An Investigation into Bone Form

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The control of bone form is particularly important in orthodontic studies. Generally it is agreed that the size and shape of a bone, or a portion of a bone, has biological significance when considered in a functional context. Moreover, using the functional terminology described by Moss and Young,[1] the classically named bones of formal osteology may be disregarded. This is because it is demonstrable that skeletal units are sometimes composed of adjacent portions of several bones, e.g., calvarium or orbit, and even more frequently, the skeletal units comprise but one portion of a formal bony element. In the first instance the term macroskeletal unit is used, whereas the latter are classified as microskeletal units. For instance, the mammalian mandible has been shown to comprise in reality of a number of microskeletal units, e.g., alveolar, coronoid, angular, basal, etc., whose size, shape, position and even existence are relatively independent of each other.

An important correlate to functional analysis is the demonstration that changes of form, i.e., of size and shape, as well as the growth, and even the maintenance in being, of all skeletal units are secondary to, and depend in all instances upon, the primary morphogenetic influences of their functional matrices. Intrinsic, i.e., genetic, factors play only an initiatory role at best,[2] while extrinsic (functional or environmental) factors derived from activity, growth and the very presence of functional matrices are primary and almost totally predominant morphogenetically. Alteration in the form and spatial position of any skeletal unit is always secondary, compensatory and mechanically obligatory to primary alterations of its related functional matrix.

The present investigation was undertaken to compare the bones between inbred strains of rats maintained under standardized environmental conditions, i.e., with similar functional matrices, differing only in their genotypes. This was a two-part investigation with the first part undertaken to establish which of the several bones, the mandible, scapula and femur, varied most between different inbred strains of rat, and secondly to investigate the most variable of these bones to establish whether various functional units of the most variable bone could be isolated.

Material and Methods

Animals

A total of 300 adult male rats were included in this investigation. The weight of each rat was within two standard deviations of the mean. They were derived from equal samples of six inbred strains: Wistar, Birmingham A, Irish Piebald, Sprague Dawley, MNR and Lister Hooded. These strains were selected for no other reason than their availability. All the rats were adjudged quite healthy and had not been subjected to previous experimental procedures. After weaning at twenty-one days, the animals had been fed on the same diet ad libitum, and had been maintained under standardized and rigidly controlled environmental conditions.

After weighing, the animals were killed and their right mandibles, scapulae and femora dissected and macerated. When all the bones were grouped together, it was not possible to distinguish between the mandibles, scapulae or femora of the various strains from visual inspection, so that for all intents and purposes the various bones were similar in each inbred strain.

Measurements

Prior to measurement, each bone was orientated in a standard manner, and various dimensions were selected for their ease in measurement. Using dial calipers, the following dimensions were measured for each mandible, scapula and femur.

Mandible

1. Overall length: distance between the posterior point of the angular process and anterior point of the interdental septum.
2. Length of ascending ramus: distance between the most anterior and posterior margins of the ascending ramus.
3. Height of coronoid process: distance between the horizontal plane and tip of the coronoid process.
4. Height of condyloid process: distance between the horizontal plane and tip of the condyloid process.

Femur

1. Overall length: minimum distance between the most superior aspect of the head and most inferior aspect of the internal condyle.
2. Length from greater trochanter: distance between the most superior aspect of the great trochanter and most inferior aspect of the external condyle.
3. Anteroposterior diameter at shaft centre.
4. Mediolateral diameter of shaft centre.

Scapula

1. Overall length: distance between the middle of the glenoid fossa and vertebrion (point on the vertebral border midway between the two ridges terminating the scapula spine).
2. Breadth of infraspinous fossa: distance between the inferior angle and vertebrion.
3. Breadth of supraspinous fossa: distance between the superior angle and vertebrion.
4. Maximum width of scapula blade: distance between the most superior point on the superior angle and most inferior point on the inferior angle.

Repeat measurements of the various bones showed that any inconsistency in measurement was not only insignificant statistically, but was also on too small a scale to affect the results of the investigation.

Statistical Analysis

Initially the mean bony dimensions were compared by univariate statistical techniques (t tests) to estimate the overall trend of the results. Univariate analysis of the data was limited, however, since it enabled only one dimension of a bone to be considered at any one time. To compare bones as a whole, i.e., as complete biological units, differences between individual dimensions of bones have to be mentally summated. The closeness of mean measurements between inbred strains of rats is taken to be indicative of similarity of bone form. Yet it is clear that small absolute differences, which are in opposite directions, may have quite a profound effect on the overall shape of the actual bone. Thus to treat a bone as a biological unit and not an inventory of separate measurements, a multivariate statistical technique must be employed. Moreover, with multivariate analysis each bone measurement has a context of other measurements of the same bone and the dimensions of each bone have a context of the particular population of inbred strain of rats, thereby providing a coherent matrix as a background for interpretation. In fact, a matrix of variation and covariation is the mathematical basis for such a multivariate analysis.

An example of a multivariate statistical technique is the discriminant function which provides a single axis or line of orientation using all the information on two groups. It is constructed so that all individuals of the two groups are segregated as completely as possible. A canonical analysis of discriminance, the type of analysis used in this investigation, is essentially a multiple multivariate discriminant analysis upon which is superimposed an eigen value analysis so as to elucidate the contribution of each measured variable to the discriminant functions. In a canonical analysis of discriminance, a number of linear functions are constructed which are chosen to space out the means (or centroids) of the given groups to the greatest possible extent. Usually this results in the major part of the spacing of the groups being expressed by means of only a small number of such mathematical functions, perhaps by three or less, so that the data can be represented by a one, two or three dimensional figure.

The data were first transferred onto punched cards and a computer program used which enabled not only the basic statistical data (mean and standard deviation calculated for each strain) to be printed out, but also plotted for each variate intrastrain standard deviations against corresponding means and calculated a linear regression to fit most nearly the resulting array of points. Plots of standard deviations against corresponding means showed that the standard deviations fluctuated randomly irrespective of the value of the means. The data were then fed back into the computer, and later sections of the same program brought into operation to produce, by the general method described by Gower,[3] canonical analyses of (a) all the dimensions of the mandibles, scapulae and femora combined, (b) all the dimensions of the mandibles combined, (c) all the dimensions of the scapulae combined, and (d) all the dimensions of the femora combined.

The program yielded a print-out not only of the coordinates of each strain of rat (centered around an overall mean of zero) in the various orthogonal dimensions of the canonical space, but also loadings by which the means of each variate must be multiplied, the products summed and a given constant added or subtracted to produce the canonical coordinates. It also provided a table of latent roots of the matrix from which the canonical axes were derived (these gave a measure of the total proportion of the total variance contained in each axis), the distance of each strain from the centroid of the whole constellation in the multidimensional canonical space, and a table of squared generalised distances (D^2) between all pairs of strains.

The results of the various analyses were examined by the standard method of plotting the positions of the various strains in relation to pairs of those canonical axes which individually provided a marked measure of separation between available strains. Only the first two canonical axes showed any real discrimination between the various strains in this investigation. Also for each analysis, the central point for each strain was circumscribed by a circle of radius 2.15 standard deviation units, thus including approximately ninety per cent of individuals.

Results (1)

As summarised in Table 1, univariate analysis between pairs of inbred strains of rat showed considerable intra- and interstrain variation. There was also no tendency for the dimensions of a bone in one strain to be consistently different from those of others. Whereas the greatest overall length of the mandible occurred in the MNR strain, the Irish Piebald strain showed the greatest height of the coronoid process. Similarly, the overall length of the femur was greatest in the Wistar strain, whereas the MNR strain showed the maximum width of the scapula blade. This emphasises the limitations of univariate statistical analysis in that no overall tendency was evident in any dimensions.

As shown in Table 2, there was significant discrimination between the various strains from canonical analysis of all the mandibular, scapular and femoral dimensions combined (Fig. 1) with the first two canonical axes separating the strains. In contrast, discrimination was considerably reduced between the strains from canonical analysis of the dimensions of the mandibles, scapulae and femora considered separately. Nevertheless a similar pattern of discrimination between the inbred rat strains emerged from each analysis. In addition, there was a greater degree of discrimination between the strains from analysis of the mandibular, compared with analysis of the scapular or femoral dimensions. The eigen value analysis, however, which is superimposed on the canonical analysis, showed that no one mandibular dimension accounted for more discrimination between the inbred strains compared with others.

In view of the greater degree of discrimination between the various strains from analysis of the mandibular compared with analysis of the scapular or femoral dimensions, further measurements were recorded on each mandible to obtain a more accurate definition of this bone.

Materials and Methods (2)

A total of thirty-four dimensions was measured from each mandible. Following standard orientation, lateral radiographs were made of each right mandible. Subsequently, these radiographs were projected at a standard magnification of ten times, and the following points identified: (a) most anterior point at the symphysis; (b) most superior point at the symphysis; (c) most inferior point on the upper border of the incisor region of the mandible; (d) most anterior point on the alveolar edge of the coronoid process where it cuts the occlusal surface of the first molar; (e) most inferior point on the body of the mandible; (f) point at which the anterior edge of the coronoid process cuts the occlusal surface of the first molar; (g) most postero-inferior point on the outline of the molar dentition; (h) most superior point in the masseteric notch; (i) tip of the coronoid process; (j) most inferior point in the sigmoid notch; (k) most superior point on the condyle; (l) most posterior point on the condyle; (m) most anterior point on the posterior border of the ascending ramus; (n) most posterior point on the angular process of the mandible.

From these fourteen points and their vertical projections onto the standard horizontal plane, thirty-four dimensions of the mandible were measured with dial calipers. These dimensions provided an accurate metrical definition of each mandible. In addition, this metrical profile could be readily subdivided, so that the incisor, molar, condyloid, coronoid and angular regions of the mandible could be isolated. The following canonical analyses were then performed: (a) analysis of all the mandibular dimensions; (b) analysis of all the mandibular incisal region dimensions; (c) analysis of all the molar region dimensions; (d) analysis

TABLE 1 MANDIBULAR, SCAPULAR AND FEMORAL DIMENSIONS OF DIFFERENT INBRED STRAINS OF RAT

	WISTAR		BIRMINGHAM A		IRISH PIEBALD		SPRAGUE DAWLEY		MNR		LISTER HOODED	
	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.	$\bar{x}$	S.E.
MANDIBLE												
Overall length	23.9	0.15	23.3	0.21	23.2	0.18	22.0	0.16	24.1	0.16	21.7	0.21
Length of Ascending Ramus	9.8	0.20	8.3	0.18	12.1	0.19	11.1	0.20	9.6	0.14	8.3	0.15
Height of Coronoid Process	12.6	0.17	11.2	0.15	14.0	0.15	13.3	0.26	12.0	0.17	11.2	0.14
Height of Condyloid Process	9.9	0.13	9.2	0.15	13.0	0.16	11.8	0.21	11.9	0.14	10.7	0.15
FEMUR												
Overall length	33.3	0.20	31.3	0.19	32.4	0.21	31.2	0.20	32.1	0.16	31.4	0.18
Length from Greater Trochanter	32.6	0.19	31.0	0.14	29.1	0.14	28.3	0.19	32.1	0.19	31.2	0.15
Antero-posterior diameter of mid-shaft	3.7	0.13	3.0	0.13	3.6	0.10	2.9	0.14	3.3	0.17	3.0	0.12
Medio-lateral diameter of mid – shaft	4.1	0.13	3.7	0.12	4.1	0.16	3.9	0.13	3.9	0.13	3.3	0.14
SCAPULA												
Overall length	25.1	0.19	23.6	0.17	24.9	0.19	24.0	0.13	24.2	0.19	23.6	0.18
Breadth of Infra - spinous fossa	2.4	0.13	2.3	0.13	2.3	0.23	2.2	0.28	2.2	0.20	2.1	0.19
Breadth of Supra - spinous fossa	3.4	0.14	3.3	0.16	3.6	0.27	3.5	0.21	3.6	0.14	3.5	0.16
Maximum width of scapula blade	5.9	0.16	5.7	0.15	6.0	0.15	5.7	0.13	5.8	0.15	5.6	0.23

$\bar{x}$ = mean dimension (mm) ; S.E. = standard error.

TABLE 2 CANONICAL COORDINATES BASED ON ANALYSIS OF MANDIBULAR, SCAPULAR AND FEMORAL DIMENSIONS

STRAIN	ALL MANDIBULAR, SCAPULAR AND FEMORAL DIMENSIONS		ALL MANDIBULAR DIMENSIONS		ALL SCAPULAR DIMENSIONS		ALL FEMORAL DIMENSIONS	
	AXIS 1	AXIS 2	AXIS 1	AXIS 2	AXIS 1	AXIS 2	AXIS 1	AXIS 2
Wistar	4.15	1.32	3.77	0.81	0.02	-1.29	0.86	2.80
Birmingham A	2.25	-3.94	0.88	2.26	3.56	1.33	-2.52	1.06
Irish Piebald	-3.59	3.01	-2.46	-0.56	-3.96	0.07	0.77	-0.32
Sprague Dawley	-5.69	-1.46	-4.66	0.20	-2.05	1.91	-1.82	-1.17
MNR	2.20	3.41	2.32	-2.35	0.23	-1.57	3.02	-0.71
Lister Hooded	0.69	-2.34	0.15	-0.35	2.19	-0.31	-0.32	-1.67

All coordinates in standard deviation units.

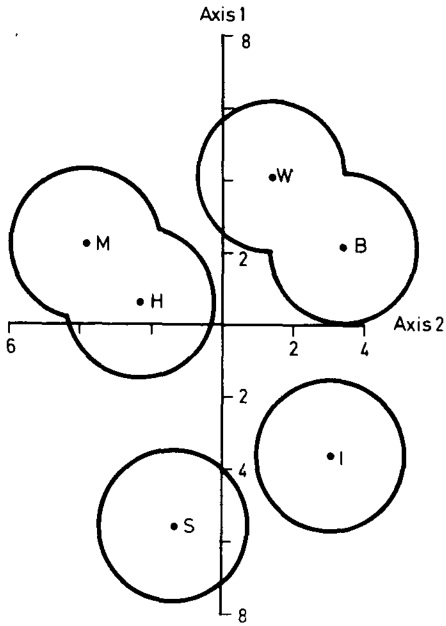

Figure 1. Canonical analysis of all the mandibular, scapular and femoral dimensions combined showing centroids for the various inbred strains and their ninety per cent confidence limits. Capital letters represent the inbred rat strains: W, Wistar; B, Birmingham A; I, Irish Piebald; S, Sprague Dawley; M, MNR; H, Lister Hooded.

of the coronoid region dimensions; (e) analysis of the condyloid region dimensions; (f) analysis of the angular region dimensions.

Repeat measurements of a random selection of mandibular radiographs showed that any error of measurement was statistically insignificant and on too small a scale to affect the outcome of the comparisons.

RESULTS (2)

Univariate analysis of the data showed no tendency for the mandibular dimensions of one strain of inbred rat to be consistently different than those of others. In contrast, when all the mandibular dimensions were combined, the first two canonical axes showed significant discrimination between the various strains (Table 3, Fig. 2). The

TABLE 3 CANONICAL COORDINATES BASED ON ANALYSIS OF MANDIBULAR DIMENSIONS

DIMENSIONS	WISTAR		BIRMINGHAM A		IRISH PIEBALD		SPRAGUE DAWLEY		MNR		LISTER HOODED	
	AXIS 1	AXIS 2	AXIS 1	AXIS 2	AXIS 1	AXIS 2	AXIS 1	AXIS 2	AXIS 1	AXIS 2	AXIS 1	AXIS 2
All dimensions	13.09	5.27	16.64	0.75	-4.44	-2.67	-2.49	-11.83	-6.34	1.68	-16.48	6.81
Incisor region	6.42	2.42	5.87	-1.83	-2.53	-0.19	-4.74	1.49	-0.92	-1.86	-4.10	-0.02
Molar region	7.42	2.95	8.61	-0.55	-2.60	-1.30	-1.63	-3.61	-1.52	-0.07	-10.27	2.58
Coronoid region	5.61	1.58	5.80	0.75	-0.93	0.19	-1.04	-4.73	-1.99	-0.26	-7.45	2.48
Condyloid region	2.53	0.30	2.41	-2.97	-2.38	0.78	3.40	1.93	-1.75	0.63	-4.21	-0.67
Angular region	3.79	1.21	5.27	2.43	-2.31	-0.70	1.65	-5.50	-3.24	1.71	-5.16	0.85

All coordinates in standard deviation units.

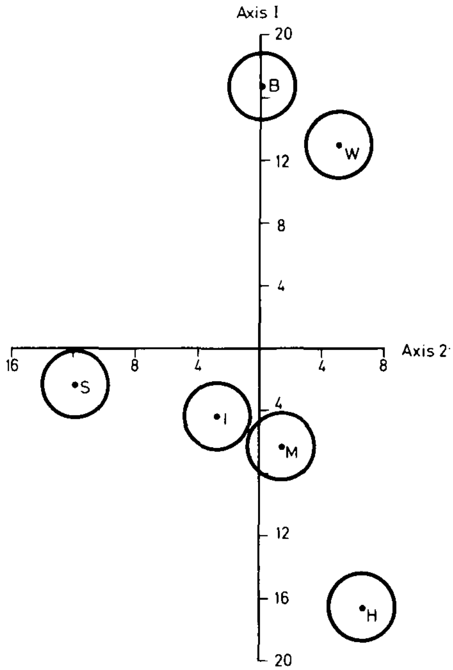

Figure 2. Canonical analysis of all thirty-four mandibular dimensions.

eigen value analysis, however, showed that there was no one mandibular dimension which contributed more to the discrimination between the various strains compared with others.

The degree of discrimination between the strains was much reduced from analysis of all the dimensions combined from the various different functional units or regions of the mandible (Table 3). This is illustrated in the following figures: Figure 3 which is based upon analysis of the incisor region of the mandible; Figure 4 which is based on the molar region of the mandible; Figure 5 which is based on analysis of the coronoid region of the mandible. Although the actual pattern of discrimination between the strains differed slightly according to which group of mandibular dimensions were included in the analysis, there were no marked differences in the degrees of separation between the various strains

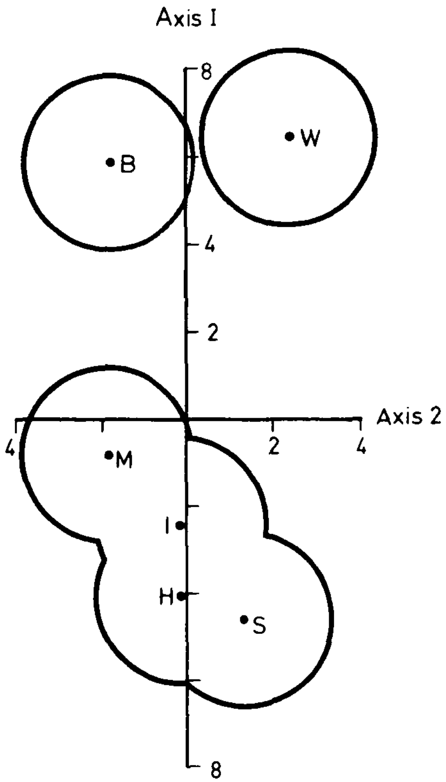

Figure 3. Canonical analysis of all the incisor region mandibular dimensions.

from analysis of the dimensions of one particular region compared with other regions.

These analyses were all based upon the comparison of mandibular size and shape combined. To determine differences in mandibular shape only, rather than both size and shape combined, each of the dimensions was expressed as a regression of the body weight of each animal. Subsequently, each of the canonical analyses was repeated and showed a similar pattern of discrimination between the strains compared with analysis of the "raw" mandibular dimensions. In other words, comparison of mandibular shape resembled the comparisons of mandibular size and shape combined.

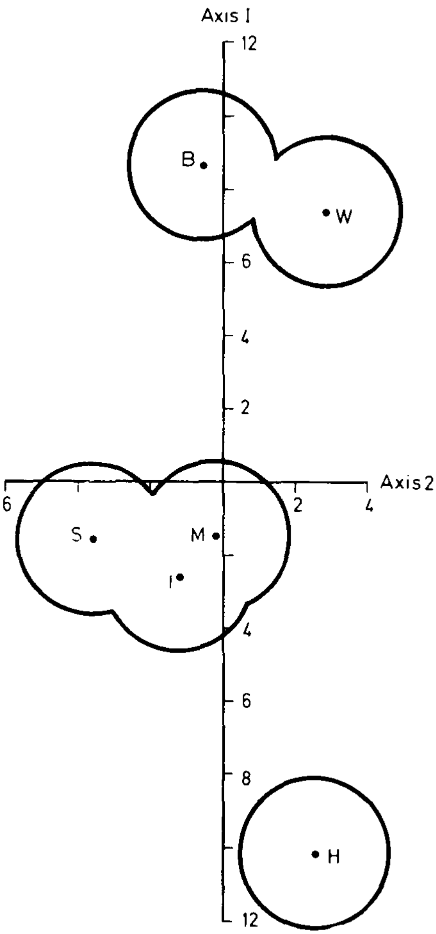

Figure 4. Canonical analysis of all the molar region mandibular dimensions.

Discussion

This investigation showed that analysis of the mandibular dimensions produced a greater degree of discrimination between the strains compared with analysis of the scapular or femoral dimensions. The reasons for this are obscure. The forms of the mandible,[4] scapula[5] and femur[6] have been shown to be affected by changes in muscle function. Nevertheless, in this investigation the rats were all male, had been maintained under standardized environmental conditions and all were of similar body weight. Hence it was

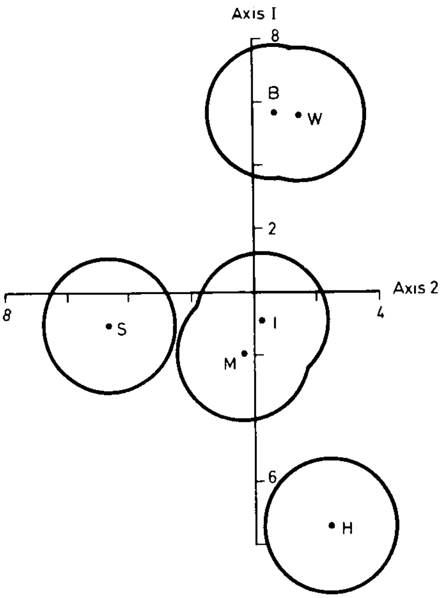

Figure 5. Canonical analysis of all the coronoid region mandibular dimensions.

considered that the functional matrix of each bone was very similar, the only difference being the genotype. Hence genetic factors presumably accounted for the greater discrimination between the strains from analysis of the mandibular compared with analysis of the scapular or femoral dimensions.

The rodent mandible consists of two bones separated by an unfused amphiarthrosis. As a freely movable skeletal lever, the mandible has a number of muscles attached to it in several ways, indirectly by periosteal and directly by intraosseous insertion. Thus certain regions of the mandible are directly related to a muscular functional environment. Functionally, the mandible may be said to comprise the following major skeletal units: (a) alveolar, which is related to the teeth; (b) coronoid, related to the temporalis muscle; (c) angular, related to the medial pterygoid and masseter muscles; (d) basal, which is related to the inferior alveolar neuromuscular triad; (e) condylar, related to the function of the movement at the temporomandibular joint: this process also corresponds to alteration in the form, function and spatial position of the orofacial capsule within which it arises, grows and is passively translated in space; (f) symphyseal, this is a macroskeletal unit composed of several independent microskeletal units, including the chin, genial tubercles, etc.,[7] and whose form has been shown to be independent of the form of other mandibular skeletal units.[8] Generalizing, therefore, mandibular form is an accurate reflection or the resultant of all the functional demands placed upon that "bone" at all times, so that functional matrices alter and skeletal units respond. Furthermore, it is well-established experimentally that removal or functional incapacitation of the functional matrix, e.g., the temporalis muscle, leads to either a partial or total disappearance of a particular skeletal unit, e.g., the coronoid process.[9] Similar data relating to other muscular processes of the mandible (or any other bone for that matter) confirm that the very existence of a skeletal unit depends upon the morphogenetically primary operational demands of its related functional matrix.

On the basis of massive evidence, Gruneberg[2] postulates that osseous morphology is not under direct genetic control. Rather it appears that it is the functional matrices which are the greatest of such activity. In this investigation considerable discrimination was noted between the various inbred rat strains from analysis of all thirty-four mandibular dimensions. In contrast, when the dimensions of the various skeletal units of the mandible were analysed, the degree of discrimination between the strains was reduced, yet there were only slight modifications in the patterns of discrimination between the strains depending upon which mandibular dimensions were included in the analysis. The reasons for this are obscure. On the one hand it may be that the mandible consists of a single bony unit or entity and the total functional matrix as a whole varies between the various inbred rat strains. On the other hand the data could equally suggest that although the mandible comprises a number of skeletal units, the functional matrix of each unit differs in a similar manner between each of the inbred strains.

Since the data from the present investigation are equivocal, it is apparent that further data are required to elucidate the genetic control of the mandible.

SUMMARY

The mandibles, scapulae and femora from 300 adult male rats from six inbred strains were measured. The results showed that there was a greater degree of discrimination between the inbred strains from analysis of the mandibular dimensions than from the scapular or femoral dimensions. Subsequently, an additional thirty-four dimensions on each mandible were measured. Multivariate analysis, however, showed that there was no one region of the mandible which revealed a greater degree of discrimination between the strains compared with others.

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