GENETICAL STUDIES ON THE SKELETON OF THE MOUSE

XXI. THE GIRDLES AND THE LONG LIMB BONES

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(With Seventeen Text-figures)

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Introduction

Earlier papers of this series on inbred strains of mice have been mainly concerned with variants of the axial skeleton and of the skull (Grüneberg, 1950*a*, 1952; Searle, 1954*a*, *b*; Truslove, 1952; 1954; Deol, 1955). More recently, variations of carpus and tarsus have also come to light (Grüneberg, 1956; Truslove, 1956). The present paper completes the systematic survey of the skeleton (scapula, clavicula, humerus, radius, ulna, os innominatum, femur and tibio-fibula). Unlike most of the variants studied previously, which were all-or-none characters of the quasi-continuous kind, the differences described in this paper are mainly such that all, or nearly all, the animals of one inbred strain (A, C 57 BL and CBA) differ from those of another. They are thus detectable in small samples, but easily overlooked except in side-by-side comparisons. Like the facial characteristics in man, they are easily recognizable by the trained eye, but they are difficult to measure, and it is easier to illustrate the differences by drawings than to describe them in words.

For each of the inbred strains mentioned, five brother-sister pairs, each from a different pair of parents and each between 60 and 65 days old, were examined; the females were all virgins. In addition to these thirty papain preparations, some alizarin clearance preparations of the same inbred strains were also examined. In the case of the papain preparations, groups of bones were mounted together on a plasticine-covered plate; for instance, all the fifteen right scapulae of the males; in this way it is possible to discover features which are consistent within a group, but different between groups. Camera lucida drawings (original magnification about 10, reduced to a magnification of $3\frac{1}{3}$ for publication) were made of bones from the right side; only a fraction of these are reproduced in this paper. The specimens drawn show features characteristic for the strain as well as individual variations. Only the strain differences will be described in the text. The anatomical terminology is that of Bateman (1954).

Observations

General differences between the strains

The bones of the C 57 BL mice are generally massive and stoutly built; those of the A strain are delicate and elegant in outline; the CBA skeleton is more or less intermediate in this respect. The C 57 BL skeletons are largest and the A skeletons smallest; this is not brought out in most of the drawings, as skeletons of nearly comparable size were selected for illustration. In all three strains the bones of the males tend to be larger than those of the females. This is most marked in C57BL, perhaps because in that strain the sex difference in size develops particularly early (Howard & Sander, 1945).

Scapula (*figs.* 1–4)

The scapula of C57BL is much thicker than that of the other two strains. This applies particularly to the margins and to the base of the spina scapulae with the result that the thin parts of the fossa supraspinata and the fossa infraspinata are much reduced in area (see left column of Fig. 1). In CBA and A the thin area of the fossa supraspinata is usually subdivided by a thicker spine of bone and thereby becomes more or less heart-shaped; in C57BL the thicker spine is continuous with the thickened base of the spina scapulae.

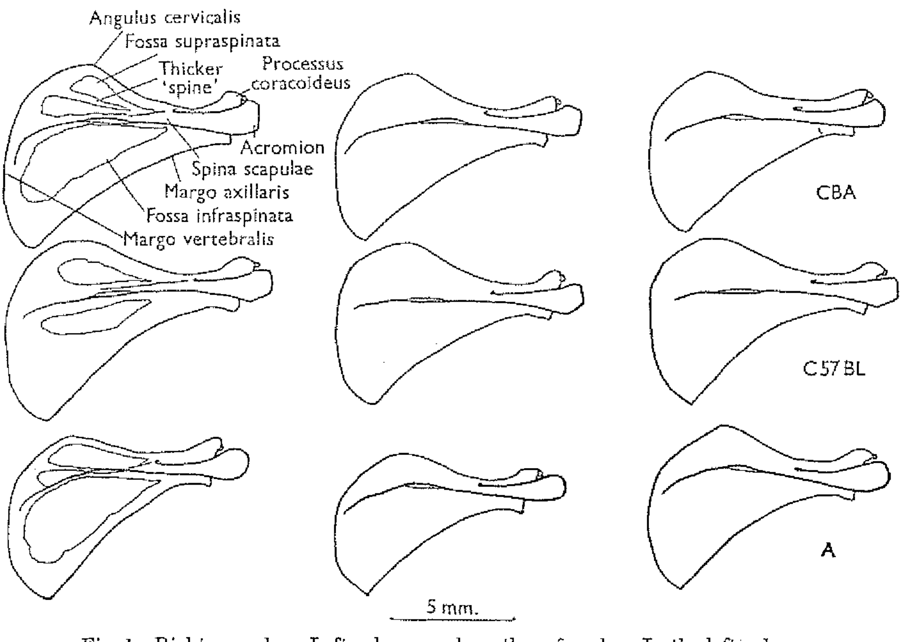

Fig. 1. Right scapulae. Left column males, others females. In the left column, the outlines of thickened and thin areas of the fossae have been indicated.

The outline of the scapula differs in all three strains. The differences are easy to see, but difficult to describe verbally. In C57BL the vertebral margin forms a rounded and continuous curve with the cervical margin. In CBA and particularly in A, the margin is truncated at the angulus cervicalis, with the result that the fossa supraspinata is reduced in size. This is best seen in Fig. 2, where the outlines of an A and a C57BL scapula have been superimposed. The spina scapulae is straighter in C57BL than in the other two strains and meets the margo vertebralis approximately at a right angle instead of curving in a caudal direction as in CBA and A.

The shape of the acromion is consistently different in the three strains; this is better seen in Fig. 3 than in Fig. 1; the latter was drawn with the fossa subscapularis flat on the plasticine and thus shows the acromion slightly on a slant. The acromion is rounded in outline in A, but angular in different ways in CBA and C57BL. In C57BL, the distance from the tip of the acromion to the tuberositas spinae is greater than in the other two strains, the tuberositas being closer to the margo vertebralis scapulae in that strain (Fig. 4). In C57BL the incisura spinoglenoidalis is smaller than in CBA and A and the height of the spina is correspondingly decreased. As a result of this reduction, the portion of the spine below the tuberositas spinae has a more gradual slope. There are also subtle inter-strain differences as regards the processus coracoideus. The scapulae of the three strains thus differ from each other in many characteristic and consistent ways, and it is perhaps no exaggeration to say that they differ as much from each other as three human faces. What applies to the scapula is true to a greater or lesser extent for all the bones studied in this paper.

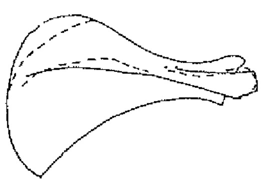

Fig. 2

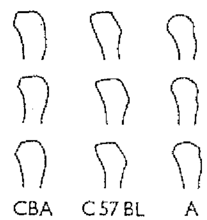

Fig. 3

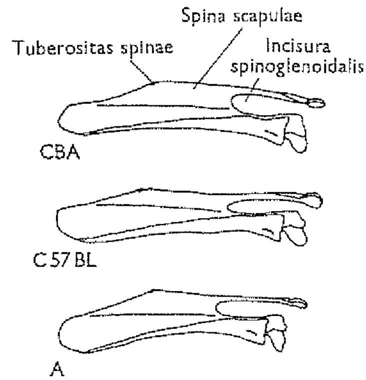

Fig. 4

Fig. 2. Scapulae of C57BL (solid line) and A (broken line) superimposed.

Fig. 3. Acromia of scapulae of three mice each of the CBA, C57BL and A strains. Lateral aspect. Top row males, others females.

Fig. 4. Scapulae of CBA, C57BL and A females in ventro-posterior aspect.

Clavicula (Figs. 5 and 6)

In C57BL the extremitas acromialis is blunter than in CBA and A. In the latter strain, the bone is more delicate and the extremitas narrower than in CBA. The shaft or corpus of the bone is more enlarged towards the extremitas sternalis in C57BL and A than in CBA. The claviculae of CBA are more strongly curved than those of the other two strains, as can be seen when the extremitas acromialis and sternalis in Fig. 5 are connected by a straight line. The characteristic shapes of the fossae claviculae in the three strains are shown in Fig. 6.

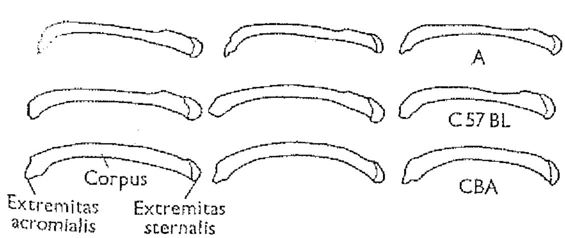

Fig. 5. Claviculae of A, C57BL and CBA in posterior aspect. Males.

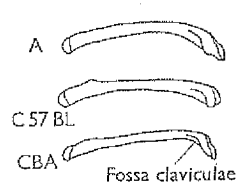

Fig. 6. Claviculae of A, C57BL and CBA in dorsal aspect. Males.

Humerus (Fig. 7)

All ten A mice examined showed a perforation of the thin partition between the fossae olecrani and supratrochlearis; this perforation was present either on one side only or bilaterally and usually of considerable size. In the other two strains similar perforations are uncommon; when present they are small. Although the humerus of C57BL (like other bones) is larger than that of the other strains, the trochlear articular area is smaller in its proximo-distal axis. When the proximal end of the bone is examined with the epiphysis removed, the indentations are deeper in C57BL than in CBA and A. Though there is some individual variation, the shape of the crista humeri is different in the three strains. In C57BL the distal attachment of the crista to the shaft of the bone flares more gradually than in the other two strains. Both the anterior and the distal margins are straighter in CBA than in the other strains where the margins are rather more curved (Fig. 7, right).

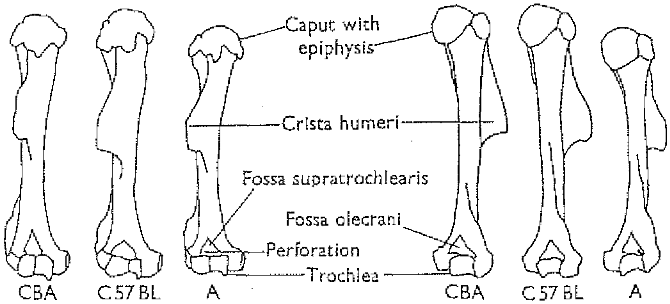

Fig. 7. Humeri of CBA, C57BL and A males. Anterior aspects on the left, posterior on the right.

Ulna and radius (Figs. 8 and 9)

As would be expected, to match the smaller trochlear area of the C57BL humerus, the ulna of these animals has a smaller incisura semilunaris. By contrast, the processus olecrani is more massive and larger and shows less concavity on its dorsal surface, and there are some minor differences in the shape of the processus anconeus. The differences between CBA and A are slight. The same applies to the radii of the three strains. The differences concern mainly the modelling and position of the tuberositas radii; the latter

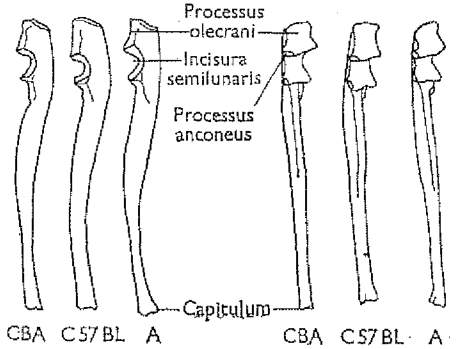

Fig. 8. Ulnae. Medial aspect (left) and dorsal aspect (right) of CBA, C57BL and A males.

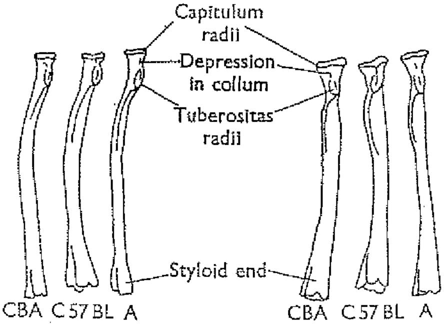

Fig. 9. Radii. Medial aspect (left) and dorsal aspect (right) of CBA, C57BL and A males.

is somewhat closer to the capitulum in C57BL, i.e. the collum is shorter. The styloid end of C57BL has the deepest indentations. As in general, the radius of C57BL is the most massive and that of A the most slender of the three.

Pelvic girdle (*Os innominatum*) (Figs. 10–13)

Two variants of the pelvis have been described previously. They are a vascular variant recognizable in papain preparations as the foramen acetabuli perforans in the CBA strain (Grüneberg, 1952; see also Figs. 10, 11) and the dyssymphysis ischio-pubica of the C57BL

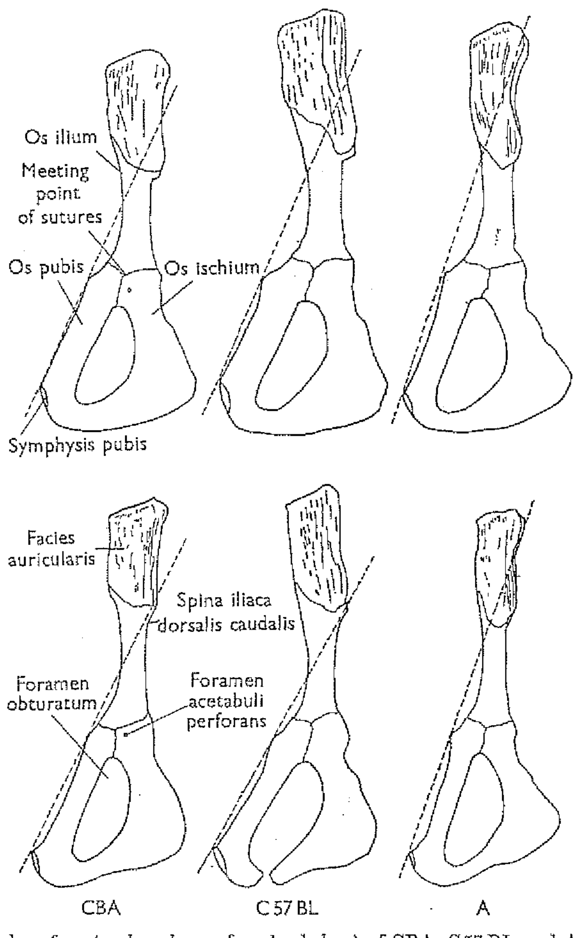

Fig. 10. Pelves. Internal surface (males above, females below) of CBA, C57BL and A mice. In this and the following drawing, note the foramen acetabuli perforans in both CBA mice and the dyssymphysis ischio-pubica in the C57BL female.

strain (Grüneberg, 1952; also figs. 10 and 11, female pelvis). In addition, there are characteristic interstrain differences which present considerable difficulties of verbal description in view of the complicated form of the pelvic girdle in three dimensions.

In A strain mice, the longitudinal axis of the os ilium as seen in internal and external views of the os innominatum (Figs. 10, 11) is very roughly in line with the ramus ossis pubis; if a straight line is drawn along the anterior margin of the ramus as shown by the broken lines in fig. 10, it passes through (or very near) the crista iliaca. In the other two strains, a line similarly drawn cuts the ilium considerably more caudally. This is partly due to the fact that in A mice there is a greater degree of torsion of the ischio-pubis in relation to the os ilium. In Fig. 12 the pelves are shown with the ventral surface uppermost and with the width of the ilium in as perpendicular a position as possible; the torsion of the ischio-pubis of the A mouse is indicated by the larger size of the foramen obturatum and the fact that more of the acetabular cavity is visible.

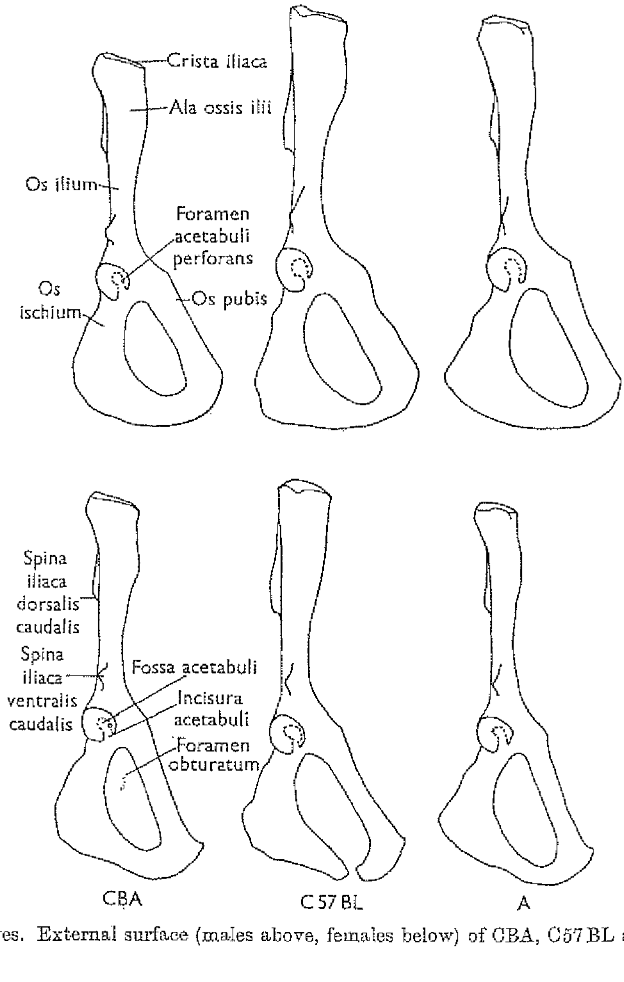

Fig. 11. Pelves. External surface (males above, females below) of CBA, C57BL and A mice.

It may be mentioned here that there are some interstrain differences in the shape of the fossa acetabuli. It seems to be shallower in A mice, and there is a smaller incisura acetabuli in the C57BL strain.

The internal surface of the ischio-pubis in C57BL is more deeply concave, especially near its posterior margin. The pubis and the corpus of the ischium are thicker and the symphysis pubis is larger in the same strain.

The different and characteristic shapes of the cristae iliacae in the three strains are shown in Figs. 12 and 13. That of C57BL is strongly bent and forms a deep trough on its internal surface. In A there is a prominent 'bump' in the middle of the external surface. The ala ossis ilii is thicker in C57 BL animals and the porosity of the articular surface is more evident. Ventral and dorsal spines are larger and heavier in this strain. The spina iliaca ventralis caudalis seems smaller in the A skeletons and is generally less pointed at the tip in CBA.

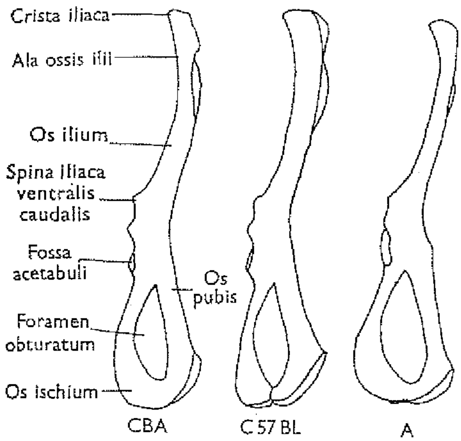

Fig. 12. Pelves of male CBA, C57 BL and A mice. Ventral aspect.

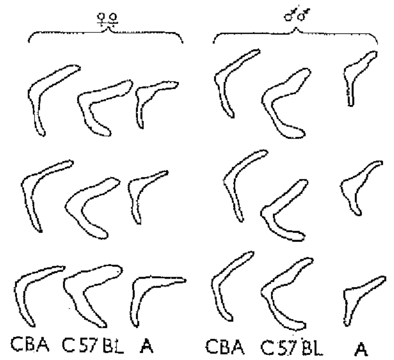

Fig. 13. Outline of the cranial views of the cristae iliacae of female and male pelves of CBA, C57 BL and A mice.

Femur (Figs. 14 and 15)

The mushroom-shaped caput of the femur, minus its epiphysis, has a finely granular surface in all three strains. In A strain mice, a narrow flange of the same granular appearance extends down the collum femoris. No such structure is present in the two other strains. A similar structure was found in all twenty-five pairs of femora of F_1 animals from a cross between A and CBA. In the F_2 generation, the majority of the animals showed an extension of the granular area on to the collum but the shape was variable.

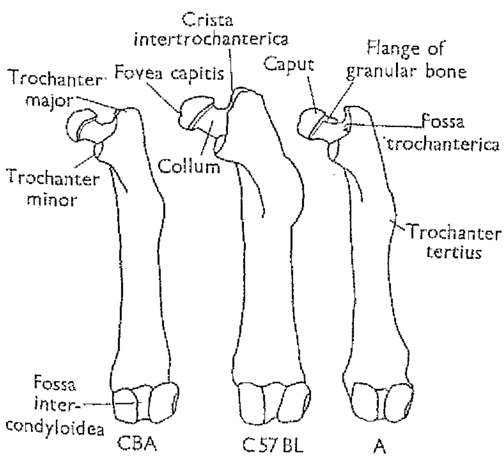

Fig. 14. Femora of female CBA, male C57 BL and female A mice. Ventral aspect.

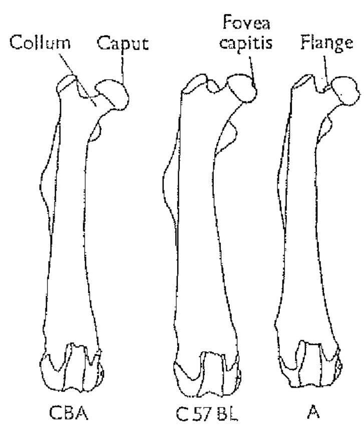

Fig. 15. Femora of CBA, C57 BL and A females. Dorsal aspect.

Most frequently it was pointed at the tip and had a wider base connecting it with the granular bone of the caput as in the purebred A mice. In some cases it was quite insignificant and in a few completely lacking. As there was thus no sharp segregation, no attempt has been made to go into the details of this situation.

On the subepiphyseal surface of the caput there is a small but definite fovea capitis for the attachment of the ligamentum teres femoris in both the A and C57BL strains. In CBA animals it is considerably less obvious. A similar depression may be seen on the surface of the epiphyseal cap in all three strains, but it is shallower in CBA. This may reflect a reduced size of the ligamentum teres femoris in CBA.

Another very noticeable feature is the prominent crista intertrochanterica in C57BL mice which together with a very large trochanter major and minor forms a very deep fossa trochanterica in this strain. The smallest trochanters, major, minor and tertius, are found in the A strain. The fossa intercondyloidea femoris is deepest in C57BL and shallowest in A mice. As in other long bones, the shafts of C57BL femora are most massive, those of A femora slender. However, there is little difference in the size of the epiphyses.

Ossa cruris (Figs. 16 and 17)

There is a small but consistent difference in the size of the crista tibiae which is largest in C57BL and smallest in A mice. In C57BL the crista attaches to the shaft by a broader base and rises more nearly perpendicularly from it, especially in its proximal part. In both CBA and A mice the free margin of the crista has more of a tendency to curve laterally around the tibia. This accounts for the difference in the shape of the tuberositas tibiae illustrated in Figs. 16 and 17. Of all the three strains only C57BL mice have the characteristic lateral bulge or tuberculum a short distance below the caput seen in fig. 17; it was very prominent in seven out of the ten skeletons examined, less noticeable in three.

Distally, the fossa anterior is least obvious in A and most marked in C57BL mice. The latter also have a definite tuberosity of bone which forms a small hillock or ridge proximally near the edge of the fossa; a similar tuberosity, though somewhat smaller, is present in CBA while the structure is absent in A mice.

The caput fibulae differs considerably in size and shape in the three strains. It is largest in C57BL, surprisingly broad in A, but thin in the antero-posterior direction in CBA. The curvature of the shaft of the bone varies somewhat between the strains. In A mice, the fibula resembles the femur and other long bones in that the shaft is very slim, but the caput is of about normal size.

Alizarin preparations

Examination of twelve skeletons of each of the three strains (21-36 days old) gives support to the observations made on adult skeletons. Differences are mainly due to age, e.g. the characteristic shape differences of the acromion have not yet become obvious. The fusion between ischium and pubis seems to occur earlier in CBA than in A mice.

The articulation of the ilium with the sacral vertebrae also varied somewhat in the three strains. In the twelve C57BL skeletons only one of the vertebrae shows contact with the internal surface of the ilium, in CBA one and a portion of a second, while in A there is variation among the skeletons, although the majority (10 out of 12) show articulation with only one, as in the case of C57BL.

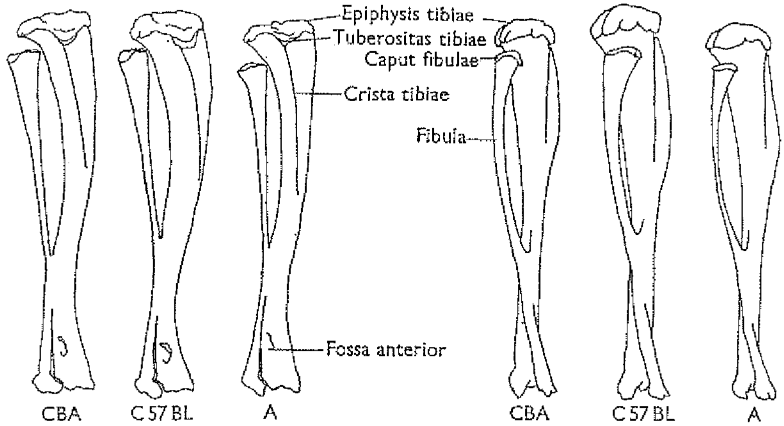

Fig. 16. Ossa cruris. Tibiae and fibulae of CBA, C57BL and A females. Anterior aspect on the left, posterior aspect on the right.

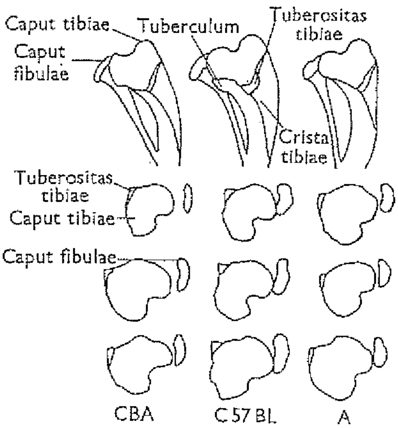

Fig. 17. Ossa cruris of CBA, C57BL and A; all females except bottom row. Proximal anterior (top row) and proximal (articular) aspects.

DISCUSSION

Since Grüneberg (1950*a*) reported in the first paper of this series on minor variations of the vertebral column between strains, a mass of data on the subject of variations has been accumulated, both for wild mice (Weber, 1950) and for in-bred strains including those used in this study. Most of these studies have been concerned with the skull and vertebral column. This paper contributes to the list of variations by a description of differences between three inbred strains in the girdles and long bones. Each of the bones examined has been found to possess features characteristic for the particular strain to which it belongs. These differences are sufficiently numerous and distinct in most cases to enable the sorting out, from a mixture of bones, of those belonging to a particular strain. A mixed sample of scapulae or of pelves, clavicles or femurs from these strains, for instance, could be sorted out fairly accurately into its components and though one might not be equally successful with all the long bones individually, an A, C57BL or CBA mouse could almost certainly be identified as such by examination of these bones as a group. In other words a strain can be recognized by its skeletal characteristics as accurately as a person by his or her facial features. The skeleton is not exceptional in this respect; recent work by M. D. Froud (unpublished) has shown that the same three inbred strains differ from each other in numerous features of the arterial system. Presumably the same would be found for any aspect of anatomy or physiology subjected to systematic study.

The characteristics distinguishing the three strains for the girdles and long bones are of various types. Some are general differences which apply to the skeletons as a whole, such as the larger size of C57BL bones and the more elegant contours of the A strain. Others are apparently localized, including certain size differences, for example the smaller incisura semilunaris of the ulna and the correspondingly smaller size of the articulating trochlea of the humerus noted in C57BL mice. Here the localized size differences between strains are in the opposite direction to that characterizing the skeleton as a whole. Differences in pattern are also both general and localized. However, it may well be that the localization of an effect is apparent rather than real in view of our ignorance concerning mechanisms of growth. Among the latter a few may be considered to be of the presence-absence type, for example the flange of granular bone on the caput of the femur and the perforation between the fossae of the distal end of the humerus.

The chain of causation of these various characteristics can only be guessed at. Variation in general rate of development might explain some of the differences observed. It is generally recognized that CBA mice develop more rapidly than those of the A strain. This could possibly account for the delayed fusion of the ischium and pubis noted in the latter. Some of the later effects on bone growth may be secondary to those on muscles. Carter (1951) attributed the lateral deviation of the ischium in luxate mice to changes of muscle action.

Some of the differences observed have parallels in other inbred strains or mutant genes. The tuberosity of bone on the tibia near the fossa anterior found in C57BL and CBA but not in A skeletons is an example. This ridge is the point of origin of the annular ligament which, in old animals of the inbred strain C3H may ossify to a considerable extent (Grüneberg, 1950*b*). Also in the case of the mutant, shaker with syndactylism (*sy*), Grüneberg (1956) has reported effects on the long bones, some of which, for example the slender character of the shaft of the humerus and the outline of the medial border of the scapula, are similar to, but more extreme than, interstrain differences noted in this study.

In view of their consistent occurrence within inbred strains, the characters studied must be considered genetically determined. No detailed study has been made of the method of inheritance of any of the variations noted in the girdles and long bones, but a preliminary survey of F_1 and F_2 skeletons from crosses of CBA males and A females indicates, for two characters of the A strain (the flange of granular bone on the head of the femur and the perforation between the fossae olecrani and supratrochlearis of the humerus), that the interstrain difference is not caused by a single pair of genes. While multifactorial inheritance is indicated, extensive further investigations would be required before any accurate conclusions could be drawn. It is possible that these characters may belong to the group of quasi-continuous variations, examples of which have been reported upon in earlier papers of this series. In fact most of the skeletal variations studied by previous contributors to this series were of the all-or-none type, or could at least be treated as such; as a consequence, interstrain differences for these characters were statistical and expressed in terms of percentage incidence of a given feature in the various strains. The differences discussed in this paper seem to differentiate most mice of one strain from most mice of the others, with little or no overlapping between the groups. There is no doubt that the same type of variation could be detected in those parts of the skeleton which have hitherto been examined mainly for discontinuous variants. Indeed, as pointed out by Grüneberg on several occasions, it is to be expected that the discontinuous variants are extreme expressions of metrical interstrain differences. Now that such differences have been identified in the limb skeleton, it may be profitable to re-examine the axial skeleton and the skull in the hope of identifying some, at least, of the continuous variables underlying quasi-continuous characters.

SUMMARY

The girdles and long bones of 10 individuals of each of the three strains A/Gr, C57BL/Gr (Subline VI) and CBA/Gr have been examined and consistent differences between the strains have been noted and described. Some of these are presence/absence differences, others are variations in size and/or shape of localized portions of particular bones. A positional difference of the ilium in relation to the ischio-pubis has been observed in the pelvis. Finally there are general differences in size, C57BL skeletons being on the whole the largest and heaviest of the three strains. Preliminary observations indicate that the presence/absence variants are probably not dependent on single gene differences between the strains. No genetic analysis of the other types of variation has been attempted, but the consistency with which they occur indicates that they are genetically determined.

The author is indebted to Prof. Hans Grüneberg, F.R.S., for suggesting this problem and to both Prof. Grüneberg and Dr Gillian M. Truslove for their interest and help throughout the investigation.

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