

ORIGINAL

CHANGES in the length of tail bones, growth plates and bone remodelling of mice maintained at 33°C and 8°C were studied using microfocal radiographic technique. The uses and applications of the method in biomedical diagnosis and research were also discussed.

The term X-ray microscope has been used to describe a form of apparatus which uses one or more electron lenses to focus an electron beam onto a target from which the X-rays are emitted. The microfocal radiography (MFR) is a modified form of X-ray microscope and is widely used in biomedical research. It allows many facilities such as direct X-ray enlargements, continuous operation with visual presentation, videotape recording for subsequent playback and analysis, simple production of stereo pairs, low and harmless X-ray dosage to organs and tissues and the immediate selection of target element during operation for improvement of contrast.

MFR has been used in many biomedical areas to examine a wide variety of organs and tissues. In this communication it is used to study the effects of high and low experimental environmental temperatures on the structural details of tail bones of mice. The use and applications of the technique will also be explored.

MATERIALS AND METHODS

Two strain A. albino mice were used. From the age of 25 days one

The use of Microfocal Radiography in Experimental Bone Growth and its Applications in Biomedical Diagnosis and Research

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of these mice was maintained at 33°C and the other at 8°C. The details of these experimental environmental temperatures were described previously (1). At the age of 40 days, the mice were killed by deep ether anaesthesia and their tails were dissected, cut and radiographed using Microfocal X-ray unit, type XM30 M (5, 6). Photographic enlargements were

obtained from these radiographs and the 14th caudal bone was selected for examination.

RESULTS

The 14th caudal vertebra from the hot (33°C) reared mouse is long, cylindrical and thin-walled bone (see figure). Fine trabecular bony network fills the entire segment particularly at the ends. Thin bands of radiotranslucency indicating the sites of the growth plates are seen at the ends of the bone. The foramina of the nutrient vessels appear as small rounded spaces at the centre of the bone.

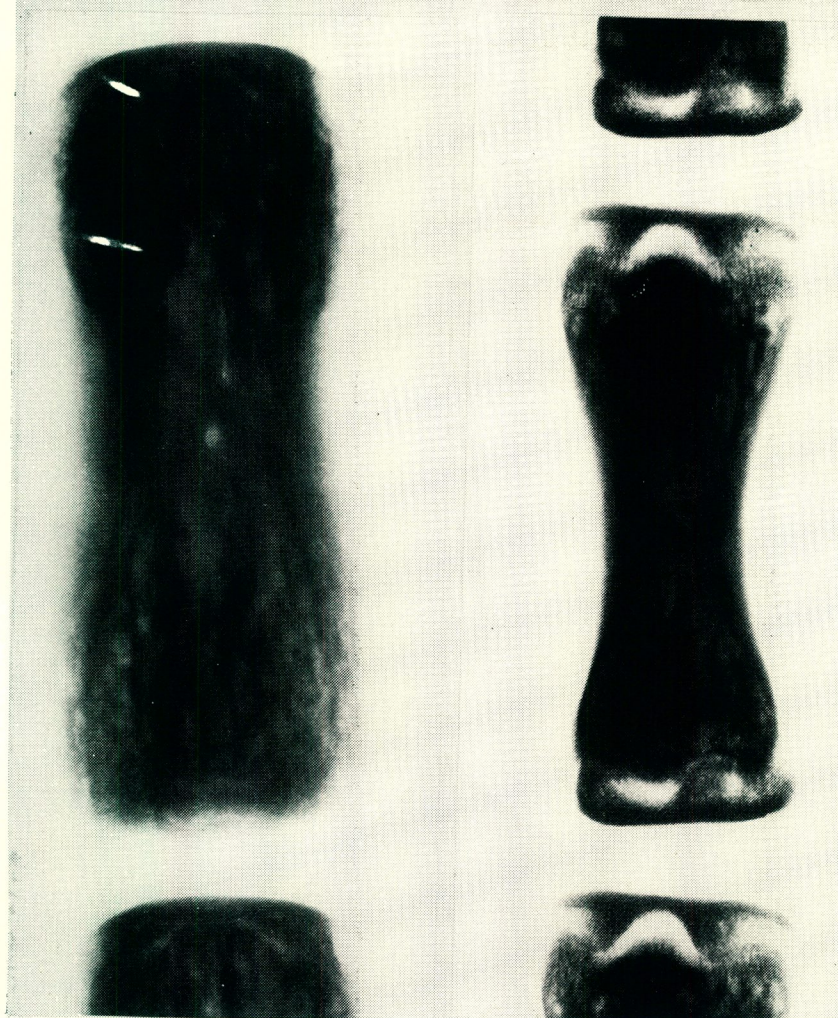
The 14th vertebra of the cold (8°C) reared mouse appear shorter than that of the corresponding hot (33°C) reared animal. It is also thicker at the ends and thin in the mid-diaphyseal part i.e. "waisted" diaphyses. The cortices are thick, particularly at the centre of the bone. The bone, especially the metaphyses, is filled with coarse trabecular bony network. At the ends, smooth wavy bands of radiotranslucency indicating the sites of the growth plates are seen i.e. the metaphyses have not yet fused to the epiphyses. The foramina of the nutrient vessels are inconspicuous.

DISCUSSION

Biomedical research employing MFR has been predominantly used in the fields of histology and angiography. Most works provided a qualitative study but a considerable amount of quantitative analysis

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Photographic enlargement (X 50) of a microfocal radiograph of the 14th caudal vertebrae of the hot (33°C) (left) and cold (8°C) reared mice at the age of 40 days.

has been undertaken particularly in histology (6, 16, 19). Microradiographic works of bone included the study of structural details and organization of bone (6), mineralization of dental tissue (17), abnormalities of enamel and dentine (7), bone mineral density (16), quantitative variations in single osteon (10), changes of mineral density with age (12), osteoporosis (19), effect of ischaemia on bone structure (11) and many other bone and joint diseases (6). The microfocal radiographic technique can be applied to nondecalcified as well as decalcified preparations. It can therefore provide immediate pathological diagnosis on nondecalcified tissue especially bone, cartilage and teeth and the appropriate line of therapy can be established without

histopathological diagnosis which require a long time for decalcification. In the present study the effects of environmental temperature on the tail bones of mouse was studied and MFR was used to demonstrate the structural bony changes. These include changes in length of tail bones, thickness of growth plates and bone remodeling.

The results of this study indicate that the higher the temperature, the longer is the tail bone. Different temperatures may influence the growth rate of tail bones in two ways : (i) changes in the rate and number of cell division occurring in the germinative zone of the growth plate (ii) changes in the rate of differentiation, as well as the size of the differentiated cartilage cells of

the plate. These changes may in turn be influenced by the direct effect of temperature on the metabolic activities of these cells. Therefore, the factors which account for the longer tail bones in the hot reared animal are the increased and accelerated rates of cell division in the germinative zone as well as the rapid differentiation of the cartilage cells along the epiphyseal columns. On the other hand the short tail bones in the cold reared mouse is due to reduced and slowed rates of cell division as well as cartilage differentiation.

The thickness of the growth plate bears a constant relationship to the amount of bone produced (15). Thus the "hot" growth plates, which contributed considerably to epiphyseal growth in length and consumed most of their growth potential during 15 days of exposure to hot temperature, matured earlier than the "cold" growth plates and appeared as thin bands of radiotranslucency. On the other hand, the growth plates of the cold reared mouse which contributed little to epiphyseal growth appeared as wide, smooth and wavy bands. This is related to the amount of retained growth potential.

The environmental temperature was found to affect bone remodeling. Thus the "hot" bone showed cylindrical diaphyses while the "cold" bone showed "waisted" diaphyses. This can be explained by assuming that apposition of external surface bone accretion is more affected by the cold condition than epiphyseal growth. In addition, the "cold" bone showed thicker diaphyseal cortical bone and more and thicker woven bone in the marrow cavity. This again can be explained by assuming that only in the cold condition is removal of bone on the endosteal

surface seriously impaired. However, there seems to be another factor responsible for the production of thick cortical bone in the cold condition. This is to fill the rather inactive bone marrow cavity in which cellularity is greatly reduced (2). On the other hand the appearance of thin trabecular cortical bone in the hot condition is to accommodate the active and highly cellular bone marrow.

Other areas of histology employing MFR (8, 18) include thyroid pathophysiology to determine whether the iodine content is detectable between the thyroid follicular material and the cellular layer surrounding the follicle, lung pathology to detect asbestos bodies and the iron containing accretion on the end of the asbestos fibre, demonstration of congenital heart defects, kidney pathophysiology to reveal deposition of minerals, embryology to study the development of small mammals, entomology to study the structural anatomy of larvae and insects of wide range of species and in botanical studies.

The second major field employing MFR is microvascular morphometric angiography (14) especially in the study of human muscle, lung, skin, stomach, intestine, jaws, teeth, bones and kidney. It is therefore of value to study the vascular disorders of these organs and tissues. Moreover its application in the angiographic study of neuroanatomy and neurophysiology together with brain (CAT) scan images provide a better correlation (13). Since the MFR technique produce no tissue damage, the sections from all these organs can subsequently be used for routine histological processing.

There are many other applications for MFR. Thus in archaeology it has been used to study the

fine structural details of fossils and sedimentary rocks (9), excavated vessels and pottery (4) as well as human and animal bones to demonstrate their age and growth patterns characteristic of communities surviving on marginal diet. It has also been used in the field of fine art to study the techniques of paste artists, paint strata and the detection of forgeries (3).

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