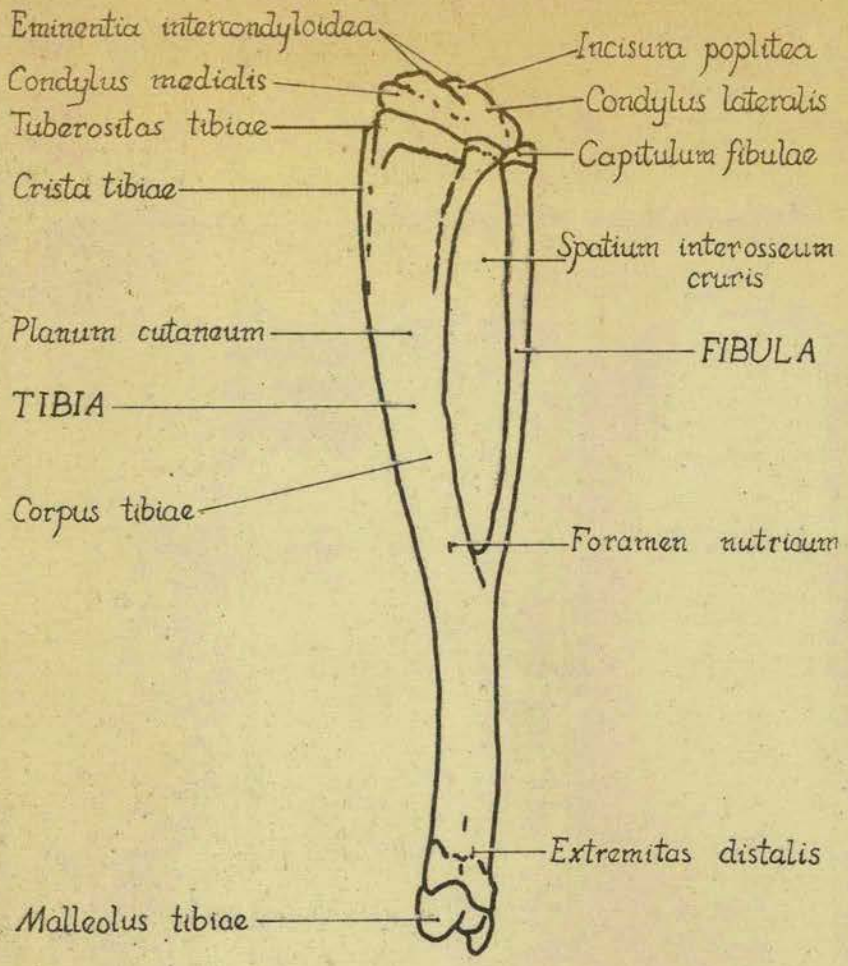
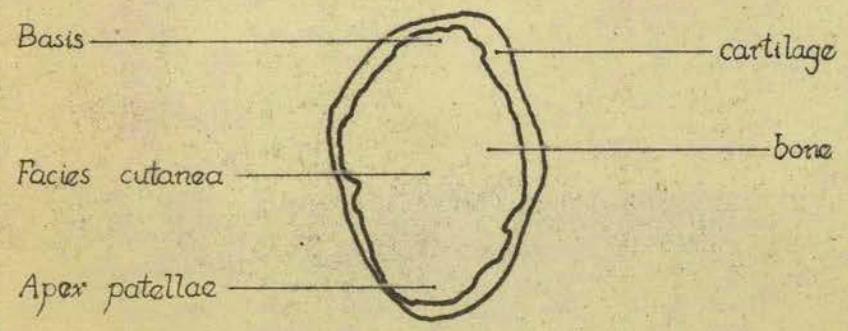
OSSA CRURIS

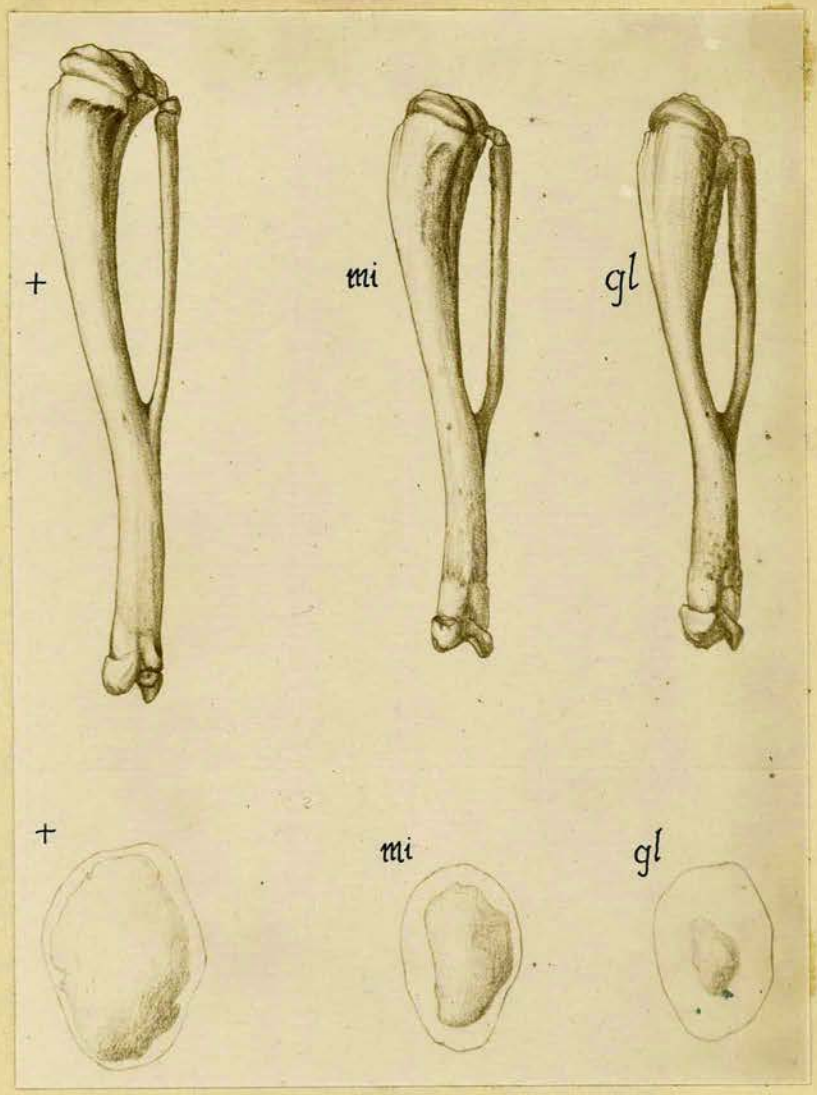
medial aspect



PATELLA

anterior aspect

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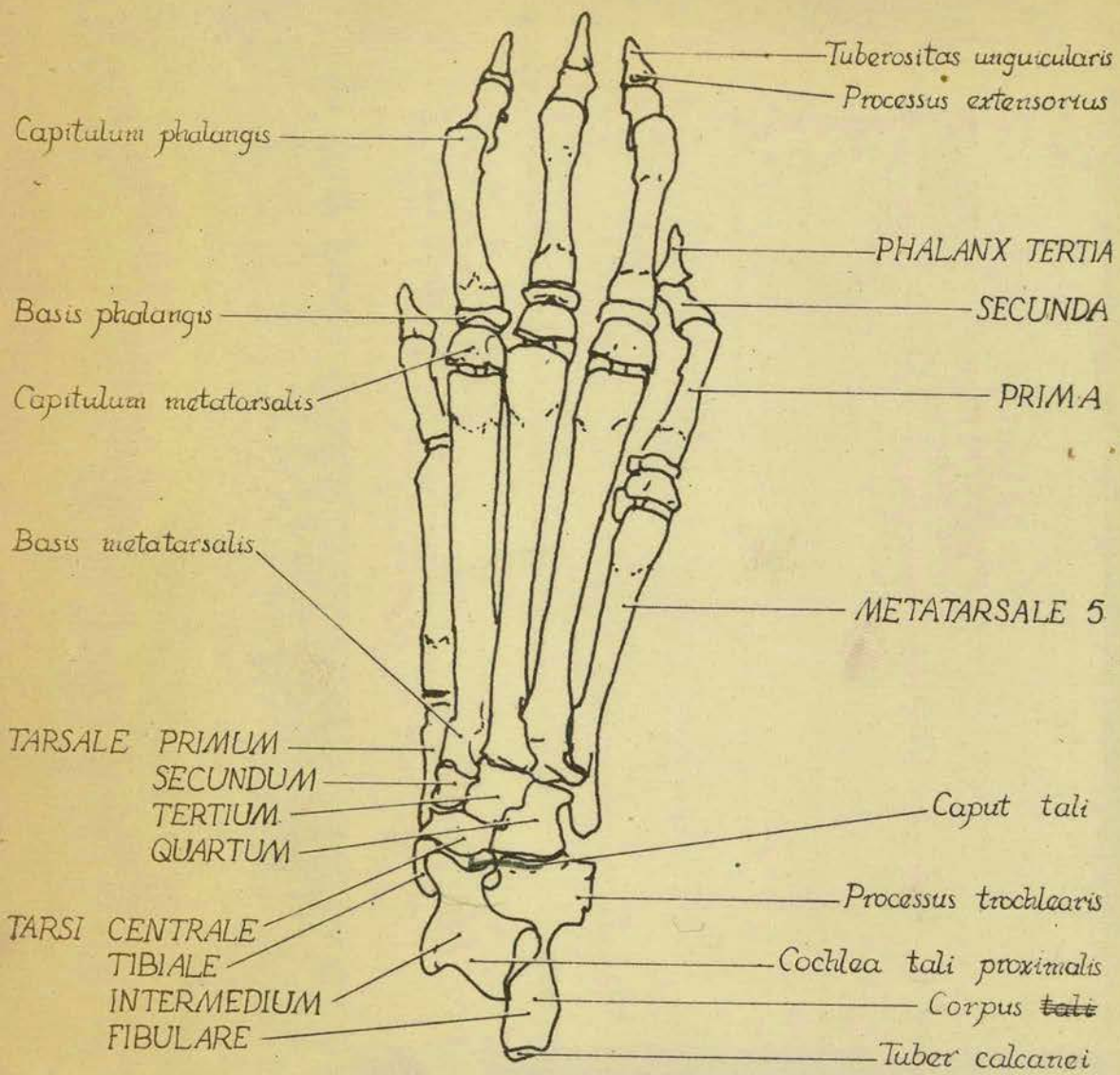
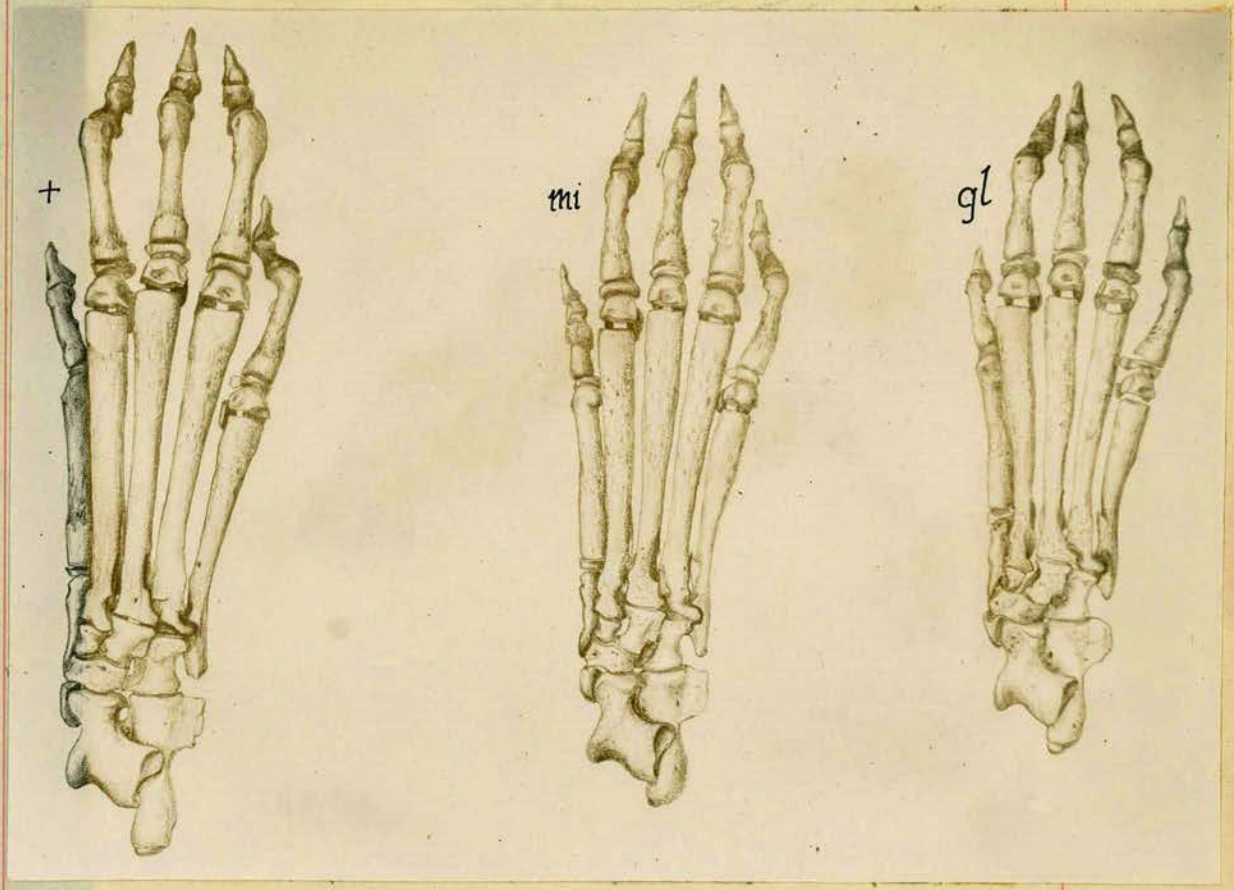
PES

dorsal aspect

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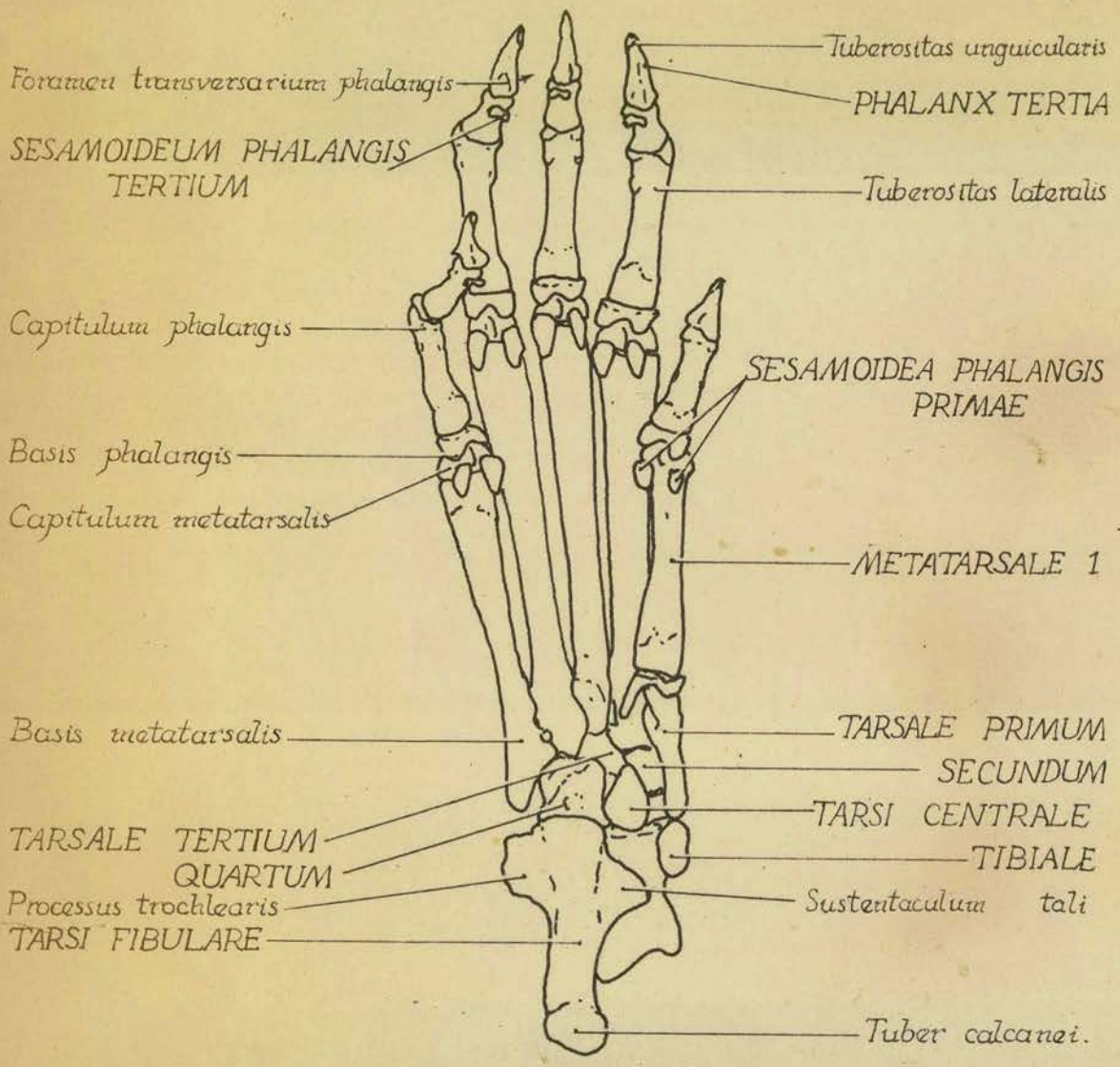
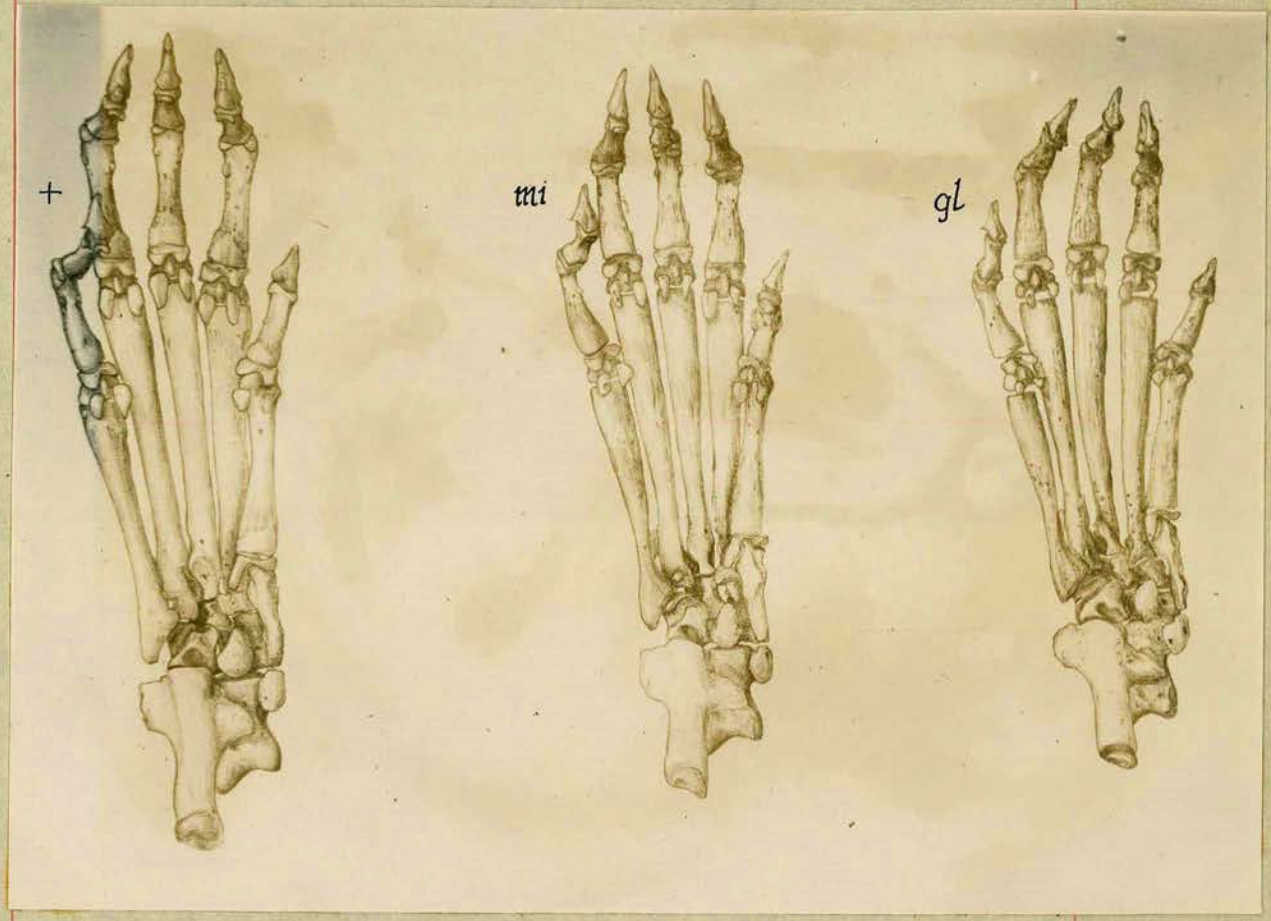
ventral aspect

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Part II: MILK YIELD IN MICE AND THE SELECTION FOR MATERNAL PERFORMANCE.

CONTENTS

MILK YIELD IN MICE AND THE SELECTION FOR MATERNAL PERFORMANCE.

1. INTRODUCTION.

The study of the inheritance of quantitative characters was the core of a new science of statistical biology called by its founder, Galton, "Biometry". Its concern was the manner of transmission from parent to offspring of metrical characters, such as height, which varied almost continuously throughout their range of variation. Eleven years after Galton had published an account of his findings and techniques Mendel's publication on the laws of inheritance was discovered. But the approaches and conclusions of the two authors were so different that it seemed impossible for the two new theories of inheritance to be reconciled. Nevertheless, Mendel's work was acclaimed by a body of biologists who later termed themselves Geneticists. The essence of the success of the Mendelian genetics was the ability to recognise opposed pairs of characters and to follow their inheritance together. Mendel's laws of inheritance were originally no less abstract than Galton's and might have won no greater favour had there not been found in the parallel behaviour of the Mendelian factors and the chromosomes a biological mechanism for the transmission of/ those characters.

This discovery might have brought about the total eclipse of Biometry but for the fact that neither genetics nor biometry could trespass far on the other's field in the study of inheritance. The myriads of subtle differences within the quantitative characters were not generally amenable to qualitative classification, and so were beyond the bounds of the Mendelian approach; conversely, qualitative characters could not be placed on the same single scale for biometrical investigation. Thus the two sciences of heredity survived together for some years. But the disharmony between them was finally removed in 1918 by Fisher, who reconciled the biometrical mode to the chromosomal theory of inheritance. Not only did he demonstrate that some features of blending inheritance could arise in quantitative characters in spite of the particulate nature of the genes, but thereby simultaneously explained the maintenance of variance in the characters studied by the biometricians. And later it was shown that the phenomena of dominance, interaction, pleiotropy and linkage which were originally thought to be peculiar to the Mendelian factors were also exhibited in some degree by the genes affecting quantitative characters. (For reviews and details see Fisher, 1918; Grüneberg, 1943; Mather, 1949; and Richey, 1950).

Nevertheless, the fusion of biometrical and Mendelian genetics meant little more than a pact of non-aggression, for biometry had inherited from its founders, Galton and Pearson, a framework of statistical technique which placed an intellectual barrier between the two branches of genetics. Nor did the

link between biometrical and Mendelian genetics bring about any simplification of the biometrical method, for it was clear that the majority of quantitative differences between individuals and strains were the consequence of the joint action of an indefinite number of individually unrecognisable genes and on this account Mendelian analysis could not be substituted for the biometrical. Even in those few instances where the differences (or the greater part of them) could reasonably be attributed to the segregation of a single pair of genes (e.g. Wriedt, 1930, on the butterfat per cent difference in Red Dane and Jersey cattle) the environment so modified the expression of the genotype that questions of "penetrance" and "expressivity" arose which again rendered the character available to statistical treatment. Thus, under the leadership of Fisher, Wright and Haldane, new statistical techniques were rapidly invented to tackle the ever increasing number and complexity of problems associated with the inheritance of quantitative characters. As a result of the technical differences, the separation of biometrical and Mendelian genetics is now almost as complete as the separation of Biometry and Genetics before 1918.

The necessity of using the biometrical method has had unfortunate consequences in the application of genetics to animal breeding. For biometry has become a mathematical rather than a biological subject, and, in spite of the paucity of experimental data, the theoretical contributions to animal breeding have developed enormously.

In this unhappy state of affairs, the practical animal breeder cannot be expected to have much faith, let alone understanding, of the intricate formulae which have been evolved; nor can it be hoped that the theoreticians, entangled by their abstruse mathematical problems, will apply themselves to the more pragmatic tasks in animal breeding. In this light, it appears desirable to conduct experiments selecting for no-matter-what quantitative character in order to test the validity of some of the more general hypotheses which have been postulated.

It is perhaps the aim of every selection experiment in animals to link theory and practice in animal breeding. But the present experiment had additional aims. The experiment was conceived as part of the research programme of the Animal Breeding and Genetics Research Organisation, in which agricultural breeding experiments are conducted on a model scale in the hope that the more profitable lines of research in the farm animals themselves may be indicated. Mice are obviously the most suitable animals for these experiments, since they are the easiest and cheapest and most rapidly reproducing of those animals which have, by virtue of being mammals, a physiology which parallels that of farm animals. The special problem which the present experiment was designed to investigate was the "physiological limit". This concept has arisen from the experiences of several experimenters (among them Mather and Harrison, 1948), who have found it impossible to increase the performance of their selected

stocks long before genetical sources of variation were thought to be exhausted.

As the inheritance of body size was already being investigated in this laboratory, a new character was chosen. This was "lactation performance" (Falconer, 1947). In his paper, upon which the present work was founded, Falconer gave evidence that "lactation performance" was highly heritable and highly variable. In fact, it seemed an ideal character on which to select towards the unexplained "physiological limit". The high yielding stock, when established, was to be subjected to intensive genetic experimentation. It was also hoped ultimately to establish a stock of highly productive mice which would be better suited for fundamental research on the physiology of lactation than existing stocks of laboratory animals. In addition, the stock would be of exceptional value in any mouse laboratory for using as mothers or foster mothers in rearing delicate mutants. It was essential that the selection be carried out in a closed population so as to reduce the chance of any improvement being diluted by extraneous "blood", but the breeding population had to be of sufficient size that little chance could arise of random fixation of genes by too drastic rates of inbreeding. A selection programme was designed which sacrificed simplicity of statistical analysis of the progress to the attainment of maximum rates of improvement.

However, towards the end of the second year of this experiment, it became clear that no continued progress was being made in spite of some initial success. The problems then confronting the author were:-

(i) Was there anything inherent in the character that would make it impossible to improve it?

(ii) What genetic systems could allow no improvement although there were correlations between daughters and dams? and (iii) Why were the initial stages of selection apparently successful?

These, then, are the three specific studies around which the present account is centred. In consequence, the investigations into maternal performance in mice have followed two distinct courses; the more important work being concerned with the inheritance of performance and its response to artificial selection. An account of this aspect of the work, which includes plausibly heritable effects of environment, is deferred until the third section, while the genetic problems are also the centre of the Discussion. Running concurrently with this programme was a series of successive experiments which were involved in determining the nature of the character being selected and some environmental influences to which it was subject. The account of these experiments now follows.

2. SUBSIDIARY EXPERIMENTS ON THE CHARACTER SELECTED.

(a) MATERNAL PERFORMANCE AND MILK YIELD.

It must be admitted that the character which was selected - "maternal performance" - was intended to be a measure of the potential milk production of the lactating mouse, and for this reason was originally called "lactation performance" by Falconer. It was the total weight at twelve days of litters of eight suckling mice, and in the present experiment was corrected for the age of the dam.

(i) The use of the young to measure a character of the mother.

1. The reason for weighing litters.

So wide a departure from a direct measure of milking capacity requires justification. The present method was adopted because no more convenient measure could be found. Hand milking was, of course, out of the question. And the attempt of Cox and Mueller (1937) to milk rats mechanically was not encouraging. They could not regularly obtain milk even after separating the dams from their young for 16 hours, and while the responses of those which gave milk were very variable, 10% of the rats gave no milk at all. Even in this "mechanical" milking manipulation of the breasts by the operator of the machine was of vital importance for the production of milk, and it appears that the yields these authors obtained measured the uncertain skill of the operator rather than the capacity of the rat. The animal's psychological inhibitions in regard to mechanical milking cannot

be removed by anaesthesia for nervous stimulation from the nipple is essential to the production of milk (Gaines, 1915). While the inhibitions might be circumvented by injection of posterior pituitary hormone (Folley, 1947b, c), such treatment might remove one of the major possible paths to selective improvement and it is unlikely that more than one of the ten mammary glands could be fully drained. This is undesirable since, from macroscopic appearance of the glands, the production of one gland cannot be regarded as representative of the mouse's total capacity (Richardson and Cloudman, 1946; Little and McDonald, 1945, and Latyszewski, unpublished). The alternative approach is to use the young themselves as the milking machine. This is open to the objection that the young may vary so much in their capacity to milk the dams, that any measurement based on them ceases to be a measurement of the dam's performance. However, MacDowell, Gates and MacDowell (1930) found that the amount of milk supplied must always normally be the limiting factor in the extent of growth of the suckling mouse - for by special methods of giving the young extra milk they were regularly able to increase their weight at 12 days by 100%. This is no less than ten times the standard deviation in weight at this age. Enzmann (1933) made the best correction possible for the metabolic losses of each suckling mouse by splitting the litters and using each half as the control for the other when on alternating 6-hourly suckling shifts. But this technique would be intolerably laborious for a selection programme in which the performances of large numbers of mice must be simultaneously measured. Cowie

and Folley (1947) compromised by measuring the young at 5 and 11 days, which covers the straight part of their sigmoid growth curve. But Falconer argued that this method only increased the errors, not the accuracy, of measurement and recommended the use of a single measurement at 12 days. This occurs at the height of lactation on the day the young open their eyes and one or two days before they touch solid food. Falconer's measurement of lactational performance was adopted with slight modification for the present experiment.

While Falconer's measurement may be less subject to errors of estimation, it does not necessarily follow that it is measuring the same character as Cowie and Folley's, and it was therefore desired to discover to what extent birth-weight influenced the weight at 12 days. The results of four calculations (Table I) made at widely different intervals in the present experiment showed that the correlation between total birth- and 12-day weights of the litter was unimportant and confirmed Falconer's belief that a correction for birth-weight contributed only to the errors of estimation. Only one of the calculations showed a significant correlation between the recorded performance and the total birth-weight of the litter which was suckled ; but in all, the variance in total birth-weight was so small that the results of the correction would be quite unimportant.

2. Intrinsic variation in the growth rates of young.

It was next desired to verify by direct observation Falconer's contention that intrinsic variation in the rates of growth of the young themselves made no important contribution

to the measurement of performance. In this regard, it should be noted that Falconer concluded that while in general the young had little control over their 12-day weights, maternal performance (or as he called the identical character, lactation performance) was affected by the degree of inbreeding of the young. This apparent contradiction in his results may be due to a misinterpretation. For Falconer attributed a certain relative consistency in the successive performances of his mice to the continuity of their constitution, and did not consider the possibility of it arising from the consistency of the average genotype of the litters which each mouse suckled. Supposing that a mouse's performance is entirely a manifestation of the genotypes of the young she suckles, then, if she suckles an average of $4\frac{1}{4}$ litters (Falconer's data) and an average of eight mice in each litter, the "mean square" between mice is expected to be no less than 35 times greater than the mean square between performances of the same mouse - Table II. Falconer's analysis (Table III) of the variance ratio was not 12.3, but only 3.27. Thus the heritability of the young's growth rate can be as low as $h^2 = 0.12$ for the data to accord well with Falconer's analysis (Table II). It is concluded that the repeatability of a mouse's performance cannot be considered in evidence of a preponderance of maternal determination in the 12-day weights of the suckled young and more direct evidence is required. Now, MacArthur (1949) noticed considerable effects of the young's genotype on the 12-day weights of the litters in reciprocal crosses between his large and small lines; and Butler and Metrakos (1950) have shown

that in fostering from birth between MacArthur's large and small and A s train mice the origin of the suckling mouse plays an only slightly less important role in determining their weights at 12 days than differences between the s trains of dams from which they suckled. But the authors did not show whether the growth capacity of the young was conditioned by their prenatal environment or by their genotype.

Once at the beginning, and again at the end of the present experiment, the variation in the weights of individual young at 12 days was analysed into a maternal component of variation and a component intrinsic to the young themselves. In each experiment a total of 160 mice were weighed from 20 litters of eight young each. In the earlier experiment (Table IV) the maternal source (that is the milk source) was found to be nine times greater than the suckling's source of variation, in determining the 12-day weight of the suckling. But the measurement of maternal performance is based not on a single individual but on the combined weight of eight young, with the result that the variation due to the young becomes only 1/70 of that due to the mother. On the second occasion, the maternal component accounted for only 8/9ths of the total variation in maternal performance.

Intra-litter competition. A portion of the variation in growth rates of the young was found to result from an extreme competition within litters for the milk (or other forms of maternal attention) from the dam. Thus while there was generally no observable tendency for the heavier litters at birth to grow faster (Table I)

the largest mouse at birth in each litter was almost invariably the largest at twelve days. The intensity of this competition is reflected by the magnitude of the intralitter regression of 12-day weight on birth weight - $b = 4.7 \pm 0.83$. It is remarkable that the competition was just as extreme in litters of two, where the regression was calculated as $b = 3.5 \pm 0.82$.

Genetic control of growth rate. Except in cases of extensive fostering differences in birth-weight were not likely to account for much of the intralitter variation in 12-day weight and it was necessary to determine to what extent this residual variation was under genetic control. The question was especially important in regard to the selection experiment for it could well happen that the selection was more effective in increasing the inherent rate of growth of the young, than in raising the true maternal performance of the dam.

The problem was solved by seeing whether there was any tendency towards uniformity in the performances of dams mated to the same male as a result of the genetic uniformity of half-brothers and sisters compared to the genetic diversity of unrelated litters. But the contribution of the sire to the growth of his young was found to be insignificant even at the 20% level of probability (Table V). Thus there could be little doubt that, except for purely environmental errors in estimation, the 12-day weights of litters in the present experiment were entirely under maternal influence.

(ii) Is performance a measurement of prenatal or postnatal maternal effect on the growth of the young?

Having verified that "performance" was a maternal

character, it was then necessary to discover whether it really was a measurement of lactation (or more generally, postnatal maternal attention), or whether it included to a greater or lesser degree a prenatal effect of the mother. To this end a special experiment, called the "Multiple Fostering Experiment", was designed. In this, dams giving birth on the same day to eight or more mice were grouped together in fours. Each dam was allowed to suckle two of her own young, and six others, two from each of the other three dams of the group. Each suckling mouse was weighed at birth (the birth-fed weight) and again at twelve days, and the variation in the 12-day weights, which were corrected for intralitter competition, was analysed within each group of four dams into prenatal and postnatal components. The account of the pooled results of this experiment is given in Table VI. There was found to be no significant contribution to the weights of the young from the interaction between prenatal and postnatal conditions so this sum of squares (4) was added to the residual sum of squares (5) to estimate a new "error" term (6) against which the contribution of prenatal and postnatal conditions were compared. It must be concluded that the contribution of the intra-uterine environment was of more importance in determining the 12-day weight of the suckling mouse than was the postnatal component.

An attempt was then made to correlate the unknown prenatal effect (which was not manifest by differences in birth-weight) with the dam's postnatal effect, which was presumably mostly lactational performance. The mean 12-day weight of mice <u>born</u>

of the same dam was compared with the mean 12-day weight of the mice suckled by that dam. The calculation was made within the groups of four females so that no difference between the mean 12-day weights would arise through differences in the average treatment of the litters. The average within group correlation was calculated as minus 0.04. It had 17 degrees of freedom and cannot be regarded as significant. In view of the greater effect of the intra-uterine environment compared with the lactation of the dam on the 12-day weight of the litter and especially because of the lack of correlation between them, Falconer's term "lactation performance" has been replaced by the more inclusive term "maternal performance".

The present account now ceases to have much relevance to lactational studies in cattle, but may still be of use in regard to the study of maternal effects on the growth of pigs, sheep, etc. Of course, its value as an account of an experiment in selection is unassailed. Some account is now given of factors which affect maternal performance and reveal something of its physiology.

(b) PHYSIOLOGICAL VARIATES ASSOCIATED WITH PERFORMANCE.

(i) The number born.

It is a common observation that there is surprisingly little difference in the individual weights of mice suckled in very different sized litters, and in view of this compensation in milk production for the number suckled there is to be expected some prepartum preparation of the milk supply in connection

with the size of the litter which will be born. In short, it is reasonable that there should be a high correlation between fertility and performance. An additional contribution to the correlation might be expected from the extent to which both fertility and performance are aspects of maternal vigour. Yet the regression of the performance of mice suckling litters of standard size (eight) on fertility is exceedingly small - b = 1.5% per mouse born; P = 0.015. This situation is probably not different from that attained in a pure line (C57) where b = 2.2% and P = 0.01 - 0.001. But in another pure line (CBA) the regression was insignificant. Thus the mouse makes very little preparation for suckling larger or smaller litters, but very rapidly regulates her lactational output after the litter is born. In fact, it is highly likely that the total milk yield is in direct proportion to the total suckling stimulus since Folley (1947c) has commented on the necessity of the suckling stimulus both in maintaining lactation and in causing "let-down". Moreover, Falconer, noted very little change in the average weight of the individual at twelve days whether they came from litters of one or eight. On the other hand, the prepartum preparation for lactation is of all-or-none character. Jeffers (1935) found that both follicular oestrin and luteal "corporin" stimulate mammary gland growth; but the <u>number</u> of follicles and corpora have negligible effect on the subsequent lactation (maternal performance). Similarly he found that the release of tension in the full-term uterus was sufficient to cause the onset of lactation, though the retention of one

embryo, or even its substitution by a lump of wax, could prevent lactation. Folley's belief (1947a) that the placentae have an effect on lactation is in accord with Asdell's and Salisbury's (1933) observation that pseudopregnancy (in which there can be no placental secretions) has an effect on the development of the mammary glands comparable to the first half of real pregnancy.

(ii) Correction for numbers suckled.

Much of the variation in the growth of young mice up to twelve days is dependent on the size of the litter suckled. Parkes (1926) was the first to observe this source of variation in the weights of the suckling mice and Falconer made a correction for it and constructed a conversion chart for translating the weights of litters of all sizes from one to fifteen into a standard measure of "lactation performance". Recognising the improbability of the good fit of Falconer's chart to all mouse stocks, every attempt was made to secure that each mouse suckled a litter of eight - fostering or removing young from birth as necessary. Contrary to the expectations from the findings of other workers (Butler and Metrakos, 1950; Howard, personal communication), there were found only negligible effects of fostering within the present stock (Table VII). Nevertheless, Falconer's chart was useful during the first year of the present experiment for the few aberrant cases when litter size deviated from eight. But it became early apparent that this system of measurement was unsuitable for the present stock, since the females suckling small litters had unusually high performances. Accordingly, it was modified so that there resulted no greater

variation between the performance of the same female suckling different sized litters as compared with that of females suckling litters of constant size. The new chart was therefore adopted at once and all previous performances corrected where necessary. All figures of maternal performance in the present account are given according to the new chart. The performances of mice suckling litters of eight are measured in terms of the weight of their litters expressed as a percentage of the mean weights of litters of eight of the mice Falconer measured at Cambridge; the performances of mice suckling other sizedlitters are expressed in equivalent units. For litters of eight the 200% point is exactly double the mean (100%) weight.

(iii) Weight and performance: the mouse's heritage.

The level of maternal performance of a mouse is at almost all times quite strongly influenced by its own weight, even as early as three weeks (b - 2.3% per gm.; P = 0.001). Although there is considerable variation in the weights of mice at three weeks both within and between families, it does not appear to be under genetic control, and the correlation with performance must be regarded as entirely environmental. Nor is the correlation an indication that three week weight is a measure of the dam's genotype for performance. For when the regression is calculated as the partial regression of performance (P) on weight (3) independently of variations in the dam's maternal performance (D) its value is little altered - $b_{P3.D} = 1.9$, P = 0.05, while the converse regression is small and insignificant - $b_{PD.3} = 0.1$, (P = 0.4). It is, however,

clear that variations in three-week weight are not merely components of the environmental errors of performance, but must be regarded as part of the mouse's heritage. The positive correlation between weaning weight and performance in mice is strange in view of Dickerson and Grimes's (1947) opposite observation in pigs in connection with economy of gain and performance. And it is in malaccord with MacDowell, Gates and MacDowell's record (1930) of postweaning compensation in the growth rate of mice if the preweaning growth rate has been abnormal. However, the anomalous situation in the present stock is traceable to peculiarities in their management. At three weeks the mice are weaned into storage cages which hold five mice each. They are not removed until they are mated some four to ten weeks later. There is thus ample opportunity for the competition which existed within litters dependent on differences in birth-weight to be continued in the storage cages, but this time dependent on differences in the weaning weights of the mice. This is clearly indicated by two pieces of evidence. The first is that three-week weight exerts no influence on performance independently of its effects on six-week weight. Thus $b_{p3.6} = 0.7\%$ per gm., and $P = 0.4$, whereas $b_{p6.3} = 2.1\%$ and $P = 0.001 - 0.01$. The absence of an independent effect of three-week weight on performance is remarkable in view of the development of the mammary glands which occurs between birth and puberty (Richardson, 1947). Secondly, if members of different families are regarded as part of the same population there appear no significant differences between the average performances of the mice from the different storage cages

($P = 0.1$). This, of course, is to be expected since the storage cages are all established within six days and are all treated alike - being placed close together in the same room and cleaned and replenished with food and water once weekly. Yet if the analysis is made within families, very important between cage differences appear. In fact, the component of variation due to differences between storage cages had even greater effects than the sum total of the remaining causes of variation which exist within families - Table VIII. In order to make this analysis possible the families were split up into groups of twos and threes for distribution to the storage cages. The results are interpreted as showing that the dominant family in one cage (probably by virtue of their higher weaning weights) may be the underdogs in another. Competition is invoked to account for the internal compensation. In fact peck order does not appear to be a facet of social behaviour peculiar to the domestic fowl.

The mouse's heritage. The partial regressions of performance on the weight at three and six weeks reveal the importance of the mouse's heritage. In view of the relative insignificance of the genetic control of performance the effect of 3-week weight on performance must be practically an environmental one. So also must the effect of 6-week weight associated with variations in 3-week weight and the additional factors which become operative between three and six weeks. Clearly, the period from three to six weeks is a sensitive period in the development of the potential performance of the mouse, especially since it

corresponds with the period of most active development of the virgin mammary gland (Cowie, 1949, in rats; Latyszewski, unpublished, for mice). Consequently, the period might well repay extensive experimentation, and experiments which are designed strongly to affect the rates of growth within this period are already under way.

The path through which weaning weight influences performance has been diagnosed as the competition for food and water, or for dominant position generally, that occurs within storage cages; and obviously, the largest mouse introduced to the storage cage has the best opportunity of attaining this position. It has also been shown that variations in the weights of mice at twelve days within each litter was strongly dependent on the birth-fed weights of sucklings. As, in general, the majority of mice from the same litter and of the same sex were weaned into the same storage cage, it appears not at all unlikely that a very large portion of the environmental variation in performance can be traced right back to intra-uterine differences in the environments of the recorded mice. These observations strongly emphasise the importance of the mouse's heritage, particularly its maternal heritage, in influencing the level of its own performance. Indeed, one begins to wonder what part gene segregations play in the inheritance of maternal performance. Moreover, it becomes quite plausible that differential intra-uterine conditions for the mice result in accumulatory size differences which ultimately largely determine the level of maternal performance.

Dickerson and Grimes (1947) have reported that in pigs the contribution of the maternal heritage (direct nutritional effects of the dam) is equal, but in opposite direction,, to her genetic (transmitted) contribution to the efficiency of the gain in weight of her offspring, so that the offspring-dam regression was zero, whereas the offspring-sire regression was 0.23.

In view of the possible heritable effects of weight on performance, no attempt was made to correct for them. However, all performances were corrected to a standard age. The regression of performance on age was low but quite significant ($b = 0.13\%$ per day; $P = 0.01$). The correction was of some importance when comparing the performances of mice from first and fourth litters and especially when comparing the mean performances of different periods and generations.

(iv) The interval effect.

While the genotype for performance is constant throughout the life-time of any particular mouse, a portion of the environmental influence on performance is likely to vary from one measurement of it to the next. Thus the more measurements of performance that are made, the more likely it is that an average environment is encountered and therefore the more nearly the average performance will reflect the genotype for performance. In the present experiment mice were allowed to have from one to four performances recorded.

Unfortunately, it soon became apparent that all performances after the first were subject to additional and

important variation due to the somewhat irregular, but very strong effect correlated with the interval between successive litters (Fig. 1). This is known as the "interval effect". Contrary to the belief of Crew and Mirskaia (1930) that lactation and pregnancy are alternative events in the mouse, it was normal for mice of the present stock to be pregnant while suckling. Moreover, concurrent pregnancy in no way affected the level of the current lactation (maternal performance) nor did the potential or actual level of lactation in any way affect the duration of the current pregnancy. Thus the first performances of pregnant and non-pregnant mice were identical, and the length of pregnancy was unaffected by the number of young suckled. This observation is contrary to commonly accepted belief since Kirkham 1918-19. Under normal conditions concurrent pregnancy had detrimental effects on the subsequent lactation only when the interval between litters was less than 24 days. (It is not known whether this reduction is effective through the pre- or post-natal conditions of the litter's development). And in the majority of cases, when the interval was more than 24 days but less than 32 or so, the second performance benefitted from the concurrence of pregnancy and the previous lactation to such an extent that it was sometimes 20% better than the first.

However, the beneficial effects of interval did not increase indefinitely and between 32 and 38 days of interval there occurred a sudden break in its positive effects so that the second performance was again frequently below the level of the first. (Fig. 1). But with even more attenuated intervals

the second performance again gradually improved.

The problems of discovering the time of initiation of the interval effect (whether from the termination of the previous lactation, or from the birth of the next litter, or from the conception of the following litter) and of interpreting the break in its effect, were the subject of investigation of three successive experiments of which an account is now given.

Now in normal conditions intervals of from 20 to 36 days were 100% correlated, either positively or negatively with the length of the dry period and pregnancy. (The state of pregnancy lasted from 20 to 36 days when the concurrent lactation was terminated not later than 21 days). But mice which did not conceive at the post partum oestrous had gestation periods of never more than 19 days following from an oestrous period some time after the termination of their first lactation. Because both concurrently and non-concurrently pregnant mice displayed the effect of interval, variation in the length of pregnancy was originally excluded as an effective factor of the interval effect. The problem therefore simplified to an investigation of the relative importance of interval itself and of dry period on performance.

But the results of three successive experiments designed to investigate this matter, failed to give a sure answer. By terminating the first lactation at 12 and 18 days and at the birth of the next litter in three groups of about 45 mice each, it was discovered that dry period or foreshortened lactation had accumulative effects on performance only when the dry period or foreshortening of the first lactation was less than three or

four days; for there was no difference between the performances of mice suckling for 12 and 18 days even though the mean intervals were identical. But no effect of interval could be traced within the groups of mice which, apart from having naturally varying intervals, were treated alike.

In the second experiment mice suckled four or eight young for 12 days or "continuously". Great differences between first and second performances were observed through differences between the treatments (Table IX), though again there was no observable effect of interval on the performances of mice treated alike. Table IX shows that lighter milking (i.e. suckling four as against eight) does not itself increase the second performance, so the effect observed in the first experiment must be due to dry period and not to lighter (that is foreshortened) first lactation. Nor is continuous light milking equivalent to heavier milking for a shorter period which is followed by a dry period, even though the total yields were about equal, (Table IX). In fact it appears from Table X that the dry period is even more important to the more lightly milked mice than to the more heavily milked ones which were suckling twice as many young. However, this may be an artefact resulting from a tendency for the mice suckling eight young to dry off naturally even when presented with continuous suckling stimulus in the form of the provision of fresh 12-day old young every six days or so.

The importance of a short rest or dry period between the termination of a lactation and the initiation of the next is

fully appreciated in the farming world. Dry period is responsible for the maintenance of the level of first performance, probably because the involution of the glandular tissues which follows drying off enables the heightened development of the gland responsible for the next lactation.

In the third experiment the variance in interval was increased experimentally by remating the mice at varying times after a short first lactation of 8 days only. In this way, a range of interval from 28 to 41 days, normally associated with the break of the interval effect and the fall in second performance, was caused to be associated with dry periods of from 20 to 32 days which under the conditions of non-experimental mice are associated with the second rise in the performance. The important factor behind the interval effect should therefore have been diagnosed by the sign of the correlation of interval and performance. But whereas the regression coefficient was expected to be $b = \pm 3.3$, with standard error only 0.46 (as calculated for the untreated mice from the selection line in which the total sum of squares for interval was identical with the present experiment) no interval effect was again observable - $b = 0.21 \pm 0.49$! In this last experiment there could be no question that there was sufficient variance in interval for the effect, if present, to be observed, and it had to be concluded that the interval effect was not initiated at the termination or the previouslactation or at the birth of the previous litter. The solution lay in the varying length of pregnancy and pseudopregnancy. For whereas in normal

conditions the length of pregnancy, or the combined length of pseudopregnancy and real pregnancy, vary with interval, they were held constant by the technique of the present experiment. It is therefore most probable that, apart from the effects of the first 3 or 4 days of dry period, the interval effect is really a pregnancy and pseudopregnancy effect. This conclusion is supported by Asdell's and Salisbury's observation (1933) that pregnancy hormones, and in particular, corpora luteal hormones which are secreted throughout the lactation interval, caused massive development of the mammary glands of the rabbit. Moreover, it is known (Richardson, 1947) that pseudopregnancy in bitches may lead to extreme development of the mammary glands, even to the extent of promoting a profuse lactation. To account for the break and the second rise in the performance curve in regard to interval, it must be further presumed that the pseudopregnancy contributes to the interval effect less effectively than real pregnancy. This assumption conforms with Asdell's and Salisbury's comment that pseudopregnancy in the rabbit is only equivalent to the first half of pregnancy as far as their effects on the development of the mammae are concerned. Lastly, it may be stated that no other variable associated with interval can explain the peculiarities of the interval effect. But, in spite of the circumstantial evidence supporting this hypothesis of the cause of the interval effect, the idea is not open to direct experimental proof; and in view of the inability to vindicate the suggestion, the development of

the mammary glands during the reproductive and lactational intervals of the mouse would be a profitable ancillary study.

The importance of the interval effect for the present is that it introduces into all measurements of performance other than the first a certain and unknown degree of error. The analysis of the interval effect in six successive generations shows that any correction for it cannot be relied upon to give good results from generation to generation, Table XI. In fact, it was possible that even after correction for interval, the average of first and second performances may not be as good a measure of a female's genotype for performance as the first alone. When there was no effect of interval the regression of second on first performance was found to be as high as $b = 0.81$, while in the selected line, even after correction, the regression was found to be only $b = 0.39$. As no correlation between first performance and interval could be discovered, it was concluded that the first performance alone was a safer measurement of the genotype, or permanent phenotype, for performance than was the average of the first two. Therefore, all data presented on the selection experiment are given in terms of the first performance only.

(v) Management.

In the course of investigating the interval effect, some managemental aspects of performance came to light which might have a bearing on the management of dairy herds, for a most striking dependence of the level of performance of succeeding occasions on the level of lactation in the preceding lactations

was revealed. The mice were allowed to have as many as five lactations providing they were concurrently pregnant in all of them - providing, in fact, they conceived at the immediately post-partum oestrus on every occasion. The present account is concerned only with these mice which always suckled litters of eight. These were divided into two groups - 28 suckling for 12 days only in each lactation, while another 24 were made to suckle almost continuously by repeatedly fostering on them until the birth of the next litter some 12-day old mice whenever their present litter was 18 days old. In neither group did all the mice survive the experiment; but only the best survived five 12-day long lactations, whereas only the worst (judged by the average first performance of the survivors at each lactation) survived five "continuous" lactations - Table XII. The detrimental effects of continuous suckling are even greater in depressing the successive performances of the surviving mice. The average level of performance was increasingly low, although the length of the interval between the successive litters remained practically constant (Table XIII). Moreover, far fewer mice of the continuous suckling group survived. There were only two left in this at the fifth lactation, whereas eleven survived the five lactations of twelve days' duration only. The groups were started with about equal numbers each: 24 and 28 respectively. Many mice were thrown out of the continuous suckling group because they failed to suckle the litters to which they gave birth. There can be no doubt that several others simply failed to conceive at the post-partum oestrus, and it is even

possible that this oestrus was suppressed. There can be no question then that continuous milking has extremely detrimental effects on the total life-time yield of the lactating animal, and that it is definitely advantageous to allow at least a short dry period. Not only does this allow the maintenance of a high performance in each lactation, but it results in higher fertility; i.e., a large number of lactations within a given period.

The results of this experiment tell something more about the nature of maternal performance. Contrary to general expectation, it is not a measure of the general health or vigour of the mouse, rather performance and general vigour are negatively correlated. For it is apparent from the above experiment that only the worst performers survive the more exacting conditions. It is as though high maternal performance meant that some other aspect of vigour had to be sacrificed. This is further indicated by the phenomenon which has occasionally been observed that mice rearing remarkably heavy litters for the number suckled suddenly die in the height of lactation. It is evident that mice which give least of their reserves to maternal performance are most capable of surviving conditions which make the greatest demands on their systems through this path. And if this surmise is true it justifies to some extent the dairy cattle breeder's ingrained habit of paying attention to details of conformation (in so far as they reflect other aspects of vigour) as well as to lactational performance.

(vi) Summary.

To sum up: both first and second maternal performances

may be subject in high degree to environmental variation. Competition, which might be traced right back to differences in the birth weights of mice suckled by the same female, and certainly to differences in the weights of mice stored in the same cage after weaning, is a major factor controlling maternal performance. The period immediately after puberty is sensitive to environmental conditions, in respect to the determination of future performance. The level of the second performance is subject to additional sources of variation - notably to differences in the length of the pregnancy or pseudopregnancy preceding it. Variations in dry period are effective in varying the performance only if the dry period is less than a certain minimum which is probably not more than three or four days. The length and intensity of the earlier lactations affect subsequent performances only in so far as they lead to excessive strain on the lactating female.

3. THE SELECTION.

(a) INTRODUCTION.

(i) Techniques.

1. The selection programme.

The method of selection, of which an account is now given, was not designed on any rigorously scientific basis, for this was impossible while certain parameters such as heritability and repeatability of performance were unknown. Moreover, rigidity and simplicity of the programme which would have allowed accurate and easy statistical analysis of the response were sacrificed to the main aim of the experiment - to select efficiently and vigorously up towards the supposed "physiological limit".

The selection was carried out in a closed population of sufficient size to give low rates of inbreeding. The selection was made in two quite separate stages - in a "test" and a "repeat" period in each generation, though in the first generation only the second stage selection was made. During the "second stage" females were selected purely on their own performances. Culling occured on two or three successive occasions. Firstly on fertility. Infertility could not be tolerated as it might become impossible to measure the performances on eight suckling young, and in the long run would only reduce the intensity of selection. So females giving birth to less than six young in their first litters were regularly discarded, and their young were used to make up

other litters to the required number of eight newborn young. At the second culling about half the females were discarded, with their litters, on the basis of the female's own first performances. The spaces vacated by the discarded females were allocated to storage cages for weaning the first litters of the surviving females. In the earlier generations, before the inadequacy of the "interval correction" was appreciated, there was a third culling on the average of the corrected first and second performances of each mouse. Where fertility was dangerously low the final selection was based on an arbitrary index combining average performance and average fertility. The finally selected group consisted of about the top quarter of breeding females. As the selected females had already been mated and had reared young, the selection consisted merely of saving their young. Thus all females were killed, even when selected, after rearing two litters to weaning age. The weaned litters of unselected females were killed so as to leave space for the weaning and storage of the surviving litters and for the setting up of the "test" matings.

The possibility of attaining a much higher rate of progress through males than through females by mating in harems, together with the impossibility of measuring the males' performances directly, made it appear desirable to alternate the mass selections of females in the "repeat" periods of each generation, just described, with other periods of selection, designed to put the selective advance through males on a more reliable basis. The high heritability expected from Falconer's

work allowed that, owing to the system of mating unrelated mates, the female's performance might not be an adequate measure of her sons' genotypes. Progeny testing was rejected on account of the high rates of inbreeding which would result if the progeny test was used efficiently. The alternative was to select males on the average performance of their sisters. The period in which the performances of these sisters, born in the first litters of the selected dams, were measured, constituted the "test generation".

While their sibs from the second litters were growing to maturity, three daughters (where possible) from the first litters of the selected dams were mated to sons of the very best dams. (This enhanced the probability that their progeny would contribute to the next generation). The family average performances so obtained were used as indices of the brothers' genotypes for performance. Males to be used as sires in the next population - the "repeat generation" were taken from only the very best families. A further, though slight, selection, based on the family averages, was also made in the female line. The regression of sisters on the average performance of three sisters differs little from the daughter-dam regression whatever the heritability (Table XIV). The formula for the regression of unmeasured sisters on their family average is obtained from Robertson and Rendel,(1950). By culling all but the best tested families which might otherwise have been included in the next population - the "repeat generation" - the breeding population in each generation was effectively increased

by about 50%. This was achieved without using any additional space and without appreciably increasing the generation length; the effect of inserting the "test" generation before establishing the finally chosen, or "repeat" generation was to increase the length of each generation by only two weeks from a minimum of thirteen weeks. The best tested females were brought forward into the repeat generation where they were remated if necessary. The combination of the mice of the repeat period with those from the test period which were brought forward constituted the "selected generation" - abbreviated to G_S.

By way of summarising the technique of selection some figures are given. Each "test generation" consisted of about 45 daughters of the best 15 females, of the preceding generation. No female was represented by more than three daughters, a few by only two or even one. Each of the daughters had its performance recorded, however infertile. On the average performances for each family then available, two or three families were selected to provide the majority of the 15 males required for the following period - the "selected generation". The remaining males were taken from other good families if their use would considerably reduce the rate of inbreeding. The best 9 or 10 families were selected to provide the 75 females required for the "selected generation". The very best females who were measured in the "test generation" were added to the "repeat generation" to complete the "selected generation". Usually not more than 5 females were so brought forward.

The "selected generation" consisted of the 75 females

from the best tested families mated in harems of five females each to males from the best two or three families. Females giving birth to five or fewer young were discarded at once. Thirty females were allowed to have second litters, and of these fifteen were finally selected on an arbitrary combination of their individual fertility and maternal performance. Their families were then tested in the "test period" of the next generation by three of their daughters (where possible) from the first litters.

At no time was any direct selection made on males.

It has been suggested that the efficacy of selection can be improved in two ways neither of which, however, has been adopted in the present experiment. Each aims at the better estimation of the genotype for the character being selected. Hazel (1943) suggested constructing a selection index which breaks the selected character into its component parts, both correlated and uncorrelated and weights each according to its heritability, and multiple genetic and multiple phenotypic correlations. Hazel's example was the selection of pigs for economic value. But in a similar way maternal performance might have been selected on at least four component traits - weight of mother at one or at several ages, fertility, and pre-natal and post-natal performances. Of course, at the beginning of this experiment very inadequate data was available for the construction of such an index. However, the author distrusts the efficacy of such indices for one reason - that it

has frequently been observed that genetic correlations are not stable under selection. Thus MacArthur (1949) reports a correlated response in fertility to the selection for large and small body size in mice; but the response was limited to the first three or four generations only. Falconer (unpublished) has observed a similarly limited change in the maternal performance of his mice which were being selected for large and small body size, although fertility and tail length were altered more systematically. These results can be interpreted as demonstrating the pleiotropic effects of a few major genes (amongst many minor ones) which affect both characters and which, owing to their importance, become rapidly fixed so that no further correlated changes occur. But Mather's account (1942) of infertility which developed in two lines of flies selected in opposite directions for bristle number defies such an explanation. Whatever their interpretation, these changing correlations vitiate the principles of Hazel's index so far as it relies on correlated measurements.

The second proposal was by Lush (1947). He suggested that an animal's breeding value can best be estimated if the phenotypic values of his near relations are also known. These are weighted positively or negatively according to calculated parameters. The application of this method, "combination selection" requires the estimation of heritability. After three years of selection for maternal performance in mice, this statistic is still unknown so that the method is inapplicable. A compromise however has been found in selecting <u>alternately</u> on

individual and family merit - the two methods which, if properly used simultaneously, are the method of Lush's combination selection.

The selection programme was simplified when it became apparent during the seventh generation that no progress was being made and the author's attention was diverted from the attainment of a high line of mothering mice to finding reasons for the failure of improvement. The intensity of selection was relaxed, so as to enable selection to be carried in both directions, and selection was practised in the female line only; family selection was dropped.

2. The calculation of the maximum possible improvement - MPI.

In each generation selection was practised at two different levels; first there was in each "test" period a selection on family average performance, and this was followed by a "mass" selection of individual females. In the calculation of the MPI - the maximum possible improvement - from the beginning to the end of the experiment, these two phases of selection must be kept clearly separated. A slight complication arises from the confinement of the selected character to females only, but this problem can for the moment be overlooked.

Following the example of Rendel and Robertson (1950) the MPI (maximum possible improvement) which is related to the selection differential in a way to be explained shortly, is calculated from its components, the I values. The genetic improvement from test to repeat periods of the same generation has two paths of origin, $I_{F♀}$ and $I_{F♂}$ which are respectively the

phenotypic superiority of families supplying females and of families supplying males over the average of families measured in the test period. Similarly, the genetic improvement from the repeat period of one generation to the test period of the next also has two paths of origin, I_{QF} and $I_{\sigma F}$, which are respectively the phenotypic superiority of females and males producing test families over the level of the population from which they came. Figure 2 shows a hypothetical plotting of these four components of the total MPI. The thin line in the figure represents that part of the improvement which applies to the measured mice (i.e. the females) in each period.

Table XIV shows that the regression of daughters on dams is the same as the regression of future sisters on the family average when heritability is unity. When $h^2 = 1$ the improvement is expected to equal the MPI. Thus, in the calculation of MPI both dam and family selections are weighted by one half. In this respect the MPI differs from the selection differential. For in calculating the latter, the selection of families, being the only operative path for improvement from test to repeat periods, would be weighted by unity, thus tending to overestimate the intensity of the selection attempted at this step. However, as far as the mass selection is concerned MPI = selection differential. Because the selection of females is only one of the two equally forcible paths through which improvement could be made (the other being the selection of males) I_{QF} is also weighted by one half when estimating the selection differential.

It is apparent from the diagram, Fig. 2, that the

maximum possible improvement in females from test to repeat periods is given by the formula:

$$MPI_{TR} = \tfrac{1}{2}I_{F♀} \quad \ldots\ldots\ldots\ldots\ldots 1$$

and similarly, that the maximum improvement between test periods of adjacent generations cannot exceed

$$MPI_{TT} = (\tfrac{1}{2}I_{F♀} + \tfrac{1}{2}I_{F♂})/2 + \tfrac{1}{2}I_{♀F}$$

$$= (I_{F♀} + I_{F♂} + 2I_{♀F})/4 \quad \ldots\ldots\ldots 2$$

From equations 1 and 2

$$MPI_{RT} = MPI_{TT} - MPI_{TR}$$

$$= (I_{F♂} + I_{F♀} + 2I_{♀F})/4 - 2I_{F♀}/4$$

$$= (I_{F♂} - I_{F♀} + 2I_{♀F})/4 \quad \ldots\ldots\ldots 3$$

Each component (I) of the selection is, of course, weighted in proportion to the number of mice recorded in the next period on account of that selection. For example

$$I_{F♀} = S(P'.n)\ /Sn - \bar{P}$$

where P' is a selected family's average performance; n the number of sisters recorded in the next period; and $\bar{P}$ the mean family average for the period. S stands for the summation of all the terms in the series.

The summation of the values of formulae 1 and 3 over the whole period of selection gives the line for the maximum possible improvement in the performances of females. This is drawn in Fig. 3.

3. The method of avoiding inbreeding.

Matings were arranged between the least related animals of the breeding populations. Females from the same family were mated as much as possible to different families of males, and at least to different males of the same unrelated family, so as to keep down the rate of inbreeding in more distant generations. Attempts were made to keep the inbreeding coefficients of females and their young as nearly as possible the same within each period, so that no correction for inbreeding was necessary within generations. Relationship and inbreeding coefficients were calculated as "genetic covariances" and "variances" according to Lush's method (Emik and Terrill, 1949). Tables of these coefficients were prepared for all possible matings between the selected families in each generation.

(ii) Management of the stocks.

1. Management.

Matings were made simultaneously in each period when the youngest mouse was at least six weeks old. The mice were mated in harems of five females to each male, and eighteen days afterwards the most pregnant females were transferred to individual cages and were examined every day for the birth of the litter. The male was kept with the most pregnant female until 24 hours after giving birth, or if a vaginal plug was seen at birth, the male was transferred forthwith. The male stayed with the last female to give birth to her first litter until he was required at the postpartum oestrus of one of the harem giving birth to a second litter.

At twenty-one days the litters from selected parents were weaned into storage cages. These contained five mice (in the earlier part of the experiment, eight) of the same sex from the same or different litters and of almost the same age. Harem, rearing and storage cages were of the standard pattern used in this laboratory. The cages were cleaned once a week (in the earlier generations twice weekly if considered "dirty") and replenished with food and water. Cages were placed at random in respect to the expected performance of the females they contained. The temperature of the mouse house was kept as near as possible to 20° Centigrade with a daily variation of about two or three degrees but occasionally reaching +9 to -7°C.

2. An effect of the male on fertility.

An interesting consequence of mating the females in harems was the discovery that the first mating in each harem was usually the most fertile. The analysis was confined to the period from 20 to 26 days after putting up the harem, but very few births occurred on the twenty-first day, and none on the twentieth, it apparently taking two days for the harem to settle in. Now, as the oestrus cycle of the mouse usually lasts about five days, each mouse in the harem was given a chance to come on heat once only - so that effects of possible correlations of "reluctance" and "inclination" to conceive with fertility were excluded.

The results (Table XV) show that the first litters are usually 1½ mice larger than the others which are of about the same level. There are two possible explanations:-

That the more avaricious females hasten the onset of

oestrus on the introduction of the male, and therefore tend to be the first to be mated. It must be further assumed that avidity is associated with fertility;

Or, that the male's store or ejaculate is exhausted by the first mating and that only the current production is available for subsequent matings. In this case it must be assumed that fertility is proportional to the quantity of ejaculate.

The first suggestion is the less plausible since, if the order of mating reflects the order of avidity (and fertility), it is to be expected that there should be a steady decline in the average fertility of first to fifth matings. Moreover, sexual appetite and maternal capacity are not necessarily correlated. Evenness of the fertility of all subsequent matings and the contention that fertility is proportional to the quantity of ejaculate is supported by Fischberg's opinion (verbal communication) that the size of the vaginal plug reflects the degree of fertility.

(iii) The initial population.

The initial population (G_1) was composed of two distinct groups of mice. The smaller was a select group (the FM's) of twenty females and their mates which were being bred as foster mothers on account of their general mothering ability - high fertility, and high performance. Their pedigrees were not all well known and most had been recently derived from the "Fancy". The other group (the N's) comprised 61 females and their mates and were the product of a four-way cross between highly

inbred strains of mice (CBA, A, C57 and RIII). Owing to the nature of the cross the N's were virtually members of a single large family and were considered 50% related to each other. In order to obtain clear statistics from analyses of variance, these two stocks were kept separate in the first two generations. However, the "foster-mother" group were so superior to the others, that in G_{3TEST} they and a few hybrids were the only females to be measured. A few males from the four-way cross were used in this generation in order to reduce the rate of inbreeding. Not until the fourth generation did the young become homogeneous, and not until G_5 did the parents form a homogeneous population. The mice segregated for the following mutant genes:- a, a^t, A^W, b, c, d, p, s, si, and "Umbrous" (U).

(b) RESULTS.

The response to selection for a quantitative character is believed to depend upon the importance of its additive genetic variance. This is the portion of its variance which is attributed to those actions of the segregating genes which involve no interaction, either within loci (dominance) or between loci (epistasis). These non-additive actions of the genes reduce the rate of improvement expected from the total genetic variance of the character, and in most analyses are lumped with the strictly environmental variance to form a component attributable to non-fixable variation. This procedure is justified by algebraic analysis of the correlation between parent and offspring, to which the possible rates of progress under selection are

obviously related. Mather (1949) has shown that if the correlation between parent and offspring is expressed in terms of additively genetic variance, D, the variance due to dominance deviations, H (epistatic variation may be treated in essentially the same way) and the truly environmental variance, E,; then only D enters into the numerator, whereas D, H, and E all contribute to the denominator. The portion of the total variation which results from fixable or additively genetic variation defines a quantity termed "heritability".

The results of selection must also depend on the degree of selection, which has been shown to be best estimated by the "maximum possible improvement" or "MPI". The product of heritability and MPI gives the expected improvement.

Now, in spite of very definite selection (MPI from the beginning to the end of the selection programme was 66.5%, and from G_{4TEST} to G_{10} was 40.2%) there was only a short initial period of success which was followed by a long phase in which no further permanent progress was made (Fig. 3). It is therefore desired to measure heritability by independent methods, and if it is not found to be deficient, to determine in what respects current theories concerning advance under selection require refinement.

(i) Heritability.

Heritability can be measured in different ways according to which of two theoretically equivalent definitions is used. It may be defined as

(1) The proportion of the total variation in the

character which is attributable to additive genetic variation. It is usual to ignore non-additive action of genes because it is difficult to take permanent advantage of their action even with special techniques of selection. This formula can be represented as

$$h^2 = G/(G + E)$$

where G and E are the additively genetic and "environmental" components of variance respectively.

Alternatively, heritability may be defined as

(2) the regression of genotype on phenotype. The phenotype is the average phenotype of each pair of parents. Their corresponding genotype or breeding value is measured as the average phenotype of their progeny. Because regression estimates are unaffected by the errors of estimate of the "dependent variate" (i.e. the performance of the progeny) and of selection of the "independent variate" (i.e. of the parents) (Eisenhart, 1939) except in so far as they alter the errors of estimate of the regression coefficient, offspring-parent regressions are ideally suited to the estimation of heritability in selection programmes. The second definition can be represented by the formula:-

$$h^2 = b_{gp}$$

The identity of the two expressions can be shown as follows (Alan Robertson, verbal communication). Let G and P be the additive genetic and the phenotypic variances, and g and e genetic and environmental components of the phenotype p. Then

$p = g + e$.

$$\text{Now } b_{gp} = \left[S(g - \bar{g})(p - \bar{p})\right] / S(p - \bar{p})^2$$

$$= \left[S(g - \bar{g})(g + e - \overline{\bar{g} + \bar{e}})\right] / S(p - \bar{p})^2$$

$$= \left[S(g - \bar{g})(\overline{g - \bar{g}} + \overline{e - \bar{e}})\right] / S(p - \bar{p})^2$$

Providing that animals with the best genotypes are not treated differentially (i.e. that g and e are not correlated) and in the present experiment they were not, positive deviations of g from $\bar{g}$ are as likely to associate with positive as with negative deviations of e from $\bar{e}$, and conversely, so that

$$S(g - \bar{g})(e - \bar{e}) = 0.$$

$$\text{Hence } b_{gp} = S(g - \bar{g})^2 / S(p - \bar{p})^2$$

$$= G/P$$

$$= G/G + E$$

Estimates of heritability are peculiar to the population in which they are estimated. For example, in a pure line, where $G = 0$, h^2 must also equal 0. But this does not mean that the character is always environmentally determined.

1. Heritability by analysis of variance (definition 1).

(a) The products of four-way crosses are especially suited for estimating heritability by analysis of variance. Each pure line in the cross may be considered as contributing a gamete picked at random from the original population from which the lines were drawn. In this light, F_1 animals can be regarded each as a synthetic, random-bred animal; the mating of two

different F_1's derived from the different crosses as the mating of two animals picked at random from the original population; and the next generation (which for convenience will be called an "F_2") as constituting a single random-bred family. The variation encountered within each F_1 is entirely environmental, whereas the difference between their means is a rather uncertain estimate (since it has only one degree of freedom) of the importance of genetic differences in the original population. The variance of the "F_2" includes genetic variation. In normal populations only half of the genetic variation is apparent within families, the other half accounting for the genetic differences between families. Thus, in the present analysis the differences in the variances in the F_1 and "F_2" populations is equal to $\frac{1}{2}G$; The variance within the F_1's, E.

It will be remembered that the products ("F_2") of a four-way cross between CBA, C57, A, and RIII lines contributed to the initial population of the present experiment, so that the above analyses are possible in this experiment. The analyses, calculated separately for the F_1's and the "F_2", are presented in Tables XVI and XVII. Each F_1 consisted of ten mice with two recorded performances each; the "F_2" consisted of twenty mice also with two performances each. The constitutionsof the Mean Squares are given in terms of I, P, C and G. G is the genetic component of variance as before, and the others are all part of the environmental variance. I is the variation which cannot be traced to any origin except temporary environmental fluctuations in performance; P is the variation attributable to consistent

differences between first and second performances of all mice; and C is the enduring effect of environment on each mouse. In the "F_2" the differences between mice contain a genetic as well as the environmental component of variance such that $M = C + \frac{1}{2}G$.

From the formula $M = C + \frac{1}{2}G$,

$$G = 2(M - C).$$

Substituting from 2 and 5,

$$G = 2(35.78 - 13.15)$$
$$= 45.26.$$

Now the environmental variance affecting the mean performance of each mouse is given by

$$E = I + C$$

As I differs in the two analyses it is calculated as the Error MS within analyses $= (1931.04 + 2728.51)/(18 + 19)$

$$= 125.93$$

whence $E = 125.93 + 13.15$

$$= 139.08$$

Now $h^2 = G/E+G$

$$= 45.26/(139.08 + 45.26)$$
$$= 0.25$$

This estimate of heritability is, however, most unreliable as neither C nor M contributed significant variation to the between mouse mean square. Thus the actual values obtained for these components must be very poorly estimated and subject to

considerable variation. Moreover, conditions do not appear to be consistent between the F_1's and the "F_2" since the calculated values of P and I differ widely in the two analyses.

G as estimated from the Mean Square between crosses in the F_1's was rather more accurately estimated, though, having only one degree of freedom, is unlikely to reflect the level of genetic variation in general. The calculation of heritability from this F_1 data (1, 2 and 4) gives

$$h^2 = 23.5/(23.5 + 13.15 + 107.3)$$
$$= 23.5/143.95$$
$$= 0.16 \ .$$

It seems from these estimates that heritability is in fact low, and Falconer's conclusion that most or all of the variation in performance was genetic does not seem to be borne out by the present analysis.

(b) Heritability was again estimated by the analysis of variance when the selected line had become more homogeneous. Table XVIII gives the results of the analysis for the "selected" generations 2 - 5 included. The G_{TESTS} were not included as they contributed nothing to the between litter variance. The analysis is made in terms of F and L - the family and litter components of variance. It is seen that there is no significant difference between the performances of sisters born in different litters. Thus the between litter component of the between family mean square can be ignored, and F is calculated as (207.56 - 110.39)/4.62 = 21.03.

Now F, the between family component of variance, is composed of $\frac{1}{2}G + MP$, where G is the total genetic variance in a randomly bred population, and MP is the variance due to environmental factors, especially the maternal performance of their dams, which affect the performances of sisters similarly. G and MP could not be separately estimated because too few dams from the same harem were selected to allow the separate estimation of S ($\frac{1}{4}G$) and D ($\frac{1}{4}G + MP$) from the performances of their daughters.

The within family variance (I) in a random-bred population contains half the genetic variance together with the environmental variance other than the MP factors. That is,

$$I = \frac{1}{2}G + E \quad .$$

In lieu of any estimate of the MP factors, these must be regarded as contributing negligible variance. This is not an altogehter unreasonable hypothesis in view of the apparent absence of effects distinguishing litters from one another in spite of the considerable variation in successive performances of females when not corrected for the "interval effect". On this hypothesis,

$$F = \frac{1}{2}G$$

Whence $E = I - F$

$= 89.4$

and $G = 2F$

$= 42.0$.

Now $h^2 = G/G+E$

$= 42.0/131.4$

$= 0.32$

This estimate, which is based on significant variance between families, is of the same order though a little higher than the other two, but nevertheless is no where near attaining the high level suggested by Falconer. Yet, in view of the inclusion of MP factors in the estimate of G, this last estimate of heritability may be a little overestimated; but underestimated if the effects of the MP factors are negatively correlated with the dam's maternal performance (Dickerson, 1951).

2. Heritability by regression (definition 2).

The regression of genotype on phenotype, which is the second definition of heritability, may be restated as the regression of offspring on mid-parent. In the present experiment the male's phenotype cannot be directly measured, but as the pairing of males and females within the breeding populations is in no way assortative, the deviations of the mid-parent phenotype from the mean is regarded as half the deviation of the dam's phenotype from the mean of dams. Thus $h^2 = 2.b_{dd}$.

Heritability has been estimated in this way from the pooled data obtained within each period from G_{3TEST} on G_2 to G_{10} on G_9 inclusive. Its value for the high lines was estimated as $h^2 = 0.36$ but was insignificant ($P = 0.10$). The estimation of the significance of regressions when the dependent variable (daughters) has many more degrees of freedom than the independent variable (dams) raises some statistical problems. On Mr Alan Robertson's suggestion each dam was included once for each of her daughters in the calculation of the regression; but for the estimation of its significance, the within family sum of squares for the

daughters was removed from the residual sum of squares, which was calculated in the normal way. The new residual SS then had the degrees of freedom appropriate to the number of dams. The analysis is presented in Table XXVII.

When it became apparent that no continued progress was being made, it became necessary to test whether there was a genetic barrier to improvement or whether there was a total lack of genetic variation for maternal performance. If there was merely a genetic barrier to improvement, it should be possible to "disimprove" the character by selecting the worst instead of the best dams. In $G_{8, 9 \text{ and } 10}$ the dams were divided into high and low halves and the regression was calculated separately for the low lines. The results were

$$b = 0.45; \quad P = 0.01 - 0.001.$$

Although the regressions in the high and low lines appear widely different, they are not at all significantly so. The errors of estimating both are so high that the probability of the difference between them arising by chance is as much as 0.15.

The estimated heritability for the low lines is extraordinarily high for a quantitative character, and conforms with Falconer's conclusions of the importance of genetic variance for the same character. $h^2 = 0.90$ and within the fiducial limits set by a statistical probability of $P = 0.05$, may vary between 0.32 (which is also unusually high) and 1.48 (which is biologically impossible).

The inconclusiveness of the differences between the several estimates of heritability presents a dilemma in their

interpretation. The results cannot be interpreted as showing that there is definitely a barrier to improvement, or that the daughter-dam regressions in the high lines should definitely be reflected by rapid improvements. And in discussing these results the two alternative must be left open.

. The pragmatic estimation of heritability.

Even if the estimates of heritability in the high line by either of the above methods were significant statistically, improvement of the breed average in response to selection cannot necessarily be assured. For neither of the above techniques can reveal environmental or genetic trends between generations which might vitiate the expected gains. It is thus at least theoretically possible to have high heritability and selection differential without improvement. The pragmatic estimate of heritability can only be that which measures the actual improvement in response to selection. In so doing, it measures the fraction of the phenotypic improvement in the parents which is of heritable origin (compare method 1) and it also measures the regression, though the between generation regression, of offspring on parent (compare with method 2). In so far as this method measures the amount of progress made in the past, it is useful in estimating the amount of progress to be expected in the future. But the method is only applicable when the environment is thought to be held constant from one generation to the next (as in the present experiment). Even resort to this method gives no conclusive result in the present experiment. For while the response in the first four periods of selection

suggested a heritability of almost 100%; one period in which $h^2 = +100\%$ separated it from an enduring phase in the experiment when $h^2 = 0$, see Fig. 3. (The irregularity in this last phase caused by the drop in the first performance of $G_{7\text{Repeat}}$ mice is attributable to some quite temporary environmental effect, for the second performances were again up to the normal level - 121%). This result, far from solving the present dilemma, only presents new problems, but further treatment of this subject is deferred until the Discussion.

(ii) Visible genes and maternal performance.

It is current belief that quantitative characters are determined by the joint action of a large number of genes with individually small effects. It is also believed that the segregation of any one of the majority of these genes is barred from phenotypic expression by the smoothing out of the discontinuity of genotype not only by the segregation of the other genes affecting the character, but especially by the continuity of environmental effects. Yet it is the common hope, and in some cases the sole hope of quantitative geneticists and animal breeders, to find the most important member of the series - to find a gene which might raise performance by five or even ten per cent. If families are sufficiently large it may be possible to detect the presence of such genes by the anomalous distribution of within family variances. The families of mice recorded in the present experiment were too small to allow this technique to be practiced, but the visible genes for which the stock segregated were investigated for

associated major effects on maternal performance.

Grüneberg (1943) has extensively reviewed the mouse literature in evidence of Mendelian factors associated with quantitative characters. Some of their effects seemed almost certainly to be pleiotropic effects of the colour genes themselves (in particular the effects on adult size at the brown locus, and less certainly at the dilute locus). Differences between the birth weights of Mus musculus and bactrianus (Green's data, 1931a) seemed to be attributable to one or more recessive genes for small size affecting the growth rate of the young mice (Table XIX andFig. 4). But gene differences affecting the adult weights of these species showed no dominance deviations (Fig. 4 and Table XX; Green 1931b). Vetulani's data (1930), reproduced in Table XXI from Grüneberg (1943), on the differences between "birth-fed weights" of hybrids between two strains of Mus musculus, indicates a dominance of the large strain's genes in their effects on the young, but recessive action in respect of their action on the mothers (Fig. 5).

In the present experiment the genes a, a^t, "A^t", b, c, d, p, s, "U" and their corresponding +'s were each investigated for a pleiotropic or linked effect on maternal performance. Each recorded mouse from the first to the tenth generations was classified at these seven loci on its phenotype and the effects of each segregation was examined within each period. On inspection, only the segregation of +/b was seen to affect performance at all, and this effect was somewhat irregular (Fig. 6). For in the earliest and latest periods brown phenotypes

were associated with slightly higher performances than the blacks, but in the intermediate generations their performances were far worse. This obviously cannot be a simple pleiotropic action of the genes black and brown, and it is most unlikely that it is due to some violent environmental interaction. As the frequency of the other visible genes remained fairly constant, there can be no question of genic interaction. Mr Alan Robertson has suggested an ingenious explanation - that the brown gene itself is a strongly "minus" performance gene, but was introduced into the foundation stock in coupling with an even stronger "plus" gene. In the course of successive generations, this association of brown with the plus gene was broken down so that the brown gene was able to show its true effect on maternal performance. It should be noted, however, that there is a little irregularity in the manifestations of the brown gene in regard to performance in the very last generations, so that the author prefers to regard the anomalous behaviour of the brown gene as a fortuitous occurrence of no significance (Table XXII).

(c) DISCUSSION.

Results like those of the present experiment, in which an apparent limit to selective advance was soon reached, are too commonplace to be lightly dismissed. The significant feature is not so much that this particular experiment has failed, but that current genetic theory has failed. In the past, workers in quantitative genetics have usually considered the genotype of

selected populations to be adequately expressed in terms of the additive actions of genes, and usually regarded contributions of the non-additively acting genes to be insignificant. But it is becoming increasingly clear that the expected progress may be vitiated by such action, and in too many selection experiments, both in the laboratory and in the field, limits to improvement have been encountered long before genetic sources of variation were thought to be exhausted. For example, Chapman, Sierk, Dickerson, Terrill and Lush (1951) report an inability to improve pigs in respect of characters which have long been selected in one direction, and the maximum rates of progress predicted by Rendel and Robertson (1950) for yields in British breeds of dairy cattle contrast unfavourably with the actual progress made in twenty years in Indian Zebu cattle (Hammond, 1947). It is the purpose of this discussion to eliminate some of the possible causes for the present failure indefinitely to improve the stock, and to suggest special breeding programmes, which might be generally applicable, to overcome the actual causes of the failure.

But the nine generations of selection for maternal performance in mice have given enigmatic results. For while there has always been an observable offspring-parent regression, there was an abrupt differentiation between a period of unparalleled success and a period of complete failure. This extreme heterogeneity of the response must reflect some special cause for the improvement in the earlier generations and two questions are therefore presented for discussion:- first: To what cause can

the initial improvement which took place steadily over the first four periods of selection be attributed? and secondly: Why was there subsequently a complete failure to improve the stock?

(i) The initial success.

Naturally, a genetic source for the initial improvement is first tested. It can be seen from Fig. 3 that during the period of success the response was almost the maximum that could be expected from the extent of the selection made. In fact, the heritability during this period seems to have equalled unity. This is most unusual for a quantitative character, but was predicted by Falconer (1947) for the identical character, though in an entirely unrelated mouse population. Besides the ratio of the response to the extent of selection, heritability can be measured by the regression of daughters on dams, or by the repeatability of family performances. When $h^2 = 1$, the value of both of these regressions is expected to be 0.5 (Table XIV). And the average of these regressions, estimated for the period of success from G_{2Test} to $G_{3Repeat}$ inclusive, did in fact equal 0.5. The average of the appropriate regression coefficients weighted according to the corresponding selection differentials, I, is not very different. The details of the component regressions are given in Table XXIII.

While the average heritability of performance can explain the improvement, the significance of the deviations of the component regressions from the expected value of 0.5 makes it most improbable that it was truly a response to selection; and the gradual hybridisation which occurred between the two foundation stocks during this period, was investigated as a possible alternative or contributary factor to the improvement in the initial populations. The expected

heterosis could be effective either by raising the performance of the dams, or in raising the growth rates of the young (Falconer, 1947). To test these hypotheses, the mean performance of each period was plotted against the proportion of hybrid females (Table XXIV), and the females suckling hybrid litters were compared within each period with those which were not (Table XXV), but neither classification revealed any beneficial effect of hybridisation. In fact, probably neither of the original stocks was sufficiently inbred to show a sudden release of inbreeding depression, which is almost certainly a major corollary to heterosis. On another occasion, mice from the fourth generation were hybridised with a number of other stocks. The result was a depression of the performance of the hybrids relative to the purebred stock of the same generation, but their fertility was greatly improved.

(ii) The subsequent failure.

The doubtful success of the initial selections renders all the more fascinating the study of the succeeding phase during which not the slightest response was achieved.

1. The possible loss of genetic variance.

The simplest hypothesis to account for the subsequent failure of the selection is that there was no more genetic variance. This does not mean that no segregating gene affected maternal performance, for an insignificant genetic variance would obtain if a very large number of loci affected the character, more or less equally, especially if the allelic frequencies were

widely different. On this hypothesis the influence of the dam on the maternal performances of her daughters must be attributed to "C" factors - i.e. environments peculiar to litters or families. While an insignificant "between sire" component of the variances of daughters would have confirmed the absence of genetic variation, the available data for this analysis was too small to render the calculation reliable.

Nevertheless, a positive daughter-dam regression was observed and even if the concept of the absence of genetic variance is adhered to the failure to improve must still be explained. In this case the regressions (especially the highly significant regressions in the downwardly selected lines) must be interpreted as reflecting the reality of the mouse's heritage, and of the reality of the transmittability of maternal effects. Now, whereas in the case in which the heritable portion of maternal performance is entirely of genetic origin the upper limit to the correlation of daughters and dams is 0.5; if it is of entirely maternal environmental origin there is no reason to place the limit below unity. In this case, although the selection through the male line is quite ineffective, the "maximum possible improvement" attains a new high level of 114.3% since the beginning of the experiment, and 69.5% since G_{4Test} (Fig. 7). On this basis, not only does the response appear more disappointing, but it is also at greater variance with the improvement expected from the regression estimates.

2. <u>The physiological limit</u>.

There are geneticists who believe in a physiological limit

and have suggested it as the clue to the failure to obtain continued selective improvement in the present experiment. For example, Brody and Nisbet (1938) have shown that the efficiency of milk production in stock rats and the best dairy cows is ostensibly at the same level when measured in terms of the calorific exchange between food intake and milk output. Here, then, may be a true physiological barrier to improvement of efficiency, though not to output, which is the character being selected in the present experiment. Nevertheless, the apparent equality of rats and the best dairy cows may be entirely spurious, in view of the great variation even in the efficiency of production of the same animal in the course of its lactation. Obviously towards the end of its lactation an animal's efficiency must be lower than at its height, and between lactations the efficiency is zero. It is argued that had the rats been measured over a longer or a shorter period the gross efficiencies of the two species would have been widely different, and there would have been no evidence of the physiological limit. The concept of the physiological limit is not well defined, but is invoked to explain the failure to improve a breed under selection when there is no reason to believe that the genetic variance in the character has been exhausted. The belief appears to be that there is an uppermost limit to the character which no member of the species can exceed either by genetic or environmental agencies. It is at once apparent that species differences can transcend these barriers. Thus, while 10 gms. per day may be the maximum output for a mouse (adapted from Enzmann, 1933), the limit for a dairy cow is

certainly not less than 60 kilograms (adapted from Garner, 1944). Nor is there evidence that the barrier within the mouse species was a physiological one. For not only was the average performance in one period as high as 127%, but there has been one animal with a first performance of 160% followed by a second performance of 174%. In short, the idea of a physiological barrier is a dangerous and sterile concept. And even the adherents to the concept cannot use it to explain the failure to maintain the level of performance above 120% in the later generations.

To sum up:- The suggestion that there may have been no genetic variance in the character is unable to elucidate the anomalies of the results to the selection and aberrant genetic situations must be turned to for their explanation. It was concluded, after describing the results of the selection programme, that no definite decision could be taken whether maternal performance was heritable or not in the high selection line, and that both possibilities would have to be considered when further discussing these results. The simpler assumption is that the low and the high line regression coefficients are not significantly different from one another and reflect real heritability intermediate between 0.90 and 0.36. The response to selection in the low line presents no difficulty as it was in accord with the estimated heritability from the daughter-dam regressions; but some explanation must be found to account for the failure to improve the high line after $G_{3Repeat}$.

3. Inbreeding depression.

If the average heritability is accepted at its face value, the failure to improve must be attributed to an accumulative force which exactly counterbalances the intrinsic genetic improvement from generation to generation. Random effects of environment can be excluded as being most unlikely to have such a colossal effect and inbreeding is the only factor which might bring about a comparable situation. Inbreeding has been known to depress the maternal performance of mice. Falconer's estimate of the effect was a depression of 7% for each 10% rise in the inbreeding coefficient. And Parvaneh (unpublished) has shown that the character in an unselected offshoot of the present stock was subject to a similar depression. However, the present author has been unable to discover any effect of inbreeding on the performances of the mice in G_{3Test} in which period alone the measured females were highly variable in their degrees of inbreeding although their young were inbred to an almost uniform extent; and it is quite possible that the coefficient of inbreeding has never attained a sufficiently high level to affect the performances, though neither Falconer nor Parvaneh had observed any curvilinearity in the effect of inbreeding. Nevertheless, if Falconer's correction for the coefficient of inbreeding is made, it is seen (Fig. 8) that had the population been sufficiently large for inbreeding to be totally avoided, there might have been improvement reasonably in agreement with the expectation from the other estimates of heritability. On the other hand, it can never be known to what extent those

animals have been selected which were not subject to inbreeding depression - and therefore to what extent such an adjustment would have over-compensated for the effects of inbreeding. While the correction for inbreeding tempers the anomalies of the results it is by no means the panacea, and the possible curvilinearity of the daughter-dam regressions must still be interpreted and its bearing on techniques of selection considered.

The extent of the curvilinearity of the regressions and of heritability is not known though it seems almost certain that the heritability in the high lines of the $G_{3Repeat}$ approaches zero. If this is so, the results of the analyses of the variance of performance between the high line families show the magnitude of the potentially heritable variance, whereas the response to selection shows the inability to harness it for improvement. It is not impossible for such a situation to arise even if all the genetic variance is additive, although more likely causes are some forms of non-additive gene action. These possibilities will now be considered in turn.

4. Balance between artificial and natural selection.

It has been suggested in connection with other experiments (Mather, 1945, in connection with the inheritance of bristle number in Drosophila, and a selection for large and small wing and body size in Drosophila carried out by Robertson and Reeve, in press) that an antagonism between the effects of natural and artificial selection may account for the failure to improve the breed beyond a certain level. There has certainly been ample scope for natural selection to be an effective factor in the

present experiment since there was a great deal of sterility and infertility particularly in later generations when as many as half the mated females did not survive the initial cullings to have their performances recorded. However, for natural selection to be at all effective in reducing the rate of progress under artificial selection, it must be shown that high performances are correlated with sterility and infertility. The former cannot be tested at all, since performance can only be measured in fertile animals. But in connection with the second condition it has been shown that fertility and performance are not negatively, but slightly positively correlated. On this evidence the natural selection for fertility should actually have favoured the improvement of the stock's average performance. There were too few deaths between the birth of a mouse and the recording of its own performance for natural selection to have had any effective control over performance through differential rates of death before recording. In conclusion, the possible antagonism between natural and artificial selection cannot be held in explanation of the failure to improve the present stock, and other causes must be searched for. The solution may be found in the non-additive action of genes.

5. Non-additive action.

Skew distribution on the natural scale - directional dominance. The apparent difficulty in improving the stock may be removed by altering the scale of measurement. This would only be justified if it can be shown (as in this instance) that the distribution of performance departs from normality. In Fig. 9 an accumulative

histogram is plotted for the departures of each recorded mouse from the mean of its period from G_1 to $G_{6Repeat}$ inclusive. The variances of the mice above and below the mean are quite different- 49.0 and 75.0 respectively - although the differences in terms of standard deviations are by no means so marked, being only 7.0 and 8.7 respectively. Thus some slight difficulty in improving the stock might be expected to be encountered. However, the skewness of the distribution of phenotypes is not evidence of a skewness in the distribution of genetic variation alone and the curvilinearity of heritability cannot certainly be explained in this way.

In fact, if the skewness manifests itself equally in the genetic and environmental sources of variation so that at all levels of performance G/(G + E) = a constant, a relatively greater response (when measured as a fraction of the selection differential or MPI) is expected in the high lines as compared with the low lines. Thus Table XXVI shows theoretical corresponding values of phenotypes on the natural, skew, scale, and on the transformed, normal, scale (cf. Fig. 10).

If G/G+E = 0.5, then on the <u>normal scale</u> parents selected with a 200% performance will have progeny of 150%

and h^2 = response over selection

= 50/1000

= 0.5 .

On the other hand, in natural units the same selection appears as:

Mean = 80

Parents = 140

Offspring = 120

and h^2 = response/selection

= 40/60

= 0.67 .

It is clear that mere skewness in the scale of measurement in the direction observed cannot possibly account for the observed curvilinearity of heritability by regression, or for the failure to obtain selective improvement.

It is unlikely that the foreshortening of the distribution curve is due to a limitation to the intrinsic growth rates of the young when supplied with unlimited quantities of milk, for there were exceptional mice in a litter of two which averaged 11.7 gm. each at 12 days. Had each mouse in a litter of eight grown to the same extent the dam's performance would have been 205%. On the other hand, no mouse has been so small at 12 days that the dam's performance would be less than 50%. It is seen that the observed skewness in the range of the growth capacity of the young is the reverse of the skewness observed in the actual performances. The latter, therefore, must be attributed to real limitations in the contributions of the dam herself.

Normal dominance. Normal dominance is an aspect of non-additive gene action which must be considered in view of the curvilinearity of heritability it can produce. Dominance is not an unknown phenomenon in the genes controlling quantitative characters and is usually invoked to account for heterosis. Haldane has shown

that there is increasing difficulty in selecting for a dominant gene when its frequency exceeds 50%; and conversely, for selecting a recessive when its frequency is less than 50%. On the other hand, Wright (1949-50) claims that dominance deviations have to be very large before they detract significantly from the additive genetic variance. For example, even in the case of full dominance, when the homozygote is indistinguishable from the heterozygote, the additive genetic component still accounts for two-thirds of the total genetic variance. Only in the case of directional dominance - when the desirable genes are either all recessives or all dominants - is dominance likely to raise a real barrier to improvement. Thus Wright points out that in the case when all the genetic variance is due to a large number of recessive genes the offspring-parent regressions will approach zero, although the intrafamily correlations may be a little larger. This situation is perhaps not very different from that encountered in the present experiment, and must be borne in mind when the solution to the difficulties is discussed later.

Heterosis: dominance, overdominance and epistasis. As indicated in the previous paragraph, dominance deviations become a serious barrier to improvement only when they render the heterozygote at any locus superior to either homozygote. This condition is known as overdominance, and is manifest as heterosis when two unrelated lines are crossed. Whereas dominance and epistasis are concepts familiar to the Mendelian geneticist, so that no examples need be given, overdominance is a concept

which has arisen out of biometrical studies. Overdominance, however, is not unknown in regard to Mendelian loci, and the actions of the genes at the Brachyury locus in mice may be taken as a typical example. Both T/T and t/t genotypes are embryonic lethals causing death at implantation, while the heterozygote, T/t, though tailless, lives for an almost normal adult life-time (Gluecksohn-Schoenheimer, 1938). Thus, concerning its effect on longevity, the brachyury locus exhibits overdominance. The existence of overdominance at many loci affecting a quantitative character is almost impossible to prove, for overdominance is not the sole genetic system responsible for heterosis, and both normal dominance and epistatic interaction between loci may also contribute to heterosis.

Bruce (1910) has shown how normal dominance deviations may produce heterosis in the hybrids between two segregating stocks to the extent that the "F_1" is superior to the mid-parental value though below the better parental stock. But heterosis of the type that the hybrid is superior to both parents can result entirely from normal dominance. For example, if two pure parental strains differ at n loci at each of which the dominant gene, G, contributes U units to the phenotype and the recessive gene, g, zero units, and supposing, moreover, that each strain has at least one or k dominant genes so that $k > 1$ (the other strain having n-k dominants), then the phenotypes of the two parental strains and the F_1 respectively would be (n-k)U, kU, and nU.

Epistasis may result in heterosis if the two parental

strains carry between them the complementary genes of the epistatic system. In the simplest case of two interacting loci a and b , one strain is homozygous AAbb, the other being homozygous aaBB, and the F_1 forms the epistatic combination AaBb. If the parental genotypes contribute nothing to the phenotype, then the superiority of the F_1 is entirely due to epistatic interaction. In fact Richey (1950) has been careful to point out that the economically important heterosis in maize breeding in America is largely of this type. The average cross between the inbred maize lines is only superior to the respective parental lines (heterosis from dominance), and is not superior to the original outbred stock from which the lines were derived. It is clear that the original outbred stock segregated for a number of undesirable rare recessives. The different recessives which have been fixed in each of the separate lines show neither in the random-bred stock nor in the average cross between the lines. This degree of heterosis is unimportant economically since the hybrids, which are costly to produce, are not superior to the outbred stock. Nevertheless, occasional crosses show a productivity far above the general level of the crosses, just as occasbnal individuals show a productivity far above the level of the average individual. This phenomenon is known as "nicking" and manifests the genetic phenomenon of epistatic interaction. Whereas the genetic basis of the excellence of the exceptional crosses can be proved, the performance of the exceptional individuals are so unrepeatable even among the members of their own families that it is impossible to differentiate between the

chance effects of environment and the chance recombination of the segregating genes. Nevertheless, by analogy with the crosses, it seems at least probable that many of the better members of the random-bred populations owe their superiority to their epistatic genotypes.

Epistasis has not yet been demonstrated to determine the quantitative characters of farm animals and poultry, although it need not necessarily be an uncommon phenomenon. Its existence is most easily demonstrated by setting up a number of crosses between inbred lines, and if the performances of the crosses bear little relation to the average breeding value of the parents, then the importance of epistasis is clearly demonstrated. But no inbred lines are available in farm animals, and if the crosses are made between randomly bred animals, the chances are too remote that an epistatic genome will be formed so regularly by a particular cross as to render this family significantly aberrant from the average genotype of its parents. In addition, the rare epistatic genotypes due to the chance segregation of genes are confused with rare phenotypes due to the chance effects of environment. Moreover, in such an experiment, epistatic genotypes occurring occasionally within families add to the error variation against which the epistatic deviations within families are compared. This situation, or rather an insufficient departure from it, probably explains the failure of Seath and Lush (1940), Johnson, Bartlett and Copeland (1940), and Lerner and Demster (1948) to demonstrate "nicking" in cattle and poultry.

Unlike dominance both epistasis and overdominance may present real barriers to selective improvement in outbred populations. In the case of overdominance it is obvious that little progress can be made, since the desired heterozygote cannot be transmitted through the gamete. But overdominance would not make downward improvement any easier than upward improvement for the chance mating of the alternative selected homozygotes would frequently re-establish the undesired heterozygotes. It seems unlikely, then, that overdominance is the explanation of the difficulties in the present experiment.

On the other hand, the epistatic situation can in certain circumstances explain both the difficulties in improvement and the ease in disimprovement. The interaction of the epistatic genes may be quite heritable if the complementary loci are few in number, and especially if the complementary genes have high frequencies. In this case they segregate together sufficiently frequently for their joint action to be indistinguishable from the action of an additive gene with lowered penetrance, and will contribute negligible non-additive genetic variance. But when the interacting genes are more numerous, or the frequency of the effective alleles lesser, the chance of all the complementary genes segregating together is remote, and great difficulty is encountered in transmitting the epistatic action from generation to generation. On the other hand, since the loss of only one of the interacting genes is sufficient to destroy the combined action of the whole epistatic group, disimprovement is remarkably easy. It is for

these reasons that epistasis is regarded as the most plausible explanation of the situation found in the later stages of the selection programme.

Deviations from optimum on an underlying scale. One last form of non-additive gene action must be considered. This is Wright's conception (1935) of the highest expression of a character depending on an intermediate genic frequency on some underlying scale. Under this system gene frequencies above and below the optimum both cause a depression of the observed character. It results in an overwhelming contribution to the total genetic variance from interaction which lowers heritability in both upward and downward directions. The system cannot therefore account for the anomalous results of the present experiment.

(iii) Conclusions:- Possible means to further improvement.

The difficulties which have been encountered in the present experiment in the improvement of maternal performance are unfortunately of common occurrence in the field, and the suggestions made here to enable the further improvement of maternal performance in mice in particular may be generally applicable for attaining improvement of the stubborn characters of the farm animals themselves.

Faced with the possibility of being unable to obtain any marked improvements by ordinary genetic means, the possibility of improvement by controlling the environment becomes of greater importance. This facet of animal improvement is naturally of

greater relevance to the farmer who has scope for little genetic improvement within his limited herds. A priori, it should be possible to make great strides forward by managemental techniques as anything from 70 to 100% of the considerable variance in maternal performance is attributable to environmental causes. And in this connection it has already been suggested (Section 2) that artificially increasing the growth rate between three and six weeks of age might well result in increased performance, or at least in increased efficiency of production. However, this optimism must be tempered in view of Wright's failure (Wright and Chase, 1936) to relegate as much as 89% of the environmental variance in the spotting of guinea pigs to anything more definite than "accidents of development".

Even if improvement can be achieved by environmental measures, the possibility of genetic improvement is still attractive on account of the indefinite response which should be available to suitable techniques. For it cannot be stated with certainty that natural selection has yet found a limit in its diverse fields of improvement. Moreover, unlike improvements due to improved management, no further effort is required to maintain the genetic improvements once they are fixed. The belief in the effectiveness of the selection with outbreeding for genes with small effects and additive in their action (for these alone can be fixed in outbred populations) is a legacy of the neo-Darwinian school's interpretation of natural evolution. But Simpson (1944) emphasises the importance of the violent changes in genotype which might be expected to be associated

with rapid inbreeding in the development of new species in the course of natural evolution. This is the sort of progress which is wanted by animal breeders, and the answer to the barriers to improvement encountered in the laboratory and in the field may be the adoption of courageous breeding programmes paralleling the population structure which Simpson believes is responsible for "quantum evolution".

From the considerations of possible non-additive actions of genes only two plausible sources have been discovered of the failure to improve performance in the later generations. These are inbreeding depression, due to the action of a large number of different and individually rare recessive genes with deleterious effects on performance; and epistasis. The evidence for the curvilinearity of the response to upward and downward selection, which is supposed to be the result of such genic action, is not yet perfectly established, but an experiment is under way which is designed to test for such action more efficiently. This experiment requires the production of inbred lines (three generations of brother-sister mating should be enough for this purpose) which are intermated in all possible ways (Schmidt, 1922 on polyallel crosses). If a number of progeny are measured from each mating it is then possible to obtain the relative values of additive and non-additive genetic variance (Lerner, 1950). If the latter is found to be considerable, then special techniques will be adopted.

Supposing that the non-additive contribution of the genes is proven, two roads are then opened to progress. Either the

problem can be circumvented by searching for additive variance over a much wider field; or it can be surmounted by so adapting the selection programme as to utilise the effects of dominance and epistasis.

The evidence from the short-lived correlated responses in the early phases of selection indicate that segregating major genes of additive action can be rapidly spread through the entire population, by selection. It suggests a new technique - of selecting the best animals from the most variable families in all available mouse stocks. In this way a number of different major additive genes may be picked out which, when combined in a foundation stock or introduced into the selected line, may be selected together and so carry the level of performance far above the highest level obtained in the present programme. There is but one snag - it is far from certain that major genes with additive action in the families from which they were derived would continue to act additively in other gene milieux; for at least some of these genes may be the unfixed last member of an epistatically acting group.

It may therefore be better to surmount the difficulties presented by considerable non-additive action of genes and to adapt the breeding programme to take advantage of such action. Inbreeding is recommended as the panacea for all troubles arising from non-additive gene action. By inbreeding, the deleterious recessives are expressed in sufficient proportion to their actual frequency for them to be effectively removed from the population by selection. By inbreeding, epistatic genotypes

may become fixed and therefore heritable. Moreover, on outcrossing the residual inbreeding depression is removed, and new epistatic combinations may be formed. These may be reformed in subsequent generations by another spell of inbreeding. This programme has, in fact, already met with great success in its application to corn breeding in America where the outcrossed generation (from particular parental strains selected for their special combining ability with each other) are far better performers than any of the inbred parental strains. It is seen that a selection programme involving periods of inbreeding separated by a single outbred generation (the programme might be called "reticulate breeding") affords great opportunities of utilising not only epistasis, but also dominance, overdominance and genes which must have intermediate frequencies for optimal effects - the latter actions being accounted for in the selection of lines with special combining propensities. Thus barriers to improvement, impenetrable under normal outbreeding systems, might be effectively overcome by reticulate breeding. And there is every reason to believe that the adoption of such a system might result in new and rapid rates of progress in existing highly selected breeds of livestock which might otherwise be thought to be approaching a genetic limit to further improvement.

SUMMARY.

THE CHARACTER.

Methods of measuring milk production in mice are described and Falconer's measure is adopted. This is the weight of a female's litter at 12 days corrected for litter size and maternal age. It is shown to be a highly repeatable characteristic of the female herself. The young contribute very little variance (particularly in the larger litters) and this is environmental in origin. While genetic differences between the growth rates of the young do not appear to be important at 12 days, there are profound prenatal influences affecting the growth rates of the young. Thus the maternal prenatal component of the measurement of "lactation" is 1½ times as important as the postnatal component. For this reason the measurement has been called "maternal performance" in the present account. Pre- and post-natal components of the character are uncorrelated.

The level of a female's performance is strongly influenced by her rate of growth between 3 and 6 weeks, which appears to be a sensitive period in her development. It is suggested that maximal feeding at this time with minimal feeding at other times may result in more economic production of milk. Age only slightly influences performance.

Performances after the first are profoundly affected by the interval between births. Dry periods of 3 or 4 days are essential if the subsequent performances are to be up to the level of the first. The extremely beneficial effects of

intervals longer than 24 days is dependent on the time during which hormones of pregnancy and pseudopregnancy are secreted.

Under normal conditions maternal performances are highly repeatable on successive occasions, but under strenuous conditions of maximal lactation period subsequent performances may be negatively correlated with the first. In fact, under arduous conditions the lowest yielders are the best able to maintain regular performances of moderate level. The initially higher performers become sterile (at least temporarily), infertile, infanticidal, or may fail to suckle their young.

THE SELECTION.

In response to alternating family and mass selection for maternal performance the level at first rose steeply simulating a heritability which approached 100%. Subsequently, after an intermediate drop, no further improvement was made although there could still be definite responses to downward selection.

The question of a physiological limit is discussed.

Genetic systems which might be responsible for the stabilisation of performance are considered and inbreeding depression and epistasis are considered to be the most plausible cause for the subsequent failure. A system of alternating periods of inbreeding and outbreeding (reticulate breeding) is suggested to overcome the difficulties.

ACKNOWLEDGEMENTS.

The author is deeply indebted to Dr. D.S. Falconer for much discussion and tuition in the planning and running of this experiment, and for his efforts to improve the presentation of this account. To Mr Alan Robertson the author is grateful for removing some difficulties in statistical analysis. The author has been in receipt of a Training Grant from the Agricultural Research Council, and wishes to thank the Council for the generous facilities they offer for research at Professor C. H. Waddington's Genetics Laboratory of the Animal Breeding & Genetics Research Organisation.

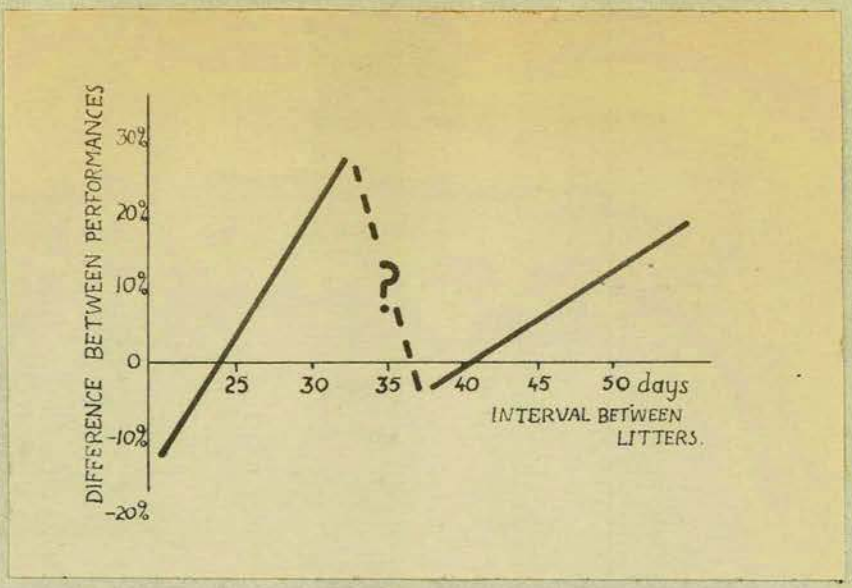

Fig. 1. The interval effect: the difference between second and first performances is plotted against the interval between the birth of first and second litters.

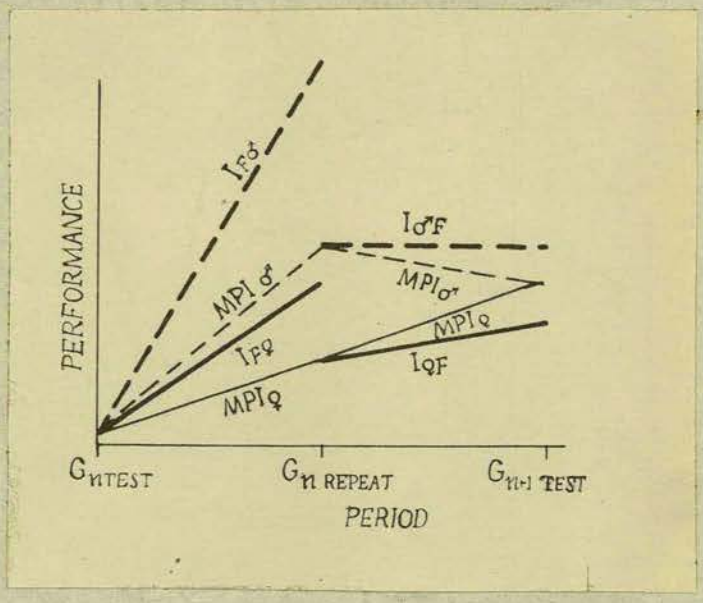

Fig. 2. Diagram of the components of selection (heavy lines) and the resulting Maximum Possible Improvement in recorded performances (MPI♀ - thin continuous line). See text.

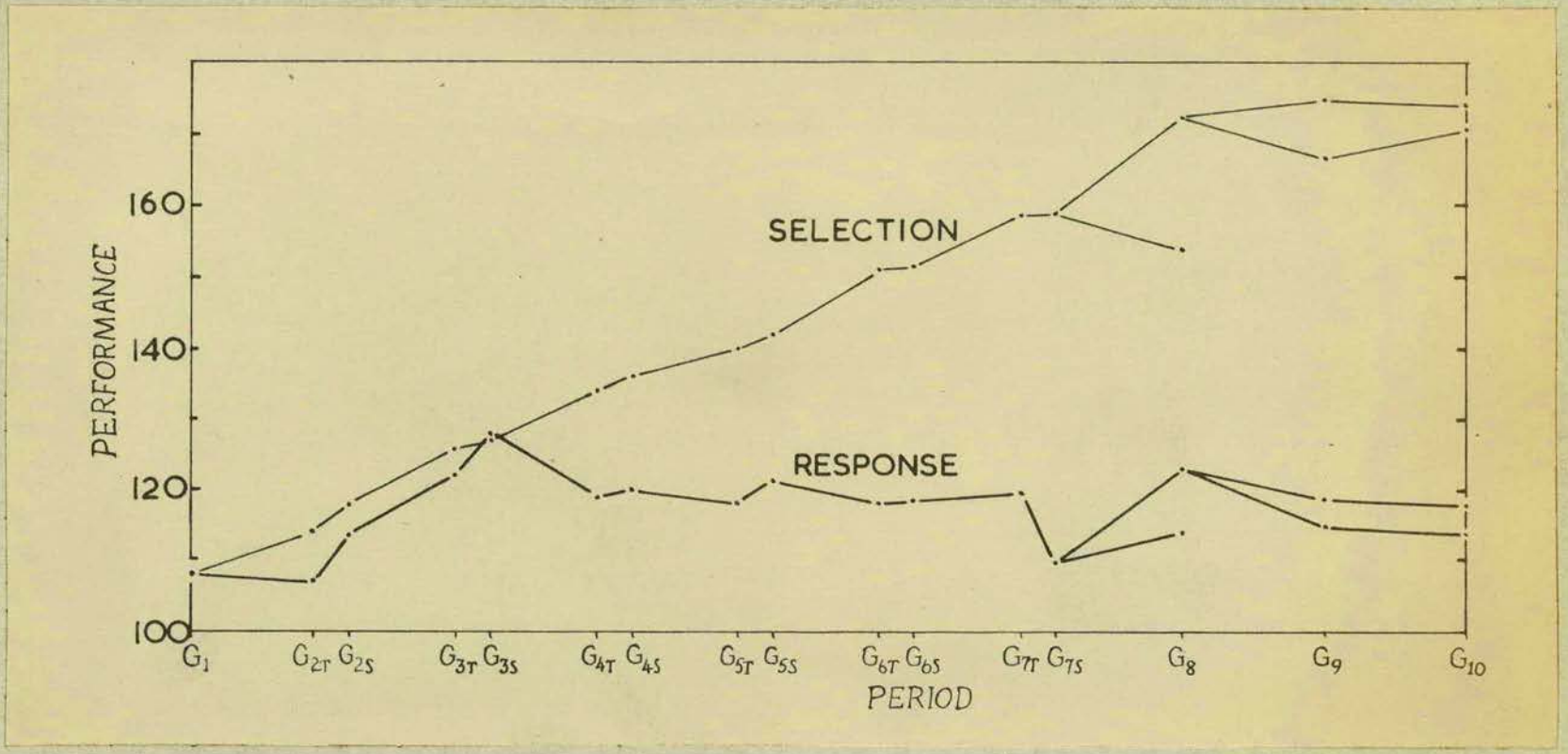

Fig. 3. The observed response to selection. The thin, upper line indicates the Maximum Possible Improvement had all the differences between selected and unselected populations been genetic and accumulative. The mean performances of the inbred lines which were used as "control" fluctuated violently from period to period and are not plotted in the figure.

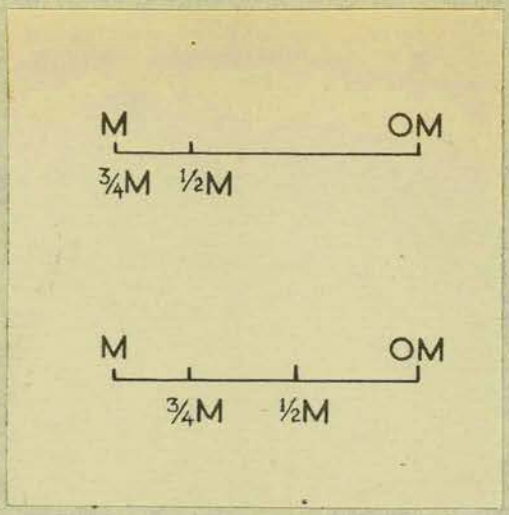

Fig. 4. Above: partial dominance of the genes for large birth weight differentiating Mus musculus (M), Mus bactrianus (OM) and the hybrids (½M) and backcross progeny (¾M). Note the absence of dominance in the genes affecting adult size - below. Cf. Tables XIX and XX.

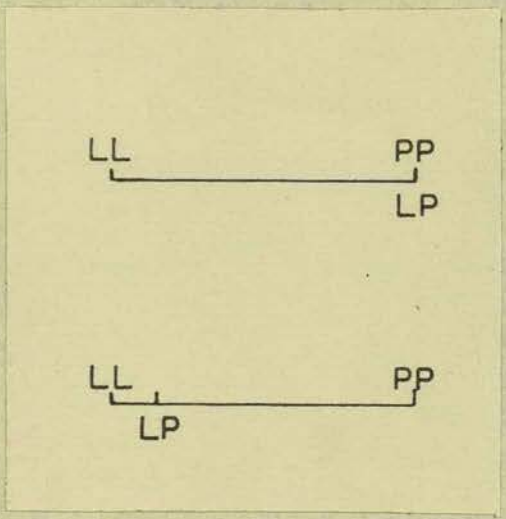

Fig. 5. Dominance of genes differentiating a lilac strain (LL) and a piebald strain (PP) of Mus musculus in regard to birth weight. The upper diagram refers to the maternal component and shows the large (L) genome to be recessive. The lower diagram refers to the young's component and shows the L genome to be almost completely dominant.

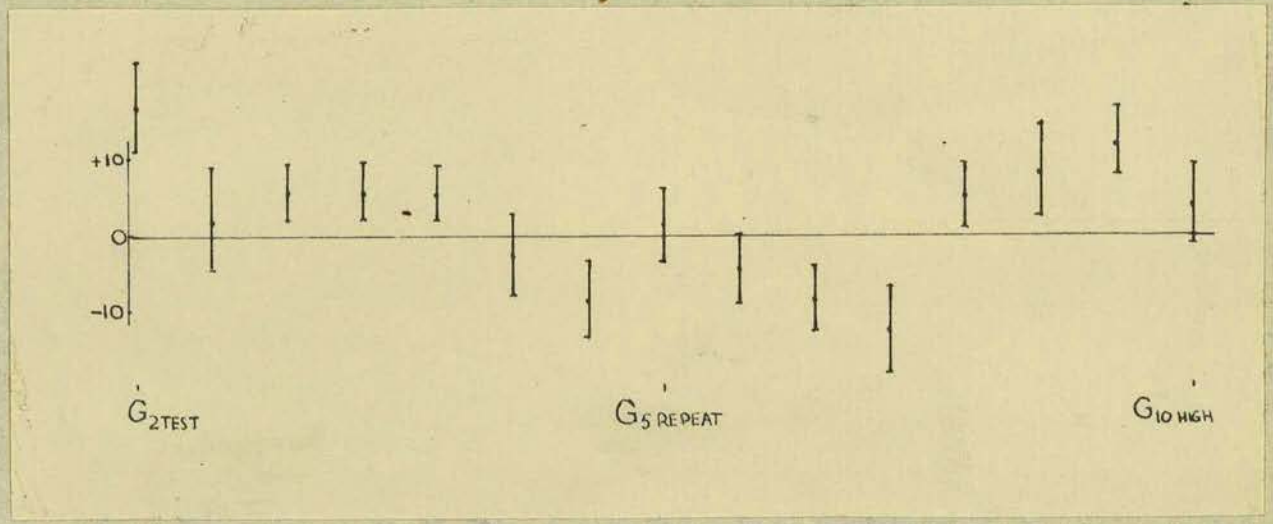

Fig. 6. The difference between the average brown, and the average black, mouse's performance (± one standard error of the difference) for each period.

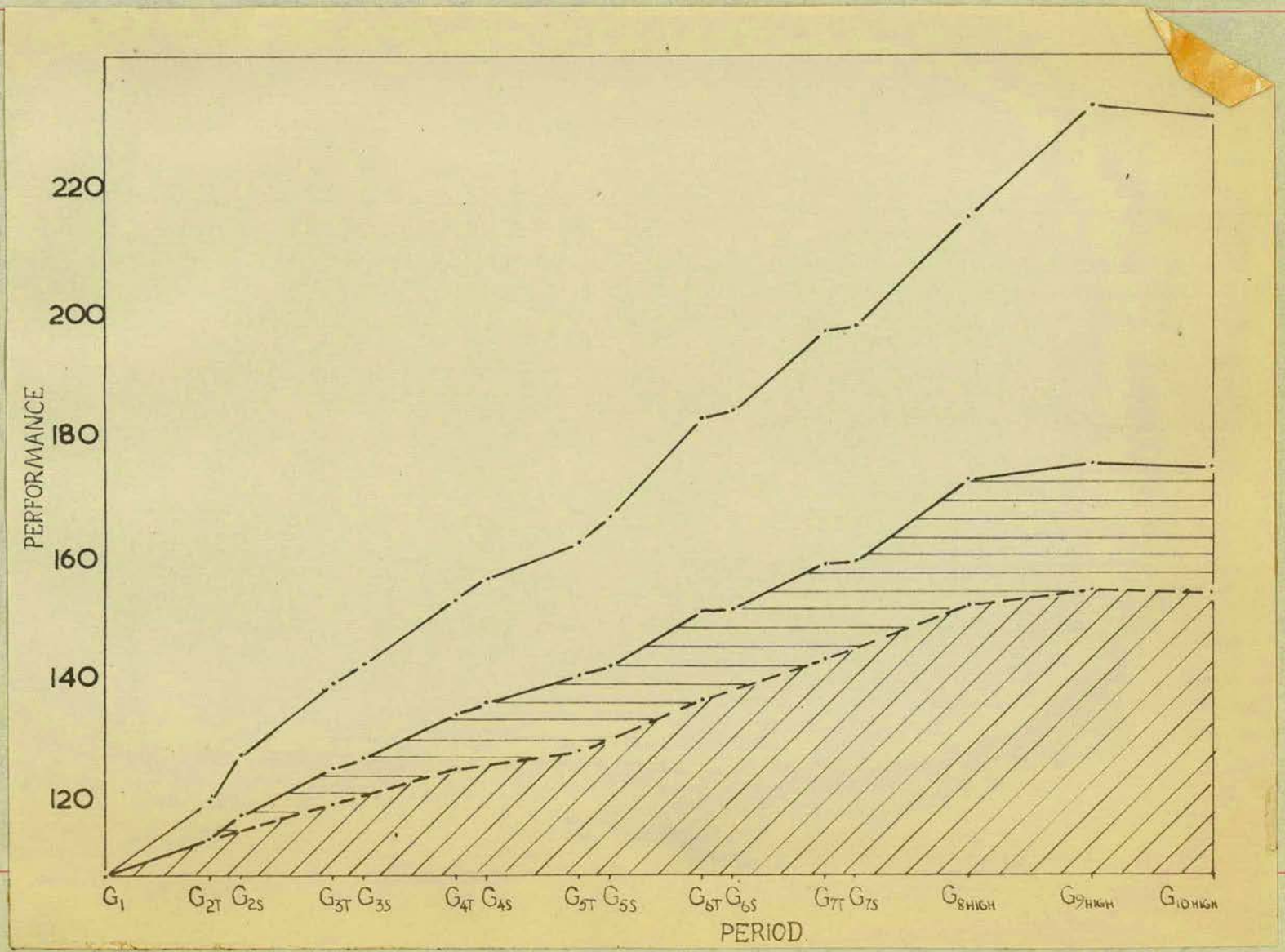

Fig.7. The accumulative Maximum Possible Improvement when response is conditioned only by maternal environment - upper line; and when performance is entirely genetically determined - shaded area. The diagonally hatched area indicates the portion of the latter due to mass selection of females; the horizontally hatched area, the portion due to family selection.

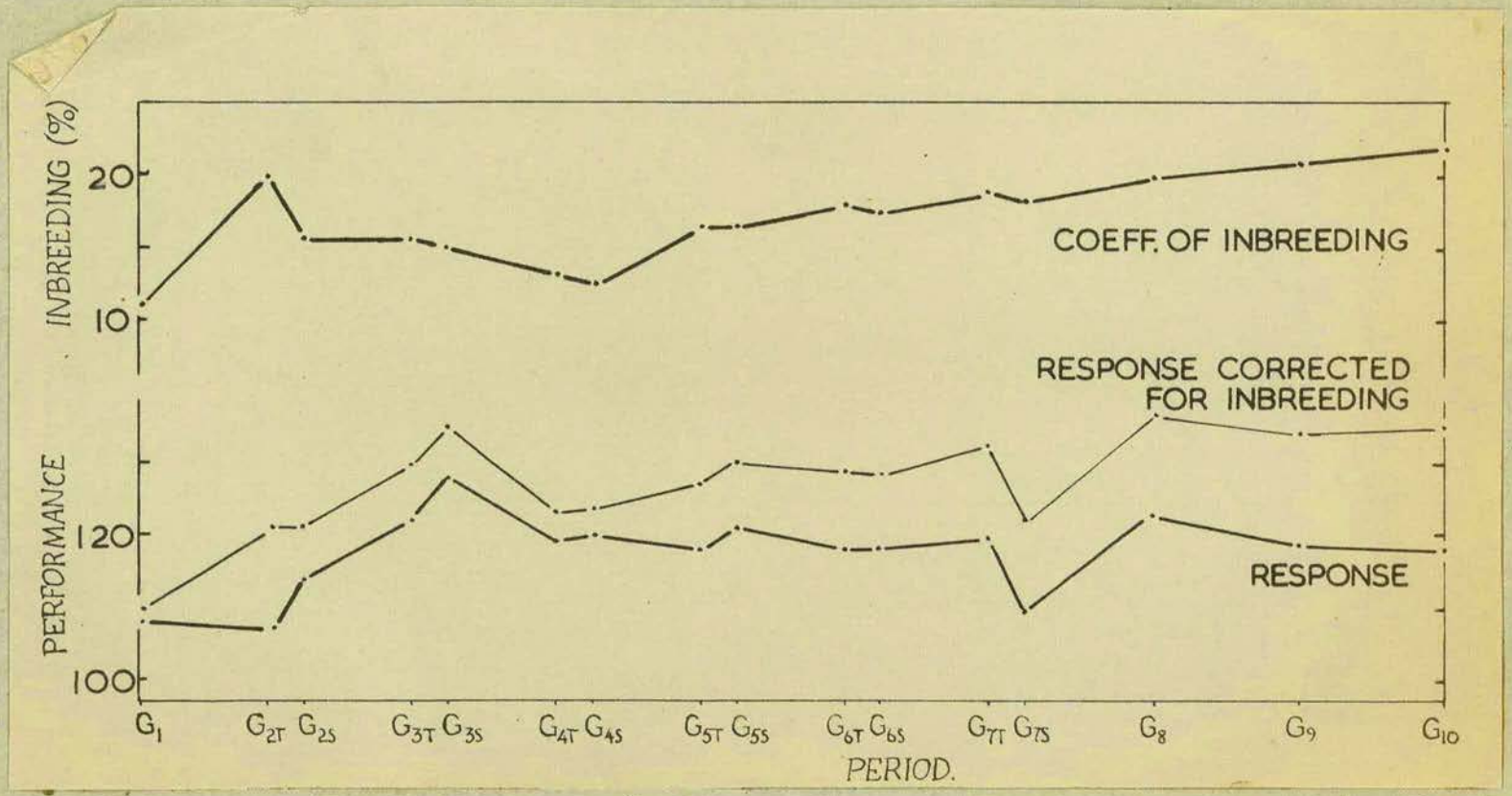

Fig. 8. The observed response to selection corrected for inbreeding depression, middle line, compared with the observed response, lower line. The average coefficient of inbreeding was reduced in the earlier periods by selecting the FN stock which was less inbred than the N stock, and by hybridisation between the two.

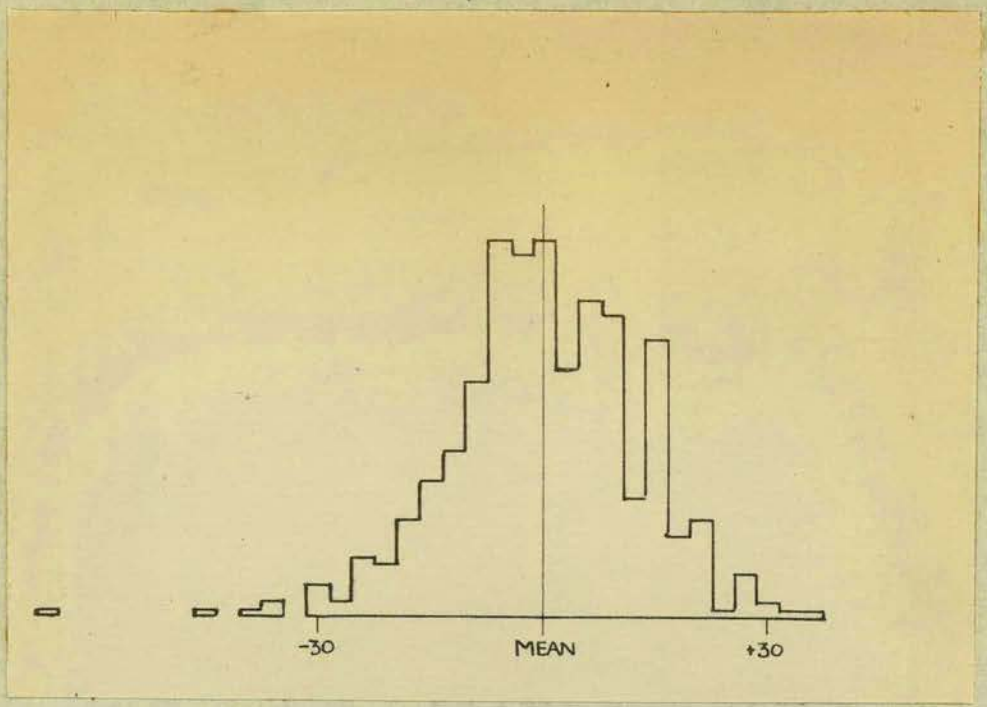

Fig. 9. The performances of 462 mice plotted as deviations from their period mean.

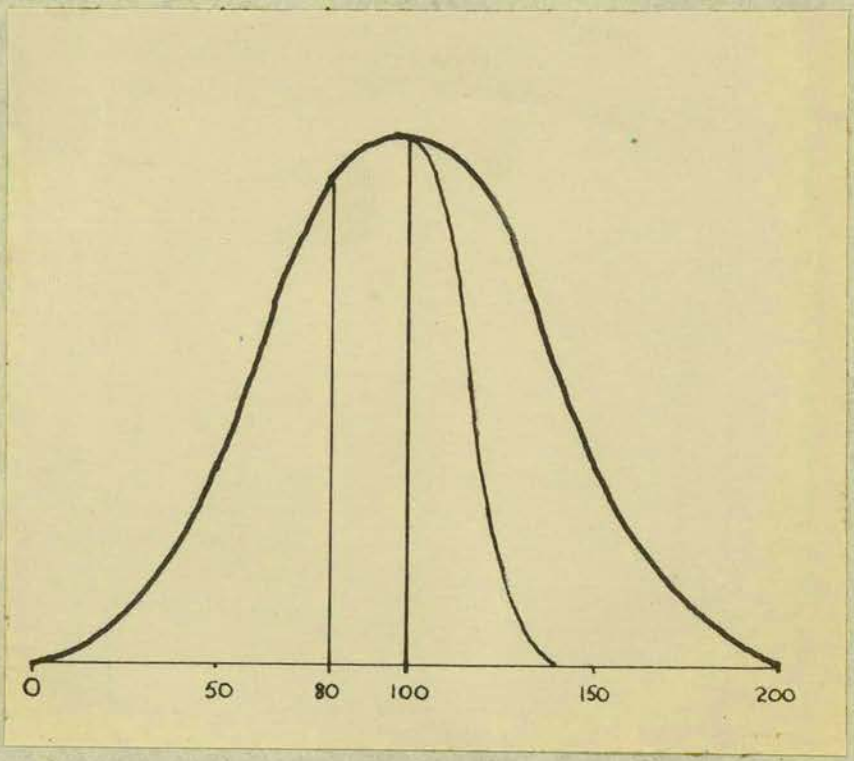

Fig. 10. Hypothetical distribution curves of a normal and a skew population. See text.

TABLES.

Table I. The correlation between maternal performance and the birth weight of the suckled litter.

Type of litter	Own	Own	2 own; 6 others from 3 other ♀♀.	Own
Correlation	0.11	0.18	0.08	0.50
Degrees of freedom	56	64	18	18
Significance: P =	>0.1	>0.1	>0.1	0.05 - 0.01

Table II. Hypothetical analysis of variance of performance assuming no maternal contribution. E = environmental component of variance; G = genetic component of variance, in the growth rate of the suckling mice. 8 mice per litter; 4¼ litters per dam.

Source of Variation	Falconer's equivalent	Variance	Mean Square	Variance Ratio E = G	Variance Ratio E = 7G
Between young within litters	-	$E + \frac{1}{2}G$			
Between litters of same mother	Within mice	$\frac{(E + \frac{1}{2}G)}{8}$	$E + \frac{1}{2}G$		
Between means of families	Between mice	$\frac{(E + \frac{1}{2}G)}{8 \times 4.25} + \frac{1}{2}G$	$E+\frac{1}{2}G+17G$	12.3	3.27

When $E = 7G$, $h^2 = G/E+G = 1/8 = 0.12$.

Table III. Analysis of the variance of the performances of 33 mice each with three or more lactations (Falconer's data). To be compared with Table II.

Source of Variation	Sum of Squares	D.F.	Mean Square
Between mice	15753	32	492
Within mice	16263	108	151

$F = 3.27$

Table IV. The importance of intrinsic differences in the growth rates of the sucklings (I) compared with the maternal contribution (L) to the measurement of maternal performance. An analysis of the variance of the twelve-day weights of 160 mice in 20 litters of eight each within and between litters.

Source of Variation	D.F.	Sum of Squares	Mean Square	Contents of Mean Square
Total	159	104.44	0.657	
Between litters	19	94.68	4.983	I + 8L
Within litters	140	9.76	0.070	I

$F_{litters} = 4.983/0.70 = 70$; $P < 0.001$

Ratio of variation due to L and I in measurement of performance

$= (4.983 - 0.070)/0.070$

$= 70.$

Table V. The effect of the sire on the rate of growth of his suckling young as manifest by his effect on the performance of his mates. The effect is insignificant at the 20% level of probability, so there is no evidence of genetic control of the young's growth rate.

Source of Variation	D.F.	Sum of Squares	Mean Square
Total	65	10229.82	
Between sires	32	5446.32	170.2
Within sires	33	4783.50	144.9

$F_{sires} = 170.2/144.9 = 1.17. \quad P > 0.20$

Table VI. The Multiple Fostering Experiment. The relative importance of prenatal and postnatal maternal components of the competition-corrected twelve-day weights of 180 mice.

	Source of Variation	Sums of Squares	D.F.	M.S.	Component
1	Total within groups	171.14	174	-	-
2	Between prenatal conditions	42.91	18	2.384	0.22
3	Between postnatal conditions	27.49	18	1.527	0.11
4	Interaction	44.01	51	0.863	0.73 . . 6
5	Between sibs within postnatal litters	56.73	87	0.652	

Variance ratios

Component tested	Mean squares compared	F	D.F.	P
INTERACTION	4:5	1.32	51 & 87	0.1
PRENATAL MATERNAL	2:6	3.27	18 & 138	0.001
POSTNATAL MATERNAL	3:6	2.09	18 & 138	0.01

Table VII. The mean twelve-day weights (gm.) of fostered and unfostered mice from the Multiple Fostering Experiment

	Fostered	Unfostered
Av. 12-day weight	7.40	7.49
Number	136	44

Table VIII. Analysis of the within family variation in maternal performance within and between storage cages.

Source	Sum of Squares	DF	Mean Square	Constitution of Mean Sq.	Component
Total within families	14460.87	59	-		
Between storage cages	10110.36	24	421.26	E + 1.49S	S = 199.3
Within storage cages	4350.51	35	124.30	E	E = 124.3

Variance ratio: $F_{24:35df} = 421.26/124.30 = 3.39$; $P = 0.001$

Table IX. Results of the second experiment on interval effect. Figures given in the table are the average differences between second and first performances of the mice in each experimental group.

Number Suckled	Lactation period 12 days	"Continuous"
4	14%	-12%
8	11%	1%

Table X. The effect of the dry period on mice suckling four and eight young respectively. The dry period is even more effective in raising the performance of the lighter yielders than of the more heavily milked mice.

No. suckled	Difference between second performances associated with the greater dry period.
4	25%
8	14%

Table XI. The heterogeneity of the interval effect from generation to generation.

Source of Variation	Sum of Squares	D.F.	Mean Square
Deviations from average regression	26160	149	175.57
Deviations from generation's regression	24198	144	168.04
Deviations between generation's regressions.	1961	5	392.40

Test of significance of variation in regressions of different generations.

$$F = 392.40/168.04$$

$$= 2.34$$

$$P_{5:144} = 0.05$$

Table XII. The mean first performances of the mice which survived to have 2nd, 3rd, 4th and 5th performances recorded. Those which suckled for 12 days only in each lactation are compared with those which suckled continuously.

Period of lactation	All 1sts	Survivors to 2nd	3rd	4th	5th
12 days	126.0	128	130	130	130
"Continuous"	120.1	124	121	114	107

Table XIII. The average levels of successive performances of the mice surviving the experiments to the 2nd, 3rd, 4th and 5th lactations. The mean length of interval was constant at 23-24 days.

Period of lactation	Survivors to 2nd	3rd	4th	5th
12 days	139	144	144	138
"Continuous"	124	121	97	81

Table XIV. Expected values of daughter-dam regressions and family repeatability (the regression of unmeasured sisters on an average of three measured sisters) when heritability is 100% and 25%.

Heritability	Regression: Daughter on dam $b_{dd} = \frac{1}{2}h^2$	Regression: Sister on family average $b_{S\ fam\ av_3} = (\frac{3}{4}h^2)/(1+\frac{1}{2}h^2)$
100%	1/2	1/2
25%	1/8	1/6

Table XV. Comparison of the average fertilities of first and subsequent litters born in harems of five females each within the first 25 days of the males and females being put together.

Litter order	1	2	3	4	5
Average litter size	8.16	6.69	6.48	8.13	7.0
Number of litters	38	26	21	8	2
Average litter size	8.16	6.82			

Significance of difference between fertility of 1st and remaining litter: P = 0.01

Table XVI. Analysis of the variance of the performances of two groups of F_1 mice.

Source of Variation	Sum of Squares	D.F.	Mean Square	Contents of Mean Square	Values of Components
Total	5358.04	39	137.4	-	
Between crosses	604.51	1	604.5	I+2C+20G	G = 23.5 ... 1
Within crosses	4753.53	38	125.1	-	
Between mice	2404.17	18	133.6	I+2C	C = 13.15 ... 2
Between 1st and 2nd performances	418.32	2	209.2	I+10P	P = 10.19 ... 3
Error	1931.04	18	107.3	I	I =107.3 ... 4

Variance ratios.

$F_{mice} = 133.6/107.3$

$= 1.2$

$P > 0.2$

$F_{between\ crosses} = 604.5/133.6$

$= 4.5$

$P = 0.05$

Table XVI. Analysis of the variance of performance in the "F_2" mice. (The value of P becomes negative through sampling error and must be regarded as zero).

Source of Variation	Sum of Squares	D.F.	Mean Square	Contents of Mean Square	Values of Components
Total	6955.42	39	178.32	-	
Between mice	4088.15	19	215.17	I+2M	M = 35.78 ... 5
Between 1st and 2nd performances	138.76	1	138.76	I+20P	P = -0.24
Error	2728.51	19	143.61	I	I = 143.61

Variance ratio.

$$F_{mice} = 215.17/143.61$$

$$= 1.5$$

$$P = 0.20$$

Table XVIII. Analysis of variance of the performances of the mice of the "select" populations from G_2 - G_5 included.

Source of Variation	Sum of Squares	D.F.	Mean Square	Components of Mean Square	Values
Total, within period	19915.59	154	-		
Between families	6226.87	30	207.56	I + 2.54L + 4.62F	F = 21.03
Within families	13688.72	124	110.39		I =110.39
Between litters	3321.38	28	118.62	I + 2.54L	L = 4.18
Within litters	10367.38	96	107.99	I	

Variance ratios.

$F_{litters}$ = 118.62/107.99

= 1.10

P = 0.20

$F_{families}$ = 207.56/110.39

= 1.88

P = 0.01

Table XIX. Dominance of the genes affecting birth weight differentiating Mus musculus (M) and Mus bactrianus (B, OM). Sexes averaged. Adapted from Grüneberg, 1943; Green's data, 1931a. Comparisons are made within ranks or columns.

Constitution of young	Constitution of Mother M	0.5M	OM
M	1.37		
0.75M	1.37	1.32	
0.5 M	1.34		1.35
0 M			1.13

Phase of substitution	Effect of Substitution (a) on mothers.	(b) on young.
M - .75M	-	0.00
M - .5 M	+0.05	
.75M - .5 M	-	0.03
.5 M - 0 M	-	0.22
M - OM	-0.01	-
	1.5M= 0.04	
	M = 0.03	M = 0.25
	No dominance	Note dominance

Table XX. The genes differentiating Mus musculus and bactrianus in regard to adultsize exhibit no dominance. Adapted from Grüneberg, 1943; Green's (1931b) data.

Constitution of young	Constitution of Mother M	0.5M	0M
M	30.4		
0.75M	26.6	26.6	
0.5 M	20.2		20.3
0 M			14.2

Phase of substitution	Effect of Substitution (a) on mothers.	(b) on young.
M - 0.75M		3.8
M - 0.5 M	0.0	
.75M - .5 M		6.4
.5 M - 0 M		6.1
M - 0 M	-0.1	
	1.5M -0.1	
	M -0.1	M 16.3
	No effect	No dominance

Table XXI. The effect of the large versus small genes differentiating the birth weights of two strains of Mus musculus (L = large; P = small). The action of the large genes is recessive in regard to the mothers; and is dominant in regard to the young. Adapted from Grüneberg (1943); Vetulani's data (1930).

Constitution of young	Constitution of Mother LL	LP	PP	Phase of Substitution.	Effect of Substitution (a) on mothers	(b) on young
LL	1.41			LL - LP	0.10	0.02
LP	1.39	1.29 1.28	1.29	LP - PP	0.00	0.13
PP			1.16	LL - PP	0.10	0.15

Table XXII. The analysis of the variance of performance as determined by the segregation of black and brown phenotypes.

Source of Variation	D.F.	Sum of Squares	Mean Square
Total	708	153254.6	-
Between + and b	1	707.5	707.5
Within genotypes	707	152547.1	215.8
Between periods	17	13447.1	791
Within periods	690	139807.5	202.6
Interaction	17	7118.7	418.7
Error	673	131981.3	196.1

Variance ratios.

$F_{interaction}$ = 418.7/196.1 = 2.13 P = 0.01

F_{genes} = 707.5/215.8 = 3.28 P = 0.05

$F_{periods}$ = 791 /202.1 = 3.90 P < 0.001

Table XXIII. Regression coefficients of daughters on dam, and sisters on family average for the period of selection during which progress was made. Selection differentials are given so that the total probable improvement can be calculated.

Regression	Periods	Regression coefficient	Standard error	Probability of equalling 0.5	Selection differential
Daughters on Dam	G_{2Test} on G_1	1.47	0.36	0.01	I_{QF} 12.0
	G_{3Test} on G_2	0.10	0.37	0.30	I_{QF} 11.6
Sisters on Test	$G_{2Repeat}$ on G_{2Test}	1.02	0.08	< 0.001	I_{FQ} 7.6
	$G_{3Repeat}$ on G_{3Test}	-0.35	0.12	< 0.001	I_{FQ} 3.2

Average regression	0.56
Weighted average	0.74
Total probable improvement	25.4%
Actual improvement	19.0%

Table XXIV. The evidence for heterosis in the females of the early generations :- varying proportions of hybridity in each period cannot contribute to the improvement made.

Period	No. of hybrid females	Proportion %	Mean perfce for period	Mean for hybrids	Mean for pure breds
G_1	0	0	108	-	108
G_{2Test}	0	0	106	-	106
$G_{2Repeat}$	0	0	113	-	113
G_{3Test}	12	30	123	119	124
$G_{3Repeat}$	9	25	128	125	129

Table XXV. The evidence for heterosis in the growth rates of the suckling young. The table shows the average performances of mice suckling hybrid litters (of the cross FM x N), "half-hybrid" litters (of the cross FM/N x N or FM x FM/N or reciprocally) and purebred litters (of the crosses N x N or FM x FM).

Period	Purebred litters	Half-hybrid	Fully hybrid
G_1	108	-	-
G_{2Test}	110	-	106
$G_{2Repeat}$	116	-	110
G_{3Test}	122	120	126
$G_{3Repeat}$	127	125	128

Table XXVI. Hypothetical equivalent performances on the natural (skew) scale and the transformed (normal) scale. For evaluation of the effects of skewness in both genetic and environmental components of variance (G/G+E = a constant on the natural scale) on heritability estimated from the rates of response to selection (cf. Fig. 10).

	Performance			
Transformed scale	50	100	150	200
Natural scale	30	80	120	140

Table XXVII. Test of significance of the daughter-dam regression in maternal performance. (b = 0.18).

Source of Variation	Sum of Squares	D.F.	Mean Square
Total, within periods	97080.63		
Within families	56589.72		
Between families	40220.91	155	
Regression on dams	732.34	1	732.34
Residual	39488.57	154	256.42

Test of significance of the regression:-

$$F = 732.34/256.42$$

$$= 2.86$$

$$P = 0.10$$

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