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THE EFFECTS OF CHANGES IN THE ENVIRONMENTAL TEMPERATURE ON THE GROWTH OF BONE IN THE MOUSE. RADIOLOGICAL AND MORPHOLOGICAL STUDY

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Summary.—Groups of 25-day-old mice were kept at 33°, 21° and 8° for up to 195 days. Measurements and observations on length, width, gross and microscopic structure using radiological and histological techniques were made on central and peripheral bones. Tail bones of animals kept at 33° grew longer and faster than those in the cold but also closed their epiphyses earlier. The diaphyses of "hot" vertebrae were cylindrical but "cold" and "control" vertebrae were of narrower diameter in their mid-diaphyses compared to their distal ends producing a "waisted" appearance. The "cold" vertebrae in addition showed thickened cortical bone and more woven bone in the marrow cavity. These changes were interpreted as indicating a disproportionate sensitivity of external apposition of cortical bone to cold. The internal remodelling of bone as the vertebrae grew was only affected by the coldest conditions and accounted for the thickened cortex and denser woven bone in the marrow cavity.

THE FACTORS which control and influence the growth of bone have been reviewed by many authors (Rang, 1969; Sissons, 1971; Potts and Deftos, 1974; Pritchard, 1974). It is generally agreed that longitudinal bone growth is chiefly controlled and regulated by local intrinsic factors which originate from the cellular elements of the skeletal model (Harrison and Clegg, 1969; Rang, 1969; Noel and Wright, 1970, 1972; Owen, 1971; Moss, 1972; Enlow, 1973; Pritchard, 1974; Kember, 1978). Other local factors are, however, equally important (Enlow, 1973).

It has been shown in an earlier study (Al-Hilli and Wright, 1983) that the tails of mice reared at higher environmental temperature have longer tail bones than those of litter-mates reared at lower temperatures. Thus the tail bones of the hot-reared mice grew rapidly and attained their greatest length during the first 3 weeks of exposure to the environmental condition and then ceased growth earlier than the tail bones of the cold-reared mice which grew slowly and for a long time.

The aim of the present study is to investigate the morphological changes that accompany these different rates of growth in peripheral bones.

MATERIALS AND METHODS

Strain A albino mice of both sexes inbred (brother-sister mating) at King's College Hospital, London SE5, were used. They were weaned at the age of 25 days and were divided into 3 groups maintained at 33° (hot group), 21° (control group) and 8° (cold group). The details of these experimental environmental conditions have been described previously (Al-Hilli and Wright, 1979).

Radiological study.—Mice of various age groups were used:

1. At the age of 40 and 195 days groups of mice maintained at 33°, 21° and 8° (8, 5 and 6 animals respectively) were used. The mice were anaesthetized with ether and immobilized with 'Cellotape' with their ventral side down on wrapped X-ray film (Kodirex, Kodak Ltd.). Using a portable diagnostic X-ray set (Model K, type 5, Newton Victor Ltd, London and Motherwell) the tails of these mice were

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radiographed. The physical factors involved were 60 kV, 10 mA, 101·5 cm f.s.d., 0·25–0·5 sec exposure. From the radiographs photographic enlargements were made and the tail bones were examined and measured using a microscope (magnification ×10) and a stage micrometer with 0·1mm divisions.

2. At the age of 92 days, groups of mice maintained at 33° and 8° (13 and 13 animals) were killed. Their feet were amputated, laid flat and cellotaped to the X-ray film, radiographed and enlarged as for the tails. From the enlargements the third metatarsal bones were examined and measured under the microscope.

3. At the age of 116 days the lower limbs of groups of mice maintained at 33° and 8° (8 and 11 animals) were radiographed. From these radiographs the femora were measured.

Gross study.—At the age of 40 days, groups of mice maintained at 33°, 21° and 8° (6, 6 and 5 animals) were killed and their tails were carefully dissected and examined using low-power stereoscopic microscope. The 3rd and 14th caudal bones were particularly examined.

Histological study.—Mice of various age groups maintained at 33°, 21° and 8° (6, 5 and 6 animals) were used. They were killed and their 14th caudal vertebrae were dissected, fixed for 1–2 days in 10% formol saline, decalcified in 10% formic acid in 10% formol saline, processed, embedded in wax and then cut a 4 μm and stained with haematoxylin and eosin.

RESULTS

Radiological results

From the photographic enlargements of the radiographs taken at the age of 195 days the mean caudal numbers were found to be 29 in the hot- and cold-reared mice and 30 in the tails of the control mice. The free caudal vertebrae (*i.e.* the bones external to the body, number 4 *et seq.*) in the hot group appeared longer and more cylindrical than those of the cold group, which were shorter and thinner in the mid-diaphyseal parts. The bones of the control group lay midway between the bones of the hot and cold groups, with thin mid-diaphyses, but were longer than those of the cold group and shorter than those of the hot group.

Figure 1 shows the mean vertebral lengths of the 3 experimental groups at the age of 40 days (15 days after the change of environmental temperature). The 1st caudal vertebra was marked as the one lying between the posterior tip of the iliac bones of the pelvic girdle. The general pattern in the 3 groups was similar, however, from the 4th caudal bone to the tip of the tail, the vertebrae of the cold group were considerably shorter than those of the corresponding control and hot groups. A very small difference was seen between the last sacral and the first 4 caudal vertebrae of the 3 groups.

Figure 2 shows the mean vertebral lengths of the hot and cold groups expressed as a percentage of the mean vertebral lengths of the control group. The mean vertebral length of the hot group lies above the 100% line, while that of the cold lies consistently below. In the more distal tail bones (from the 4th caudal bone outwards) the discrepancy increases to a maximum of 120% between the hot and control bones and 79% between the control and cold bones.

Alternatively because the vertebrae of the cold group were shorter than those of the control and hot group, these vertebrae could be regarded as a "control" group for the other groups.

Figure 3 shows the mean vertebral lengths of the hot (33°) and control (21°) groups expressed as a percentage of the mean vertebral lengths of the cold group (8°). Again, the general pattern between the hot and control groups was similar and the differences between the last sacral and the first 4 caudal vertebrae were small. The maximum percentage difference of 126% was seen between the terminal tail bones of the control and cold groups and 150% between the hot and cold groups.

Table I shows the lengths of the femora and 3rd metatarsal bones of the hot and cold groups. A small difference was seen between the lengths of the femora of these groups. A significant difference was seen between the lengths of the 3rd metatarsal bones of these groups.

Gross results

There was no gross morphological differences seen between the 3rd caudal vertebrae of the 3 experimental groups. It

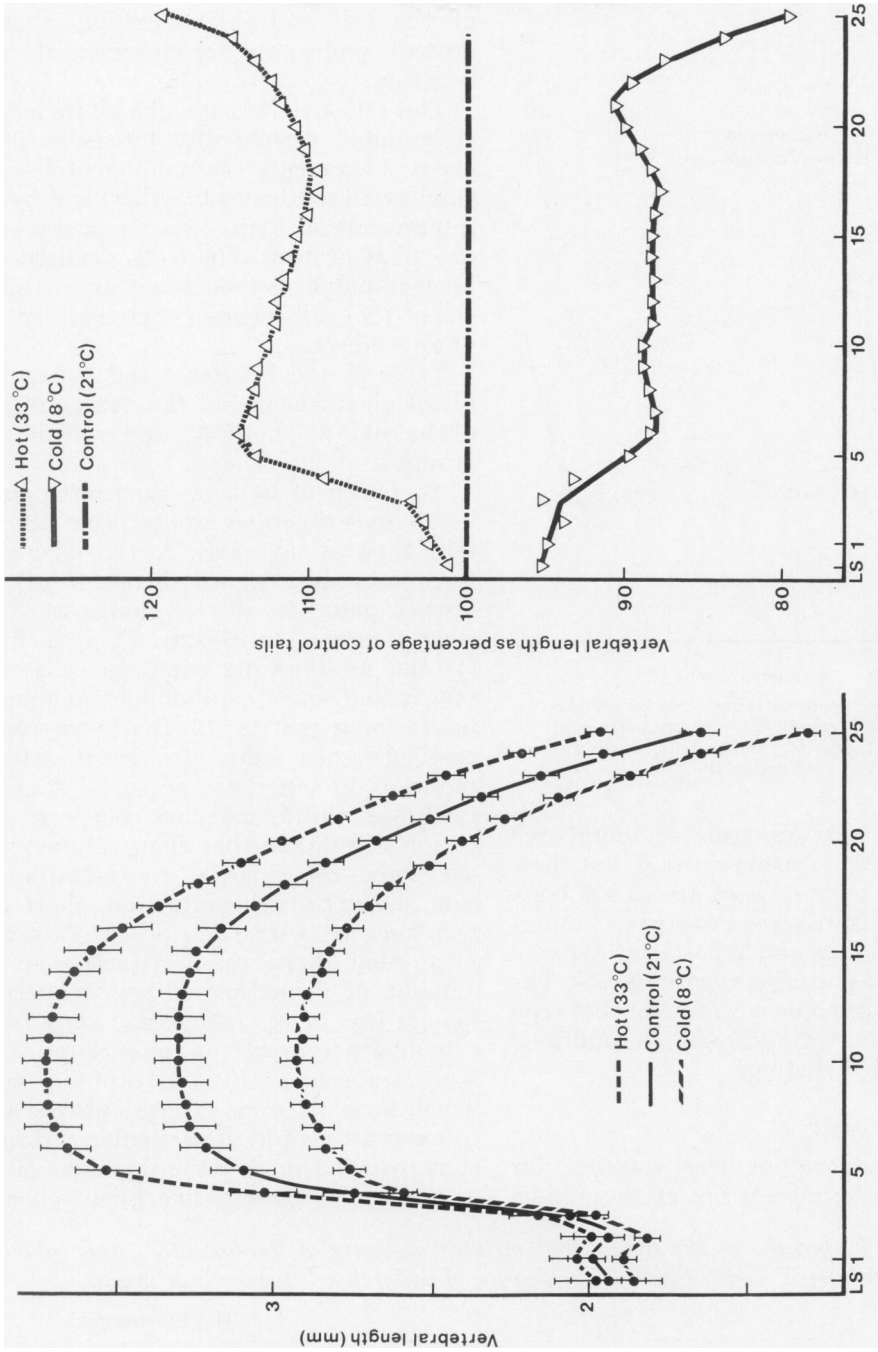

FIG. 1.—The mean lengths of the last sacral (LS) and first 25 caudal vertebrae of the cold (8°), control (21°) and hot (33°) groups at the age of 40 days.

FIG. 2.—The mean vertebral lengths of the hot (33°) and the cold (8°) groups expressed as percentages of the mean vertebral lengths of the control group (21°).

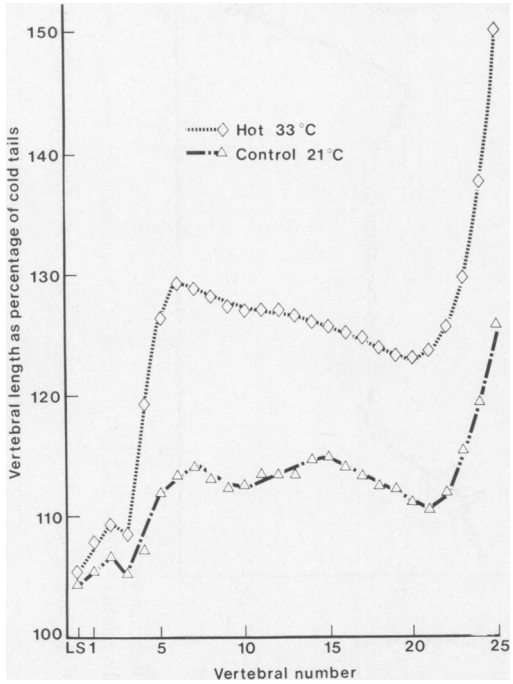

FIG. 3.—The mean vertebral lengths of the hot (33°) and control (21°) groups expressed as percentages of the mean vertebral lengths of the cold group (8°).

is made of a body (centrum), a neural arch which forms the vertebral canal, but there is no neural spine. There are also 6 bony processes: 2 transverse processes, 2 anterior zygapophyses and 2 posterior zygapophyses and 2 posterior zygapophyses. The main gross differences were seen between the free caudal vertebrae, which confirmed the radiological findings.

Histological results

The 14th vertebra was selected for examination because it lies at the middle of the tail and might exhibit average growth under any environmental temperature.

The 14th caudal bone like all its fellows is bounded peripherally by dense fibro-elastic ligaments (which encircle and connect all tail bones together) and by the intervertebral discs on its distal and proximal surfaces. These discs consist of a nucleus pulposus (which act as a cushion when the tail bends) encircled by the nucleus fibrosus.

Table II and Figures 4 and 5 show the histological features of the 14th tail bone of the cold (8°), hot (33°) and control (21°) groups at different ages.

At the age of 40 days, the growth plates in all experimental groups were thicker than those of older ages. At the older ages, the main histological features of the growth plates in all the groups were the signs of epiphyseal closure. These include: (1) the proliferating cartilage cells were scanty and set in an abundant amount of intercellular matrix; (2) the hypertrophic cartilage cells were also scanty, being reduced to an average of 1–2 cells' thickness; (3) the cartilage cells were seen to form groups rather than columns; (4) the bony trabeculae on the metaphyseal side of the growth plate were short and anastomosed with each other. This may mean that there was a reduction in the amount of formation of new trabecular bone; (5) more ostoclasts and fewer osteoblasts were seen on the surfaces of the bony trabeculae; (6) the capillary tufts which form the zone of erosion were seen to extend towards the articular cartilage, thus reducing further the thickness of the growth plate. The zone of erosion appeared

TABLE I.—*The lengths of the femur and metatarsal bones of the hot* (33°) *and cold* (8°) *groups.* P *indicates the significance of difference between these groups*

	Femur		Third metatarsal	
	Hot (33°)	Cold (8°)	Hot (33°)	Cold (8°)
No. of Cases	8	12	13	13
Age of animals	116 days		92 days	
Range	11·8–13·0 mm	12·5–13·8 mm	7·3–7·8 mm	6·2–6·5 mm
Mean	12·55 mm	21·96 mm	7·48 mm	6·38 mm
S.d.	0·45	0·41	0·16	0·12
P	0·05		<0·00001	

TABLE II.—*Main microscopic findings of the 14th caudal bone of the animals in the 3 experimental groups*

	Hot (33°)		Control (21°)		Cold (8°)	
No. of animals	3	3	3	2	4	2
Age of animals (days)	40	165	40	200	40	420
Growth plate						
1. Mean thickness (μm)						
Centre	55	76	82	67	235	88
Periphery	59	47	106	67	147	118
2. Average number of cells						
Proliferative zone	3–4	1–3	5–6	2–4	4–6	2–3
Hypertrophic zone	2–3	1–2	3–4	1–2	8–10	1–3
Diaphysis						
1. Mean total length (mm)	3·58	3·82	3·15	3·53	2·33	2·78
2. Cortical bony walls	Very thin, non-lammelar		Thin, less lammelar		Very thick, lammelar	
3. Thinning at mid-diaphysis	Smooth		Smooth		Very thin	
4. Bony trabeculae in bone cavity	Fine traversing the bone		Occasional and fine		Thick, coarse	
5. Mean thickness at mid-diaphyses (μm)	86	94	129	148	386	451
6. Mean thickness at metaphyses (μm)	57	62	115	125	257	312
7. Bone marrow	Very cellular		Fatty, moderately cellular		Fatty, less cellular	

irregular and osteoclasts were seen on the surface; (7) areas of hyalinized acellular matrix were evident extending through the growth plate. This may indicate inactivity of the cells of the growth plate. At the metaphyseal surface of the plate, the cartilage appeared basophilic indicating calcification; (8) the articular cartilage shows many irregular perforations which are filled with fatty marrow. In some cases, complete ossification of the articular cartilage was evident and a "bridge" of bone was seen connecting the body epiphyses to the trabecular bone of the metaphyses.

Epiphyseal closure occurred at an earlier age in the growth plates of the hot group than in those of the control and cold groups.

DISCUSSION

The changes in tail length brought about by changes in environmental temperature (Sumner, 1913; Sundstroem, 1922; Ogle, 1934; Harrison, Morton and Weiner, 1959; Harrison and Clegg, 1969; Chevillard, Portet and Cadot, 1963; Barnett, 1965; Noel and Wright, 1970; Pritchard, 1974; Al-Hilli and Wright, 1979) have been demonstrated to be almost entirely due to differences in the lengths of the tail vertebrae that lie outside the body. The unlikely possibility of a change in the number of vertebrae has been excluded, although there was relatively more change in the terminal bones than in the proximal or mid-tail bones. The sacral, 1st 4 caudal vertebrae and the long bones such as the femur, which lie inside the body of the mouse, are little affected by changes in the environmental temperature (Al-Hilli and Wright, 1980). These are internal bones and their temperature will approximate that of the internal temperature of the body (Noel and Wright, 1970; Al-Hilli and Wright, 1980). Thus the morphological characters of the 3rd caudal bones is not different in the tails of the mice exposed to the 3 environmental temperatures under study. On the other hand, the growth of bones other than the tail vertebrae which lie outside the body of the mouse, such as those of the feet, may also be influenced by changes in the environmental temperature (Sumner, 1913, 1915; Sundstroem, 1922; Barnett, 1965; Al-Hilli and Wright, 1980). These observations are seen here as a clear indication of local control of growth.

It has been debated whether the factors which determine the growth of tail bones

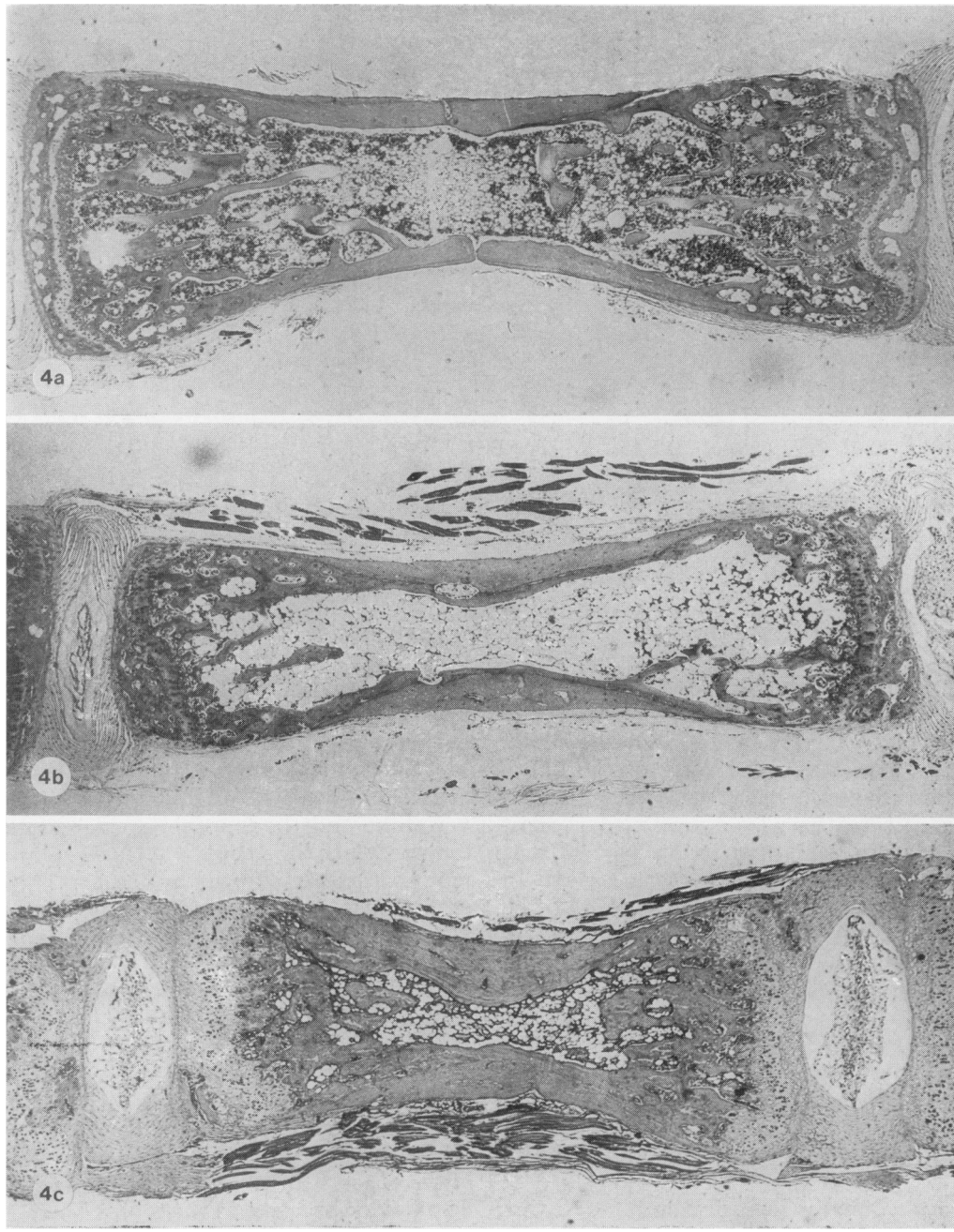

FIG. 4.—Photomicrograph (H. & E.) of the 14th caudal vertebrae of the hot (33°) (A), control (21°) (B) and the cold (8°) (C) groups at the age of 40 days. ×30.

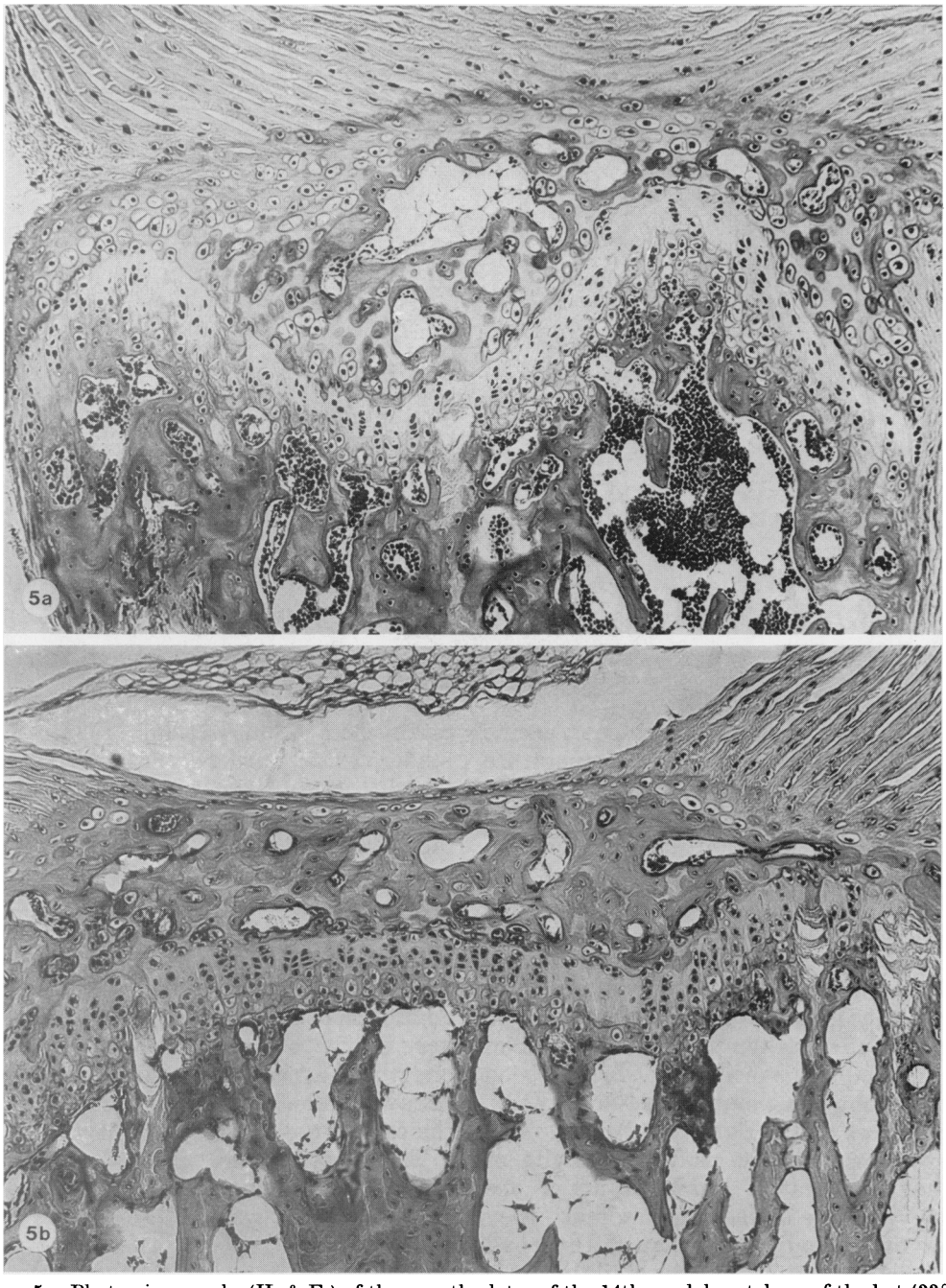

FIG. 5.—Photomicrographs (H. & E.) of the growth plates of the 14th caudal vertebrae of the hot (33°) (A), control (21°) (B) and cold (8°) (C) at the ages of 165, 200 and 420 days respectively. × 120.

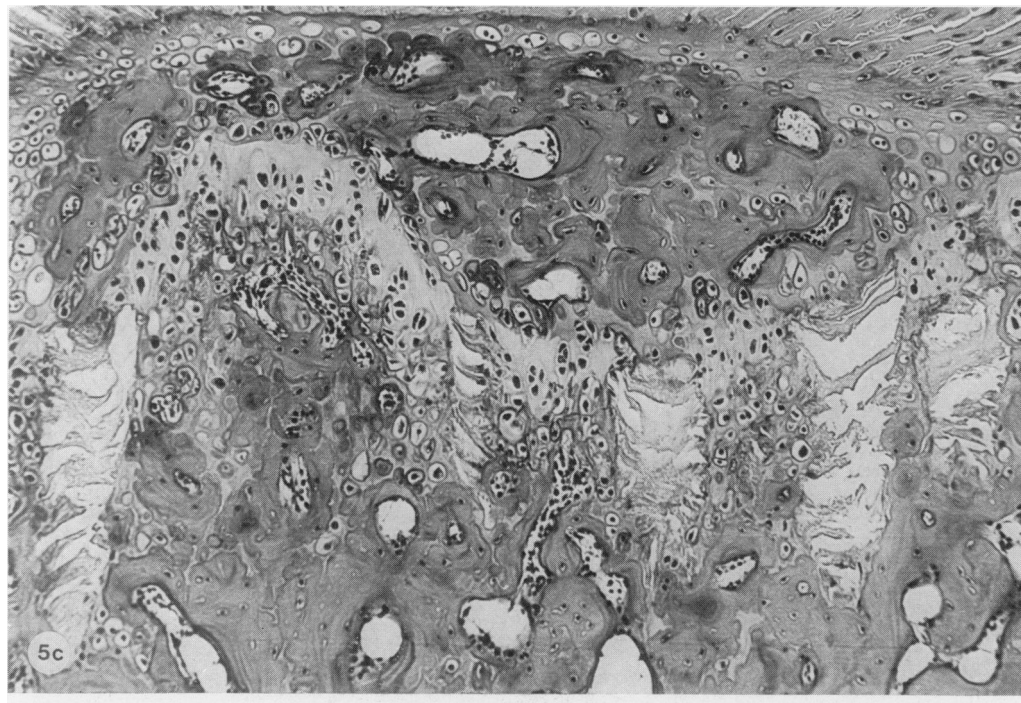

FIG. 5.—(*cont*)

in the mouse and rat represent specific adaptation to environmental temperature (Chevillard *et al.*, 1963; Harrison *et al.*, 1959; Harrison and Clegg, 1969) or local effects of the environmental temperature on the growth of bones.

In the cold environment, the shortening of tail length in relation to body size (Ogle, 1934; Barnett, 1965; Al-Hilli and Wright, 1979) indicated that the effect of temperature on the growth of tail bones was due to the local effect of temperature rather than to an adaptive mechanism. It has been shown from transplantation studies (Felts, 1961; Noel and Wright, 1972) that when the tail bones were grafted to a site of higher temperature, such as under the kidney capsule of the same animals, they grew faster and longer than if they had been left to grow on the tail in their natural position. In the renal capsular space, the tail bones were disconnected from their original conditions and were independent of environmental changes.

In the mouse, the importance of the tail as a thermoregulatory organ is well established (Sundstroem, 1922; Scholander, 1955; Barnett, 1965; Al-Hilli and Wright, 1980). The tail is not homoeothermic (Harrison and Clegg, 1969) and in fact its temperature is several degrees lower than that of the body at the usual animal house temperature of 21° (Sumner, 1913; Noel and Wright, 1970; Al-Hilli and Wright, 1980). In this work, the tail bones were left to grow on the tail and the environmental temperature was raised very nearly to that of the body. Therefore one would expect these "hot" tail bones to behave similarly to those transplanted to the capsular space of the kidney.

The "hot" bones showed cylindrical diaphyses, while both the cold and control bones showed "waisted" diaphyses. This can be explained by assuming that apposition of external surface periosteal bone is more affected by cool or cold conditions than epiphyseal growth. In addition, the "cold" bones showed thicker diaphyseal cortical bone and more and thicker woven bone in the marrow cavity. This can be explained by assuming that

only in the cold conditions is removal of bone (for remodelling as the bone grows) seriously impaired.

The thickness of the growth plate bears a constant relationship to the amount of bone produced (Tapp, 1966; Rang, 1969; Sissons, 1971; Moss-Salentijn, 1974). It is also related to the rate and number of cells produced. Thus the "hot" growth plates which had already completed most of their growth potential during the first 15 days in the hot room (Al-Hilli and Wright, 1979) appeared thinner than those of the control group. On the other hand, the growth plates of the cold groups which had contributed little to epiphyseal growth were thicker. This is related to the amount of retained growth potential.

The histological changes of epiphyseal closure in the tail bones of the mouse described here were consistent with the findings of Beck *et al.* (1948) on the 3rd metacarpal bone of the rat. However, the sequence of events in epiphyseal closure are not clear, nor are the histochemical changes. On the other hand, the factors which control this closure are also unknown. The generally held opinion is that it is related to hormonal changes (Rang, 1969) which arrest the proliferation of the epiphyseal cartilage cells with the result that bone formation may invade the growth plate. Clearly the closure may also be controlled by local factors which are independent of the level of circulating hormones. In this case the epiphyseal closure of the tail bones (and not any other bone of the mouse) can be accelerated by heat so as to be completed in 15–17 weeks (Al-Hilli and Wright, 1983) or delayed by cold for as long as 1 year.

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