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# Stereo-projection radiography in the study of the structural organisation of cat skull bones

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## INTRODUCTION

In the past the accuracy of conventional X-ray machines has been defined in terms of the extent to which lesions, produced either as a result of pathological conditions (Chasin, 1928; Böhmig & Prévôt, 1931; Borak, 1942) or experimentally (Ardran, 1951; Goldman, Millsap & Brenman, 1957; Bender & Seltzer, 1961) in compact and/or cancellous bone, were or were not recorded in the radiographs. In many cases the lesions were either not detected or recorded indistinctly on the radiographs. Becks & Grimm (1945) tried to establish a correspondence between the histology and radiographic appearance of morphological changes in jaw bones. They found the resolution they obtained in the film was not good enough to record in detail the alterations which were necessary for precise diagnosis of the osseous conditions. The lack of detail in the radiographs obtained from clinical machines has led a number of investigators to suggest that radiography was not a satisfactory method of examining bone structure. For example Siepel (1948) stated that X-rays were of no use in studying the trabeculation within the mandibular medullary bone compared with that obtained by dissection of a demineralised specimen. The poor resolution which is a feature of the conventional X-ray machines is due principally to (*a*) the reduction in contrast within biological specimens resulting from the large component of 'white' radiation in the X-ray spectrum due to the machines operating at high accelerating voltages (80 – 120 kV); (*b*) the large penumbral blurring which is associated with the large X-ray source size (1–0·3 mm in diameter); and (*c*) the use of fast coarse grained X-ray film.

These limitations have been largely overcome with the development of the microfocal X-ray unit. This paper demonstrates the high resolution obtained from this instrument as illustrated by an examination of the detailed organisation of the cat skull bones. Three different methods were used to demonstrate the correspondence between the detailed organisation of bone, its histology and its radiographic appearance. The projection radiographs were also used to determine the effect of increasing bone thickness and overlapping trabeculae and large vascular canals on the number of osseous structures recorded in the radiographs.

## MATERIALS AND METHODS

### *Preparation of material*

Five adult female cat skulls were defleshed, sectioned in the mid-sagittal plane. Lateral and dorsoventral stereo-projection radiographs were taken of the hemicrania. The right hemi-crania were embedded in Ward's cold setting 'Bio-Plastic' resin. Coronal sections, 3 mm thick, were cut perpendicular to the palatal process on a Bateman sectioning machine. Anteroposterior projection radiographs were taken of the sections. The surface of the slabs used for histological examination were ground and polished using graded alumina powders of 1–0·03 μm, with ethelene glycol as a lubricant. The histology of the bone was recorded photographically using dark ground and incident light illumination.

In the first method used to determine the correspondence between bone organisation and its radiographic appearance, X and Y co-ordinates were drawn on the slabs of the coronally sectioned skulls and their anteroposterior radiographs. The position and size of the trabeculae and large vascular canals were measured. The position of the slab was drawn on one of the pair of stereo-projection radiographs of the right hemi-crania. The trabeculae and large vascular canals in the bone slabs were correlated with their appearance in (*a*) the anteroposterior radiograph of the same slab, in (*b*) the lateral stereo-projection radiograph of the hemi-crania and in (*c*) the photomicrographs, which depicted their histological organisation (Fig. 2).

In the second method, X and Y co-ordinates were drawn on both anterior and posterior surfaces of the bone slabs and on the lateral and dorsoventral radiographs of the right hemi-crania. Measurements were then taken to determine the correspondence between the position and orientation of the trabeculae and large vascular canals within the skulls and their appearance in radiographs taken at right angles to one another (Fig. 3).

In the third method, the cortical bone in different parts of the skull of the left hemi-crania was dissected off with a dental drill and burr. The structural organisation thus revealed was photographed and compared with the radiographs of the same area. The angle of orientation of the trabeculae and large vascular spaces relative to a common base line was measured in the photographs and compared with that in the radiograph (Fig. 4). The size of the smallest structures recorded in the radiographs was also determined from measurements taken on both the bone slabs and the projection radiographs of the skulls.

For the assessment of the effect of the depth of bone and the superimposition of osseous structures on the number of those recorded in the projection radiographs, the slabs of the coronally sectioned skull were examined under a binocular microscope with a graticule fitted in the eyepiece. Structures which were of the same size or larger than the smallest detected in the projection radiographs were recorded for every half millimetre division along the margin of the bone slab. The depth of bone at each division was recorded. The position of the coronal sections on the radiographs was demarcated by cotton threads drawn across one of the stereo-projection radiographs and examined under the stereoscope. After adjusting for the relative increase in the magnification of the radiograph, the transects were divided into the equivalent half millimetre divisions. Starting at the same line as that of the coronal sections, the number and type of structures were recorded per division. The percentage differences in the numbers of large vascular canals and trabeculae recorded on the coronal sections and in the radiographs were then determined. Graphs were drawn expressing the number of structures not observed relative to (1) the increase in depth of bone (Fig. 5) and (2) the increase in the number of superimposed structures (Fig. 6).

### *Radiography*

Radiographic examination of the bone material was carried out first on a clinical machine (X-ray source size 0·3 mm in diameter) with the same X-ray film and processing techniques used in the preparation of the projection radiographs. The latter were prepared using a microfocal X-ray unit XX50 (Ely, 1972; Buckland-Wright, 1976, 1977). This unit is a constant potential X-ray generator with a kV range of 10–50 and delivering a current of 1 mA. The X-ray column is continually evacuated and comprises two electromagnetic lenses which throw a greatly reduced electron image of the cathode onto the side of a solid, water cooled target. The X-rays are emitted at right angles to the column through a thin aluminium window. The solid target comprises a series of rings of different elements (Al, Cr, Cu and W) which can be selected at will for their different characteristic wavelengths. In the present study the tungsten target was used with the unit operating at 50 kV and 0·5–1 mA. The specimens were brought close to the window, hence close to the source, in order to obtain a high direct magnification in a short camera with a range of × 1–100. The skulls were taken at a magnification of approximately × 2 on Cronex NDT 55 (Dupont Photoproducts) fine grained industrial X-ray film at exposure times of 90–120 seconds. The resolution is equivalent to the size of the source (Cosslett, 1957) which is of the order of 5–10 μm in diameter for an accelerating voltage of 50 kV.

The virtually unlimited depth of focus obtained with the apparatus, coupled with the progressive increase in magnification of the specimen strata, enabled stereo-pair radiographs to be produced. These were prepared by placing the skull in a stereotaxic apparatus between the port of the X-ray unit and the film and tilting the skull 5° on either side of the plane perpendicular to the centre of the X-ray beam. The detailed three dimensional organisation within the skull bones was examined by placing the stereo-projection radiographs under a Hilger & Watts folding mirror stereoscope.

## RESULTS

### *Radiology of cat skulls*

The difference between the results obtained from the microfocal X-ray unit and a 'good' clinical machine is illustrated in Figure 1. The advantage of magnification in the projection radiograph (Fig. 1 *a*) is illustrated by comparing it with the contact radiograph in Figure 1 *b*. Figure 1 *c* is a photographic enlargement of the anterior region of the skull in Figure 1 *b* to the same size as that illustrated in Figure 1 *a*. The advantage of greater resolution in the projection radiograph is illustrated in the difference between Figures 1 *a* and *c*. The reduced resolution in Figure 1 *c* is due to the large size of the X-ray source and greater penumbral blurring produced by the clinical machine. A further advantage of the projection radiograph is that it can be photographically enlarged well beyond the level possible with the contact radiograph, as the shadow image cast by the detailed osseous organisation covers a larger area of the X-ray film and is proportional to the primary radiographic magnification.

### *Histology*

From the polished ground sections, the compact bone was identified as a primary vascular type (Enlow, 1968). The bone was composed largely of circumferential lamellae (Fig. 2 *e*). The osseous structures comprised trabeculae and two categories of vascular canals (*a*) in the primary and secondary osteones and (*b*) large intra-osseous vascular canals consisting of narrow medullary spaces, resorptive spaces and immature osteones.

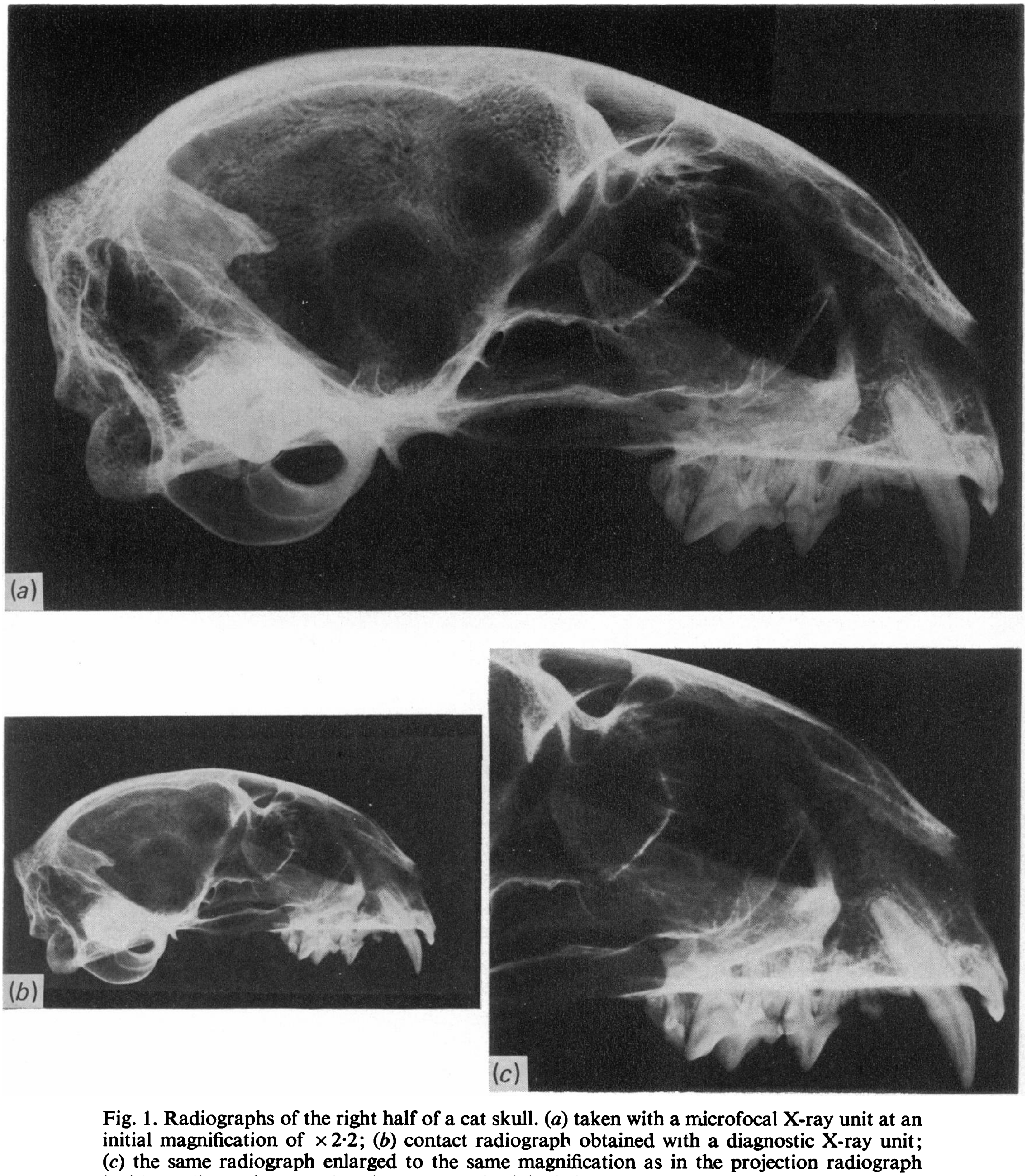


Fig. 1. Radiographs of the right half of a cat skull. (*a*) taken with a microfocal X-ray unit at an initial magnification of ×2·2; (*b*) contact radiograph obtained with a diagnostic X-ray unit; (*c*) the same radiograph enlarged to the same magnification as in the projection radiograph in (*a*). Radiographs reproduced at ×0·73 of original size.

*Correspondence between the histological and radiographic appearance of bone*

Figure 2 illustrates the results of the first method where (*a*) is the photograph of the anterior surface of the coronally sectioned slab through the maxilla at the level of the apex of the canine and shows the gross osseous organisation of the horizontal and frontal processes of the maxilla, the alveolus around the second premolar and apex of the canine. Superior to the root of the canine, a number of osseous structures are visible: (1) a vascular space with a number of trabeculae lying inferiorly, (2) a trabecula, (3) a small vascular space, (4) a second trabecula and (5) a small trabecula situated in a vascular space. The superior part of the frontal process of the maxilla comprises compact bone containing intra-osseous vascular canals. A few canals are seen above structure (5) in the photomicrograph of the ground polished section (Fig. 2*e*). Figure 2*e* illustrates the histological organisation of the bone outlined in the rectangle in Figure 2*b*. In comparing the photograph of the coronal section with its anteroposterior radiograph, the same bony structures (1)–(5) are visible (Figs 2*b*, *c*). The clarity of detail is reduced in the radiograph, (*a*) by the superimposition of underlying structures in the slab and (*b*) by the limitations of the preparation of the photograph from the radiograph. The two parallel lines drawn on the latero-medial projection radiograph of the anterior region of the cat skull (Fig. 2*d*) represent the anterior and posterior surfaces of the slab. The position of the structures (1)–(5) (Fig. 2*b*, *c*) is visible in the lateral radiograph of the skull.

Figure 3 illustrates the second method of evaluating the radiographic appearance of bone. This shows photographs of the anterior and posterior surface of a single coronal section of the skull from the maxilla in the region of the apex of the canine. Within the frontal process of the maxilla, the positions of a large vascular canal (1) running up the anterior surface of the slab and of a number of canals (2)–(6) on its posterior surface were readily visible in the lateromedial and dorsoventral projection radiographs. The third method of evaluating the radiographic appearance of bone provided a longitudinal assessment of the arrangement of the structures in the skull bones.

Figure 4 illustrates two areas of the skull which have had the cortical bone dissected off to reveal the trabeculae and large vascular canals. The angle of orientation of the trabeculae and large vascular canals was measured relative to a common base line drawn on radiographs and photographs of these areas. The structures measured are indicated in Figure 4. The measurements showed that the angle of alignment of the structures was the same in both the radiographs and photographs of the dissected bone. The mean error in the angles measured between the photographs and radiographs was $\pm 2{\cdot}5°$ for 50 measurements.

The smallest structures recorded radiographically were large vascular canals and trabeculae 60 $\mu$m in diameter. Osteones were not visible in the projection radiographs of the hemi-crania.

Despite the great depth of focus characteristic of the stereo-projection radiographs, there was a reduction in the number of structures visible in the radiographs with increasing depth of bone and the number of superimposed structures. The first set of graphs (Fig. 5) shows the effect of an increase in the depth of bone on the percentage number of trabeculae (Fig. 5*A*) and large vascular canals (Fig. 5*B*) not visible in the radiographs relative to the number recorded in the bone slabs of the skulls. These show that the effect of the depth of bone was to reduce the ability to detect trabeculae and large vascular canals by 50 % at bone depths of 4 and 1·5 mm

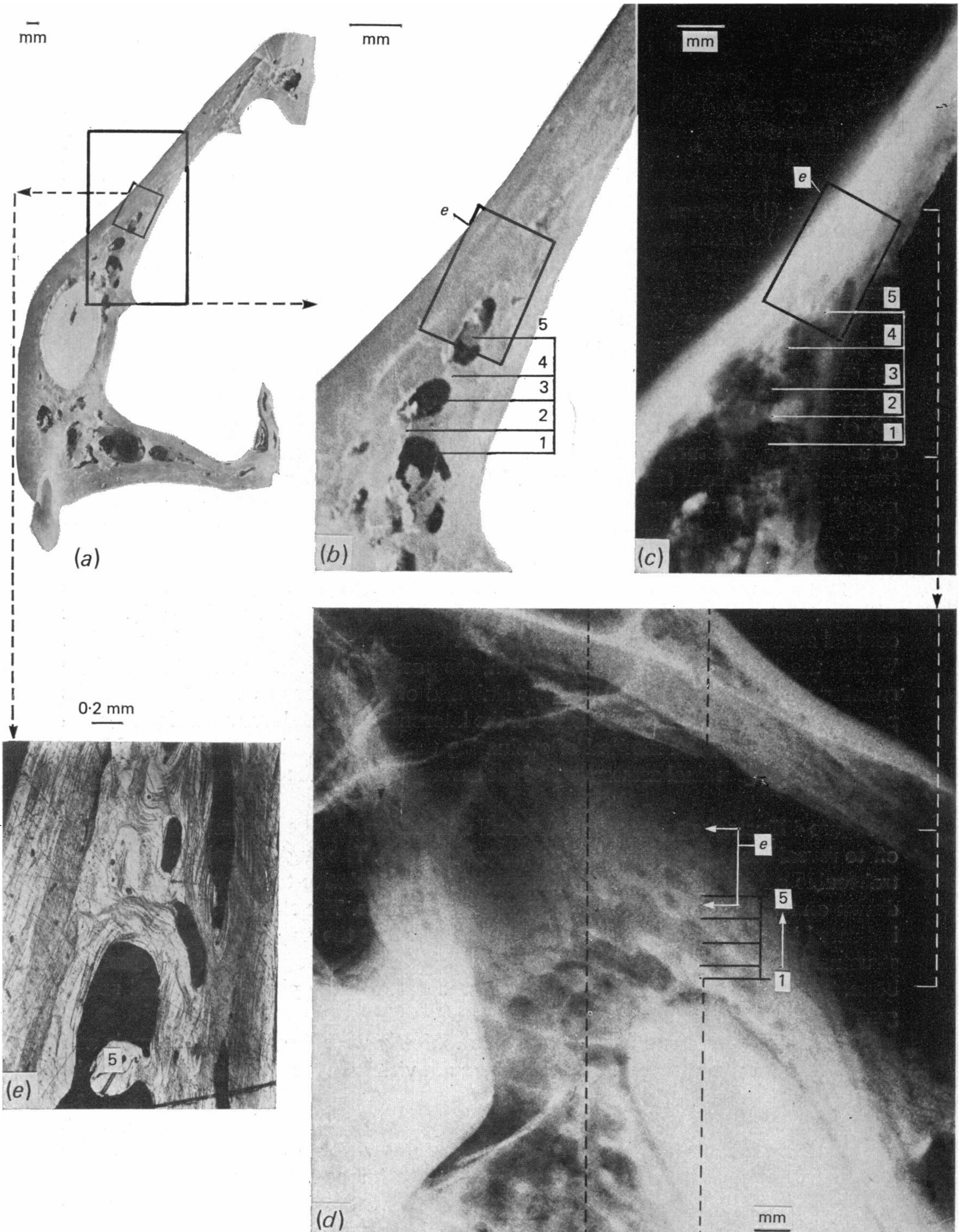


Fig. 2. Comparison of the structures seen on the anterior surface of a coronal section, at the level of the root of the canine in the maxilla, with their histological and radiographic appearance. (*a*) Photograph of the anterior surface of the slab, enlarged in (*b*) to show the bony features (1)—(5) described in the text; their radiographic appearance in the anteroposterior projection radiograph of the slab is seen in (*c*) and in the lateral projection radiograph of the skull in (*d*). The two dashed lines in (*d*) represent the position of the anterior and posterior surfaces of the

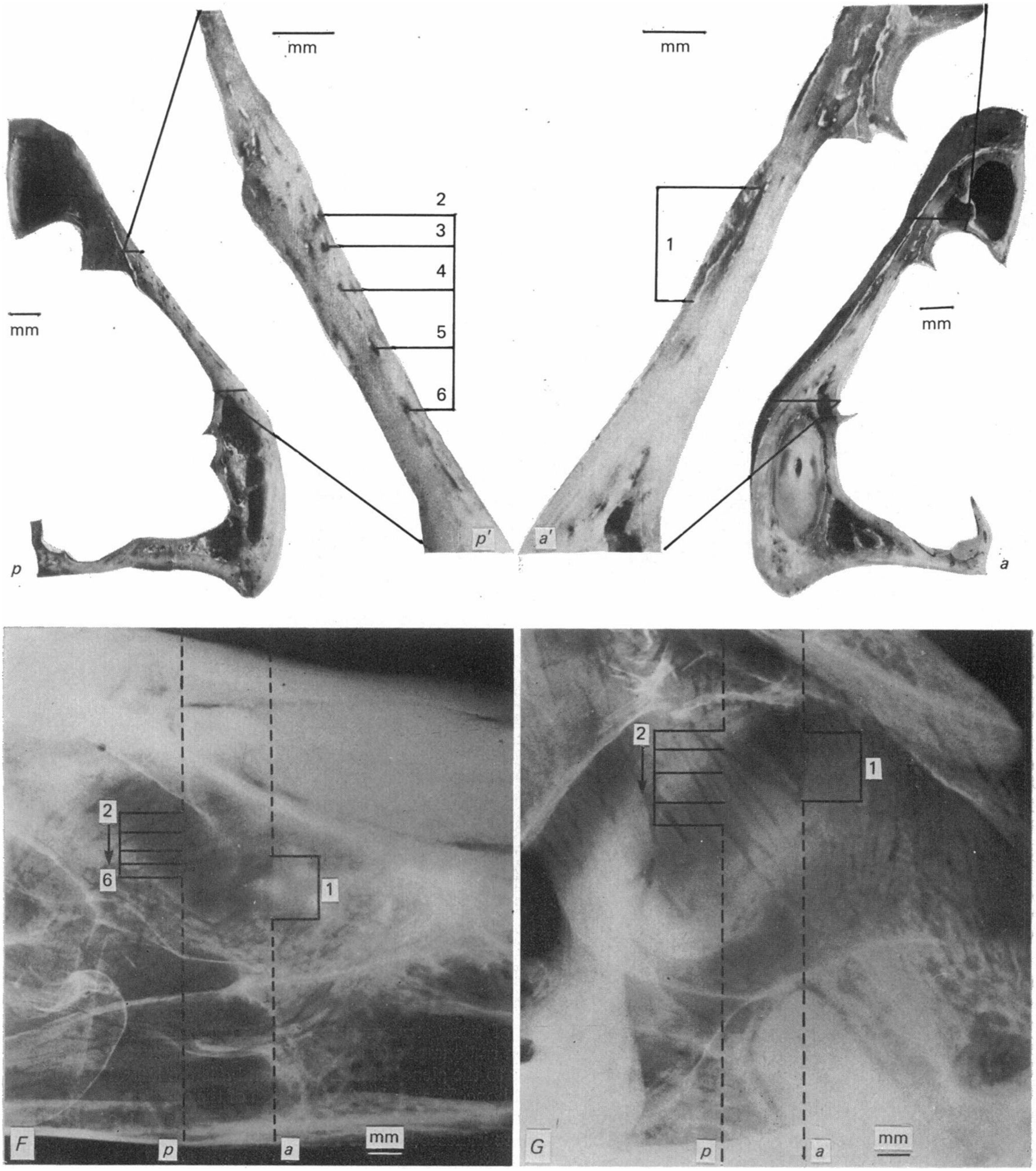


Fig. 3. Comparison between the structures seen on a coronal section of a cat skull and their radiographic appearance in the anteroposterior (*F*) and lateral (*G*) projection radiographs. (*a–a′*) and (*p–p′*) are photographs of the anterior and posterior surfaces respectively of the coronal section cut in the region of the frontal process of the maxilla. (1) is a large vascular canal on the surface of (*a′*), the position of which can be traced in the radiographs (*F*) and (*G*) at (1) on the dashed line (*a*) which represents the anterior surface of the slab. (2)–(6) are large vascular canals seen on the surface of (*p′*); their position is indicated in the radiographs (*F*) and (*G*) on line (*p*). Magnifications are indicated by the 1 mm scales.

slab photographed in (*a*). The photomicrograph of the ground polished section (*e*) illustrates the histology of the bone in the area of the bone indicated in (*a*) and includes a trabeculum corresponding to the feature numbered (5). The position of the photomicrograph is outlined in (*b*), (*c*), and (*d*). Magnifications are indicated by the 1 mm scales.

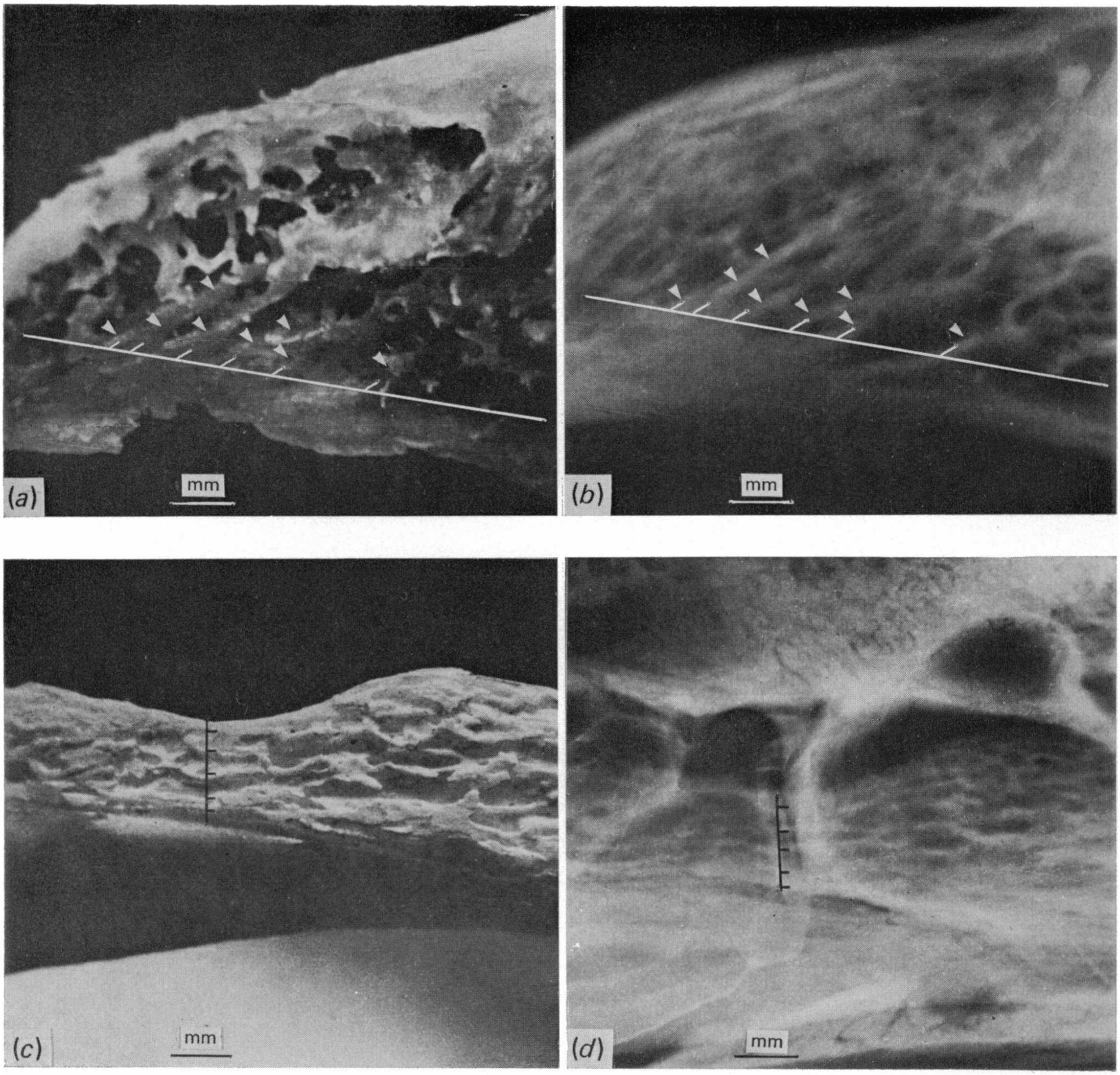


Fig. 4. Comparison between the arrangement of bony structures seen in the dissected bone (*a* and *c*) and their appearance in the projection radiographs (*b* and *d*). The region illustrated in *a* and *b* is part of the temporal bone superior to the glenoid fossa, and *c* and *d* is the lateral surface of the zygomatic process of the temporal bone. The angle of orientation of the structures indicated by the dashes and arrows was measured relative to a common base drawn on the photographs and radiographs. Magnifications are indicated by the 1 mm scales.

respectively, and that, at a thickness of 0·5 mm or less, no trabeculae or large vascular canals could be detected. Both graphs also show that, with increase in bone thickness above 4 mm for trabeculae and 2 mm for canals, the extent to which the presence of these structures is masked is reduced. This indicates that, even when the bone is very thick, a small percentage of structures will still be visible in the radiograph, but it is clear that no structures would be visible in a radiograph when the thickness of the bone was sufficient to absorb totally the X-radiation.

The second set of graphs (Fig. 6) gives an indication of the effect of an increase

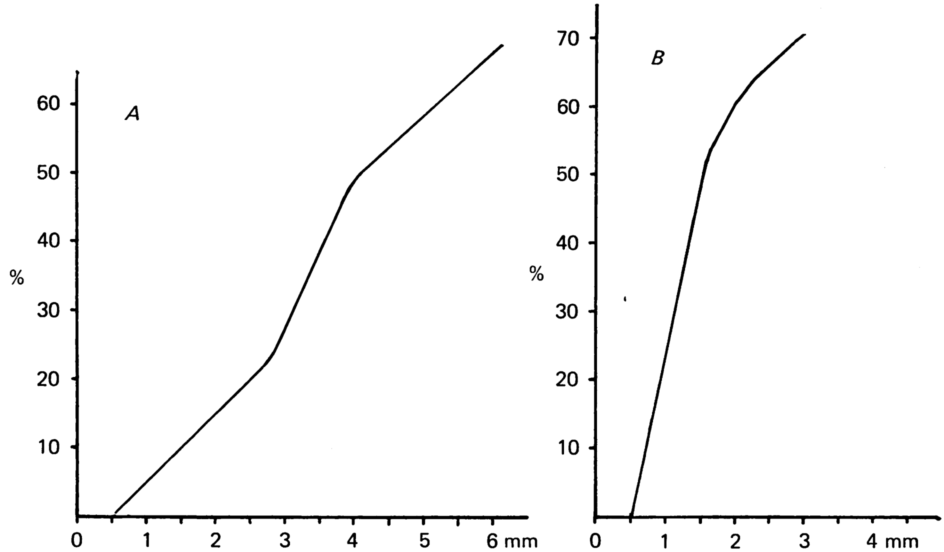


Fig. 5. Graphs of the percentage number of (*A*) trabeculae and (*B*) large vascular canals not visible in the stereo-projection radiographs (ordinate) due to the absorption of the X-rays with increasing bone thickness (abscissa).

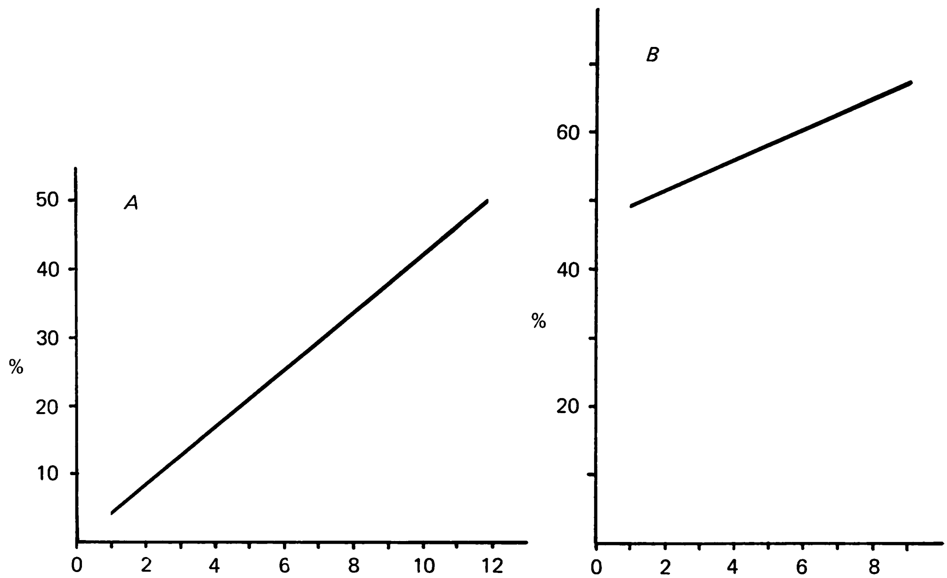


Fig. 6. Graphs of the percentage number of (*A*) trabeculae and (*B*) large vascular canals not visible in the stereo-projection radiographs (ordinate) due to the effect of superimposed structures upon the trabeculae and canals respectively (abscissa).

in the number of superimposed structures on the percentage number of trabeculae (Fig. 6*A*) and large vascular canals (Fig. 6*B*) visible in the radiographs. Figure 6*A* shows that there is a 50 % chance of a trabecula being seen in the radiograph when superimposed by 12 other trabeculae and/or canals. Figure 6*B* shows that where there are two structures overlapping a large vascular canal, there is only a 50 % chance that the canal will be visible in the radiograph.

These results show that within the range of thickness of the cat skull bones, having a maximum thickness of between 2 and 4 mm, the majority of the trabeculae and large vascular canals were recorded in the radiographic film.

Establishment of the organisation of the osseous structures in the skull bones was considerably facilitated by the use of the Hilger and Watts folding mirror stereoscope. The three dimensional view obtained enabled separation of the different specimen layers within the bones and the identity, position and orientation of the structures to be determined. Because of the visual augmentation of stereopsis, structures which were poorly defined in a single radiograph were readily discernible when the stereo-pair radiographs of the same region of bone were examined under the stereoscope. For example, stereopsis assisted in the detection of large vascular canals within the compact bone, for, as described here, their appearance within the bone was soon lost through the effects of increasing bone thickness and superimposition by other osseous structures (Figs. 5*B*, 6*B*).

## DISCUSSION

By virtue of the very small source of X-rays produced by the microfocal X-ray unit it is possible to obtain radiographs of high resolution and at large magnifications compared with conventional X-ray machines. These factors are crucial to the determination of the position and orientation of large vascular canals in compact bone and trabeculae in the spongiosa. The results obtained in this study represent a considerable advance over those obtained in earlier investigations. Becks & Grimm (1945) were able to record a reduction in trabecular density only as a slight radiolucency in their radiographs. Bender & Seltzer (1961) observed that lesions of a diameter of 1 mm in the cortical bone were not seen in the radiographs. Further, Ardran (1951) found that destruction of bone in an area of over 1 cm in diameter can occur in the centrum of a vertebral body without definite radiographic evidence of its presence. Ardran (1951) and Bender & Seltzer (1961) observed that bony lesions which appeared in radiographs taken in one plane were not always visible in radiographs taken perpendicular to that plane. The stereo-projection radiography of bone, using the microfocal X-ray unit, enabled the structures of 60 $\mu$m or greater to be identified and their position and orientation to be determined. In addition the radiographs in Figure 3 illustrate that the details of osseous structures were readily discernible in both lateral and dorsoventral radiographs. The poor definition of the vascular canals relative to trabeculae (Figs. 5*B*, 6*B*) was due to the relatively lower level of contrast between the canal and its surrounding bone compared with a trabecula. The explanation for the reduced contrast within the vascular canals is that it is due to the absorption of X-rays by the organic remains, such as connective tissue and blood vessels. The canals would be more readily visible in the radiographs were these organic remains removed through chemical treatment, thus reducing the absorption of X-rays by the soft tissues. Alternatively, the radiographic examination of living bone would be even less discriminating than in post mortem material because of the increased radio-opacity of the tissue fluid in the canals.

The use of microfocal radiography as a non-destructive method of studying organs and tissues has the advantage that the material can be submitted subsequently to alternative methods of investigation such as histology. Microfocal radiography has been applied successfully to a number of investigations, among which have been the evaluation of bone structure in relation to growth (Hobdell, 1970) and force transmission (Buckland-Wright, 1978), and further to the evaluation of tissue vascularisation (review in Hall, Röckert & Saunders, 1972). At present it is being applied to the quantification of soft and hard tissue changes associated with rheumatoid and osteoarthritis and during the preclinical drug trials in experimental animals with one or other of these conditions (Buckland-Wright, in preparation).

## SUMMARY

The relatively low resolution in the radiographs produced by clinical X-ray machines has led to the rejection, by a number of investigators, of this machine as a method of examining the detailed structural organisation of bone.

In the recently developed microfocal X-ray unit a fine beam of electrons is focussed onto the side of a water cooled target. Stereo-projection radiographs of specimens can be produced at magnifications of × 1–100 and at a theoretical resolution to the diameter of the X-ray source (5–10 $\mu$m).

The application of stereo-projection radiography to the study of cat skulls revealed details of structure not readily visible with conventional X-ray techniques. Using three different methods, the shadow images of trabeculae and large vascular canals seen in the radiographs were closely correlated with the structures observed in the coronal sections and dissected regions of the cat skulls. The smallest structures recorded from the radiographs had a diameter of 60 $\mu$m.

The effect of the depth of bone and of overlapping trabeculae and large vascular canals on the number of osseous structures recorded in the radiographs of five cat skulls was assessed. The results showed that 50 % of the trabeculae and large vascular canals were no longer visible at a bone depth of 4 and 1·5 mm respectively and when superimposed by twelve and two structures respectively.

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