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A STUDY OF FACTORS CONTROLLING AND INFLUENCING THE
GROWTH OF BONE WITH PARTICULAR REFERENCE TO THE
EFFECTS OF ENVIRONMENTAL TEMPERATURES

by

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ABSTRACT

The study is concerned with the effects of temperature on mammalian bone growth. The tail bones of the mouse were used because they tend to assume the environmental temperature at which the animals are maintained. Thus the tail vertebrae of 25 day old mice grow very slowly when the animals are placed in a cold environment (8°C), but when the animals are placed in a hot environment (33°C) the vertebral growth rate increases approximately four-fold. The change in growth rate appears to be immediate and certainly occurs in less than 2 hours and this may indicate the role of the differentiated cartilage cells in bone growth. High dose irradiation (5000 r) given to the 32 day old "cold tails" prior to being placed in the hot room does not alter the initial acceleration of growth during the first 12 hours but no growth occurs after this period. Increased growth rate after 12 hours in the un-irradiated tails is due to mitotic activity of the generative cartilage cells of the growth plates.

The growth of long bones is controlled by local mechanisms. Thus bones inside the body, including some of the proximal tail bones, are little affected by changes in environmental temperature.

The distal tail vertebrae of mice kept in the hot environment complete their development and cease growth earlier than those animals kept at lower temperatures. The tails of animals kept at 8°C continue to grow very slowly for many months although in central parts bone growth is completed normally. If these "cold animals" are placed in the hot environment at any time up to 310 days there is a spurt of growth but the final length never equals that of the animals kept constantly in the hot environment.

أَمْ يَخْشَى اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ

فِرَآنْ كَزِد

Of His worshippers,
the learned fear Him most.

Holy Koran

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CHAPTER I. GENERAL INTRODUCTION

The form of the mammalian body is largely determined by the size and shape of the skeletal system. In the adult human body the skeleton accounts for about 14% of the total body weight and with the fibro-cartilagenous intervertebral discs contributes to about 97-98% of the body height (Sinclair, 1973). The control of growth of the skeletal system has been investigated over the last 200 years by many students of growth including anatomists, physiologists, pathologists and paediatricians. Their theoretical and practical ideas about the control mechanisms of skeletal size and shape have come mainly from the study of the behaviour of the cartilage and the bone.

A. Development of Knowledge (for the bibliography cited below see Keith, 1919; Sissons, 1971). In 1727 Stephen Hales described what is generally regarded as the first scientific attempt to provide knowledge about the mode of bone growth. He simply made two small holes, half an inch apart, in the middle of the tarsometatarsal bone of a young chicken. As the limb grew the distance between the holes remained unchanged. He concluded that bone growth took place at the bone ends. This observation was confirmed by Duhamel in France and John Hunter in England.

Later, in 1736, while John Belchier was dining off a leg of pork in London he observed a red dye in the bone. The pig had been fed on the plant madder (*Rubia tinctoria*) which contains a compound similar to the dye alizarin and this had been deposited where bone was laid down and stained it red. The works which followed this famous meal were then directed towards the periosteum as a possible site responsible for the longitudinal growth of bones. Belchier, however, made no use of his observation and it was Duhamel who presented a series of experiments on fowls, turkeys, pigeons and pigs to the Academie de Sciences de Paris in 1739-1743. This was two centuries before the normal histological

structure of bone was known and almost three centuries before sophisticated autoradiographic studies. He noted that only certain parts of the bones were stained and that bones from young animals took up the dye more readily and deeply than bones of old animals. He also studied the uptake of the dye at 24, 36 and 72 hours and found that by alternating the madder diet with ordinary diet, alternating red and white rings or layers on the circumference of the bone were produced. Although he seemed to be aware of the mechanisms of longitudinal bone growth, he concluded that bones, like trees, grew in thickness by the progressive development of new bone under the periosteum.

Between 1760 and 1770 John Hunter provided a more complete description of the mechanisms of bone growth. He repeated the "marker" experiments of Hales and Duhamel and maintained that bones grew by deposition of new bone in the periosteum accompanied by bone resorption in the expanding medullary cavity. He also inserted two pellets in the tibia of a growing pig. When the tibia was fully grown the pellets remained the same distance apart. He concluded that the longitudinal growth of bones occurred at their ends.

The use of the microscope in the nineteenth century enabled the investigators to ascribe the mechanisms of bone growth to cellular processes. In the periosteal region of the long bones, the cells (i.e. osteoblasts) lay down bone by surface accretion on the pre-existing cortical bone. This type of bone formation is now known as "membranous ossification". However, the bulk of bone length is provided by the continuous proliferation of cartilage cells which are situated at the ends of the bones and known as the epiphyseal cartilage plates (or growth plates). The latter type of bone formation is now known as "endochondral ossification".

In the long bones the growth plate lies between the epiphysis and

metaphysis. Its histology has been described by many authors (Lacroix, 1951; Siffert, 1966; Ham, 1969; Little, 1973; Bloom and Fawcett, 1975 and others). The cells were artificially divided into 3 distinct zones which are commonly known as the resting, proliferative and hypertrophic zones.

B. The Control of Bone Growth

The factors which control and influence the growth of bones have been reviewed by many authors (Siffert, 1966; Rang, 1969; Sissons, 1971; Moss, 1972; Little, 1973; Potts and Deftos, 1974; Prichard, 1974 and others). It is generally agreed that longitudinal bone growth is controlled and regulated by means of local intrinsic factors which originate from the skeletal elements of the bone itself (Harrison and Clegg, 1969; Wright, 1969; Noel and Wright, 1970a, 1972; Owen, 1970, 1971; Hall, 1971; Kember, 1971; Moss, 1972; Enlow, 1973; Noel, 1973; Prichard, 1974). However, other general factors, namely hormonal and nutritional are also important. These factors not only sustain or modify the bone growth (Sissons, 1971) but also assist the action of the locally mediated factors.

a) General Factors

1) Hormonal Control. The hormonal environment is the principal physiological regulator of endochondral bone growth. The hormones of the pituitary, thyroid, adrenals, and gonads all influence the growth plate, its growth rate, pattern of growth, and time of closure. With the exception of the parathyroid hormone and calcitonin, all the endocrine hormones act non-specifically on the skeleton (Moss, 1972). The foetal hormones on the other hand, can play no part in the control of bone growth before the second month of intrauterine life, because the gonads are not formed until then. The pituitary becomes functional about the 4th month.

Because each growth plate forms bone at a specific rate, the endocrine

glands may thus influence the body proportions. Hormonal imbalance may halt the closure of the growth plates so that, in humans, the body stature and proportions may remain those of a child. On the other hand, if hormonal influences make the growth plates remain open, and growth continues, then a differently abnormal stature and shape develop.

i) Growth Hormone (for review and details see Urist, 1972). This is a polypeptide hormone secreted by the anterior lobe of the pituitary gland. Excessive secretion produces gigantism in children and acromegaly in adults while deficiency of the hormone produces dwarfism in children.

It is secreted throughout life and has very constant level during early life (Turner, 1972), although racial variations in growth hormone titre do occur (Little, 1973). For example, adult negroes tend to retain greater potential for the secretion of the hormone than adult whites.

The importance of the growth hormone in the regulation of bone growth is its role in the control of cell division at the germinative zone of the growth plate (Rigal, 1964). It plays no part in promoting closure of the plate (Rang, 1969), and probably does not act directly on the skeleton, but by means of an intermediary compound (sulphation factor) released possibly by the liver (see Potts and Deftos, 1974).

The growth hormone is also known to stimulate the biosynthesis of collagen (Prockop and Kivirikko, 1968; Woessner, 1968). This anabolic action, however, is of doubtful significance in the control of the shape and size of bones (Moss, 1972).

The studies of Harris and Heaney (1969) have suggested that growth hormone may increase the rate of mineral accretion, but it is not clear whether this effect is separate from that of stimulation of matrix formation. It could equally be argued that osteoporosis (and not osteopetrosis) is commonly seen in conditions associated with the excessive secretion of the hormone such as acromegaly.

ii) Thyroid Hormone. This hormone has a general anabolic action on the cellular metabolism. It controls the biosynthesis of collagen and thus influences bone formation and resorption (Potts and Deftos, 1974). Bone turnover is known to decrease in hypothyroidism and to increase in hyperthyroidism. The hormone has no direct effect on calcium and phosphate metabolism.

Hyperthyroidism in children is usually associated with advanced skeletal maturation and acceleration of linear growth so that it exceeds the chronological age (Schlesinger and Fisher, 1951). On the other hand, hypothyroidism in childhood (cretinism) is associated with retardation of skeletal growth, delayed appearance of ossification centres and the persistence of a thick growth plate. The changes in the growth plate in cretinism may be due to the specific action of the thyroid hormone on the metabolism of mucopolysaccharides (Rang, 1969) so that cretinism may be a form of mucopolysaccharidosis (Krompecher, 1961a, 1961b, 1962; Rang, 1969).

The relation between the thyroid and growth hormones and their effects on bone growth were studied by Simpson et al (1950) and Ray et al (1954). It was noticed that thyroidectomy lowers the growth hormone production. Therefore it is difficult to assess whether growth deficit following thyroidectomy is due to the reduced pituitary output or that of the lowered thyroid level.

iii) Gonadal Hormones (for review and details see Silberberg and Silberberg, 1971). These are also anabolic hormones but their effects vary according to the dosage. Small doses of testosterone are associated with increased growth rates, while large doses lead to growth retardation. On the other hand, large doses of oestrogens increase trabecular bone formation and result in retardation of bone growth (Silberberg and Silberberg, 1941; Gardner and Pfiffer, 1943; Tapp, 1966). Of particular interest is the selective effect of the ovarian hormones on the osteo-

blasts and osteoclasts of the bones of the pelvis and shoulder in the female (Sinclair,1973). This effect, however, which determines the feminine configuration is not clearly explained.

The gonadal hormones determine skeletal maturation and closure of the growth plate, and hence the cessation of endochondral bone growth at puberty. The mechanism of this effect is not clearly understood. Precocious puberty in children is known to be associated with premature closure of the growth plate. Conversely, castration in both male and female animals leads to delayed skeletal maturation. Thus in eunuchs some bones may continue to grow for abnormally long periods if the epiphyses are not closed at the time of puberty.

The androgens secreted by the adrenal glands are also important in bringing about skeletal maturation in both sexes in the absence of other hormones affecting epiphyseal closure. For example, congenital adrenal hyperplasia is usually accompanied by premature closure of the growth plate after a period of rapid growth.

iv) Adrenal Steroids (for review and details see Silberberg and Silberberg,1971; Little,1973). In children with asthma or rheumatoid arthritis large doses of cortisone reduce the proliferation of the cartilage cells in the growth plate and result in skeletal growth retardation. But if adrenocorticotrophic hormone is given instead of cortisone, a normal growth rate can be restored (Friedman and Strang,1966). Cortisone may not act directly on the growth plate but indirectly by depressing the pituitary gland and reducing the growth hormone level (Dent,1969).

Cortisone was also reported to stimulate the coalescence of the osteogenic precursor cells to form osteoclasts (Little,1973). It therefore enhances bone resorption (Potts and Deftos,1974).

v) Parathyroid Hormone and Calcitonin (for review and details see

Hirsch and Munson,1969; Copp,1972; Little,1973; Potts and Deftos, 1974). These hormones have no effect on the growth plate itself, but are principally concerned with the control of bone mineral homeostasis; parathyroid hormone stimulates bone resorption while calcitonin inhibits resorption.

It has been suggested that bone remodelling is regulated by two forms of control mechanisms (Harris and Heany,1969). The first is hormonal (parathyroid hormone-calcitonin) and acts systematically anywhere in the osseous tissue to subserve mineral homeostasis, i.e. homeostatic remodelling. The second mechanism reflects the effects of extrinsic mechanical forces where the mass and arrangement of the skeletal materials are altered locally at certain selective sites of the skeleton to meet the changing needs, i.e. structural remodelling.

2) Dietetic Factors

Adequate food is essential for the maintenance of normal body and skeletal growth. Malnutrition and undernutrition not only result in deficiencies of certain dietetic factors but also general retardation and cessation of growth (see Tanner,1962).

About one third of the body protein is collagen (Underfriend,1966) and about 70% of this is found in the bone where it forms the bulk of the organic matrix of the bone (Rang,1969). Thus a protein deficient diet (e.g. Kwashiorkor) is associated with retardation, even arrest, of skeletal growth and the appearance of "transverse lines of growth arrest" which are seen radiologically at the growing ends of long bones (Follis and Parks,1952; Park,1954). The bones become shorter, thinner and lighter than normal and the appearance of ossification centres will be delayed (Jones and Dean,1956,1959; Dickerson and Widdowson,1960; Le Roith and Pimstone,1973). Chondrogenesis is also disturbed (Silberberg and Silberberg,1940; Frandsen et al,1954; Acheson,1959). Accordingly,

the proliferation of cartilage cells in the growth plate is arrested and the plate becomes thin.

Certain other metabolic factors, namely calcium and phosphate (see Potts and Deftos, 1974), and vitamins D and C (see Bourne, 1972; Cousins and De Luca, 1972; Omdahl and De Luca, 1973) are also required for normal bone growth. The presence of these factors in sufficient concentration in the body is not only necessary for skeletal homeostasis, but deficiency as well as excess may result in metabolic derangements.

Vitamin D is important in bone growth because, together with parathyroid hormone, it regulates the calcium and phosphorus balance. Its deficiency results in rickets which is characterised by failure of calcification of the cartilage. The proliferation of cartilage cells is not affected, but the hypertrophic cells fail to degenerate, the growth plate becomes widened as the epiphyseal columns elongate, the uncalcified matrix cannot be invaded by the metaphyseal blood vessels and any osteoid is poorly calcified.

Vitamin C is essential for the biosynthesis and maintenance of the integrity of the collagen. Its deficiency results in scurvy which is characterised by the failure of the bone matrix formation but the proliferation and calcification of the cartilage cells remain unaffected.

Vitamin A is also an essential metabolic factor necessary for the growth and maintenance of normal skeletal structures (see Barnicot and Datta, 1972). It accelerates the histological sequences of endochondral bone growth and bone remodelling. Experimental lack of vitamin A is associated with a decreased rate of chondrogenesis, irregular cartilage palisading, and alteration in bone remodelling. Its excess, however, may lead to metaphyseal osteoclasia and diminished osteogenesis.

b) Local Control of Bone Growth

The growth cycle of the growth plate consists of 3 inter-related

processes: (1) The division of cartilage cells at the germinative zone, (2) Proliferation and cytodifferentiation along the epiphyseal columns, and (3) Maturation, selective cell death, removal of the cartilage cells and calcification of the matrix. The mechanisms which control this cellular repertoire are not clear and it seems that genetic factors play the most important part (see Hall,1971). One may postulate 3 groups of genetic factors for the 3 principal processes of endochondral ossification.

The first group of genetic factors may control the rate and number of cell divisions in the generative cartilage zone. It is evident from transplantation studies on isolated blocks of the growth plate that the cartilage cells have a considerable autonomous potential for growth (Lacroix,1951; Felts,1961; Koski and Rönning,1969; Dorenbos,1972; Noel and Wright,1972; Noel,1973; Servoss,1973 and others). This potential has been attributed to intrinsic genetic control in the growth of bones (Siffert,1966; Rang,1969; Wright,1969; Hall,1971; Moss,1972; Little, 1973; Pritchard,1974) and would simply mean that the total number of cell divisions occurring in the germinative zone of the growth plate are also genetically predetermined by the intrinsic growth property of the bone (Rang,1969; Wright,1969).

The second group of genetic factors may control the differentiation of the cartilage cells in the epiphyseal column. Using tritiated thymidine, it has been shown that these cells are unable to divide. Cell division is a process which involves changes in the DNA synthesis. The DNA of the nucleus not only transmits genetic information to the daughter cells but also determines the specific morphological and enzyme-chemical characteristics of each cell (Bloom and Fawcett,1975). The inability of the above cells to divide may be due to the loss or suppression of some of the DNA genetic code after division, i.e. they do

not require increased DNA synthesis. However, it may be that the genetic enzyme-chemical properties may be retained after division and these may in turn determine the subsequent stages of cartilage cell hypertrophy and degeneration. It also appears that the cartilage cells at every level in the epiphyseal columns are predetermined towards the completion of certain functions.

The longitudinal growth of the cartilage plate occurs as a result of cell multiplication and matrix elaboration. The formation of the matrix seems to be the function of those cartilage cells of the proliferative zone where normally an abundant amount of matrix is seen. In the hypertrophic cartilage zone the matrix is reduced to thin and narrow bands. Apparently the secretion of the matrix as early as the proliferative stage is to prepare the growth plate for the calcification which occurs just before bone formation. As a whole, the matrix stains intensely and metachromatically with basic dyes, including alcian blue, but only the matrix associated with the hypertrophic cells is periodic acid-Schiff (PAS) positive. This indicates that while all matrix is rich in acid mucopolysaccharides, glycoproteins are chiefly confined to the hypertrophic zone. This is also seen in the histochemical changes of the proliferative cells which accumulate increasing amounts of glycogen, but as these cells hypertrophy, they lose their glycogen to the matrix.

The enzyme alkaline phosphatase, on the other hand, cannot be demonstrated in the proliferative cells, but begins to appear as the cells hypertrophy, and becomes very intense as glycogen disappears (Rang, 1969; Bourne, 1972). Even the matrix around the cartilage cells gives an intense reaction for the enzyme just before the zone of calcification is reached.

The regulation of these enzyme-chemical properties of the cartilage

cells as well as their selective activity and function at a certain level in the epiphyseal column is not known. These cells probably retain their enzyme-chemical potentials after division but they only manifest this potential with certain differences in the microenvironment, possibly variations in ionic concentration between the cells and the matrix. These highly selective properties must also indicate the role of genetic control. It also seems that the different functional potentials of these cells are controlled by a negative feedback mechanism so that a particular potential can only be brought about after the completion of the preceding potential (Hall, 1971).

The subsequent stages in the epiphyseal development are calcification of the matrix, selective degeneration and death of the cartilage cells, breakdown of the calcified matrix (vascular invasion and osteoclast/chondroclast activity) and its replacement (osteoblastic activity) by uncalcified matrix (osteoid) which calcifies to form bone. These stages are possibly controlled by the third group of the local intrinsic genetic factors, which may operate via several negative feedback mechanisms so that a particular stage can only be manifested after the completion of the preceding ones.

Calcification of the matrix is known to begin just proximal to the level of cell degeneration in the form of random deposition of inorganic salts in the fibrillar matrix (Siffert, 1966; Little, 1973). In this area, the calcium crystals render the chondrocytes impermeable to metabolites so they die. On the other hand calcification of the lamellar osteoid on uncalcified bone matrix (Little, 1973) occurs in an orderly fashion with the crystal axis lining up with the longitudinal axis of the collagen fibres in the matrix (Siffert, 1966). These calcification areas, which are localised to certain parts of the epiphyseal column, may be determined by the genetic enzyme-chemical potentials of these

areas as well as factors in the microenvironment such as the concentration of alkaline phosphatase. It also emphasises the importance of calcium and other inorganic salts in the microenvironment of the growth plate and mineralisation of bones.

It is generally agreed that both the osteoclasts and osteoblasts arise from a common stem cell whose intrinsic genetic information is responsive to extrinsic factors in the microenvironment (Owen,1970,1971; Hall,1971; Moss,1972). However, the trigger stimuli to which the stem cells, the osteoclasts, and the osteoblasts may respond are not clear. It is possible that the chondrocytes transmit their messages to these cells across altered cell surfaces via an extracellular messenger such as acid mucopolysaccharides, collagen, proteins and altered ionic micro-environment. The particular chondrocytes which are responsible for this message may again be determined by their position in the epiphyseal columns.

Finally, the existence of uncalcified cartilage and osteoid in the final stages of the epiphyseal cycle needs questioning since both types share the same fate. Could not the growth plate form osteoid without the formation of the uncalcified cartilage as an intermediary step? It seems that this two-stage process is dictated in the evolution of endochondral bone formation. Perhaps interstitial growth is not possible in the osteoid because of its great affinity to calcium. On the other hand, the osteoid is not as strong as the cartilage and thus it is the site of fracture in osteomalacia. Moreover this two-stage process may be necessary to build up more materials or growth factors.

Another indication of the genetic role is seen in the work of Ring (1955a,1955b,1955c) who found that when the distal growth cartilage of the ulna in rabbits was excised and reversed so that the metaphyseal side of the plate lay against the epiphysis, bone growth occurred towards the epiphysis. He concluded that the activities of the growth

plate were predetermined and that its polarity was determined by its structure rather than its site.

The variations in racial and familial stature (see Tanner, 1962) are further clear reflections of the genetic influences on the activities of the growth plate (Rang, 1969) although a variety of socioeconomic factors is also involved. Indeed departure from the genetic mechanism and its enzyme-chemical control may result in localised (e.g. brachydactyly, syndactyly) or generalised (e.g. osteogenesis imperfecta, multiple exostosis, osteochondrodystrophies) skeletal abnormalities.

Of particular interest in skeletal growth studies is achondroplasia which is inherited in man as a single dominant trait (Cockayne, 1933). It affects all growth plates in the body and not other forms of cartilage, e.g. articular, hyaline cartilage (Rang, 1969). The skeletal anomalies in man and animals have been described by many authors (see Maroteaux and Lamy, 1964) but the cause is still unknown. In the long bones of achondroplastic mice, the growth plate was found to be made of short epiphyseal columns; small chondrocytes which were surrounded by a large amount of matrix but there was no hypertrophic cartilage zone (Silberberg and Lesken, 1975). In addition, epiphyseo-diaphyseal union was also described. It seems, therefore, that all the processes of endochondral bone formation were affected (Sissons, 1971) and that cell division in the germinative zone and the proliferation of the chondrocytes may be maintained at a slow rate. As a result the epiphyseal columns become short with the accumulation of excessive amounts of matrix. The failure of the cells to hypertrophy may be due to the absence of certain negative feedback genetic controls, referred to earlier. It is also possible that the total number of cell divisions dictated by the genetic control is limited, therefore, the growth plate matures and closes earlier than that of the non-achondroplastic animals.

It is unlikely that hormones modify endochondral bone growth in achondroplastic mice. Somatotrophin levels were found to be normal (Konyukhov and Paschin, 1967) and the almost equal sex distribution of the disease precludes the possible influence of sex hormones.

A number of skeletal anomalies are also known to occur in a group of biochemical disorders referred to as genetic mucopolysaccharidosis (see Rang, 1969; McKusick, 1972; Bornstein, 1974). These anomalies include dwarfism, abnormal facies, scaphocephaly, kyphosis, short broad hands, stubby fingers, abnormalities of the thoracic cage, joint contractures, genu valgus, joint laxity and a variety of distinctive radiological appearances. Among the genetic mucopolysaccharidoses with skeletal involvement are Hurler's syndrome, Hunter's syndrome, Sanfilippo's syndrome (polydystrophic oligophrenia), Morquio-Ulrich syndrome and Maroteaux syndrome (polydystrophic dwarfism). Congenital hypothyroidism is also regarded as a form of mucopolysaccharidosis (Krompecher, 1961a, 19691b, 1962) because of its resemblance to Hurler's syndrome. For such conditions the term "acquired mucopolysaccharidosis" was introduced by Rang (1969).

Several other heritable and chromosomal disorders with biochemical disturbances are also known to affect the skeleton. These include Gaucher's disease, Nieman-Pick disease, Albers-Schonberg disease, renal osteodystrophy, cleidocranial dysostosis, melorheostosis, hereditary multiple diaphyseal sclerosis, osteopathia striata, osteopathia condensans, hypophosphatasia, phenylketonuria, Marfan's syndrome, mongolism, Turner's syndrome, Klinefelter's syndrome and many other diseases. To avoid confusing terminology the classification approved by the Paris Nomenclature for Constitutional Disorders of Bone in 1969 (McKusick, 1972) is helpful.

The congenital hypertrophy involving one side of the body, single

limb, or bone as well as the absence of a limb (amelia) or underdevelopment (aplasia) and of antenatal damage (rubella, syphilis) represent conditions of defective growth and intrauterine dysmaturity (see Rang, 1969). They cannot be regarded as a genetic inheritable disorder.

Other Local Control Factors

1. Vascularity of the Growth Plate. It has long been noticed that increased blood flow at the growing ends of bones (e.g. nearby arthritis, osteomyelitis, hyperaemia, arteriovenous fistula, aneurysms) is often associated with overgrowth of that bone and limb length inequality. This led Trueta et al (1953, 1960a, 1960b, 1960c, 1963a, 1963b, 1964) and many other investigators (see Brookes, 1971) to suggest that the particular shape, number and arrangement of the vessels of the growth plate may determine the growth of its cartilage cells.

With the aim at correcting limb length inequality in children, the blood flow to the growth plate was successfully increased by such surgical measures as periosteal stripping, artificial arteriovenous connections and sympathectomy. The resultant bone overgrowth is more probably secondary to changes in the metabolic activities of the cartilage plate rather than primarily to increased blood flow (see Licht, 1965).

2. Mechanical Forces. The role of pressure and tension on the cellular dynamics of bone structure is debatable (see Murray, 1936; Smith, 1962a, 1962b; Little, 1973). Thus on one hand the compression forces were believed to inhibit bone growth and produce atrophy while tension (e.g. increased muscle activity) increases bone growth and leads to the formation of osseous tissue. On the other, both compression and tension were claimed to increase bone growth and the formation of osseous tissue.

It has been suggested recently that the basic architecture of the

bone is not dependent on mechanical forces and that the form of the bone is indicated as early as the embryonal mesodermal condensation (Hall,1971). Thus the long bones of the mouse were found to grow normally in vitro even if subjected to extreme longitudinal compression forces beyond those normally encountered in vivo (Monson and Felts,1961).

It seems likely that mechanical forces exert their effects on the bone architecture through changes in the blood flow and pressure of the bone marrow (Little,1973). Thus diminished muscle activity increases the marrow pressure and stimulates bone removal, but without sufficient blood flow to stimulate new bone formation. This results in "disuse osteoporosis". On the other hand increased muscle activity after a period of inactivity may result in a greater rate of bone removal than formation. This may result in temporary osteoporosis but soon after the establishment of normal blood flow and marrow pressure normal rates of bone remodelling may be restored.

According to Bassett (1971) the stressed bone exhibits measurable electrical potentials and these may change the rates of bone remodelling. Thus compression forces may lead to concavity of the bone surface, accumulation of negative electric potentials, and bone deposition. On the other hand, tension forces may lead to convexity of bone surface, accumulation of positive potentials, and bone removal.

The response of the growth plate to compression forces is entirely different from that of the bone. Thus when the forelimbs of newly born rats were amputated so as to alter the naturally occurring stresses, a relatively slight change was observed in the hind limbs (Colton,1929). Significant changes can only be produced with sustained forces greater than those experienced normally (Gelbke,1951; Sissons,1971). Such forces were found to slow or even stop the growth of the cartilage plate (Arkin and Katz,1956; Rang,1969). As a result, the plate becomes

narrow and distorted with irregular cartilage columns (Gelbke,1951).

It has also been shown that epiphyseal stapling is often accompanied by arrest of bone growth at the stapled end of the bone and overgrowth at the other end (Hall-Craggs,1968; Hall-Craggs and Lawrence,1969). Removal of the staples was found to be followed by normal growth rates at both ends. Similarly, when the proximal cartilage plate of the tibia in rats was irradiated with 2400 r, "compensatory growth" occurred at the distal tibial plate (Dawson and Kember,1974). These findings suggest the possibility of a balanced differential growth potential between the growth plates at both ends of the long bones. This may be mediated through inhibitory or stimulatory local intrinsic factors between these plates. Such factors may not only operate during stress but also control the normal growth rates of both cartilage plates.

3. Differential Growth Rate. Certain growth plates of the human skeleton, namely those of the proximal ends of the tibia and fibula and the distal end of the femur in the lower limb and the distal ends of the ulna and radius, and the proximal end of the humerus in the upper limb, grow faster and close earlier than the growth plates at the other ends of these bones. This differential growth potential has been attributed to rapid cell division, differentiation, and removal of the cartilage cells (Moss-Salentijn,1974). These phenomena again direct attention to the role of genetic control. Another factor may be that the net compressive forces at the shoulder and wrist are less than at the elbow, while the hip and ankle have higher net compressive forces than the knee (Moss,1972).

c) The Local Extrinsic Growth Factors

From the above review of the local control factors, it is important to distinguish between the growth of skeletal tissues (bone and cartilage) and skeletal organs (entire bones or systems of interrelated bones,e.g.

bones of lower limb). Thus, whereas the ability of the cartilage model to grow in vivo and in vitro unquestionably demonstrates the activity of the genetic mechanisms, the growth of any anatomical form of skeletal organs must therefore be controlled by the same direct and local genetic factors. Of course, the skeletal organs are influenced by other local factors (e.g. degree of pressure, position of the growth plate), but it is the genetic control which determines the shape and form of these organs (Hall,1971).

It has also been shown that the cell division of the cartilage cells in the epiphyseal columns as well as their differentiation, maturation and selective cell death are also genetically predetermined. These processes are, however, greatly influenced by extrinsic micro-environmental factors which act primarily on the genetic mechanisms (Moss,1972). Therefore, it appears that there are two groups of local factors. The first are the genetic (intrinsic) factors which control the growth potential of the cartilage cells and their differentiation. This means that the basic shape and form of the bone is inherited in the bone itself. The second group of factors are the microenvironmental (extrinsic) factors. These occur outside the cartilage cells, but their local effects on these cells may change the genetic mechanisms and the growth potential.

The local extrinsic factors may include local environmental variables, pH level, pressure, oxygen tension, bio-electric potentials, chalone-like inhibitors and stimulators, cyclic AMP, vasomotor control, membrane control, neurotropic control, nutrition, local enzymes and hormones, and many other factors (Enlow,1973).

The environmental variables include climatic temperature, humidity, atmospheric pressure (Harrison and Clegg,1969), acoustic waves and radiant energy. Among these variables, the environmental temperature

appears to be one of the most important local extrinsic control factors (Noel and Wright, 1970a) and its effects on the genetic growth potentials of the cartilage cells may help in the interpretation of the growth responses. It was, therefore, the aim of the present study to elucidate the effects of environmental temperature on the growth of bones.

Albino mice were selected as suitable experimental animals - (1) Because of their rapid maturity and longevity. The first few weeks of the mouse's life corresponds to several years in terms of human life. (2) Because there are many reports about the growth and development of mice. (3) Because they are hardy animals, able to survive the manipulation and environmental changes involved.

The tail vertebrae were selected as a growth model because (1) the tail can be subjected to extreme conditions without affecting other biological systems, (2) the tail is readily accessible so that any deviation in its growth rate can at once be detected, precisely measured, and further investigated by such methods as radiography, and (3) the tail contains between 27 and 30 free caudal vertebrae (Rowett, 1974). The amount of growth their growth plates can provide makes the tail a favourable organ for skeletal growth studies.

C. Local Effects of Temperature on Bone Growth

Heat has long been used to relieve aches and pains, treat ulcers and inflammation, and to promote the healing of wounds and fractures. It is also used to increase the blood flow to tissues and organs in the treatment of rheumatic diseases and skeletal and neuromuscular disorders (see Licht, 1965). The raising (or falling) temperature was also used with a view to accelerate (or slow) cell division and metabolic activities.

The effects on bone growth of changes in temperature has been studied from two points of view. Firstly using mice and rats, investigators have been concerned either to study the effects of various changes in the

ambient temperature on the growth of such peripheral organs as the tail (Sumner, 1909, 1915; Sundstroem, 1922; Przibram, 1931; Ogle, 1943; Ashoub, 1958; Chevillard et al, 1963) or the response of different animal genotypes to these changes (Harrison et al, 1959, 1963, 1964, 1969; Barnett, 1965). Most of these workers were not aware of the growth of tail bones and others used the tail to study the mechanisms of thermoregulation (Harrison, 1958; Barnett, 1965; Rand et al, 1965; Scholander et al, 1950a, 1950b, 1950c, 1955; Whittow, 1971; Yousef et al, 1972).

Secondly investigators have studied the local application of heat and cold on the longitudinal growth of bones. Scow (1949) studied the freezing effects of -10°C on the tail bones of newborn rats and reported retardation of the tail growth, deformity and angulation, failure of calcification and necrosis of the cartilage plate.

With the aim of correcting limb length inequality in children, the effect of a local rise of temperature on the longitudinal growth of bones was also studied by many experimental and clinical investigators (see Sunden, 1967; Rang, 1969). In these studies, the local rise of temperature was brought about indirectly by such measures as increasing the blood flow, nervous intervention and the implantation of various materials near the growing bone. Studies on the biological effects of direct application of heat to the growing bone were few.

Nonetheless, when the temperature of the femur in dogs and rats was raised by means of an electrically heated wire, increased longitudinal bone growth was produced (Richards and Stoffer, 1959). This effect has also been attributed to the hyperaemia demonstrated by injection studies. However, when the distal bony epiphysis of the ulna in rabbits was heated using a carbon resistor, no longitudinal bone growth was noticed (Ring and Lee, 1958).

Another positive effect was produced by Doyle and Smart (1963) who

applied moderate short wave diathermy to the distal femoral region of rats and reported an increase of bone growth of 0.4-2.8 mm. The effect was also attributed to heat and increased blood flow. According to Wise et al (1949), short wave and microwave diathermy of 75 volts may damage the bone and result in growth retardation in young rats. This amount of heat has been considered by some authors (Doyle and Smart,1963; Granberry and Jones,1963) to be too large. The clinical reports on the effects of short wave diathermy on the longitudinal growth of bones have been contradictory (Wilson and Thomson,1939; Bertrand and Trillat,1948).

The effects of ultrasonic energy on the growth of bones has received considerable attention (Bender et al,1953,1954; Ardan et al, 1957; Janes et al,1956; Herrick et al,1956). Thus the application of as much as 10 watts of ultrasonic waves for 10 minutes to the upper tibial epiphysis of growing dogs and rabbits produced no acceleration of the longitudinal bone growth, but resulted in bone necrosis and fractures (Frost et al,1953; Buchan,1970). This was attributed primarily to the temperature rise resulting from the absorption of ultrasonic energy by the bone and soft tissues (Lehman et al,1967; Dyson and Pond,1970; Hill,1972).

CHAPTER II GENERAL MATERIALS AND METHODS

A. Experimental Animals

Strain A, Albino mice, of both sexes, inbred (brother-sister mated) at King's College Hospital Medical School were used. They were allocated to the different experimental groups by using a table of random numbers based on body weight and tail length.

Cages: The animals were kept in metal cages with wire mesh tops 36 x 16 x 11 cm. Food (Oxoid Ltd., Laboratory Animal Diet, Diet 41-B-modified) and water were supplied ad libitum. The floors of the cages maintained in the hot room were covered with a thin layer of saw-dust and wood shavings. In the cold room, nests of thin non-absorbent cotton-wool wrapped around wood shavings were provided.

B. Experimental Environmental Conditions

1. Previous Attempts. When this work was started the most difficult task was the maintenance of the experimental animals in extreme environmental conditions, especially the cold temperature. Because most growth in tail length occurs in the first 4-5 weeks after birth and particularly the first fortnight of life (Noel, 1968), a significant change in the growth rate of tail length was thought to be most easily demonstrable during this period. But when one week old mice and their mothers were given ample cotton-wool for nest construction and housed at $3 \pm 1^{\circ}\text{C}$ the young mice died after 2 days. For better adaptation, "trained" pregnant female mice (15 days of gestation) were provided with thick cotton wool covers in their cages and housed at the same temperature. The mothers built nests inside the cotton-wool, but died later. In addition, 10-12 days old mice and their mothers were helped by providing nests (described above) and housed at $3 \pm 1^{\circ}\text{C}$ but these animals also failed to thrive, and died.

By pumping warm air ($20 \pm 2^{\circ}\text{C}$) at a rate of 7 litres/min into a wooden box, the temperature inside the box was raised to 8°C . When one

week old mice and their mothers were provided with a cotton-wool nest and housed in this new cold environment, the young mice survived and thrived. But this procedure did not alter the growth of the young mice because (presumably) the mothers were able to keep their young warm.

To find the optimal hot temperature, one week old mice and their mothers were kept in an incubator (Gallenkamp Incubator, Size Three, Model 1 H-100) at 36°C . The young mice died after about 1-3 days but the mothers were able to adapt to this environment. The temperature was then lowered to 33°C . Four "trained" pregnant female mice (15 days of gestation) were housed at 33°C but when they gave birth the young mice grew very slowly, were underweight and most of them died (two survived to be mentioned later). The maintenance of the one week old litter at 33°C was associated with a mortality rate of more than 45%.

2. Current Procedure. Although only about 3 cm of tail growth remained to be grown after the age of 21 days, the following standard method was used in this study: - The animals were weighed and marked by nail clipping at the age of 17-18 days, weaned at 22-23 days and housed in the allocated environmental conditions at 25 days old. The tail lengths and body weights were recorded daily and the cages were changed every week.

The three main environmental conditions used in this study were as follows:

i. The hot environment (hot room). Consisted of an incubator (described above) which is maintained at a constant temperature of 33°C . The air inside this incubator was stirred by a fan. In addition, air ($20 \pm 2^{\circ}\text{C}$) at a rate of 14 litre/min was pumped through a water-filled plastic jar which was kept inside the incubator.

ii. The temperate environment (control temperature) was that of the Animal House, King's College Hospital Medical School maintained at $21 \pm 1^{\circ}\text{C}$.

iii. The Arctic environment (cold room) consisted of a wood-walled

	HOT		TEMPERATE		COLD	
	T($^{\circ}$ C)	R.H. (%)	T($^{\circ}$ C)	R.H. (%)	T($^{\circ}$ C)	R.H. (%)
Range	32.9 - 33.2	49-55	16.8 - 32.8	30-101	7.8 - 8.5	58-84
Mean	33.01	57.71	21.38	74.08	8.04	71.00
S.D.	0.14	3.37	2.41	14.16	0.23	9.63

Table 2.1 The temperature (T° C) and relative humidity (R.H.%) of the three environmental conditions during the period February - December 1976

incubator (Charles Hearson & Co.Ltd., London SE1) fitted with a thermostat (Model C/5, Greenhouse Thermostat, Humey, Byfleet, Surrey, England) to give a constant temperature of 8°C . This incubator was kept in the cold room at an external temperature of $3 \pm 1^{\circ}\text{C}$ and the air inside it was warmed by pumping air at $20 \pm 2^{\circ}\text{C}$ directly into it.

3. The Control of Environmental Temperature. The pumping of air into the incubators not only supplied the animals with enough air to breath but also controlled the humidity level. In the cold room it dried the condensed water vapour on the inside walls which are made of metal, and helped to maintain the small rise in temperature necessary to reach 8°C .

The temperature was regulated thermostatically. In addition, the temperature and humidity were recorded and observed daily on a set of thermohygrometers and maximum-minimum thermometers. Table 2.1 shows the ranges, means and standard deviations of the daily readings at 1200 hour during the period from February-December 1976. The minor variations in temperature and humidity levels that occurred in the hot room depended on the amount, state and temperature of the water in the plastic container and the frequency of opening and closing the door of the incubator.

In the cold room the slight variations depended on the number of animals and cages housed in it at any one time. High temperature and humidity levels were observed when the maximum number of cages (8 cages) and animals (64 mice) were housed because the pumped air failed to dry all the water vapour which had condensed from the air exhaled by the animals. Attempts to increase the rate of pumped air caused the temperature inside the cold room to rise to a higher level. To avoid these variations the number of animals and cages and animals at any time was kept to a maximum of 4 cages and 25 mice.

On the other hand, the wide variations in temperature and humidity which were observed in the temperate environment were mainly due to changes

in the weather and the frequency of washing the floor of the Animal House. During the "heat wave" of May-August 1976 the indoor temperature rose to about 37°C at 1200 hour. This high temperate temperature which was similar to that of the hot room was reflected in the varied growth rates of tail growth in the temperate-reared mice.

C. Techniques, Measurements and Methods

1. Measurement of Tails. The tails were measured to within less than 0.5 mm by the graduated glass tube technique described by Howard-Flanders and Wright (1957). In this method, the unanaesthetised animal is held gently in the left hand by the interscapular skin and a graduated glass tube held in the right hand is slipped over the tail and preseed gently against the body. Slight rotation ensures a standardised optimum tube position and allows the tail to straighten in the tube. Slight variation in pressure made little difference to the measurement.

The standard tube used throughout this work was 11.5 cm in length, 0.4 mm in internal diameter and 4.3 gm in weight.

The accuracy of the tube method was tested in several ways:

i) When the tails of 24 animals were re-examined and measured by the same observer after a short interval the difference in the means between the first and second reading was 0.003 mm ($t = 0.76732$, $p = 0.54256$).

ii) When the tails of 26 animals were measured by two observers the differences in the mean tail lengths were 0.014 mm ($t = 1.37407$, $p = 0.18115$) and 0.001 mm ($t = 0.86890$, $p = 0.60300$).

iii) The individual tail bones were measured from the photographic enlargements of a series of radiographs. Taking into consideration the radiotranslucency of the terminal cartilagenous tail vertebrae and the intervertebral joint spaces no appreciable difference was encountered between the methods.

CONTROL GROUP (21°C)

Animal No.	Sex	Age in (days)	15	25	35	45	55	65	75	85	95	105	115	125	135	145	155	165	175	185
59	F		4.75	5.85	6.55	7.12	7.55	7.80	7.95	8.05	8.12	8.18	8.25	8.3	8.32	8.35	8.38	8.38	8.38	8.38
61	F		4.50	5.40	6.12	6.70	7.12	7.35	7.52	7.62	7.70	7.75	7.80	7.85	7.90	7.95	7.95	7.95	7.95	7.95
69	F		4.43	5.60	6.30	6.88	7.30	7.55	7.70	7.80	7.88	7.95	7.98	8.05	8.08	8.12	8.15	8.15	8.15	8.15
71	F		4.38	5.50	6.20	6.78	7.20	7.45	7.60	7.70	7.78	7.85	7.90	7.92	7.98	8.02	8.05	8.05	8.05	8.05
73	M		4.60	5.72	6.42	7.00	7.42	7.70	7.82	7.92	8.00	8.05	8.10	8.15	8.20	8.25	8.25	8.25	8.25	8.25
74	M		4.50	5.62	6.32	6.90	7.32	7.60	7.72	7.82	7.90	7.95	8.00	8.05	8.10	8.15	8.18	8.18	8.18	8.18
Mean			4.50	5.62	6.32	6.90	7.32	7.58	7.72	7.82	7.90	7.96	8.01	8.05	8.10	8.14	8.16	8.16	8.16	8.16
S.D.			0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.15	0.15	0.15	0.15	0.16	0.15	0.14	0.15	0.15	0.15	0.15

HOT GROUP (33°C)

50	M		4.42	5.50	6.90	7.80	8.18	8.30	8.38	8.42	8.45	8.48	8.50	8.50	8.50	8.50	8.50	8.50	8.50	8.50
51	F		4.52	5.65	7.02	7.90	8.22	8.40	8.45	8.50	8.52	8.55	8.58	8.60	8.62	8.62	8.62	8.62	8.62	8.62
60	M		4.50	5.60	6.98	7.85	8.18	8.38	7.45	8.50	8.52	8.58	8.58	8.60	8.60	8.60	8.60	8.60	8.60	8.60
67	M		4.55	5.48	6.85	7.72	8.18	8.25	8.30	8.38	8.42	8.45	8.48	8.50	8.52	8.52	8.52	8.52	8.52	8.52
68	F		4.50	5.40	6.88	7.80	8.15	8.42	8.52	8.60	8.65	8.68	8.70	8.72	8.75	8.75	8.75	8.75	8.75	8.75
72	M		4.52	5.62	7.05	8.00	8.45	8.50	8.50	8.58	8.60	8.62	8.65	8.68	8.70	8.70	8.70	8.70	8.70	8.70
75	F		4.75	5.85	7.25	8.15	8.48	8.65	8.72	8.78	8.80	8.82	8.85	8.85	8.85	8.85	8.85	8.85	8.85	8.85
76	F		4.60	5.70	7.08	7.90	8.18	8.25	8.35	8.38	8.42	8.45	8.48	8.50	8.52	8.52	8.52	8.52	8.52	8.52
Mean			4.50	5.60	7.00	7.89	8.25	8.39	8.46	8.52	8.55	8.58	8.60	8.62	8.63	8.63	8.63	8.63	8.63	8.63
S.D.			0.14	0.14	0.13	0.13	0.13	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13

COLD GROUP (8°C)

47	F		4.55	5.65	6.05	6.38	6.65	6.90	7.08	7.18	7.25	7.30	7.38	7.40	7.42	7.45	7.45	7.48	7.48	7.48
48	F		4.80	5.92	6.20	6.50	6.78	7.05	7.20	7.32	7.40	7.48	7.52	7.55	7.58	7.60	7.62	7.62	7.65	7.65
63	M		4.60	5.70	6.12	6.45	6.72	6.98	7.15	7.25	7.32	7.38	7.45	7.48	7.50	7.52	7.52	7.55	7.55	7.55
64	M		4.40	5.50	5.90	6.20	6.50	6.75	6.92	7.02	7.10	7.15	7.20	7.22	7.25	7.28	7.30	7.30	7.32	7.35
77	F		4.48	5.50	5.90	6.22	6.50	6.78	6.92	7.02	7.12	7.18	7.22	7.25	7.28	7.30	7.32	7.32	7.32	7.32
Mean			4.57	5.64	6.03	6.35	6.65	6.89	7.05	7.16	7.24	7.30	7.35	7.38	7.41	7.43	7.44	7.45	7.46	7.47
S.D.			0.15	0.17	0.15	0.15	0.15	0.13	0.15	0.15	0.15	0.15	0.14	0.14	0.13	0.14	0.13	0.14	0.14	0.14

Table 2.2 Sample of the tail lengths (cm) of 5 experimental groups measured with the graduated glass tube. The mice were weaned at 25 days of age and transferred to the hot (33°C) and cold (8°C) room at 25 days of age

Temperature	Age (days)	15	25	35	45	55	65	75	86	95
21°C - 33°C		0.93348	0.84893	0.00038	0.00001	<0.00001	0.00001	0.00001	0.00002	0.00002
21°C - 8°C		0.51793	0.70628	0.00099	0.00031	0.00010	0.00012	0.00012	0.00013	0.00011
33°C - 8°C		0.58517	0.55620	0.00007	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
Temperature	105	115	125	135	145	155	165	175	185	195
21°C - 33°C	0.00003	0.00003	0.00005	0.0005	0.00008	0.00011	0.00011	0.00011	0.00011	0.00011
21°C - 8°C	0.00013	0.00016	0.00015	0.00011	0.00009	0.00008	0.00010	0.00011	<0.00001	0.00012
33°C - 8°C	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001

Table 2.3 Significance of difference between the tail lengths of the 3 experimental groups in Table 2.2.
P values of less than 0.05 were regarded as showing a statistically significant difference

Tables 2.2 and 2.3 show a sample of the tail lengths of the three experimental groups as measured with the graduated glass tube. The tables also show some statistical analysis of the readings.

2. Radiographic Technique. The mice were anaesthetised with ether and immobilised with cello tape with their ventral side down on wrapped unexposed X-ray film (Kodirex, Kodak Ltd.). A portable diagnostic X-ray set (Model (K), type 5, Newton Victor Ltd., London & Motherwell) was used at 60 kv, 10 mA, 101.5 cm f.s.d., 0.25 to 0.5 sec exposure.

The films were then developed (4 minutes), fixed (10-15 minutes), washed (15 minutes) and dried. From these radiographs photographic enlargements were made and the tail bones were measured using a microscope (magnification x 10) and a stage micrometer with 0.1 mm divisions.

3. Irradiation Technique. The unanaesthetised mice were placed on a Perspex plate and held in small Perspex holders with the tails protruding and fixed to the plate by cello tape. The body of the mouse was covered with a layer of lead (6 mm) to prevent any mouse from receiving a whole body irradiation. The whole length of each tail was irradiated except for about 3 mm next to the body of the mouse.

The physical factors employed in carrying out the irradiation using Marconi X-ray machine were: 230 Kv, filter 0.5 mm Cu + 1 mm Al, f.s.d. 30 cm and dose-rate 323 rads/min. A total dose of 5000 rads was given to the tails in 15.5 minutes.

4. Measurement of Tail and Body Temperature. The external (skin) temperature of the bodies and tails of the mice was measured using an electric thermometer with a 1 mm exposed thermistor indicator (Ellab, Copenhagen. Supplied by Sierex Ltd., 15-18 Clipston Street, London W1). The thermocouple was applied directly to the skin under the fur.

Using the same thermometer but a different thermocouple the internal (rectal) temperature of the mice was also recorded. In this case 1 cm of

the thermocouple lead was inserted into the rectum.

The temperature of any part of the mouse was repeated several times and the readings of the indicator was left to stabilise before the final measurement was made.

5. Histological Techniques. The animals were usually killed by ether and the tail bones dissected and examined under a dissecting microscope. The bones were fixed for 1-2 days in 10% formol saline, decalcified for 4 days in 10% formic acid in 10% formol saline, and then processed and embedded in wax and cut at 4 micrometres.

CHAPTER III THE LONG TERM EFFECTS OF CHANGES IN THE ENVIRONMENTAL TEMPERATURE ON THE GROWTH OF TAIL BONES

A. Introduction

In a survey of the wild mammals of North America, Allen (1905) noted wide geographical variations in the colour and the relationship of the sizes of the animals to their peripheral parts. As one progresses from the colder northern regions to the southern warmer plains, several species developed longer peripheral parts, which included the tails, ears, feet, antlers, skulls and pelage. Other investigators confirmed Allen's rule in laboratory animals (Sumner, 1909; Sundstroem, 1922; Przibram, 1931; Ogle, 1934).

Sumner (1909, 1913, 1915) reported smaller ears and shorter tails in mice reared at a lower temperature and this was confirmed by Sundstroem (1922). Ogle (1934) also observed retraction of the scrotal sacs of the male mice living in the cold environment and distension in those living in the hot environment. Przibram (1931) interbred mice in a wide range of temperatures (between 5°C to 40°C) and noted that on progressing from the lower to the higher temperature, the tails of the mice gradually lengthened in relation to the body length. This relationship was expressed mathematically by Sumner (1909) and Sundstroem (1922).

The temperature-tail relationship has since been investigated by several workers (Ashoub, 1958; Harrison et al, 1959, 1969; Chevillard et al, 1963; Neel and Wright, 1970a) but the reason for this relationship in the mice and rats is debatable. Does it represent a specific adaptation to environmental temperature? It has been suggested (Harrison et al, 1959, 1963, 1964, 1969; Chevillard et al, 1963) that the increase in tail length in the higher temperature is an adaptive response, and that longer tails encourage heat loss by increasing the surface area. The biological function of the tail in the thermoregulation of the mouse and rat is well established (Sundstroem, 1922; Harrison et al, 1959, 1969; Barnett, 1965; Rand et al, 1965;

Hart,1971). These adaptational responses are possibly homeostatic mechanisms which are preserved in the species through natural selection because they are directed towards the maintenance of the integrity and somatic fitness of the organism. They would also, of course, be preserved in the genetic constitution of the organism. If animals who had already developed long tails because of their growth in hot environments had offspring with long tails in the temperature conditions this would be Lamarckian inheritance, that is, of acquired characteristics.

One piece of evidence supporting Mendelian genetic inheritance of adaptational mechanisms is the well known phenomenon of "hybrid vigour". This could be explained by the inheritance of two different sets of adaptive mechanisms. Harrison et al (1959,1969) showed that hybrid animals were more able to adapt to unusual heat than inbred.

Recently the tail-temperature relationship has been attributed to the direct local effect of environmental temperature on the growth of tail bones (Barnett,1965,1970; Harrison and Clegg,1969; Noel and Wright,1970a, 1972). When mice of different strains were inbred at -3°C and 21°C , the tails of the cold groups showed marked shortening, but the differences in body length between the -3°C and the 21°C groups did not change (Barnett, 1965). This effect of the cold environment may therefore be independent from that on the body length. Such effects may be due to the direct action of cold on the tail itself.

It has also been shown that when the tail bones were transplanted under the kidney capsule of the same animal they grew faster and longer than if they had been left to grow on the tail (Noel and Wright,1972; Noel,1973). This effect has been attributed to the differences in temperature between the distal region of the tail and the kidney. In the capsular space the tail bones were disconnected from their original environmental conditions and dependent henceforth on such "local growth

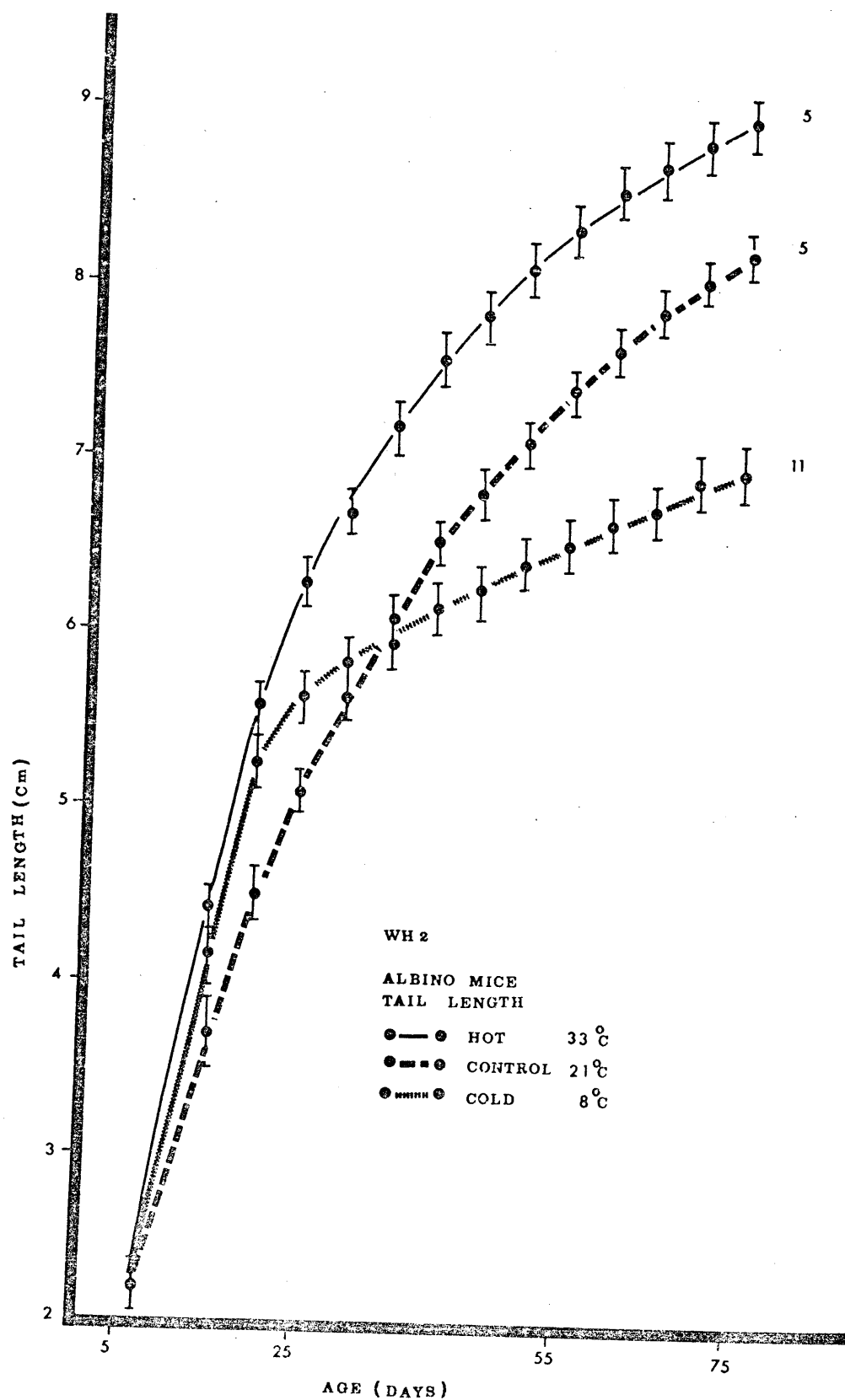


Fig.3.1 The curves of tail growth of mice kept at 33°C (hot group), 21°C (control group) and 8°C (cold group). The tails of the cold group grew at the same rate as the hot group, but the growth was slower after 20-23 days.

factors" as temperature and vascularity.

However, most authors studying the effects of environmental temperature on the mammalian body and its peripheral parts were concerned principally with the physiology of adaptation and homeothermic mechanisms (Brody, 1945; Alee et al, 1949; Scholander et al, 1950a, 1950b, 1950c, 1955; Bajusz, 1969; Whittow, 1971; Hardy, 1972; Yousef et al, 1972) and the responses of the different genotypes to the changes in environmental temperature (Harrison et al, 1959, 1969; Barnett, 1963, 1965). Studies on the biological effects of changes in the environmental temperature on the growth and morphology of the tail bones are lacking. This chapter is concerned with the study of the long term effects of different environmental temperatures on the growth of tail bones in mice.

B. Experimental Procedures and Results

1. The Long Term Effects of Environmental Temperature on the Growth of Tail Bones

a) The Selection of Age and Temperature

Four litters (21 mice) one week old were used. Eleven were kept at 8°C (cold group), 5 at the Animal House temperature of 21°C (control group) and 5 at 33°C (hot group). The cold group (2 litters) were provided with a cotton wool nest.

In addition a litter of 5 mice one week old were housed with their mother in the cold room (8°C). After 4 days the temperature inside the nest was measured.

Figure 3.1 shows the curve of tail growth of the 3 environmental groups. At the beginning of the experiment (at the age of one week) the mean tail lengths of the hot, control and cold groups were closely similar (2.42 cm, 2.19 cm and 2.05 cm respectively). The tails of the hot group grew rapidly during the first 3 weeks (1.4 mm/day) and by the age of 75 days were 0.8 cm longer than the tails of the control group which grew

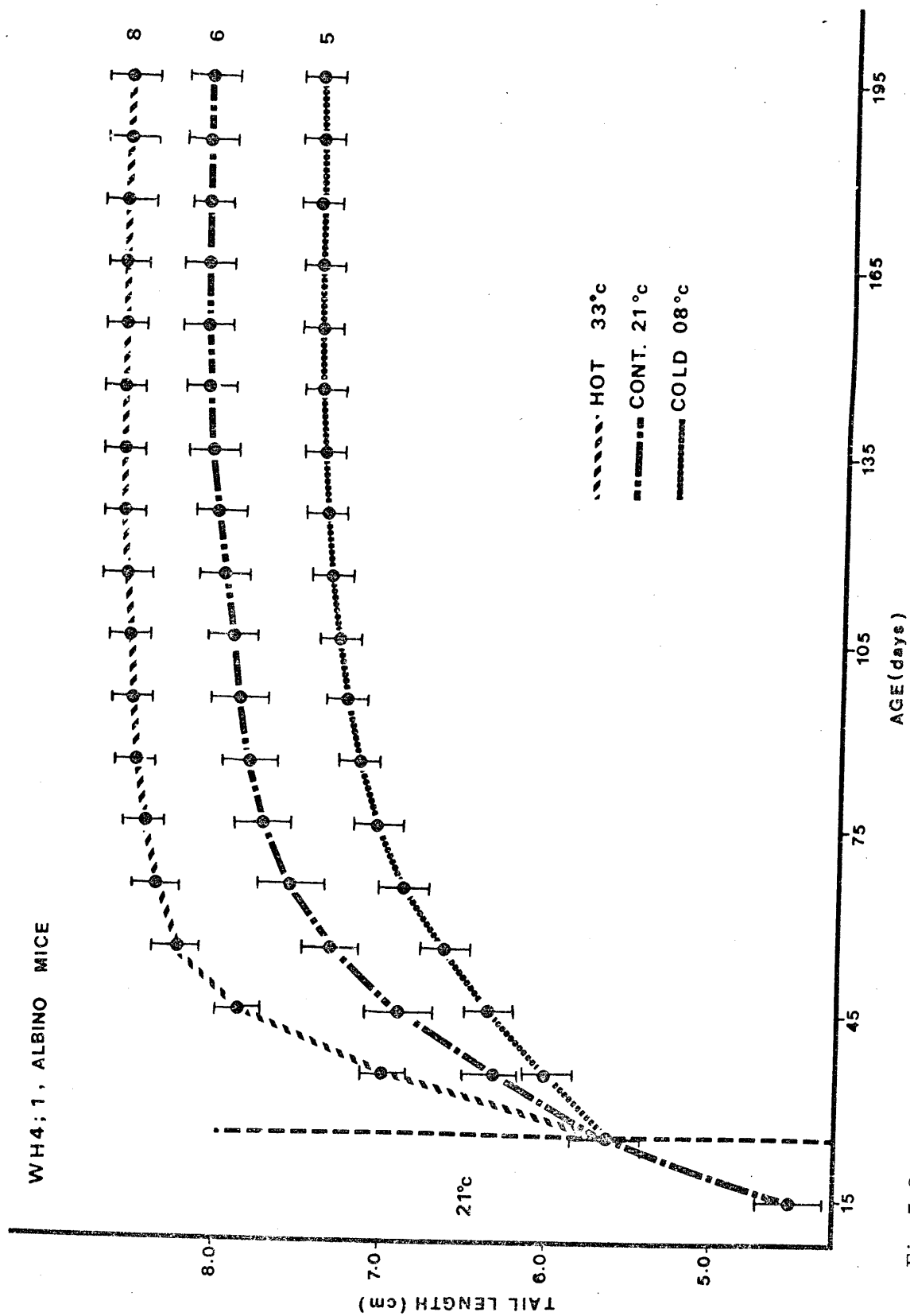


Fig.3.2 The curves of tail growth of hot (33°C), cold (8°C) and continuous (21°C)

WH4, ALBINO MICE

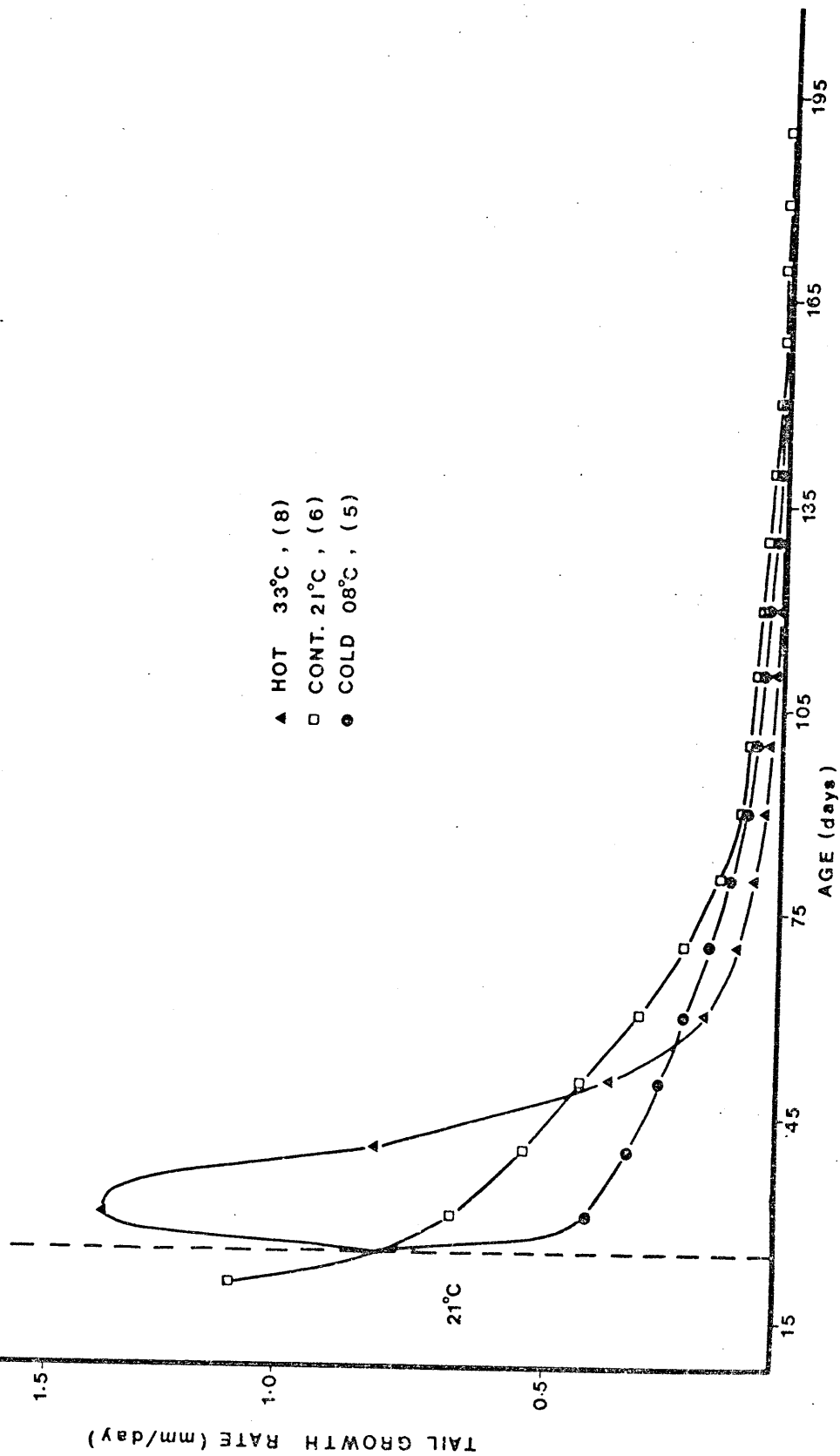


Fig.3.3 The curves of growth rate of the tails (mm/day) of the hot (33°C), cold (8°C) and control (21°C) groups. Soon after transferring the 25 day old mice to the environment.

at about 1.2 mm/day during the same period.

The tails of the cold group also grew rapidly during the above period (1.6 mm/day) but after 20-23 days the growth rate was only 0.03 mm/day. By the age of 75 days the tails of the cold group were 2 cm shorter than those of the hot group.

After 4 days in the cold room (8°C) the temperature maintained by the young mice and their mother inside the cotton wool nest was found to be 34.1°C .

b) The Long Term Effects of Three Environmental Temperatures

Four litters of mice (19 mice) were used. They were received from the Animal House at the age of 12 days and weaned at 23 days old. At 25 days old the mice were divided into hot (8 mice), cold (5 mice) and control (6 mice) groups maintained at 33°C , 8°C and 21°C respectively.

Figure 3.2 shows the curve of tail growth of the three groups. Although the mean tail lengths of these groups were equal at the age of 25 days (S.D. 0.15 cm) the tails of the mice permanently kept in the hot room grew very rapidly and by the age of 195 days were longer than the tails of the control and cold groups by about 0.5 cm and 1.2 cm respectively. They also stopped growing earlier.

The tails of the mice permanently kept in the cold room were by the age of 195 days shorter than those of the hot and control groups. They grew slowly and over a longer period and never caught up with those of the hot and control groups.

The growth of the "control tails" was midway between the tails of the hot and cold groups.

Figure 3.3 shows the growth rate of the tails of the three groups. After going into the hot room, the tails grew very rapidly, at about 1.1 mm/day, in the first 4 weeks. In the later weeks, the growth rate gradually slowed to about 0.028 mm/day and stopped growing earlier than

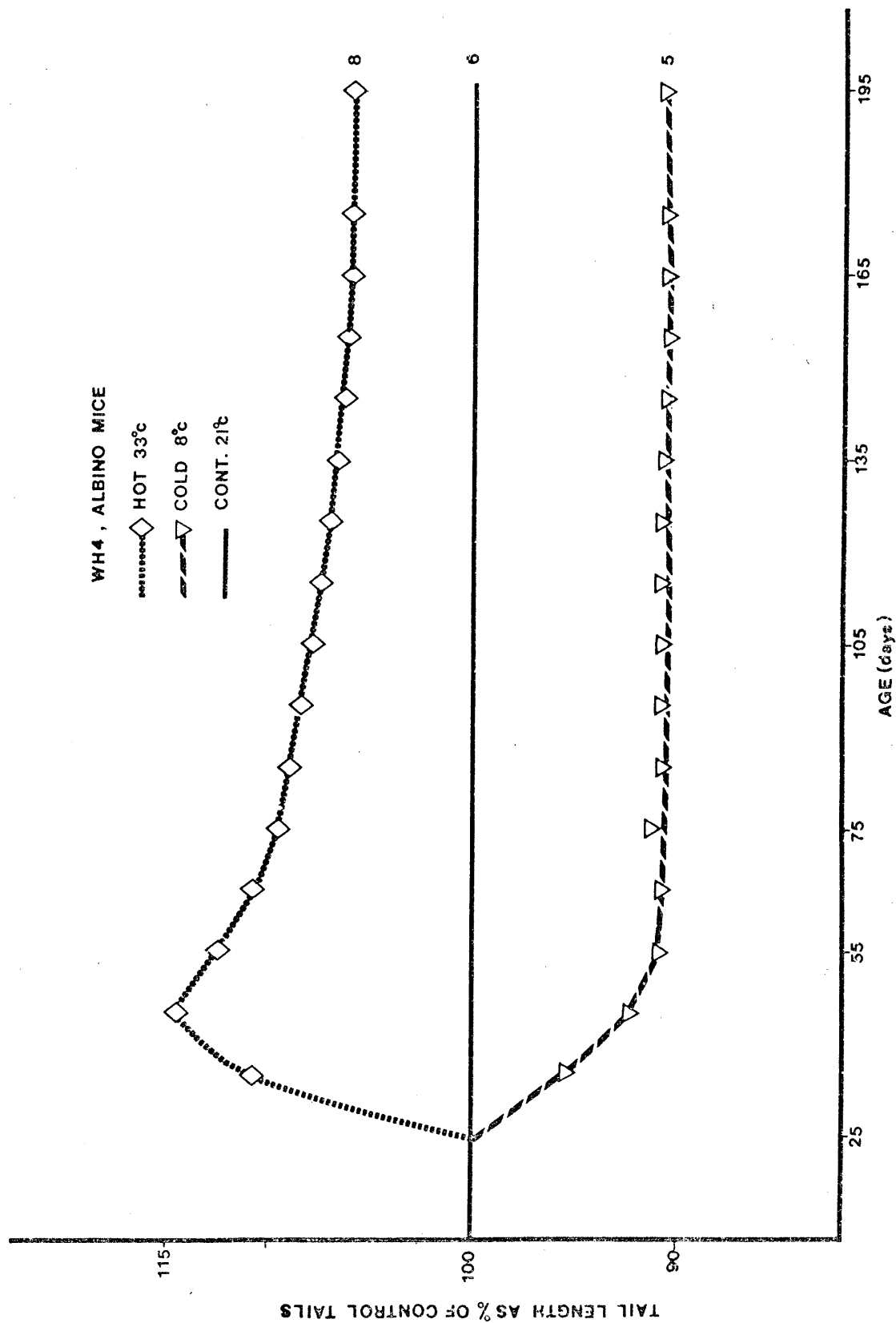


Fig. 3.4 The mean tail lengths of the hot (33°C) and cold (8°C) groups expressed as percentages of the

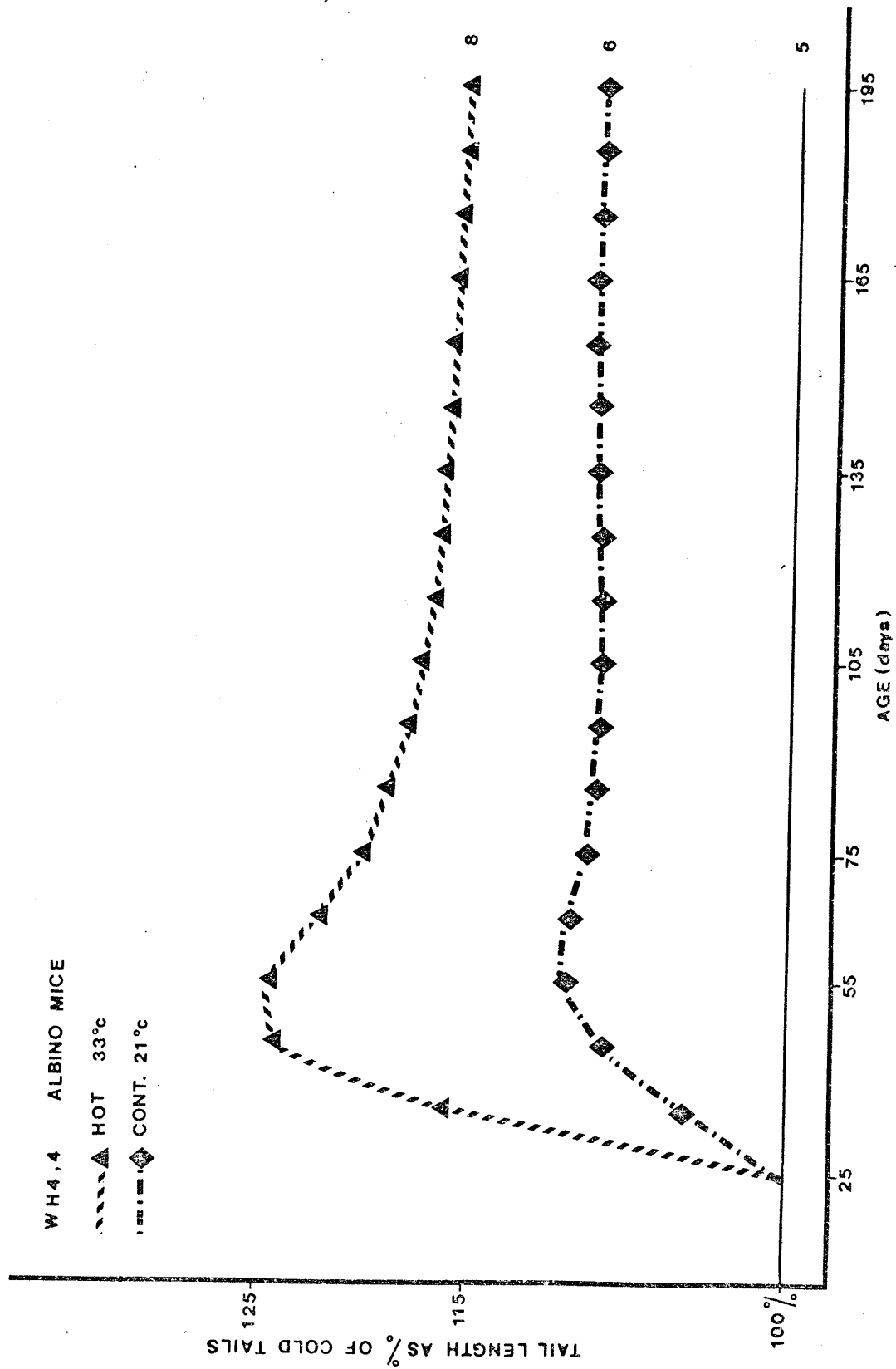


Fig.3.5 The mean tail lengths of the hot (33°C) and control (21°C) groups expressed as percentages of the mean tail lengths of the cold group (8°C)

the tails of the control and cold groups.

In the cold room, the tails grew very slowly during the first 4 weeks, at about 0.3 mm/day, and later even more slowly at about 0.057 mm/day but continued to grow over a long period.

The tails of the control group grew at about 0.5 mm/day for a long time.

Figure 3.4 shows the mean tail lengths of the hot and cold groups expressed as a percentage of the mean tail lengths of the control reared mice. The mean tail length of the hot group lies above the 100% line while that of the cold group lies consistently below. Immediately after exposing the mice to the hot environment, there was a rapid increase in the tail length, reaching a maximum of 114.5% in the first 4 weeks. Thereafter the ratio gradually diminished to a minimum of 105.9%. On the other hand exposing the mice to the cold environment was accompanied by a gradually decreasing ratio which reached a minimum of 90.6% during the first 4 weeks.

Because the cold reared mice have the shortest tails of the three groups, they could be regarded as a "control" group to the control (temperate, 21°C) and hot (33°C) groups. This is shown in Figure 3.5 in which the mean tail lengths of the hot and temperate groups were expressed as a percentage of the mean tail lengths of the cold groups. Both ratios lie above the 100% line. Soon after exposing the mice to the hot environment there was a rapid increase in tail length to a maximum of 124.6% of those in the cold group during the first 4 weeks; thereafter the ratio gradually diminished to a minimum level of 115.7% of those in the cold group.

The increasing ratio between the mean tail lengths of the temperate and the cold groups was gradual. A maximum difference of 110.5% was seen during the first 4 weeks and thereafter a constant ratio of 109.2% was maintained.

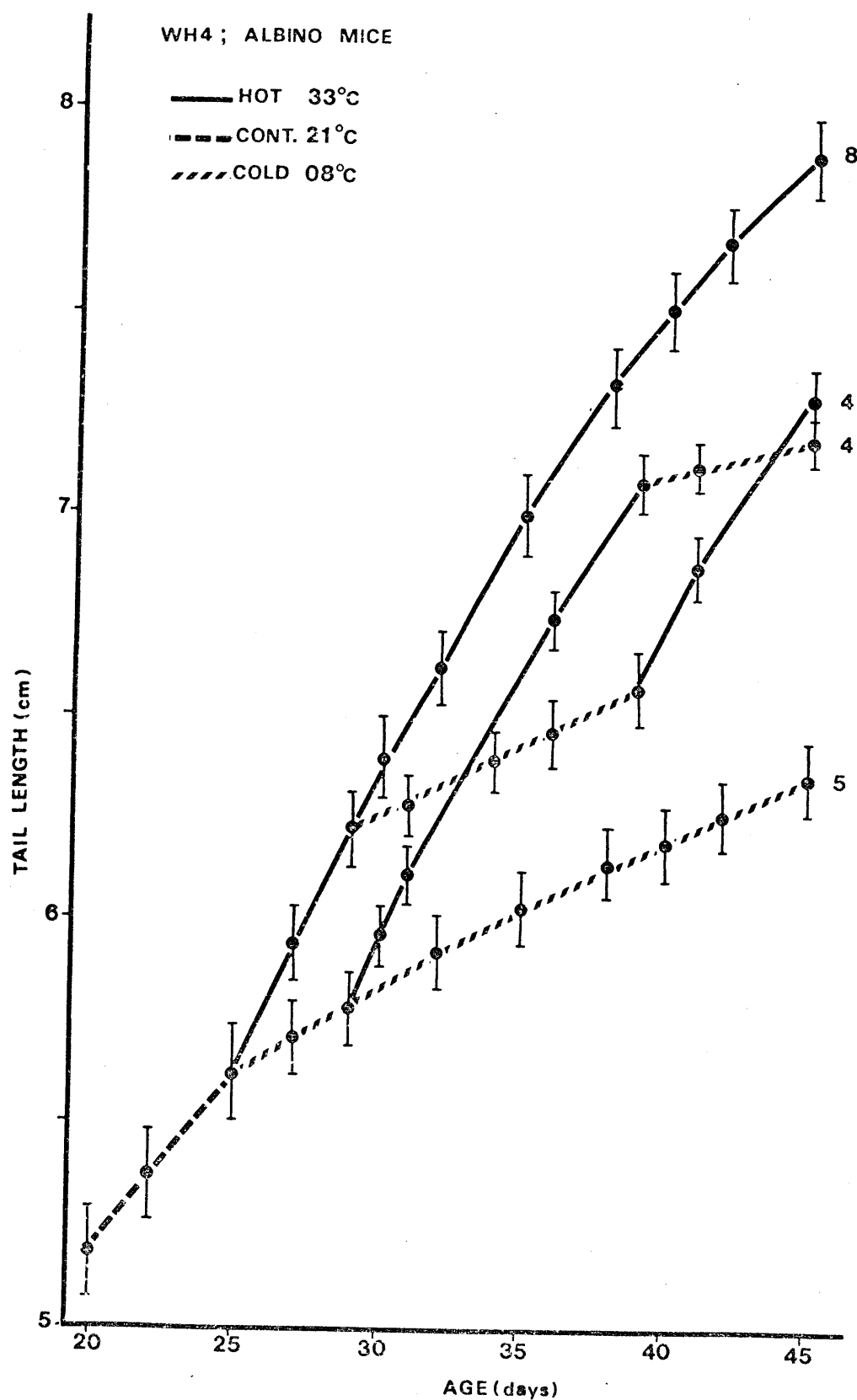


Fig.3.6 The effects on tail growth of short exposures of different temperatures in which the slow growth in the cold environment and the rapid growth in the hot environment was apparent after each change. This resulted in the appearance of graphical "parallelograms" between the tails of the hot and cold groups

Group	No. of mice	Period (days)		
		4	10	6
1 (H)	8	1.5 mm/d	1.2 mm/d	0.8 mm/d
2 (C)	5	0.4 mm/d	0.4 mm/d	0.3 mm/d
3 (H-C-H)	4	1.5 mm/d	0.3 mm/d	1.2 mm/d
4 (C-H-C)	4	0.4 mm/d	1.3 mm/d	0.2 mm/d

Table 3.1 The effects of short exposure to different environmental temperatures on the growth rate of tails. The rates, which were greater in the hot than the cold environment, were reversed by changing the temperature

The early cessation of tail growth in the hot and temperate groups relative to the still growing tails of the cold group was reflected by the appearance of the late and parallel plateau type of curve.

2. The Effects of Short Exposures to Different Environmental Temperatures

Three litters (21 mice) 20 days old were used. They were weaned at 23 days old and divided at 25 days old into 4 groups:

1. Continuous hot group (8 mice) housed at 33°C .
2. Continuous cold group (5 mice) housed at 8°C .
3. Hot-Cold-Hot group (4 mice). These were kept at 33°C for 4 days, transferred to 8°C at the age of 29 days and back to 33°C at the age of 39 days.
4. Cold-Hot-Cold group (4 mice). These were kept at 8°C for 4 days, transferred to 33°C at the age of 29 days and back to 8°C at the age of 39 days.

Figure 3.6 and Table 3.1 show the tail growth in the 4 groups. Soon after housing the 25 day old animals there was rapid increase in the rate of tail growth in the continuous hot group (1.1 mm/day) and marked reduction of the rate in the continuous cold group (0.3 mm/day). After 20 days in the hot and cold rooms, the tails of the continuous hot group were 1.53 cm longer than the tails of the continuous cold group.

In groups 3 and 4 there were also marked changes in the tail growth rate associated with the change of mice from one environmental temperature to another. This is shown in Table 3.1 in which the rates of tail growth of groups 1 and 2 during the relative periods were also added. The environmental changes produced rapid increase (the hot) or decrease (the cold) in the growth rate of the tails. These changes were reversed by housing the mice at the different temperatures.

By changing the environmental temperature a series of graphical "diamonds" and "parallelograms" were produced between the tails of the

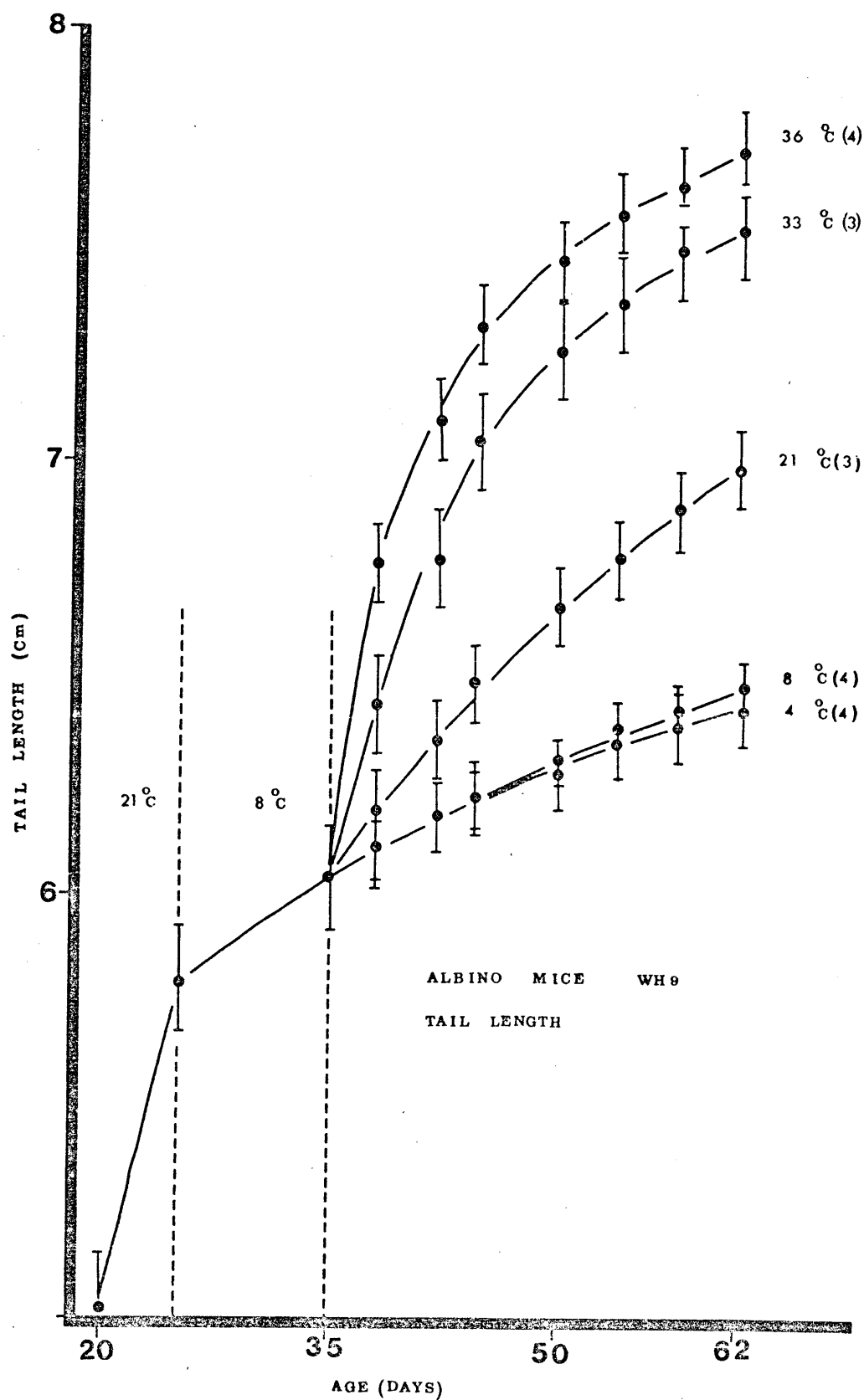


Fig.3.7 The curves of tail growth of groups of mice maintained at 36°C, 33°C, 21°C, 8°C and 4°C

Group	No. of mice	Age	20 - 25	25 - 35	25 - 38	38 - 42	42 - 50	50 - 62
		Days	5	10	3	4	8	12
36°C	4				2.43 mm/d	0.88 mm/d	0.48 mm/d	0.21 mm/d
35°C	3				1.33 mm/d	0.85 mm/d	0.46 mm/d	0.23 mm/d
21°C	3		1.50 mm/d		0.53 mm/d	0.40 mm/d	0.38 mm/d	0.26 mm/d
8°C	4			0.24 mm/d	0.23 mm/d	0.19 mm/d	0.17 mm/d	0.13 mm/d
4°C	4				0.23 mm/d	0.19 mm/d	0.15 mm/d	0.11 mm/d

Table 3.2 The effect of several environmental temperatures on the growth rate of tails

hot and cold groups.

3. The Effects of Several Environmental Temperatures

Three litters (18 mice) of 20 day old mice were used. They were weaned at 23 days old, housed in the cold room (8°C) at 25 days old and at the age of 35 days were divided into 4 groups and housed at 36°C (4 mice), 33°C (3 mice), 21°C (3 mice) and 4°C (4 mice). A fifth group of 4 mice were left in the cold room at 8°C .

Figure 3.7 and Table 3.2 shows the tail growth of the 5 groups. Soon after changing the environmental temperature the growth rate of the tails of these groups had slowed from 1.5 mm/day during the first 5 days while the animals were in the temperate environment (21°C) to 0.24 mm/day during the following 10 days in the cold room (8°C). The latter rate was also changed by transferring the mice to 36°C , 33°C , 21°C and 4°C .

The difference in the growth rate (1.1 mm/day) between the hot groups (36°C and 33°C) was only seen in the first 3 days but later on the rates were similar. Finally by the age of 62 days, the tails of the 36°C group were 1.8 cm longer than those of the 33°C group.

No difference in the growth rate between the 8°C and 4°C groups was seen during the first 8-9 days, but later on a small difference was observed. Finally by the age of 62 days the tails of the 8°C group were 0.5 cm longer than those of the 4°C group.

In most respects the tail lengths of the mice maintained at 21°C lay midway between those of the hot (36°C and 33°C) and the cold (8°C and 4°C).

4. The Effects of Environmental Temperature on the Growth Potential of Tail Bones

Two litters (12 mice) 20 days old were used. They were weaned at 23 days old and transferred at 25 days into the cold room at 8°C . The mice were then divided into 5 groups:

1. Group 1 (6 mice) transferred to the hot room at the age of 32 days.

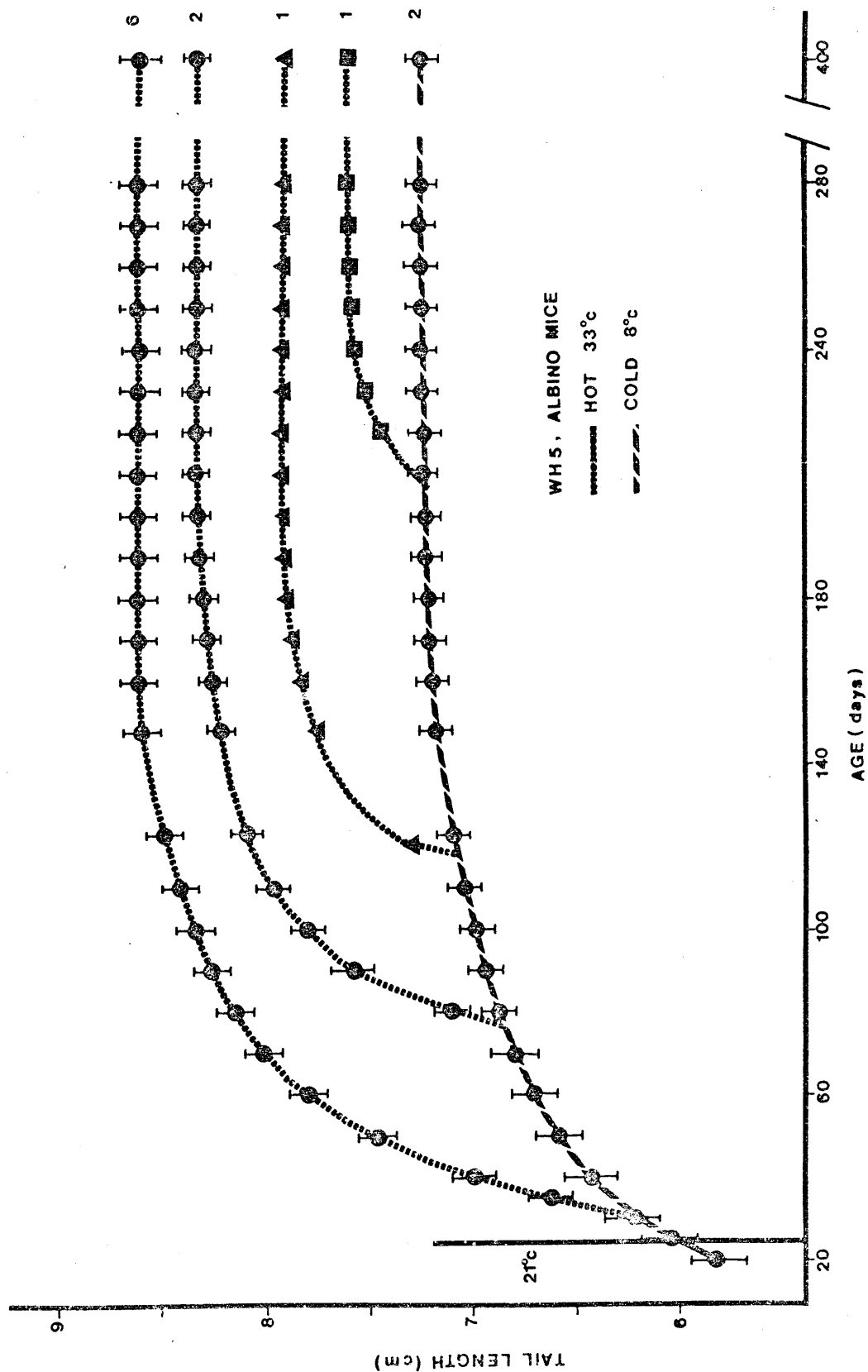


Fig.3.8 The curves of tail growth of 4 groups of mice which were transferred from the cold room to the hot room at different ages. Four series of "parallelograms" were produced

Group	No. of mice	PERIOD (months)						Age of temp. change (days)	No. of days in cold (8°C)	Age of cessation of tail growth (days)	No. of days in hot (33°C)
		1	2	3	4	5	6				
1	6	0.570 mm/d	0.147 mm/d	0.100 mm/d	0.047 mm/d	0.000 mm/d	0.000 mm/d	32	7	157	125
2	2	0.563 mm/d	0.183 mm/d	0.065 mm/d	0.013 mm/d	0.000 mm/d	0.000 mm/d	77	52	191	114
3	1	0.197 mm/d	0.043 mm/d	0.013 mm/d	0.000 mm/d	0.000 mm/d	0.000 mm/d	119	94	196	77
4	1	0.107 mm/d	0.013 mm/d	0.000 mm/d	0.000 mm/d	0.000 mm/d	0.000 mm/d	208	183	258	50
5	2	0.200 mm/d	0.083 mm/d	0.057 mm/d	0.037 mm/d	0.010 mm/d	0.005 mm/d				

Table 3.3 Rates of tail growth (mm/day) of 4 groups of mice (groups 1, 2, 3 and 4) during the first 6 months after the change of temperature from the cold (8°C) to the hot (33°C). In group 5 the mice were maintained constantly in the cold (8°C)

2. Group 2 (2 mice) transferred to the hot room at the age of 77 days.
3. Group 3 (1 mouse) transferred to the hot room at the age of 119 days.
4. Group 4 (1 mouse) transferred to the hot room at the age of 208 days.
5. Group 5 (2 female mice) remained in the cold room. At the age of 310 days one of these mice was taken to the hot room but returned to the cold room after one week. Likewise, the second mouse was also taken to the hot room at the age of 352 days but returned to the cold room after one week. In addition 2 male mice which were maintained separately in the cold room since the age of 25 days, were taken to the hot room at the age of 441 days and returned to the cold room after 10 days.

Figure 3.8 and Table 3.3 show the tail growth of the 5 groups. In groups 1, 2, 3 and 4 the rate of tail growth was increased after the animals were transferred to the hot room. This has resulted in the appearance of 4 series of graphical parallelograms between the tails of the hot reared mice. The two female mice of group 5 were followed for a long time in the cold room, and when one mouse was housed at the age of 310 days (285 days in the cold room) for one week in the hot room, no tail growth was measured. It was then returned to the cold room. The same observation was repeated when the other mouse was taken at the age of 352 days (327 days in the cold room) to the hot room. Such findings were thought to occur only in the tails of the female mice, but when two male mice were housed in the hot room at the age of 441 days (416 days in the cold room), no increase in tail length could be measured. The tails of these female and male mice were accordingly considered as the group permanently kept in the cold (8°C).

Again, the tails of the hot groups grew rapidly and attained greater

length but stopped earlier than those of the cold group. The growth rate decreased gradually in all the groups as the mice became older. The later the mice were transferred from the cold room to the hot room, the earlier was the cessation of tail growth.

The mean tail lengths of the mice in the cold room were more or less equal at any age (maximum S.D. 0.24 cm, minimum S.D. 0.08 cm). The final tail length, however, of those mice transferred to the hot room earlier in the experiment were ultimately longer than the tails of the cold group. They were also longer than the tails of the other groups that were transferred to the hot room later in the experiment. Thus the tails of group 1 were 0.1 cm longer than those of group 2 by the age of 400 days and even longer by 0.7 cm, 1.02 cm and 1.37 cm than those of groups 3, 4 and 5.

In addition, the tails of the cold group (group 5) failed to catch up with the tail lengths of the hot groups (groups 1,2,3 and 4) which themselves were unable to catch up with one another. Thus, for example, the tail lengths of groups 3 and 4 mice did not catch up with the tail lengths of group 1 and 2 mice.

5. The Effects of Environmental Temperature on the Growth Potential of Small Bodied Mice with Short Tails

Experiment 1. Effects on Mice Born in the Experimental Conditions

Hot group. Earlier in this work when the optimal experimental environment was being explored, four "trained" pregnant female mice (15 days of gestation) were housed at 33°C. When these littered, the young mice were undersized and underweight and died shortly after birth, except for two which were followed for 132 days. These also died in an accident.

Cold group. When male and female mice were inadvertently left together in the cold room at 8°C, one of the females became pregnant. The newborn mice (5 mice) died shortly after birth except for two which

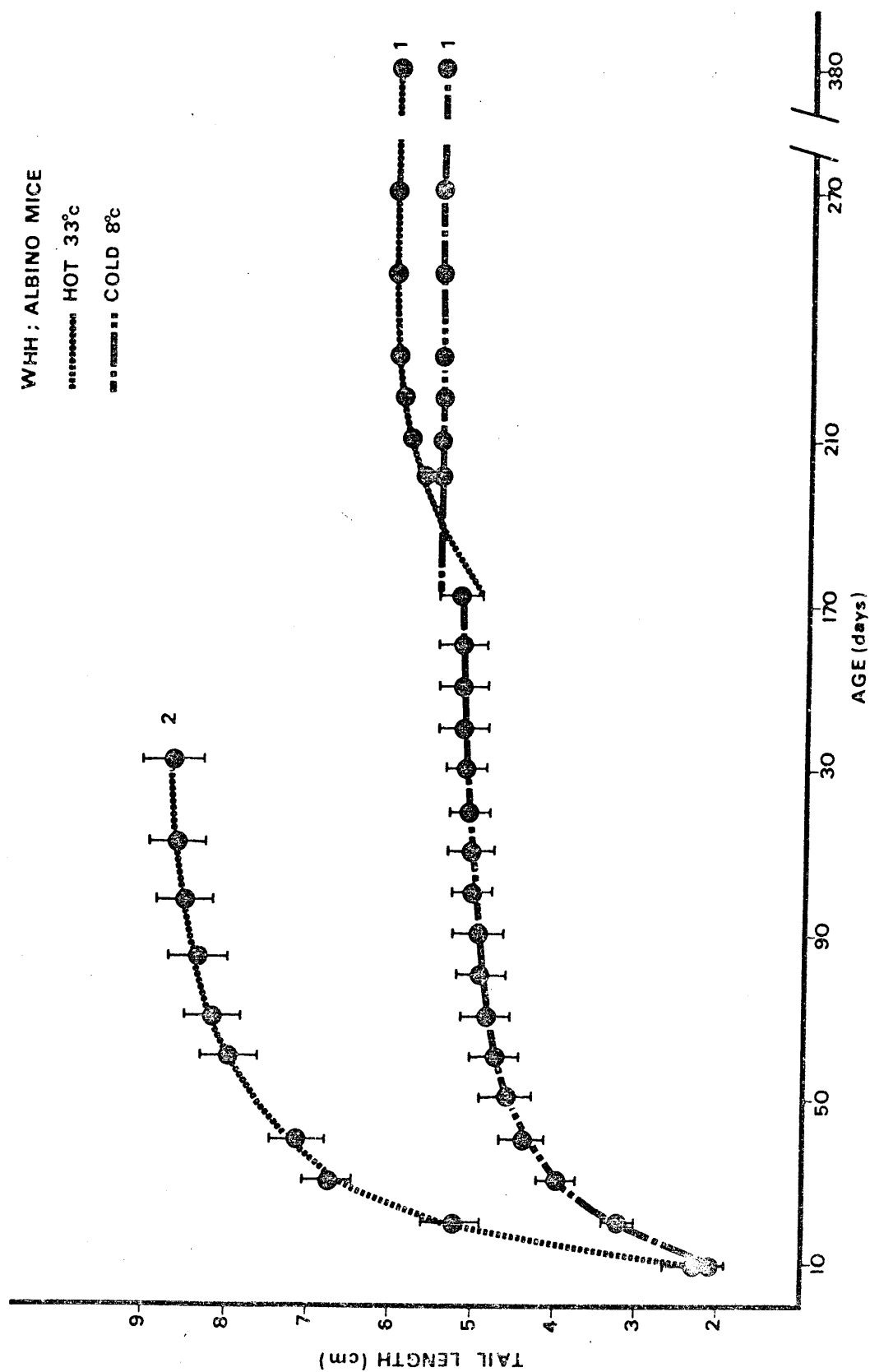


Fig.3.9 The tail growth of groups of animals which were born in the hot (33°C) and cold (8°C) rooms. At the age of 171 days one mouse of the cold group was transferred to the hot room

Group	HOT (33°C)			COLD (8°C)		
	Body Weight (gm)	Tail Length (cm)	Body Weight (gm)	Tail Length (cm)	Body Weight (gm)	Tail Length (cm)
No. of mice	2			2		
Age (days)	10	132	10	172	10	172
Range	2.2 - 2.7	14.4 - 19.4	2.2 - 2.4	12.2 - 13.7	1.92 - 2.22	4.9 - 5.45
Mean	2.45	16.9	2.35	12.95	2.06	5.18
S.D.	0.35	3.54	0.07	1.06	0.23	0.39
Growth rate:						
First 4 weeks	1.65 (1.73) gm/wk	0.171 mm/d	1.26 (1.80) gm/wk	0.081 mm/d		
Later weeks	0.54 (0.2) gm/wk	0.017 mm/d	0.29 (0.85) gm/wk	0.006 mm/d		

Table 3.4 Body weights (gm) and tail lengths (cm) of two groups of mice which were born in the hot (33°C) and cold (8°C) environments. The growth rates of body weight (gm/week) of a control hot and cold group are also shown ()

were followed until the age of 320 days. At the age of 172 days, one of these mice was taken to the hot room (33°C).

Figure 3.9 and Table 3.4 show the mean body weight (gm) and tail lengths (cm) of the mice which were born in 33°C (hot group) and 8°C (cold group).

In the hot room the growth in body weight was consistent with the control animals which were maintained at the same temperature. In the cold room the growth rates in body weight were below that of their control animals.

Although the mean tail lengths of these groups were more or less equal at the age of 10 days, the tails of the hot group grew rapidly and were finally, by the age of 132 days, about 3.51 cm longer than the tails of the cold group. The maximum growth of about 1.2 mm/day was seen in the first 4 weeks and thereafter the rate gradually slowed to about 0.129 mm/day. After 132 days in the hot room the tails had apparently not stopped growing.

The tails of the cold group grew slowly over a long period. Maximum growth of about 0.7 mm/day also occurred in the first 4 weeks and thereafter the rate was maintained at about 0.057 mm/day. At about the age of 156 days the tail of one of these mice stopped growing. When the other mouse was taken to the hot room at the age of 172 days the tail increased in length by about 1.1 cm and stopped growing after 68 days in the hot room.

Experiment 2. Effects on Mice Born in the Temperate Environment

During randomisation procedures (p. 28) the undersized and underweight mice with short tails (runts) were usually discarded from the principal experiment because they spuriously inflate the statistical variation. On one occasion, two of these animals (group 1) were followed. These runts as well as a control group (group 2) of 8 animals were weaned at 23 days

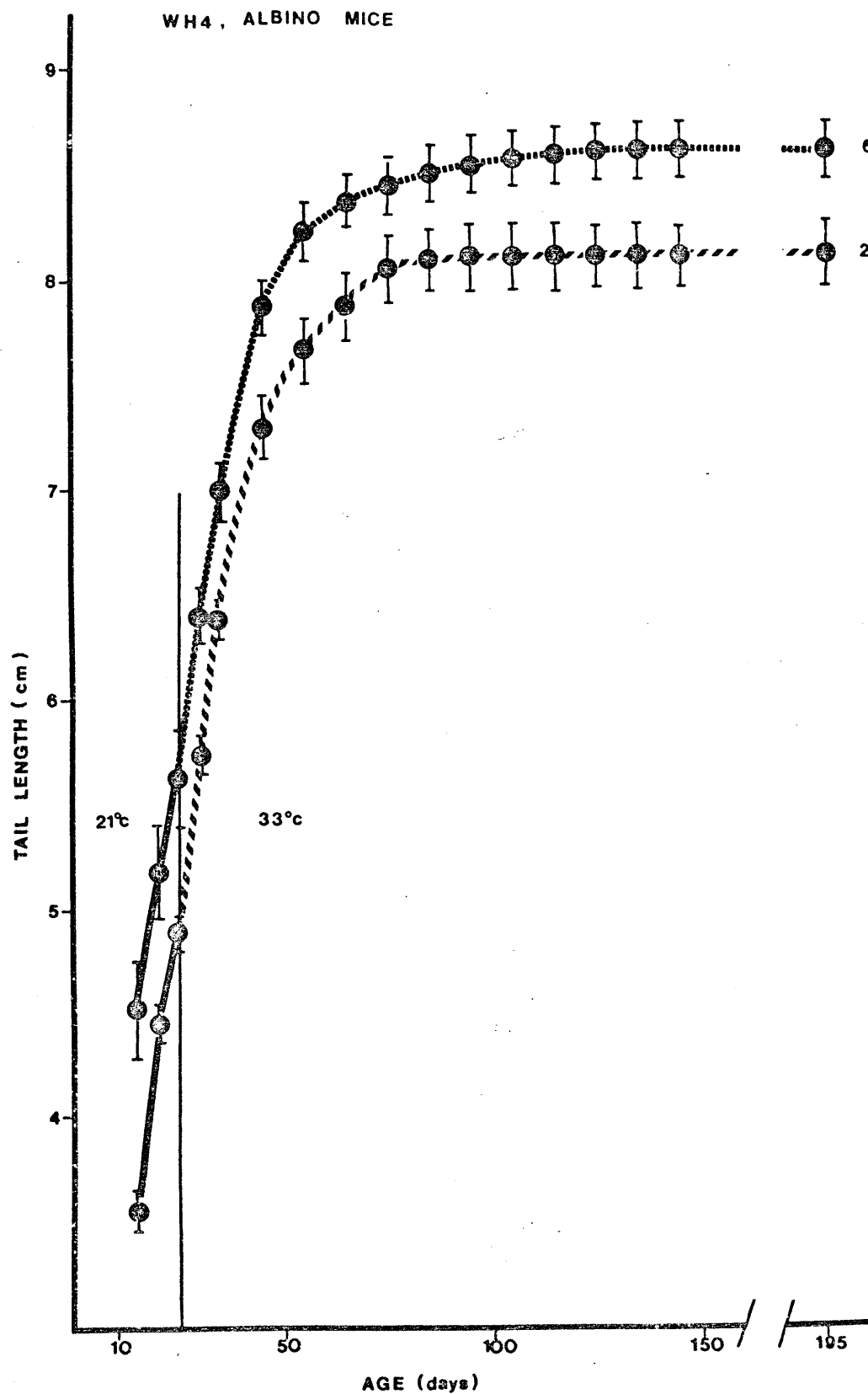


Fig.3.10 The tail growth of 2 mice with short tails and 8 control mice. Both were born at the Animal House temperature of 21°C and transferred to the hot room (33°C) at the age of 25 days

	Group 1	Group 2
Mean body weight at age of		
15 days	4.75 gm	5.68 gm
25 days	6.6 gm	9.9 gm
195 days	16.3 gm	20.8 gm
Growth rate in body weight during first 4 weeks	1.56 gm/week	1.73 gm/week
Growth rate in body weight during later 20 weeks	0.07 gm/week	0.2 gm/week

Table 3.5 Body weight of two mice with short tails (Group 1) and 8 control mice (Group 2). Both groups were maintained at 33°C from the age of 25 days

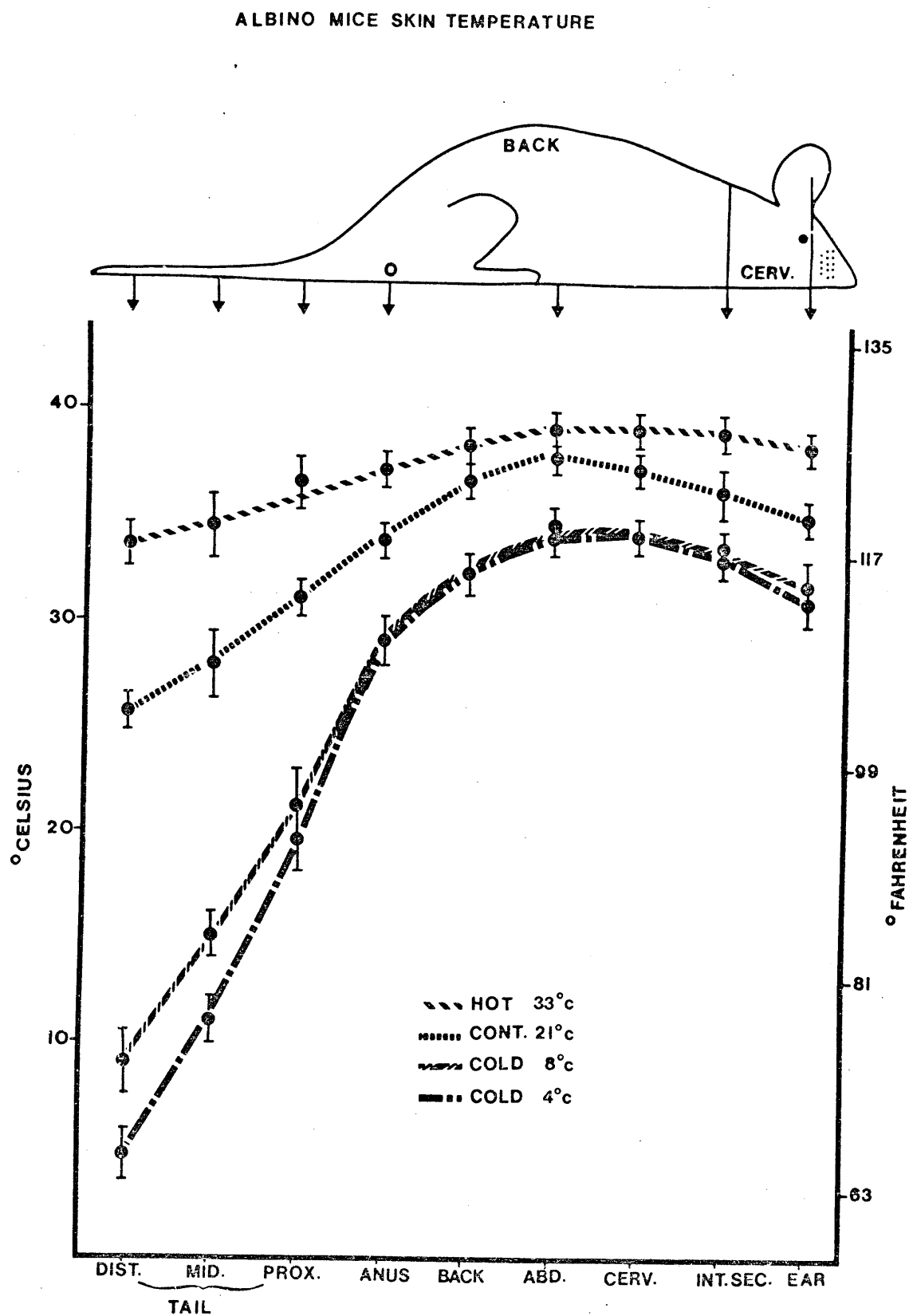


Fig.3.11 The external body and tail temperatures of groups of mice maintained at 4°C, 8°C, 21°C and 33°C. The temperatures over the tails were below that of the body

old and housed into the hot room (33°C) at 25 days old.

Table 3.5 shows the body weights of groups 1 and 2. Figure 3.10 shows the curves of tail growth of each group. The differences in tail lengths between these groups at the age of 15 days and 25 days were 0.97 cm and 0.74 cm. Soon after housing the mice in the hot room, the tails of both groups grew rapidly and finally by the age of 195 days, the tails of group 2 (which had been longer since the age of 15 days) were longer than group 1 by about 0.5 cm.

In both groups the maximum tail growth occurred during the first 4 weeks. The growth rate of group 1 (1.2 mm/day) was, however, greater than that of group 2 (0.8 mm/day). In the later weeks the rate of tail growth gradually slowed to about 0.04 mm/day in group 1 and 0.11 mm/day in group 2.

The tails of group 1 stopped growing earlier (at about the age of 77 days) than those of group 2 (at about the age of 128 days).

6. Changes in Body and Tail Temperatures

The external skin temperature of the bodies and tails of a group of mice of both sexes maintained at 33°C (22 mice), 21°C (15 mice), 8°C (12 mice) and 4°C (14 mice) were measured.

In addition, the internal (rectal) temperature of another group of mice of both sexes maintained at 33°C (14 mice), 21°C (19 mice), 8°C (10 mice) and 4°C (19 mice) were also recorded.

Figure 3.11 shows the external (skin) body and tail temperature of the 4 groups. Generally, the external temperature of the female mice were 0.4 – 1.7°C lower than in male mice. The general pattern between the 4 groups was similar, with the temperature over the tails always below that of the body. The temperatures of the tails, particularly of the distal parts, were only a few degrees higher than the surrounding environmental temperature.

	COLD (°C)		CONTROL (21°C)	HOT (33°C)
	4°C	8°C		
No. of mice	19 (12M, 7F)	10 (5M, 5F)	19 (9M, 10F)	14 (7M, 7F)
Range (°C)	33.9 - 36.8	33.4 - 37.3	34.0 - 37.8	34.5 - 37.2
Mean	35.14°C	35.24°C	36.20°C	36.24°C
S.D.	0.75	1.12	1.10	0.91
P ₍₁₎	0.00109	0.02382		0.95424
P ₍₂₎	0.00262	0.04652		

Table 3.6 Internal (rectal) temperature. P₍₁₎ indicates the significance of differences between 21°C group and 4°C, 8°C and 33°C groups and P₍₂₎ indicates the significance of differences between 33°C group and 4°C and 8°C groups. The significance between the 4°C and 8°C groups was 0.77796 (see footnote to Table 2.3)

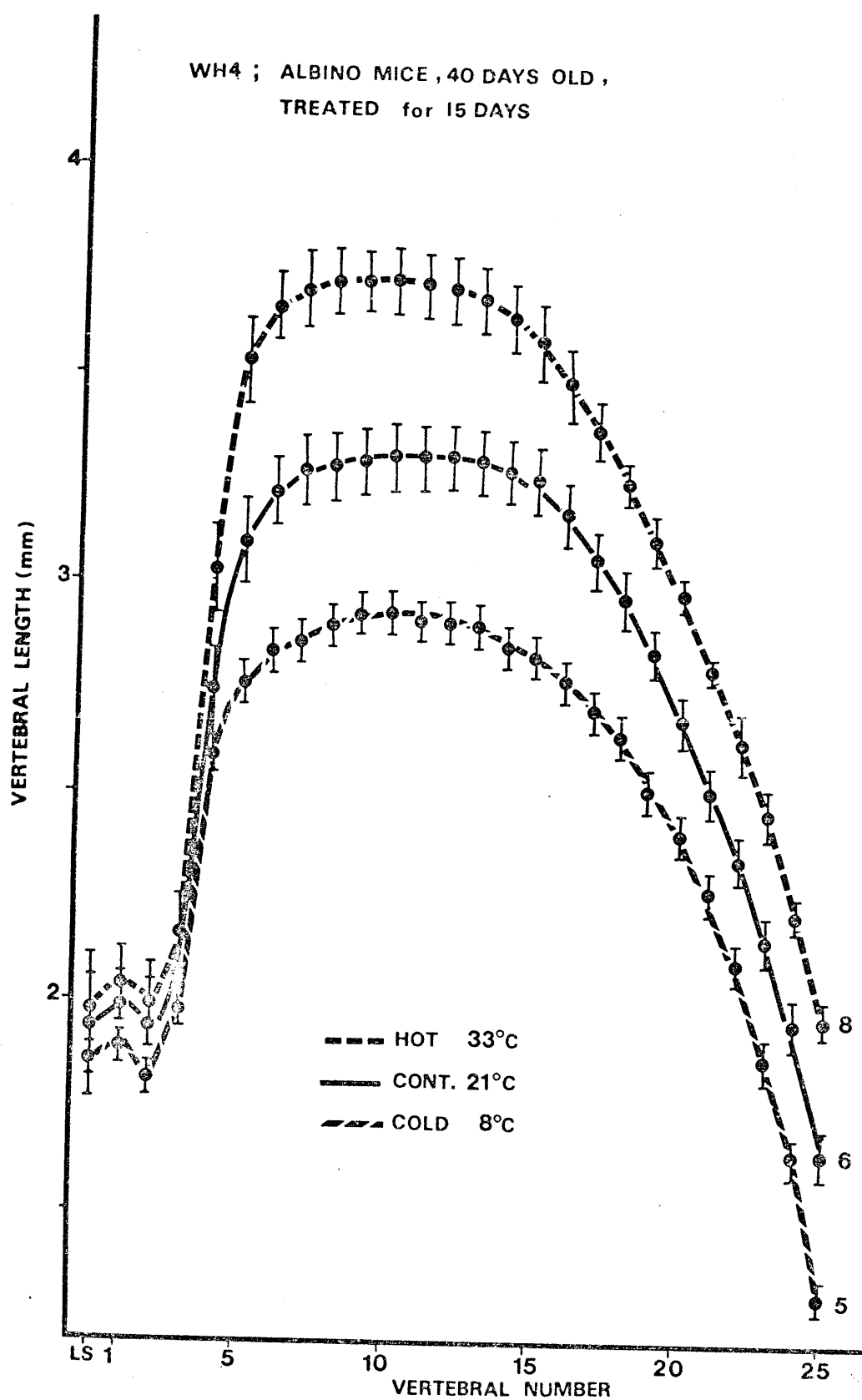


Fig.3.12 The mean lengths of the last sacral and first 25 caudal vertebrae of cold (8°C), control (21°C) and hot (33°C) groups at the age of 40 days

The maximum difference in the external temperature of the body between the 4 groups was seen over the interscapular, cervical and abdominal regions. Thus the interscapular temperature of the control group was 2.85°C higher than the cold groups and 2.6°C lower than the hot group. Small differences, 0.6°C and 0.9°C , were observed over the interscapular region and the ears of the cold groups (8°C and 4°C).

Table 3.6 shows the internal (rectal) temperature of the 4 groups. The difference in the means between the extreme cold group (4°C) and the extreme hot group (33°C) was 1.1°C . This was influenced by the predominant male group in the 4°C which had 0.8°C higher rectal temperature than the females.

7. The Effects of Environmental Temperature on the Long Bones and Tail Vertebrae

Radiological Study. At the ages of 40 and 195 days the tails of hot (33°C), cold (8°C) and control (21°C) groups of animals (8, 5 and 6 mice) were radiographed (p. 35) and from photographic enlargements of the tail vertebrae the bones were carefully examined and measured.

From the photographic enlargements of the radiographs taken at the age of 195 days the mean caudal vertebral numbers were found to be 29 in the hot and cold reared mice and 30 in the tails of the control mice.

The first caudal vertebra was marked as the one lying between the posterior tips of the iliac bones of the pelvic girdle. Figure 3.12 shows the mean lengths of the last sacral and the free caudal vertebrae of the cold, hot and control groups at the age of 40 days (15 days after the change of environmental temperature). The general pattern in the three 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 vertebrae of the three groups.

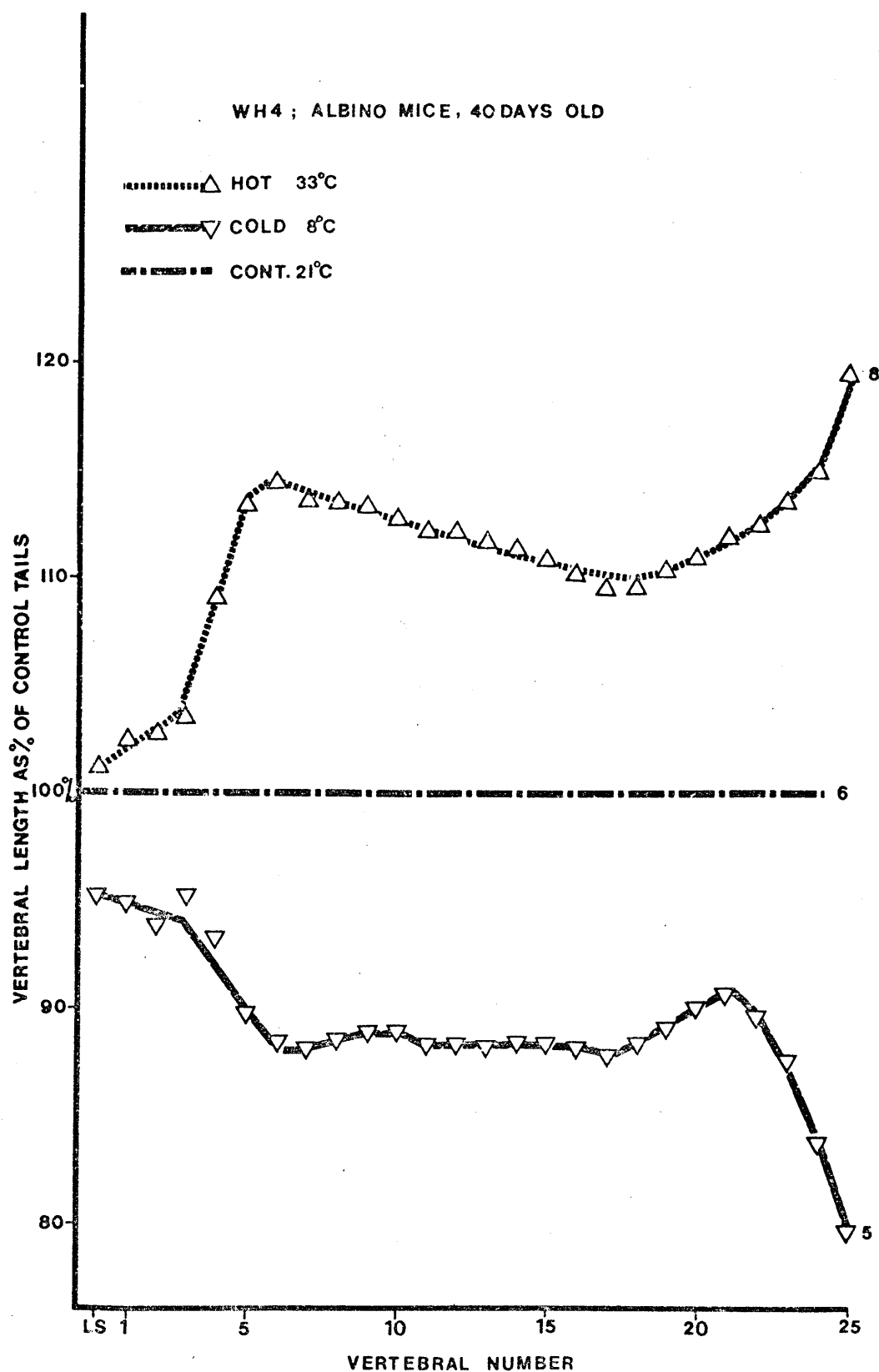


Fig.3.13 The mean vertebral lengths of the hot (33°C) and cold (8°C) groups expressed as percentages of the mean vertebral lengths of the control group (21°C)

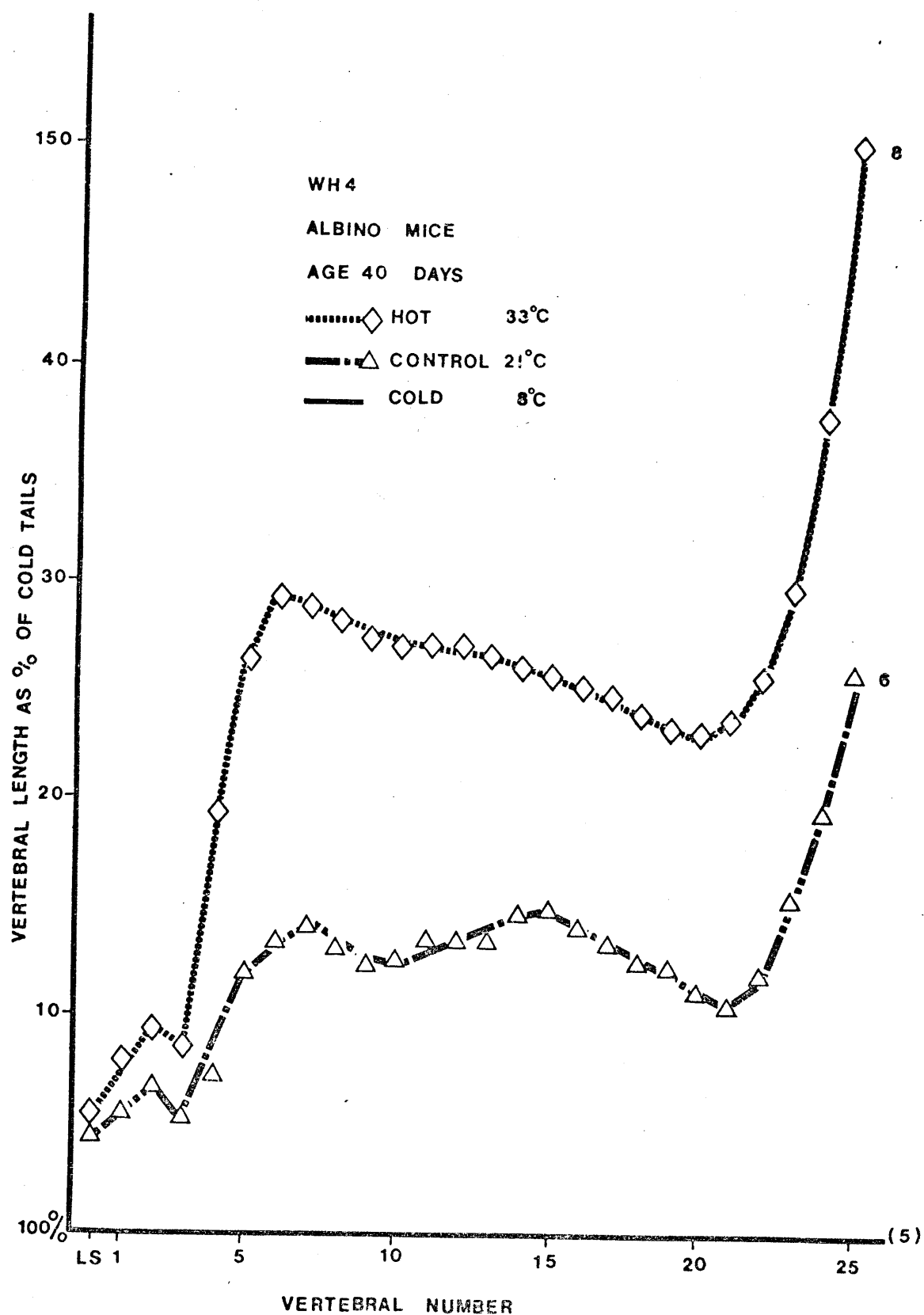


Fig.3.14 The mean vertebral lengths of the hot (33°C) and control (21°C) groups expressed as percentages of the mean vertebral lengths of the cold group (8°C)

Figure 3.13 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 group lies consistently below. In the more distal tail bones (from the 4th caudal vertebra outwards) the discrepancy gradually increases to a maximum of 129.4% between the "hot-control bones" and 79.4% between the "control-cold bones".

Because the vertebrae of the cold group were shorter than those of the control and hot groups, these vertebrae could therefore be regarded as a "control group" for the other groups. This is shown in Figure 3.14 in which the mean vertebral lengths of the hot (33°C) and the control (21°C) groups were expressed as a percentage of the mean vertebral lengths of the cold group (8°C). Again the general pattern between the hot and control groups was similar, and the differences between the last sacral and first 4 caudal vertebrae were small. The maximum percentage-difference of 126% was seen between the terminal tail bones of the control-cold and 150.4% between the hot-cold groups.

In the photographic enlargements of the radiographs of the tail bones of the three groups at the age of 195 days the free caudal vertebrae in the hot group appeared longer and more cylindrical than those of the cold group which were shorter and thinner in their 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.

The 14th caudal vertebra was selected for examination because (1) it lies at the middle of the tail and might represent the average growth of all the tail bones under any environmental temperature, (2) from the standardisation curves, the first 6-7 and the last 10-11 bones are consistently longer in the hot and shorter in the cold groups than those

of the controls, so that the average tail bone would be expected to lie between the 7th and the 18th caudal bones.

In addition the tails of one mouse randomly selected from the hot and cold groups were radiographed at the age of 40 days using Micro-Focal X-ray Unit, Type XM50M (for details and procedures see Buckland-Wright, 1976). From the photographic enlargement the 14th caudal vertebrae were examined.

Figure 3.15 shows the photographic enlargement of the 14th bone from the mice from the hot and cold groups. The vertebra from the hot reared mouse is a long, cylindrical and thin-walled bone. A fine trabecular bony network fills the entire vertebra particularly at the ends. Thin bands of radio-translucency indicate the sites of the growth plates 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 from the cold reared mouse appears shorter than the corresponding "hot" one. It is also thicker at the ends and thin in the mid-diaphyseal part. The cortices are thick, particularly at the centre of the bone. The bone, especially the metaphyses, is filled with a coarse trabecular bony network. At the ends smooth wide wavy bands of translucency indicate the sites of the growth plates, i.e. the metaphyses have not yet fused to the epiphyses. The foramina of the nutrient vessels are inconspicuous.

The femur and third metatarsal bones of a group of mice maintained at 33°C (hot group) and 8°C (cold group) were also measured. The femurs were measured from the X-ray films of the animals taken for the measurement of tail bones. The feet, however, were cut from the animal, laid flat and cellotaped to the unexposed X-ray films and then radiographed and enlarged as for the tails (p. 35). From these enlargements the third metatarsal bones were measured under the microscope.

Table 3.7 shows the lengths of the femurs and third metatarsal bones

	Femur		Third Metatarsal	
	Hot (33°C)	Cold (8°C)	Hot (33°C)	Cold (8°C)
No. of Cases	8	12	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	12.96 mm	7.48 mm	6.35 mm
S.D.	0.45	0.41	0.16	0.12
P	0.04715		<0.00001	

Table 3.7 The lengths of the femur and metatarsal bones of the hot (33°C) and cold (8°C) groups. P indicates the significance of difference between these groups

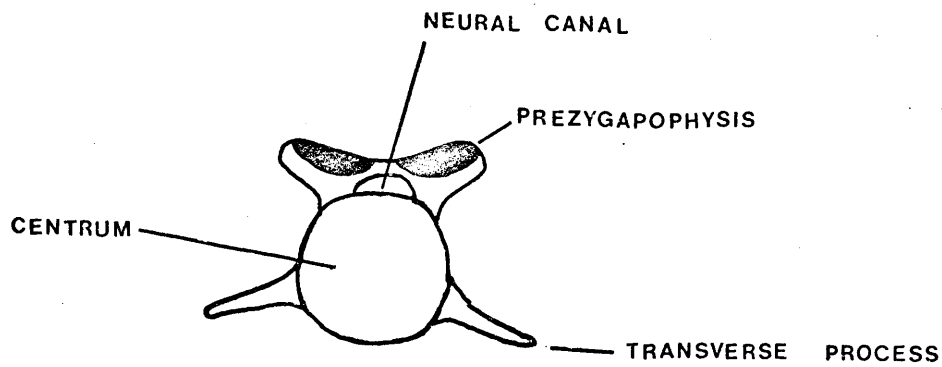


FIG. 3-16. ANTERIOR VIEW OF THE 3RD FREE CAUDAL VERTEBRA OF CONTROL - TAIL.

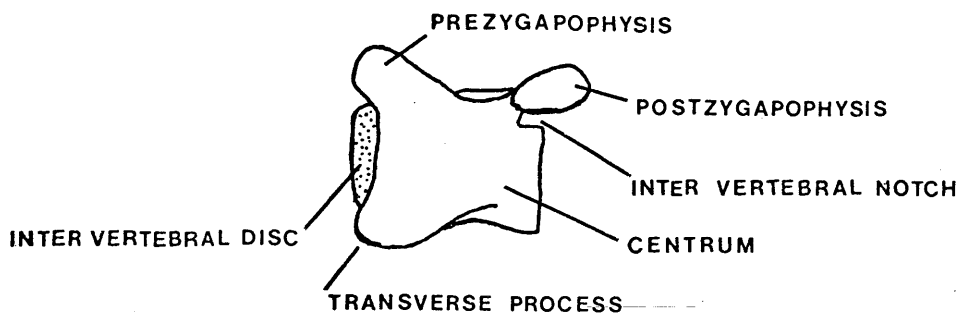


FIG. 3-17. POSTERIOR VIEW OF THE 3RD FREE CAUDAL VERTEBRA OF CONTROL - TAIL.

HOT [33°C]

CONTROL [21°C]

COLD [8°C]

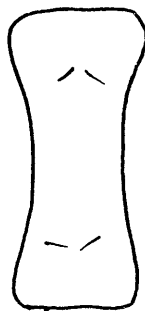
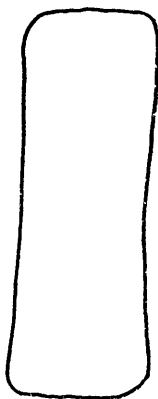


FIG. 3-18. THE 14TH FREE CAUDAL VERTEBRAE OF HOT, CONTROL AND COLD TAILS.

of the hot and cold groups. A very small difference was seen between the lengths of the femurs of these groups. This is attributed to the variations in the position of the mice on the X-ray film as well as the distance between the femur and the film. A significant difference was seen between the lengths of the third metatarsal bones of these groups.

Gross Study. The tails of a large number of mice from the three experimental groups were carefully dissected and examined under the dissecting microscope. The 3rd and 14th vertebrae of the 40 day old mice were examined.

In these groups no gross morphological differences were seen between the 3rd caudal vertebrae of the three groups. Figures 3.16 and 3.17 show the anterior and posterior views of this bone of the control group. It is made of a body (centrum), a neural arch which forms the vertebral canal, but there is no neural spine. There are also six bony processes, 2 transverse processes, 2 anterior zygapophysis, and 2 posterior zygapophysis.

The main gross morphological differences were seen between the free caudal vertebrae (Figure 3.18) which confirmed the radiological findings.

Histological Study. From paraffin sections (4 μ m) stained with H & E the 14th caudal bone of a number of animals of different ages maintained at 8°C, 21°C and 33°C were examined. This bone is bounded peripherally by dense fibroelastic ligaments (which encircle and connect all the tail bones together) and by the intervertebral discs on its distal and proximal surfaces. These discs consist of a nucleus pulposus (which acts as a cushion when the tail bones move over one another) encircled by the nucleus fibrosus. The bony segment itself is made of several flattened cells (articular cartilage) covering the ends, the growth plates at each end and cortical bony walls enclosing the bone marrow.

Table 3.8 and Figures 3.19 and 3.20 show the histological features of the 14th caudal vertebrae of the cold (8°C), hot (33°C) and control

No. of animals Age of animals	HOT (33°C)		CONTROL (21°C)		COLD (8°C)	
	3	3	3	2	4	2
	40 days	165 days	40 days	200 days	40 days	420 days
A. Art. Cart.						
1. Mean thickness	Centre 29 um Periphery 24 um	129 um 129 um	59 um 100 um	106 um 65 um	82 um 124 um	71 um 106 um
2. Average cell thickness	Centre 1-2 cells Periphery 1-3 cells	2-3 cells 1-2 cells	4-5 cells 1-3 cells	3-4 cells 1-2 cells	4-6 cells 6-8 cells	5-5 cells 5-7 cells
B. Growth Plate						
1. Mean thickness	Centre 55 um Periphery 59 um	76 um 47 um	82 um 106 um	67 um 67 um	255 um 147 um	88 um 118 um
2. Average cell thickness (centre)	Centre 3-4 cells Periphery 2-3 cells	1-3 cells 1-2 cells	5-6 cells 3-4 cells	2-4 cells 1-2 cells	4-6 cells 8-10 cells	2-3 cells 1-3 cells
C. Bone Seg.						
1. Mean total length	3.58 mm	3.82 mm	3.15 mm	3.53 mm	2.53 mm	2.78 mm
2. Cortical bony walls	very thin, non-lamellar	smooth	thin, less lamellar	smooth	very thick, lamellar	very thin
3. Thinning at mid-diaphyses	fine traversing the bone		occasional and fine		thick, coarse	
4. Bone trab. in bone cavity	86 um	94 um	129 um	148 um	386 um	451 um
5. Mean thickness at mid-diaphyses	57 um	62 um	115 um	125 um	257 um	312 um
6. Mean thickness at metaph	less fatty, very cellular		fatty, moderately cellular		fatty, less cellular	
7. Bone marrow						

Table 3.8 Main microscopical findings of the 14th caudal bone of animals in the 3 experimental groups at different ages

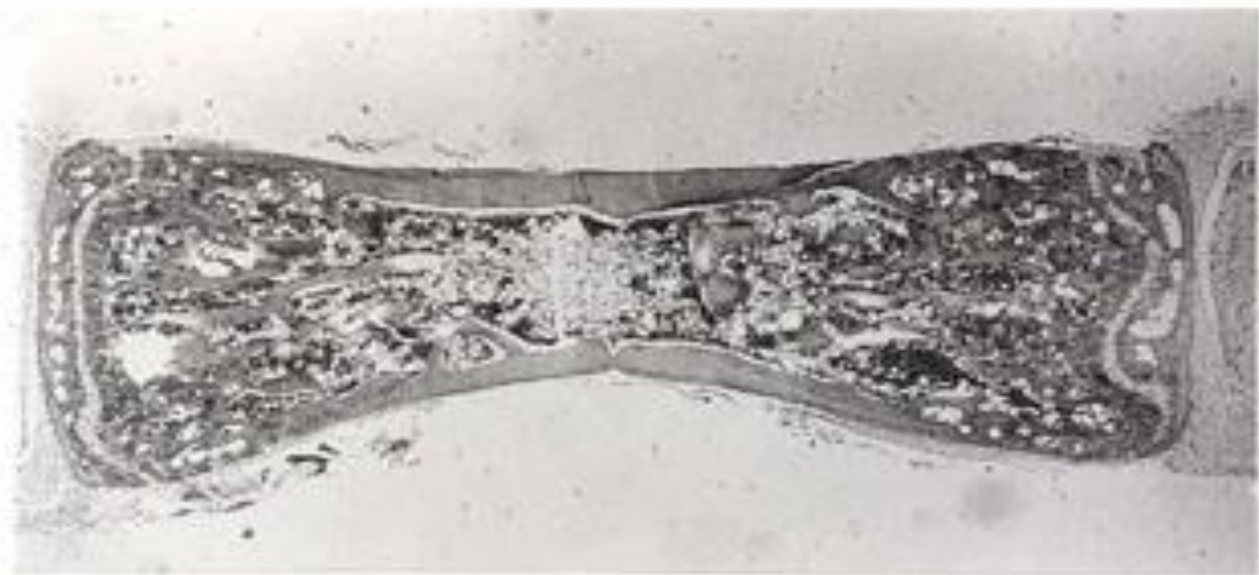
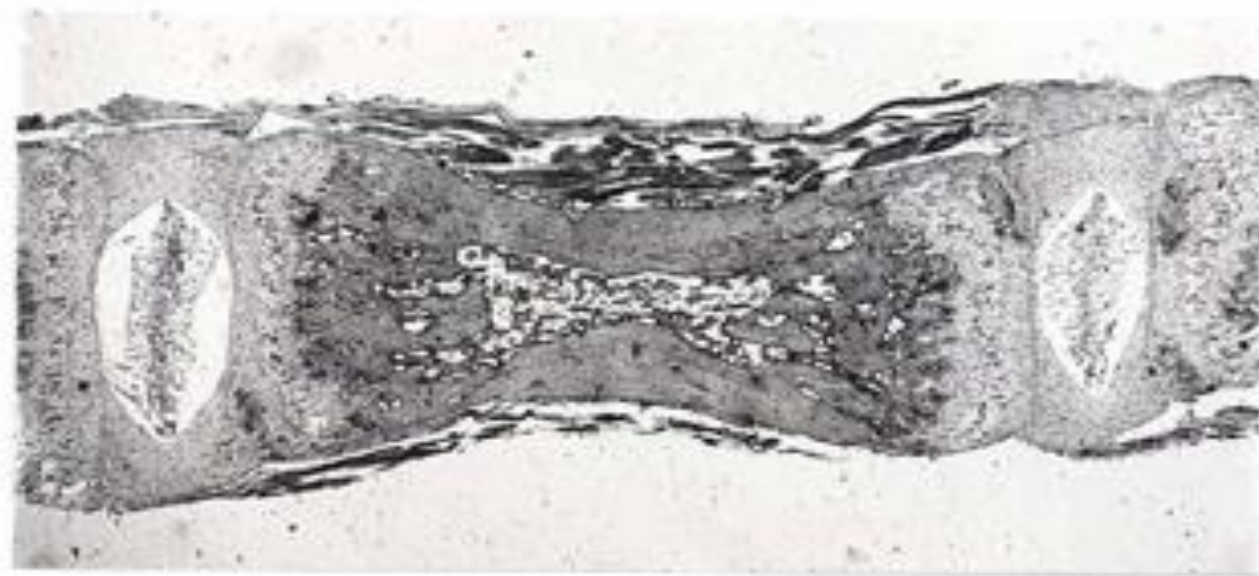
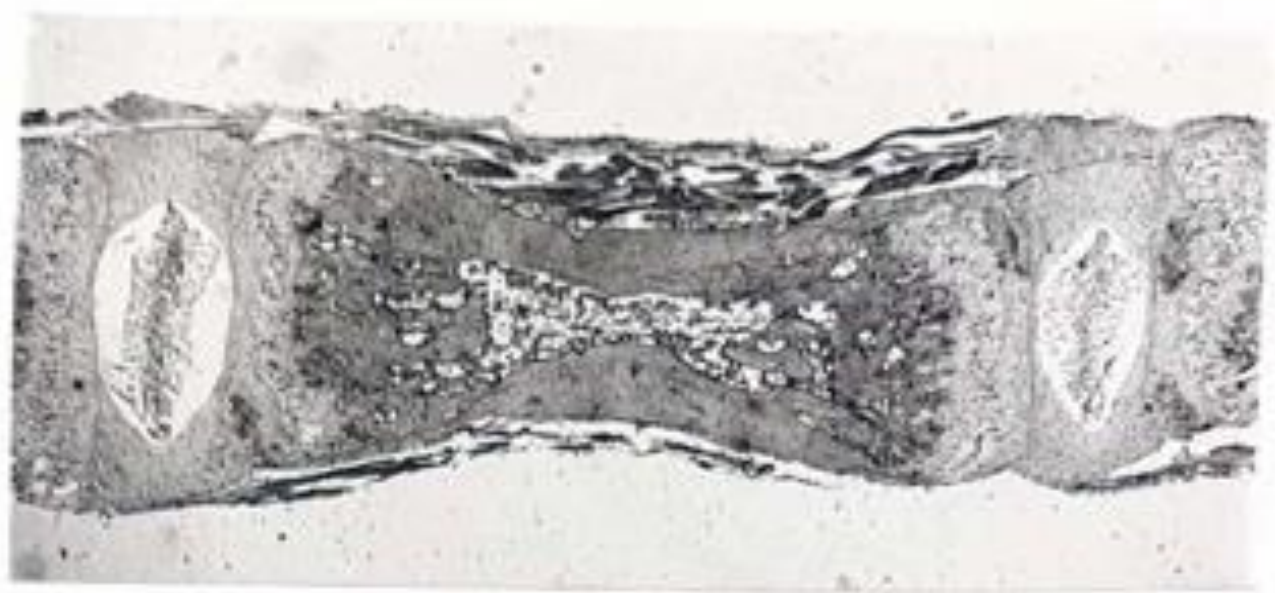


Fig. 3.19 Photomicrograph (H & E) of the 14th caudal vertebrae of the hot (33°C) (right), control (21°C) (middle) and cold (8°C) (left) groups at the age of 40 days (x 30)

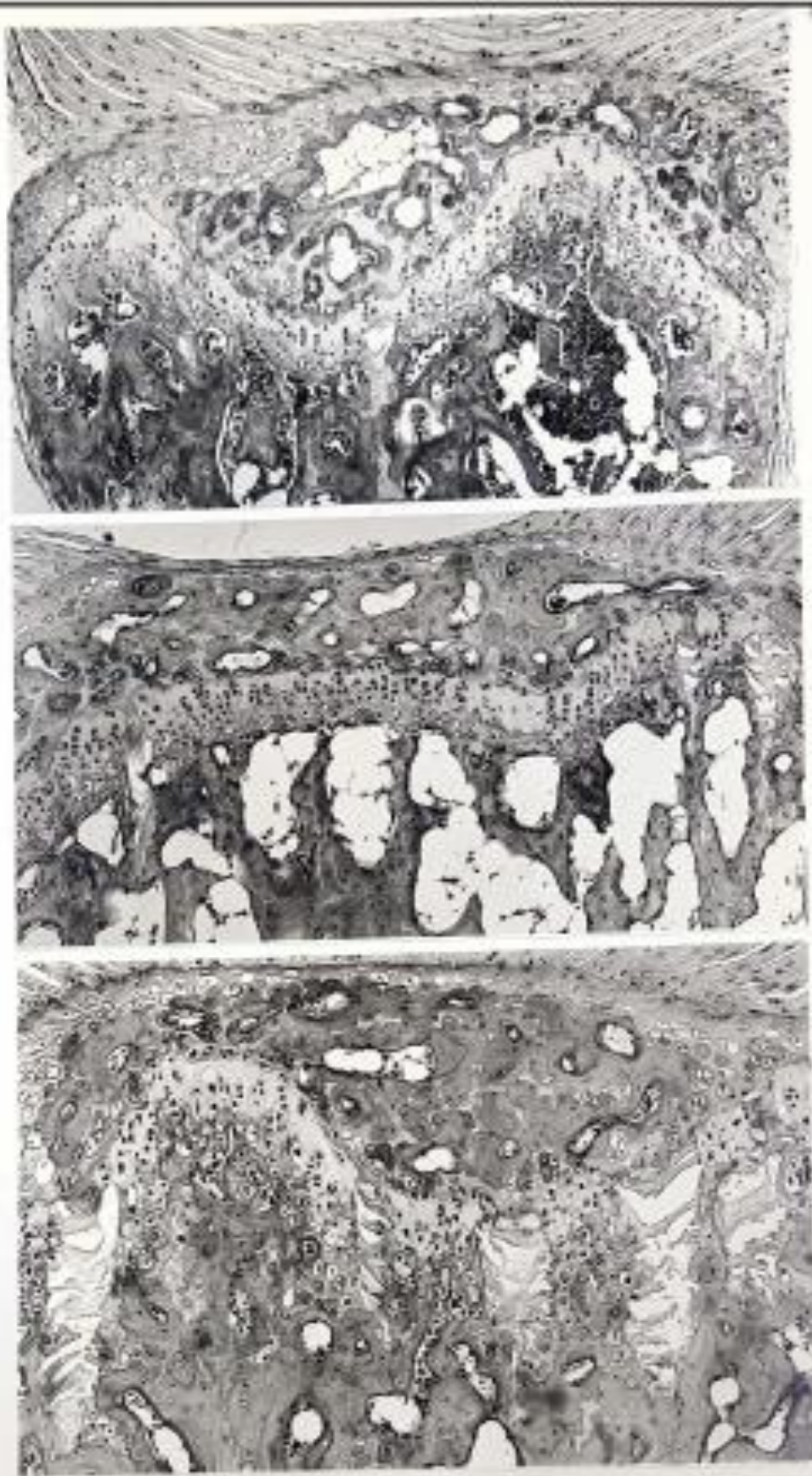


Fig. 3.20 Photomicrographs (H,E) of the growth plates of the 14th caudal vertebrae of the hot (35°C) (upper), control (21°C) (middle) and cold (8°C) (lower) groups at the ages of 165, 200 and 320 days respectively ($\times 125$)

(21°C) groups at different ages.

The vertebral lengths of this bone obtained from the radiographic measurements were found to be greater than those obtained by microscopic measurement. This is due to tissue shrinkage which occurs with the use of formol saline fixative and routine histological processing.

At the young age of 40 days, the growth plates in all experimental groups were thicker than those at older ages. At the older ages the main histological feature of the growth plates in all the groups were the signs of epiphyseal closure. These include:

(1) The proliferating cartilage cells were scanty in an abundant amount of intercellular matrix.

(2) The hypertrophic cartilage cells were also scanty, 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) Many osteoclasts and a few osteoblasts were seen on the surfaces of the bony trabeculae. This may mean that the growth plate and bone were being gradually removed and not replaced by new bone formation.

(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 also appeared irregular and osteoclasts were seen on the surface.

(7) Areas of hyalinised 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. These perforations may aid in the compression of the remnants of the growth plate and accelerate the vascular invasion. They may also help to establish the connection with the capillary tufts of the zone of erosion. In some cases complete ossification of the articular cartilage was evident and a "bridge" of bone was seen connecting the bony 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. In addition, from the radiographic results (Figure 3.15), the signs of impending closure of the "hot growth plates" were evident as early as the age of 40 days.

C. Human Clinical Observations

The following clinical cases are reproduced by kind permission of Drs.E.Hamilton (Case 1), A.St.J.Dixon (Case 2) and Barbara M.Ansell (Case 3,4 and 5).

Case 1. B.P.Z. (F). Born 3.10.1968.

- April,1969 Intermittent swelling of right knee.
- 1973 - X-ray showed increase in size of lower femoral epiphyses.

Diagnosis: synovitis.

- Jan.1975 - right leg 2 cm longer than left.
- 22.7.1975 - synovectomy: villonodular synovitis.

Manipulation under G.A. resulted in a supracondylar fracture which required P.O.P. Fourteen weeks later, a further operation to cut the adhesions between the supracondylar pouch and the patella was carried out. Five manipulations were then performed.

- Jan.1976 - flexion contracture 18° , extension lag 4° , flexion lag 85° . "Nasty gait".
- 28.6.1976 - warm bony swelling of right knee. Marked wasting of right quadriceps. Range of movements $25-70^{\circ}$. The right leg is 3 cm longer than left. X-ray: osteoporosis and irregular bone density of lower right

metaphysis.

Case 2. R.J. (F). Born 8.2.1969.

- July 1975 - fall from a bicycle. Swelling of right knee. Aspiration of right knee joint: a few cells but sterile, A.F.B. negative. Arthroscopy and biopsy: monoarticular rheumatoid arthritis.
- 27.1.1976 - right knee swollen and shows excess fluid and synovial thickening. The right knee flexed as far as the left. No flexion contracture. 1 cm muscle wasting in the right calf and thigh. The right leg 1 inch longer than left. Much of the difference in the tibia.
- 10.8.1976 - slight wasting of right thigh and calf. Trace of fluid still in right knee which is soft, cool and not tender. The distance from A.S.I.C. to right heel is 77.5 cm on the right side and 76.5 cm on the left side. Inequality is less.
- 6.10.1976 - inequality lessened to 1 cm.

Case 3. N.K. (F). Born 20.6.1969.

- At the age of 2 years 2 months: onset of monoarticular rheumatoid arthritis of right knee.
- 16.8.76 - right leg longer than left. Circumference of right knee is 3.5 cm greater than left leg. X-ray: overgrowth of femoral and tibial epiphysis. Synovectomy.
- 13.6.1977 - synovectomy

Case 4. J.H. (M). Born 16.3.1958.

- October 1966 - onset of monoarticular rheumatoid arthritis of left knee.
- 10.9.1968 - left leg is $\frac{3}{4}$ inch longer than right. Circumference of right knee 1 inch greater than right. X-ray: overgrowth of femoral and tibial epiphyses. Synovectomy.
- 6.1.1969 - left leg is $\frac{1}{4}$ inch longer than right.
- 4.8.1969 - leg lengths equal.

- At 5 years follow up: epiphyses are equal.

Case 5. J.D. (F). Born 26.9.1964.

- 1968 - at the age of 3 years 1 month: onset of monoarticular rheumatoid arthritis of right knee.
- 20.5.1972 - right leg 1 inch longer than left. Circumference of right knee 1 inch larger. X-ray: overgrowth of femoral and tibial epiphyses.
- 5.6.1972 - synovectomy
- 4.1.1973 - right leg $\frac{1}{2}$ inch longer than left
- 8.8.1974 - leg lengths equal
- 25.3.1976 - leg lengths equal

Summary of Clinical Observations

In these cases the unilateral involvement of one knee joint with rheumatoid arthritis has caused the diseased limb to grow longer than the sound limb. But after a certain period the short limb catches up with the length of the diseased side.

D. Discussion

Growth has been defined by Moss (1972) as "any change in any parameter that is measurable". This definition could be applied to any organism or any part of it. The skeletal system has three basic properties: size, shape, and age. The first (as exemplified in the tail length) and the third are measurable parameters in the ordinary and practical senses. The second (shape), is not spoken of in terms of real numbers but as a geometric disposition of numbers in a space (Medawar, 1945). In his essays "On Growth and Form", Thompson (1917) has elegantly emphasised that the different sizes and shapes of the mammalian bones can be shown to have a similar shape if they are transformed by altering the rectangular coordinates by relatively simple mathematical transformations. One can thus look at any human long bone (for example) as similar to that of any long bone in the mouse (such as the tail bones). The mathematical treatment and graphic

representations in this work are hoped to satisfy students of growth and descriptive morphologists and to reflect the underlying biological phenomena.

1. The Long Term Effects of Changes in the Environmental Temperature on the Growth of Tail Bones

When this work was started it was difficult to maintain the experimental animals in the extreme hot and cold environmental conditions. Although the early attempts were associated with many failures, the successes were used to identify the desired optimal environmental condition. The results indicated that one week old mice survive only when housed with their mothers at 8°C and 33°C. The differences in the growth rates of the tails of these mice did not, however, differ significantly during the first 3 weeks of life. In fact the tails of the cold reared mice grew faster than those of their litter-mates maintained in the hot room. This was presumably because the mothers were able to keep their young warm, as was confirmed by measuring the temperature maintained by the litter inside the cotton-wool nest. However, at about the age of 21 days, i.e. the age of weaning, the young mice were seen to occupy several sub-compartments in the cotton-wool nest away from the mothers. This made the young mice dependent on themselves for nutrition and survival and also exposed them directly to the cold temperature; hence the reduction of the growth rate of their tails after the age of weaning. Therefore, although only about 3 cm of the tail growth remained to be completed after the age of weaning (Noel, 1968), the method used in subsequent experiments was to wean the mice at 22-23 days old and transfer them into the hot and cold rooms at 25 days old. At this age the mice were found to survive temperatures as low as 4°C and as high as 33°C, but at 38°C they failed to gain weight and died.

The series of experiments described in this study amply confirms the influence and the role of the environmental temperature in the determination

of the growth of the tails (Sumner, 1909, 1913, 1915; Sundstroem, 1922; Przibram, 1931; Ogle, 1934; Harrison et al, 1959, 1969; Chevillard et al, 1963; Barnett, 1965; Noel and Wright, 1970a). Thus, all the adult mice reared at the higher temperatures of 33°C and 36°C had tails 1-2 cm longer than all litter-mates reared at 21°C, and as much as 3-4 cm longer than all those reared at the lower temperatures of 4°C and 8°C. In this way the growth of the tails conformed to Allen's rule (1905).

The effect of environmental temperature on the growth of tails was particularly marked during the first 4 weeks of exposure to the environmental conditions and especially at the higher temperatures. In this case the final length of the tail (and tail bones) was determined by changes in the growth rate and growth period. Therefore, soon after exposing the 25 day old mice to the hot environment, the tails grew very rapidly, attained a greater adult size and matured and stopped growing earlier than the tails of the control and cold groups. On the other hand, the exposure to the cold markedly slowed the growth rate of the tails but prolonged the growth period. This may account for the late maturation and cessation of growth of these tails. However, as will be indicated later on, these tail bones retain some growth potential for a very long time. The tails of the cold groups were permanently shorter than those of the control and hot groups (Harrison and Clegg, 1969).

The standardisation growth curves also confirm the changes in the growth rate and growth period. The appearance of the percentage ratio above or below the control line indicates that the tail lengths of the groups concerned are longer or shorter than those of their control group. In any case, the increasing and decreasing ratios of the tails of the hot and cold groups (growth rate) occurred mainly during the first 4 weeks (growth period) of the change of environmental temperature. The constant ratios thereafter reflect the processes of maturation and cessation of

tail growth.

During the period of maximal tail growth under the effects of changes in the environmental temperature the growth rate which accompanied a particular environmental condition was reversed several times. This was done simply by changing the environmental temperature and resulted in the appearance of graphical "diamonds" and "parallelograms" in the growth curves of the tails of the cold and hot reared mice. One is therefore able to make the vertebral bones of the mouse grow rapidly or slowly for as long or as short as desired.

There are between 27 and 30 free caudal vertebrae in the mouse's tail and 54 to 60 epiphyseal cartilage plates (Rowett, 1974). Thus a change in the tail length under any environmental condition reflects a change in these bones. Although the mechanisms by which these temperatures determine the growth rate of the tail bones are not clear, they may be through: (1) alteration of the blood flow to the growth plates, particularly the dividing cartilage cells, (2) direct action on the metabolic activities of the cells of the growth plates, or (3) direct action on the length of the mitotic cycle and/or the differentiation of the cartilage cells in the epiphyseal column; i.e. the temperature has a direct and local effect on the growth plates of the tail bones.

2. The Effect of Environmental Temperature on the Growth Potential of Tail Bones

In this work the effects of environmental temperature on the growth of tail bones were used to assess the growth potential of the growth plate. This was done by regularly transferring the mice from one "effective" temperature - the cold - in which the growth rate of tail bones was reduced, to the hot which was "potentially favourable" for maximum tail growth.

The growth potential of the endochondral bone is the capacity for the cells of the growth plate to divide and differentiate.

The growth in length of long bones, as exemplified here by the tail bones, consists of 3 interdependent processes: (1) the division of cartilage cells at the germinative zone of the growth plate, (2) the palisade differentiation of the cartilage cells in the epiphyseal column, and (3) the removal of the cartilage cells and calcification of the matrix. Thus one may postulate three possible groups of factors which may control the growth of long bones.

The first group of factors concerns the specific genetically-programmed ability of the germinative cartilage cells to divide (Siffert,1966; Rang, 1969; Wright,1969; Owen,1970; Hall,1970,1971; Little,1973; Noel and Wright,1972). This may simply mean the total number of cell divisions occurring in the germinative zone of the growth plate during the growth period. It also means that the basic shape and the final length of the bone is inherited in the bone itself and thus controlled by "local control factors".

So, when an animal is transferred from a lower to a higher environmental temperature, the process of cell production and removal is greatly accelerated. Accordingly, the definite number of cell divisions and differentiation are completed more quickly. This early completion of the cellular programme would be responsible for the earlier cessation of tail growth in the hot groups than in those of the control and cold groups which retain their potential for a long time. Again, the final length of the bone represents changes in the growth rate and growth period of the cellular programme. The hot environment has led to the achievement of the full capacity of the growth plate in a very short time.

The concept of growth potential may explain the failure of the tails of the lower temperature to catch up with those tails of the higher temperature. Likewise, it can also account for the failure of the tails of the "parallelograms" (Figure 3.8) to catch up with the tail length of one

another. It appears that the prolonged maintenance of the mice at lower temperature is associated with gradual reduction of the growth potential of the growth plate. Thus when transferred to the higher temperature only the remaining potential can be used and this results in some increase in tail length which remains less than those of the mice which were transferred at an earlier age to the higher temperature. But, from the curves and tables of growth rate a gradual reduction of the tail growth (growth potential) was seen as the mice become older. Thus one would normally expect a gradual reduction of the growth potential with age, which may be related to the degree of vascularity of the growth plates and the impending fusion of the metaphyses to the epiphyses (Harrison and Clegg, 1969).

At the lower temperature the process of gradual reduction of the growth potential of the tail bones finally ends with complete loss of the whole potential. Thus, when female and male mice which were maintained in the cold room (8°C) for more than a year were transferred to the hot room (33°C) there was no increase in tail length.

The second group of factors may determine the differentiation (epigenesis) and palisading of the cartilage cells along the epiphyseal columns before these cells are finally removed. Such factors - the "epigenetic control factors" - may possibly dictate the innate genetic control factors, and may therefore determine the final tail lengths of animals reared at different environmental temperatures, especially those of the extreme groups (4°C & 8°C and 33°C & 36°C). Accordingly, the growth plates of the tail bones may, at the lower temperature, produce smaller differentiated cartilage cells along the epiphyseal columns than at the higher temperatures. It follows that the continuous production at a slow rate in the germinative zone of the growth plate of cartilage cells which differentiate into small cells and are removed at a slow rate along the epiphyseal column may account for the shorter tail bones in the lower temperature than those of the

higher temperatures.

In the 33°C and 36°C groups (Figure 3.7), the initial more rapid growth rate of the 36°C group may be explained by the faster cell division in the growth plates of the former than those of the latter. Later on, the growth rate was similar in both groups, but because (presumably) the differentiated cells in the 33°C group were smaller than those in the 36°C group, the tail length of the first group was finally shorter.

The third group of factors which may also determine the final length of the tail bones may be related to the rate of removal of the cartilage cells, the calcification of the matrix and its replacement by bone. These processes are largely influenced by the degree of vascularity of the growth plate (Trueta, 1960a, 1960b, 1960c, 1963a, 1963b, 1964; Siffert, 1966; Sunden, 1967; Rang, 1969; Brookes, 1971; Little, 1973) which is increased in the hot and decreased in the cold environment. For example, in the hot room, the increased blood flow to the growth plate is associated with rapid production of cells at the germinative zone as well as increased removal of the calcified matrix and bone formation.

However, the degree of vascularity of the growth plate indicates the metabolic activities of this structure. Therefore, the "vascular factors" could not be regarded as "primary growth factors" directly related to the changes in the environmental temperature, but as a "secondary growth factor" related to the metabolic activities of the growth plate.

The Effects of Environmental Temperature on the Growth Potential of Mice with Short Tails

The response of the undersized and underweight mice with short tails to changes in the environmental temperature presents three problems: the body weight, body size and tail growth. Regrettably the animals maintained in the hot room died in an accident. Therefore, an analytical study of the body size similar to those of Dixon (1953) on the outliners was not possible.

The effect of undernourishment and malnutrition on the growth of the mammalian body is well known, and it seems most likely that the pregnant mice were stressed by the extreme cold and hot environments and were unable to feed their young after birth despite an ad libitum diet (MacDowell et al, 1930; Crozier and Enzman, 1935; Schultz, 1954; Harrison et al, 1959, 1969; McCance, 1959; Widdowson et al, 1960, 1962, 1963; Tanner, 1962; McCance and Widdowson, 1962; Barnett and Scott, 1963). This has resulted in permanently undersized and underweight animals. The failure of these animals to gain weight was explained by Widdowson and McCance's conclusion (1960, 1963, 1975) that the earlier in the life of the animal the undernourishment was imposed, the more serious and permanent the effects would be.

In addition, the brain (possibly the hypothalamus) probably controls the growth of the mammalian body (Tanner, 1963; Widdowson and McCance, 1975). Therefore, malnutrition occurring in the early life of the animal while the brain is still rapidly growing could permanently damage the brain control mechanisms and result in permanently undersized and underweight animals.

The variability in size, weight and tail growth in a litter of mice may also be due to insufficient uterine space or circulation (Eckstein and associates, 1955a, 1955b, 1955c) and to variability of the maternal temperature (Russell, 1948). The genetic influence seems to be equally important (Russell, 1948; Falconer, 1953; Chai, 1956a, 1956b; Biggers et al, 1958; Harrison et al, 1959, 1969).

The results of the present work confirm the findings of Harrison et al (1959, 1969) that the hot environment is a favourable condition for the small and light animals to show a positive peak in tail growth after a change of environmental temperature. Thus the tails of these animals reached a considerable length in a relatively short period. This form of "favourable growth potential" towards the accomplishment of tail length

greater than the adult length in undersized and underweight animals is mainly related to the earlier onset of the "growth stimulus", i.e. the hot environment.

The main objectives of maintaining the undersized and underweight animals in the hot environment were (1) to test the hypothesis that if the environmental temperature determines and, in case of the hot condition, stimulates the growth of tails, then the tails of these runts might catch up with the tail length of a control group maintained at the same temperature, (2) to assess the growth potential of the tail growth of these animals.

After the change of temperature to the hot environment, the tails of these mice not only showed a greater positive peak of tail growth but also ceased growing earlier than control litter-mates maintained at the same temperature. In this way the hot environment seems to have brought about the sum of the definite number of programmed cells divisions and differentiation occurring in the growth plates of the mice with short tails more rapidly to an end than in the other group, hence the earlier cessation of tail growth. This sum may be genetically determined and certainly less than that of the control group, because although the growth plates of both groups may have responded by producing bigger differentiated cartilage cells, the first group failed to catch up with the growth and tail length of the second group.

In the cold, the growth of the undersized and underweight animals which were born in the cold (8°C) represents a condition of "depressed growth potential", not only in their failure to gain weight but also in tail growth. In these animals, the slow growth rate in body weight seen during the first 4 weeks was followed by an even slower rate during the following weeks. Thus, at the age of 172 days they were lighter by 10.3 gm than those animals which were maintained in the cold room (8°C) at the age

of 25 days (p.125).

At the age of 10 days, the tails of the animals which were born at 8°C were shorter than those born at 21°C. The growth rate of the tails during the first 4 weeks was consistent with those rates of the animals maintained in the cold room at the age of 25 days. Therefore, the effect of the cold environment on the growth of the tails would be the same regardless of the age of the animals or the temperature at which the animals were born. This seems to be due to the retained amount of growth potential, because unlike those groups which were born at 21°C and housed in the cold room at the age of 25 days, the tails of the mice born in the cold did not lose any growth potential in the 21°C environment. Instead, the growth potential was retained over a long period but was finally lost.

Finally, although all the groups born in the cold and hot environments remained undersized and underweight animals from birth, the effect of these temperatures on the growth rate of the tails was independent of that on the body size and weight. This determination of the tail growth must therefore be due to the local effects of the environmental temperature.

3. Changes in the Body and Tail Temperature

Although the internal temperature of the mouse is more labile than that of other larger mammals (Brody,1945) the differences in internal (rectal) temperature in animals maintained at the different environmental conditions was insignificant. It was about 1°C lower in the 4°C and 8°C groups than in the 21°C and 33°C animals. This was, however, mainly due to the temperature of the male group in the cold (4°C and 8°C) which was 0.8°C higher than the females. These findings agree with those of most other workers (Sumner,1913; Fuller and Hiestand,1947; Lessin and Parkes,1957; Harrison and Clegg,1969) and confirms Brody's statement (1945) that among the eutherian mammals the mouse has one of the lowest temperatures.

Sumner (1913) found no difference in the internal temperature of mice

kept at 22.3°C and 5.8°C once the animals were acclimatised to the environmental temperature. Likewise, Lessin and Parkes (1957) found that the internal rectal temperature of mice maintained at an ambient temperature of 32°C was similar to that of those maintained at 22°C . However, the work of Harrison et al (see Harrison and Clegg, 1969) indicated that mice reared at 90°F (32.5°C) tended to be $1-2^{\circ}\text{C}$ higher than their control group maintained at 70°F (21.3°C). In addition, Scholander et al (1950a, 1950b, 1950c, 1955), in their studies on the adaptation and thermoregulation of a wide variety of species, have also indicated that arctic mammals and birds have an internal rectal temperature similar to that of animals in temperate climates.

The external body temperature reflects both the internal temperature of the part and the surrounding environmental temperature. This was especially seen over the cervical and interscapular regions of the cold and control groups. In these regions the underlying brown adipose tissue (p.159) serves as a heat generator. In the hot group this form of fat had transformed into ordinary white adipose tissue and thus had no thermal function. The relatively higher local temperature over the abdomen in the cold group may have been due to the thicker fur coat (p.143) which provided better insulation, but mainly reflected the internal (abdominal) temperature. It is not clear whether the small difference of 0.6°C which was recorded over the ears between the 4°C and 8°C groups was due to the local effect of the environmental temperature on the cartilage of the ears or reflected the surrounding temperature.

In this study the main difference in the external temperature was seen over the tails, particularly the distal parts which were only a few degrees higher than the surrounding environments. This also reflects the internal temperature of the tail bones as well as the surrounding environment. Thus the temperature of the tail bones was independent of the

external body and internal (rectal) temperature. Therefore, the effect of environmental conditions on the growth of tail bones must be due to the direct and local effect of the temperature on the metabolic processes of the tail bones (the growth plates). In this sense, the mouse appears to be unable to control the temperature of the tail (tail bones) because the tail closely reflects the temperature of that environment.

On emerging from the tail towards the body, the temperature of the proximal tail bones approximates to that of the body. This is because these bones are maintained and influenced by the internal temperature of the body. In this way, the temperature of the internal and proximal tail bones may be slightly raised (in the hot) or lowered (in the cold) by the heated or cooled venous return from the tail.

4. The Local Effects of Environmental Temperatures on the Growth of Long Bones and Tail Vertebrae

The changes in the tail length brought about by changes in the environmental temperatures could be due either to differences in the vertebral length or in the number of the tail vertebrae.

In this study, the effect of environmental temperature on the tail bones was demonstrated to be exclusively a change in the vertebral length and never in the number. The sacral, first 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. These are internal bones and their temperature will approximate that of the internal temperature of the body (Noel and Wright, 1970a). Thus, the morphological character of the 3rd caudal vertebra is not different in the tails of the mice exposed to the three 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 determined by changes in the environmental temperature (Allen, 1905; Sumner,

1909,1913,1915; Sundstroem,1922). These observations are seen here as another indication of the presence of local control growth factors which are selective not only to certain tail bones but also to some other bones of the mouse. In the mouse and rat, the importance of the tail as a thermoregulatory organ is well known (Sundstroem,1922; Scholander et al, 1950a,1950b,1950c,1955; Harrison,1958; Barnett,1965; Rand et al,1965) and it seems that the feet and possibly the hands and ears may act as an auxiliary thermoregulatory organ (see p.166).

It is often claimed that mice born in the cold (-3°C) tend to have fewer caudal vertebrae than normal (Barnett,1965). This has been attributed to variation in the maternal temperature (Russell,1948). However, most of the attempts carried out to study this finding used a special inbred strain of animal whose vertebral number may be genetically predetermined. In this work, the two (female) mice born in the cold (8°C) had 29 caudal bones. Another point here is that in the first weeks after birth, the distal vertebrae cannot be seen radiologically because they are made of cartilage.

In the cold the production of thick lamellar cortical bone in the tail vertebrae which in some cases fills the entire bone segment appears to be due to appositional bone growth. This may reflect an increased rate of bone accretion on the periosteal surface and decreased removal of bone on the endosteal surface. The latter factor may itself be due to diminished vascularity. The environmental temperature may therefore determine the rate of bone remodelling. However, there seems to be another factor responsible for the production of thick cortical bone in the tail bones of the cold group. This is to fill the rather inactive bone marrow cavity in which the cellularity is reduced.

In the bones of the hot group, appositional bone seems to be removed rapidly (presumably) because of the increased vascularity. In this way, the shape of the bone cavity was changed to accommodate the marked

cellularity of the active bone marrow.

The appearance of mid-diaphyseal thinning in the vertebrae of the cold group is not the function of the cold environment, because similar but delicate thinning was seen in the bones of the control groups. It may, however, be an exaggeration of the control picture produced by the combined effect of the two factors which are responsible for the "reshaping" of the tail bones of the cold group: (1) epiphyseal growth occurring at a slow rate at the ends of the bone segment and (2) appositional bone growth occurring in the walls of the bone segment between the growth plates. These factors change the shapes of the tail bones from the rather longer rounded tubular bones in the control group to the short, thick bones which are wide at their ends and narrow at the mid diaphyses in the cold group.

The environmental temperature was also found to determine the maturation processes of the tail bones. This was seen in the differences in length between the terminal vertebrae. In the bones of the cold group, which retained their growth potential for a long time, the bones appeared histologically as cartilagenous segments and were not yet calcified. In the hot group, these bones appear calcified and show normal bone structure. The effect on the terminal bones is seen here as a clear indication of the direct and local effect of the environmental temperature on the growth of tail bones.

The thickness of the growth plate bears a constant relationship to the amount of bone produced (Tapp,1966; Rang,1969; Sisson,1971; Moss-Salentijn,1974). It is also related to the rate and number of cells produced. Thus the "hot growth plates" which contributed considerably to the epiphyseal growth and consumed most of their growth potential during the first 15 days in the hot room appeared thinner than those of the control group. On the other hand, the growth plates of the cold

groups which contributed little to epiphyseal growth appeared thick.

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 Becks et al (1948) on the third 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 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 to 17 weeks or delayed by cold for as long as one year.

A reasonable hypothesis would seem to be that closure may be closely related to the amount of growth potential retained by the growth plate. Thus it appears earlier in the "hot growth plates" than the control and cold groups (Harrison and Clegg, 1969). This is presumably because the "hot bones" were able to complete all their cell divisions (and maturation/differentiation) in a relatively short time and when this is completed closure occurs.

"Boy (20) 5 ft. 7 ins., wishes to increase height to 5 ft.10 ins."

The Times, 6 November 1968

5. Limb Inequality and the Clinical Implications of Changes in Local Temperature

Inequality in length of the limbs, especially the lower limbs, has been a subject of considerable interest to the medical profession in general and to surgeons and paediatricians in particular. However, despite the tremendous amount of work describing the clinical features and the experiments designed to correct limb inequality either by shortening the long limb or lengthening the short limb (for review and details see Bertrand and Trillat, 1948; Sunden, 1967; Rang, 1969), little attention has been focussed on the effect of local heat on the growth plates.

Limb inequality has been described with rheumatoid arthritis (Ansell and Bywaters, 1956; Jayson, 1976), anterior poliomyelitis (Ring and Ward, 1958), osteomyelitis (Harris, 1930; Trueta, 1953), joint tuberculosis (Gill, 1944; Sissons, 1952), haemophilic haemarthrosis (Dennison, 1952), haemangiomas, cirroid limb aneurysm, venous stasis, arteriovenous fistula, arterial and venous hyperaemia (Harris, 1930; Harris and McDonald, 1936; Ward and Horton, 1940; Janes and Musgrove, 1950; Hutchison and Burdeau, 1954; Trueta et al, 1953, 1963a, 1963b, 1964; Hierton, 1957, 1961; Richards and Stoffer, 1959; Dickerson, 1966; Doyle and Smart, 1969; Rang, 1969; Brookes, 1971), avascular necrosis, delayed healing of fractures and reduced growth potential (Troup, 1961).

The experimental and clinical methods successfully used to stimulate the longitudinal growth of bone have been reviewed by several authors (Sunden, 1967; Rang, 1969; Brookes, 1971; Little, 1973 and others). These include direct methods such as heat, X-ray, trauma, fracture, periosteal stripping, and application of various materials to the medullary cavity as well as indirect methods such as disturbed circulation, disturbed

innervation and changes in the dynamic conditions of the bone.

In the clinical cases described in this work (p. 79) there is unilateral inflammatory involvement of one knee joint with subsequent lengthening of the affected limb. After a certain period the short (sound) limb apparently catches up with the length of the diseased side. Thus, one would postulate a local accelerating factor due to the local involvement of the joint. This factor may be that of increased vascularity resulting from the active inflammatory condition which produces a local rise in temperature over the affected joint (Ansell and Bywaters, 1956; Rang, 1969). Therefore, these clinical conditions of "hot joints" resemble the experimental conditions of "hot tails".

The knee comprises two principal contributors to the longitudinal growth of the lower limb: the upper tibial and lower femoral growth plates. These plates are also known to grow at a faster rate than the growth plates at the other ends of these bones. This selective growth potential was attributed to the mechanical and biophysical forces which act on these plates (Moss-Salentijn, 1974).

The increased blood flow through the epiphyseal and metaphyseal vessels of these plates (Trueta, 1953, 1960a, 1960b, 1960c, 1963a, 1963b, 1964) may therefore lead to: (1) rapid cell division occurring in the germinative zone of the plate leading to increased chondrogenesis, (2) increased rate of removal of calcified matrix in the zone of osteogenesis. The net result of these factors is an increased rate of bone growth. Like the tails of the hot reared animals, maturity and cessation of growth is accomplished earlier in the diseased limb than the sound one.

The catch up of the sound limb with the growth and length of the diseased limb is often attributed to compensatory growth of the growth plates (David, 1924; Trueta, 1953; Dawson and Kember, 1974) of the sound limb. This seems unlikely, because limb overgrowth occurs mainly in the affected

bone (Trueta,1953). The growth rate of the sound limb which corresponds to the "control tails" was maintained over a long period and finally nearly caught up with the length of the diseased limb (the "hot tails") which had stopped growth earlier than the sound limb. In this way, the final results were brought about by changes in both the growth rate and the growth period.

The onset of these conditions of monoarticular "hot joints" occurred during the growing period (childhood), before epiphyseal fusion. Therefore the early recognition and diagnosis of the cause of limb inequality is important from the view of orthopaedic management since the prognosis is good and complete recovery and almost complete equalisation may be expected with time.

Nevertheless, certain human races living in warm regions of the world (the pigmies of Africa) are known to be shorter than other races living in the colder regions (Scandinavian). Indeed, racial differences in height do exist (Tanner,1962; Sinclair,1973) but the climatic differences here play a minor role. Whitacre and Grimes (1959) have suggested that the faster growth of children in South Texas relative to those in Northern Texas might be due to the warmer climate of the south. But these results can be explained on the basis of socioeconomic differences (Berry and Cowin,1954; Greulich,1958; Tanner,1962). However, it is possible that the growth plates of the human bones (like the tail bones of the mice) are genetically predetermined and programmed for a definite number of cell divisions which may vary greatly from one race to another. This factor may also explain the racial variation in skeletal growth and maturity (Tanner,1962). This same factor may still explain why certain growth plates of the human skeleton grow (proximal end of tibia and fibula and distal end of femur in the lower limb and distal end of ulna and radius and proximal end of humerus in the upper limb) or close at a different rate from other plates. The genetic code must still explain why the

phalanges and toes do not grow to the size and length of femurs, tibias or humeruses.

CHAPTER IV. THE EFFECTS OF CHANGES IN THE ENVIRONMENTAL TEMPERATURE ON THE GROWTH OF TAIL BONES DURING THE FIRST 48 HOURS

A. Introduction

The growth in length of long bones as exemplified in the tail bones consists of 3 interdependent processes: cell division at the germinative zone of the growth plate, palisade differentiation of the cartilage cells and the removal of these cells towards osteogenesis. In the preceding chapters the changes in length of tail bones were investigated as changes in this cellular repertoire on the assumption that the germinative cartilage zone is capable of a definite number of cell divisions (Siffert, 1966; Rang, 1969; Wright, 1969; Owen, 1970; Hall, 1970, 1971; Little, 1973; Noel and Wright, 1972). This genetic predetermination with its consequences in bone growth and maturation, however, determined the long term responses to the environmental temperature which have been reported by several authors (Sumner, 1909, 1913, 1915; Sundstroem, 1922; Przibram, 1931; Ogle, 1934; Chevillard et al, 1963; Barnett, 1965; Harrison et al, 1959, 1969; Noel and Wright, 1970a). Reports on the immediate effects of changes in the environmental temperature which may possibly be due to changes in the second and third stages of the cellular repertoire of the growth plate (the differentiation and removal of the cartilage cells) are absolutely lacking. This chapter is concerned with the study of changes in the growth of tail bones during the first 48 hours after a change of environmental temperature. To separate the mitotic activity of the growth plate from that of the cellular differentiation of the cartilage cells a supralethal dose of X-irradiation was used.

B. Experimental Procedures and Results

1. Tail Length. Three litters (18 mice) were used. They were weaned at 23 days old and housed in the cold room (8°C) at 25 days old.

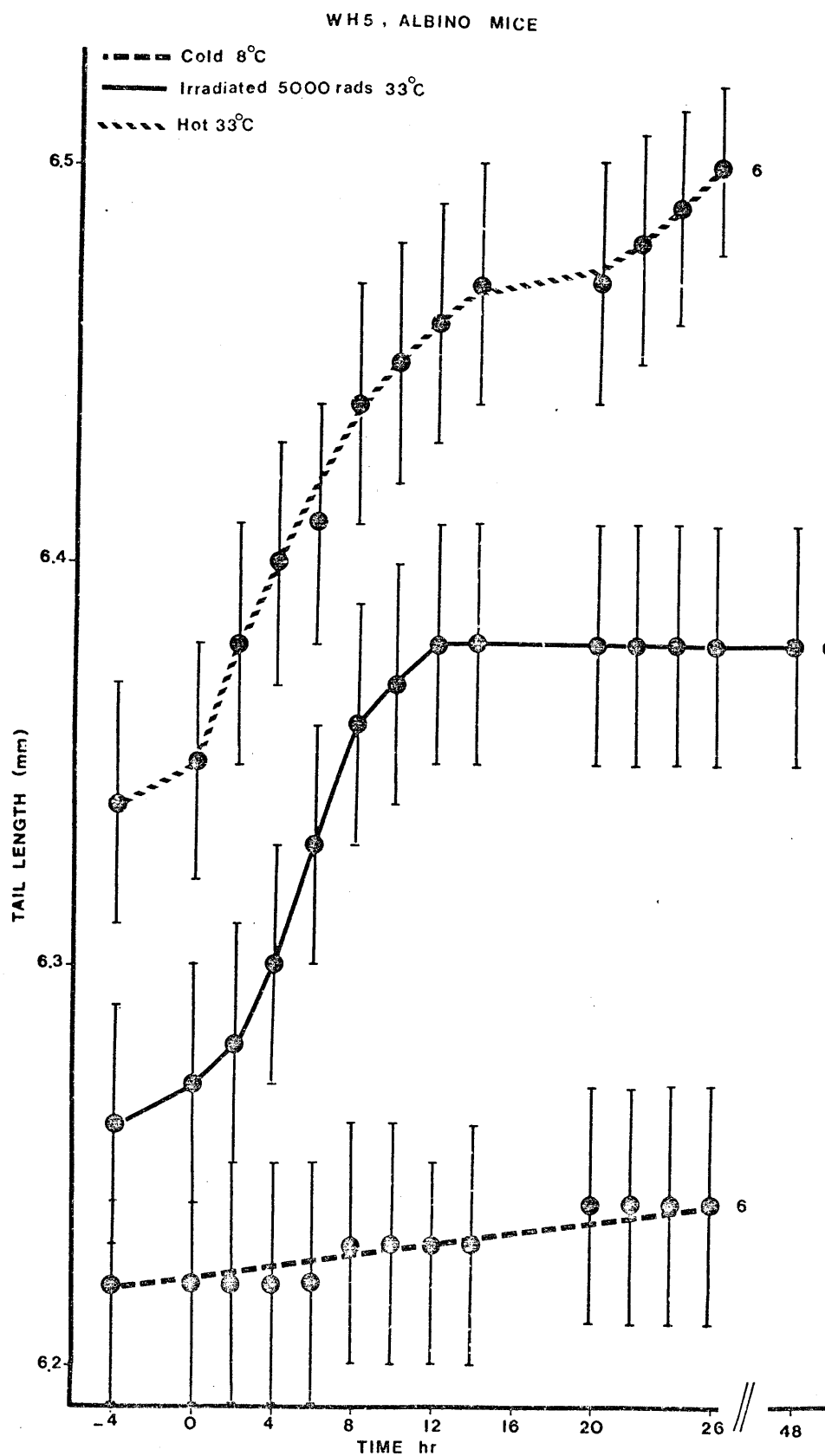


Fig.4.1 The curves of tail growth of the hot (33°C) irradiated (5000r) - hot (33°C) and cold (8°C) groups

At 1200 hour on the 31st day of age, the tails of these animals were measured. At 1600 hour (zero hour), the animals were divided into 3 groups and the tail lengths were measured every 2 hours.

- i. Cold Group (6 mice) left in the cold room at 8°C.
- ii. Hot Group (6 mice) taken to the hot room (33°C)
- iii. Irradiated Group (6 mice). The tails of this group were given an X-ray dose of 5000 r just before placing the animals in the hot room at 33°C at zero hour.

Before each tail measurement, the hot and irradiated groups were transferred temporarily to the cold room at 4°C for 10-15 minutes. After the measurement the animals were returned to the hot room (33°C).

Figure 4.1 shows the curve of tail growth of the three groups from minus 4 hours till 48 hours. Immediately after housing the animals in the hot room the tails of the hot group grew rapidly during the first 24 hours (1.14 mm). The immediate change occurred in less than 2 hours. The maximum tail growth of 1.1 mm occurred during the first 12 hours and then tail growth slowed to 0.3 - 0.4 mm during the second 12 hours. Temporary cessation of tail growth occurred between the 14th and 20th hour.

The irradiated tails also grew rapidly and exactly parallel to the "hot unirradiated tails" during the first 12 hours (1.1 mm) after transferring the mice from the cold (8°C) to the hot (33°C) room. Again, the changes in tail length occurred in less than 2 hours. Complete cessation of tail growth was seen after the first 12 hours and was followed till the 48th hour.

A linear and slow tail growth was seen in the tails of the mice which were left in the cold room at 8°C.

2. Radiology. The tails of the irradiated group were radiographed soon after the irradiation and 48 hours after housing the mice

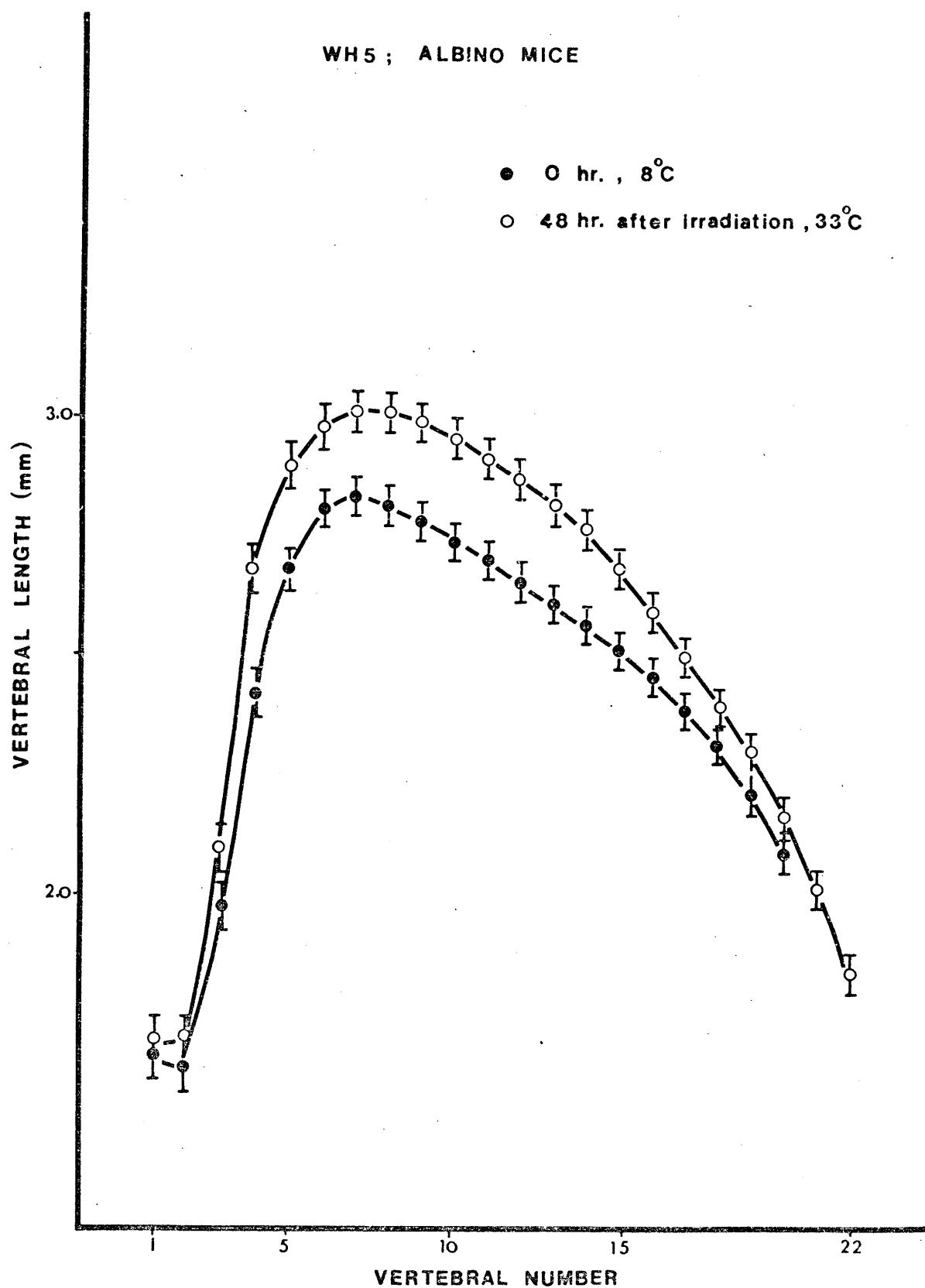


Fig.4.2 The mean vertebral lengths of the irradiated group just after the irradiation (5000 r) and 48 hours after transferring the mice to the hot room (33°C)

Group (°C)

IRR-HOT (33°C)

HOT (33°C)

Time (hr)

0

6

12

24

48

6

12

24

1. No. of animals

2. No. G.P. examined

3. Average thickness of G.P.

4. Average No. of cells in G.P.

5. Average No. of LHCC/G.P.

6. Total No. of LHCC examined

7. Dimensions of LHCC

Longitudinal Dimension

Transverse Dimension

Range

Mean

S.D.

Range

Mean

S.D.

Table 4.1 Mean histological findings in the growth plates (G.P.) of the 13th and 14th caudal vertebrae of the cold (8°C), irradiated (5000 r)-hot (33°C) and hot (33°C) groups

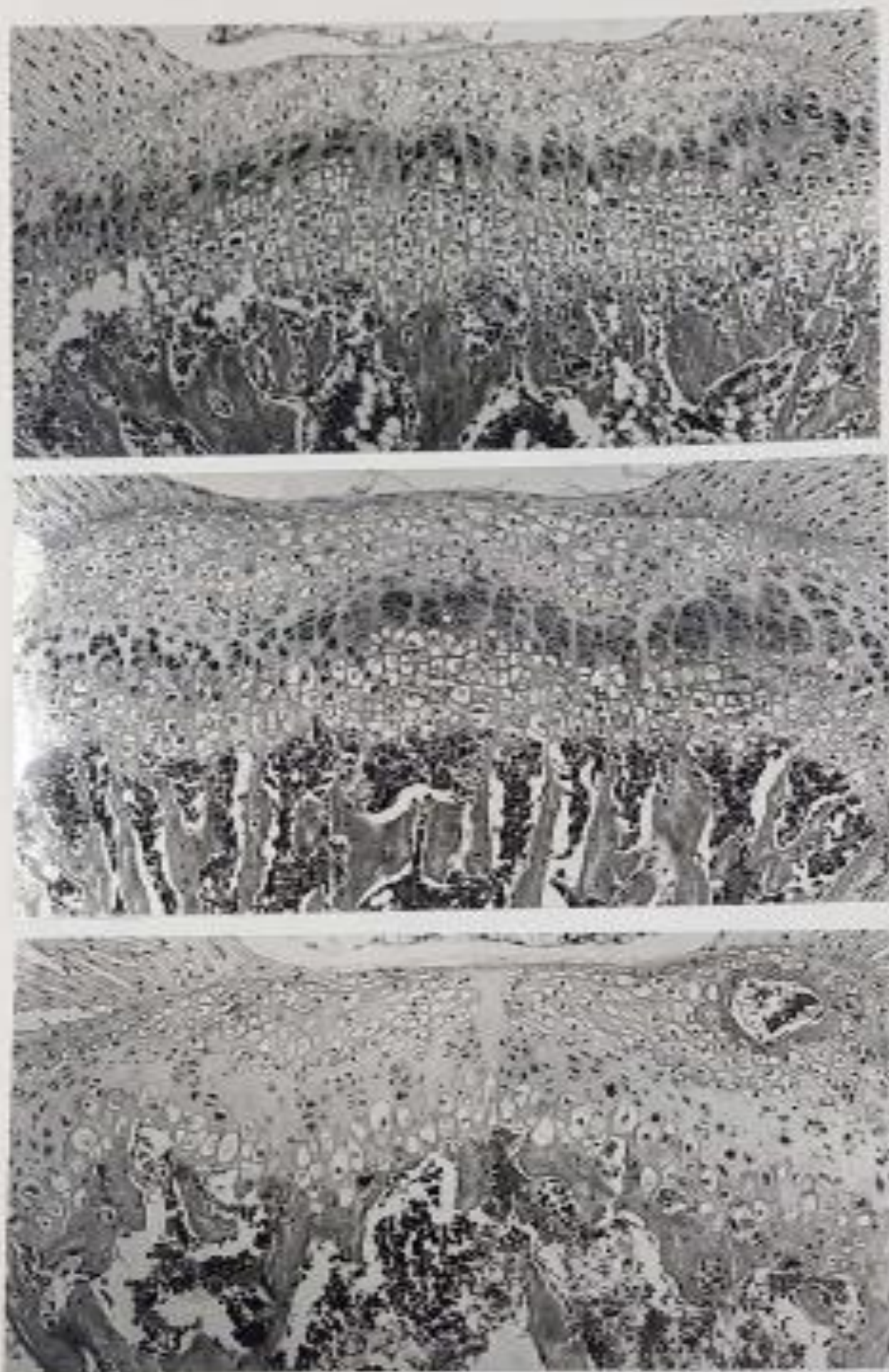


Fig. 6.3 Photomicrograph (H.E) of the growth plates of the 14th caudal vertebrae of the cold (upper) and hot (middle) groups at 24 hours and irradiated (5000 r)-hot group (lower) at 48 hours ($\times 125$)

in the hot room. From the radiographs, photographic enlargements were obtained and the tail bones were measured under the microscope.

Figure 4.2 shows the mean vertebral length of the tail bones plotted against the vertebral number of the tails just after the irradiation (zero hour) and 48 hours after housing the mice in the hot room. The general pattern of the vertebral lengths at zero and 48 hours was similar. However, from the 4th caudal vertebra outwards, the bones at 48 hours were considerably longer than those at zero hour.

3. Microscopy. At 48 hours, the mice of the irradiated group were killed, their tails were dissected and the tail bones were examined under a dissecting microscope. The 14th caudal vertebra of each animal was processed for microscopical examination and stained with H & E.

To provide comparative hot and cold groups for microscopical study, an additional group of animals (8 mice) which underwent the same changes as the principal groups were killed at 0, 6, 12 and 24 hours. The 13th and 14th caudal vertebrae of the tails of these animals were also processed for microscopical examination. The proximal and distal growth plates were examined to estimate the number of cells in each of the main zones, the thickness of these zones, and the diameters of the last hypertrophic cartilage cells (L.H.C.C.). These cells were identified as the lowest cells in the epiphyseal column with complete cell boundaries. The lower transverse border of these cells was identified as the base of the epiphyseal cartilage column.

When the tails of the mice were dissected, no gross morphological changes were seen between the tail bones of the three groups.

Table 4.1 and Figure 4.3 show the histological features of the growth plates of the three groups. The growth plate is made principally of two zones - the proliferative which includes the uppermost dividing cells and the hypertrophic cartilage cells.

In the tail bones of the hot and cold groups, a distinct line could always be seen separating the growth plate from the articular cartilage. This line becomes less distinct in the irradiated tail bones.

At any hour, the average thickness of the whole growth plate as well as the number of cells in each of the main cartilage zones was greater in the cold than the hot groups.

In the irradiated group, the columnar arrangement of the epiphyseal column was lost, and the remaining degenerated and dead cells with nuclear fragments were seen scattered throughout an abundant intercellular matrix. The hypertrophic cartilage zone is about 1-2 cells thick.

In the hot and irradiated groups, the hypertrophic cells appear vacuolar and the diameters of the L.H.C.C., particularly the longitudinal dimension, were greater than the cold group.

Taking the longitudinal dimension of the L.H.C.C. as an indication of the diameter of these cells and the growth in tail length as 1.14 mm/day in the hot group and 0.2 mm/day in the cold group, it may be possible to estimate the number of cells produced/tail/day. This may be calculated as follows:

$$\text{Number of cells produced/day} = \frac{\text{Growth in tail length/day}}{\text{Diameter of L.H.C.C.}}$$

Therefore, the estimated rate of cells produced/tail/day may be 33 cells in the tails of the hot reared mice and 10 cells in the cold group.

In the mice with tails containing an average of 29 vertebrae, only the last 24 free caudal bones (48 growth plates) which lie outside the body of the mouse were affected by changes in the environmental temperature. Accordingly, the total number of cells produced/epiphyseal column may be 0.7 cells/day in the hot group, and 0.2 cells/day in the cold group.

From these calculations it may also be possible to estimate the

average growth contribution of each tail bone/day in response to changes in the environmental temperature. This may be calculated as follows:

$$\text{Average growth of each tail bone/day} = \frac{\text{Tail growth/day}}{\text{Number of tail bones}}$$

Thus, the average growth contribution of each tail bone may be 47.5 $\mu\text{m/day}$ in the hot group and 8.3 $\mu\text{m/day}$ in the cold group. The contribution of each single growth plate may possibly be 23.8 $\mu\text{m/day}$ in the hot and 4.2 $\mu\text{m/day}$ in the cold.

C. Discussion

1. The Effects of the Hot Environment on the Growth of Tail Bones During the First 48 Hours

From the results of this study, the increase in the growth rate of the tails upon changing the mice from the cold to the more favourable hot environment occurred in less than 2 hours. This sudden and immediate effect could be due either to changes in the soft tissues of the tail such as reactive hyperaemia and subcutaneous oedema in response to the heat, or to the growth of the individual tail vertebrae themselves. To exclude the soft tissue element the experiment was repeated several times on different animals which were also kept in the cold room at 4°C for 10-15 minutes just prior to tail measurement. In these cases an increase in tail growth was measured. Therefore, the immediate increase in the growth rate of the tails following the change of mice from the cold to the hot room must be that of the growth of tail bones.

Accordingly, the sudden bone growth which occurred in less than 2 hours could be due either to rapid cell division occurring in the germinative cartilage zone, rapid cell differentiation and removal in the zone of chondrogenesis, or to sudden enlargement of the differentiated cells beyond the usual limits of cartilage cell hypertrophy.

In the growing rat the duration of the cell division of the upper

tibial growth plate was estimated at 11 hours and the transit time (from the dividing cells to the L.H.C.C.) at 2 days (Kember, 1960, 1971). Therefore, the immediate growth of tail bones after the change of environmental temperature from the cold to the hot may be due to the sudden enlargement of the differentiated cartilage cells. The mouse tail contains between 27-30 free caudal vertebrae (Rowett, 1974) of which only 23-26 bones (46-52 growth plates) lie outside the body and are affected by changes in environmental temperatures. The amount of growth of the differentiated cartilage cells of these plates particularly in the longitudinal dimension may account for the immediate growth of tail bones.

In the hot environment, the hypertrophic cells appear vacuolar, and it seems, therefore, that this environment may have altered the metabolic activities of these cells and made them capable of enlargement beyond the normal limits of cartilage cell hypertrophy.

The microscopical examination of the growth plates confirmed the concept of the linear dimensions of the hypertrophic cartilage cells. The L.H.C.C. in each epiphyseal column was selected for measurement because it represents the final stage of differentiation and maturation. Thus in 24 hours the hot environment caused the longitudinal dimensions of the L.H.C.C. of the growth plates almost to double. However, the transverse dimensions of these cells has not changed greatly compared with the longitudinal dimensions. This may mean that the environmental temperature principally affects longitudinal bone growth and has little influence on transverse growth.

The growth of tail bones in length depends mainly, however, on the continuous supply of new dividing cells at the top of the epiphyseal column. In the hot environment this is most likely to take place after the first 6-12 hours, because a steady rate of tail growth was maintained after the 12th hour. In this case the hot environment also accelerated

the process of cell division and doubled the number of cells produced in the tail bones. Accordingly, in the hot environment the period of cell division has been reduced from 11 hours (Kember, 1960, 1971) to almost half that time. In addition, the dividing cells were taken rapidly to the proliferative zone to hypertrophy beyond their usual limits and then removed. In this way the transit time was reduced from 2 days (Kember, 1960, 1971) to about 12 hours. On the other hand, the cold environment did not stop the cell division, differentiation and removal of these cells, but simply slowed these processes.

The fewer number of cells in the zones of the "hot growth plates" compared with those of the cold group may be due to their slower mitotic rate and cellular differentiation (i.e. transit time) in the cold group compared with the hot group. These processes seem to occur rapidly in the hot groups as evidenced by the fewer number of hypertrophic cells, because the "original number" was presumably removed rapidly in the zone of osteogenesis. However, soon after 6 hours, the mitotic activity of the growth plate is restored, thus the number of proliferative and hypertrophic cells was greater at 24 hours in the hot group than those of the cold group.

2. The Effect of Supra-lethal Dose of Irradiation on the Growth of Tail Bones

In the growing animal one striking effect of external irradiation is upon the growth of bones. The resultant stunting of skeletal growth was first described by Perthes (1903) on the wings of a young chicken and subsequently confirmed by many clinical and experimental observers (Cohn and Gong, 1953; Howard-Flanders and Wright, 1957; Reidy et al, 1947; Vaughan, 1962, 1971, 1973; Blackburn and Wells, 1963; Phillips and Kimeldorf, 1964; Dixon, 1967; Kember, 1967; Katzman et al, 1969; Sissons, 1971).

The principal cause of the irradiation damage is injury to the DNA of the dividing cartilage cells which renders them incapable of cell replication. This was shown by Bloom et al (1955) who found that if a heavy dose of irradiation is applied to the cytoplasm of the cardiac muscle (with the nucleus spared or shielded) of a newt, the cell could continue to divide normally. But if a very small dose is applied to the nucleus, normal cell division did not occur.

The severity of the skeletal changes after irradiation appears to be related to the dose of X-rays. It is generally agreed that a dose above 2000 rad applied to the bone could produce dramatic effects (Neuhauser et al, 1952; Cohn and Gong, 1952; Howard-Flanders and Wright, 1957; Bensted and Courtenay, 1965; Katzman et al, 1969; Vaughan, 1973 and others). It would appear that a larger dose of between 1800 and 2600 rad could produce complete and permanent arrest of the epiphyseal growth (Sissons, 1971).

To establish the importance of the linear dimensions of the hypertrophic cartilage cells in the determination of the epiphyseal growth, X-irradiation was used to block permanently and effectively the mitotic activity of the germinative zone of the plate. In this way the continuous supply of new cells at the top of the epiphyseal column was stopped and the remaining differentiated cartilage cells continued their programme towards ossification. To achieve this state, a lethal dose of irradiation (5000 r) was required to ensure the complete "sterilisation" of the growth plate. Therefore, the basic growth factor (cell division) was excluded and bone growth had to rely on the linear dimensions of the surviving differentiated cartilage cells which were not affected by irradiation.

When the supply of new cells at the top of the epiphyseal columns was stopped and the mice were taken to the hot room, tail growth

continued for only 12 hours. This growth, which was demonstrated by radiography, must have been due to the sudden increase in the longitudinal dimensions of the differentiated cartilage cells. The hot environment seems also to have reduced the transit time of the surviving cells towards osteogenesis. However, because there was no supply of new cells, tail growth ceased after the first 12 hours of the change of temperature to the hot room. The residual bone growth which was reported to occur for 10-20 days following the application of large doses of irradiation before the commencement of complete arrest of bone growth (Regen and Wilkins, 1936; Phillips and Kimeldorf, 1964; Sissons, 1971) was most likely due to the differentiated cells which escaped the irradiation damage and continued their progress towards osteogenesis. Furthermore, the cartilage cell hypertrophy may also account for the growth of tail bones after heavy dose irradiation and in vitro transplantation under the kidney capsule (Noel and Wright, 1970b) where a higher temperature than that of the tail is expected.

The microscopical examination of the growth plates also confirmed the cell dimension concept. Thus before the irradiation the growth plate may have been, as in the cold groups, of about 5-6 cells in the proliferative and 4-6 cells in the hypertrophic zone. After irradiation, the proliferative zone was reduced to 3-4 cells and the hypertrophic zone was 1-2 cells thick. This is because, upon changing to the hot temperature, the original number of differentiated cartilage cells were rapidly removed towards osteogenesis.

Like the growth plates of the hot group, the hypertrophic cells of the irradiated group appear vacuolar. Again, this may possibly be due to changes in the metabolic activities of these cells. The diameter of the L.H.C.C. has also doubled in 48 hours, but the average number of L.H.C.C./growth plate, i.e. number of epiphyseal columns/growth plate,

has been reduced. This may be due to the effect of irradiation on the normal proliferation of cartilage cells.

The irradiated bones also show the acute structural changes of X-irradiation on epiphyseal growth. These include in addition to the relation of thickness which was referred to earlier, a loss of the columnar arrangement of the epiphyseal columns, an increase in the amount of intercellular matrix and the appearance of several forms of degenerated and dead cells with fragmented and pyknotic nuclei dispersed throughout the matrix. These cells with nuclear fragments are probably cells which were about to undergo cell division but were killed by the irradiation.

The irradiation may have possibly also changed the structure of the intercellular matrix of the growth plate and made it similar to that of the articular cartilage. This may account for the line which normally separates both structures to become inconspicuous in the irradiated tail bones.

In the irradiated bones no gross morphological differences were seen between these bones and those of the hot and cold groups. This may be due to the early termination of the life of these animals. This was necessary because of the failure to gain weight and the appearance of bloodstained diarrhoea which may possibly have been due to the effect of scattered irradiation on the rectum.

CHAPTER V. CONCLUSION AND GENERAL HYPOTHESIS OF BONE GROWTH

The control of size as opposed to pattern in the mammalian skeleton is important for every member of every species. The vertebrae of the smallest species are measured in millimetres, whereas those of the largest are measured in metres, a linear factor of 1000. In terms of mass a whale's vertebra may weigh 100,000,000 mgm while that of a shrew mouse only 1 mgm. The mechanism of control of these enormous differences is not known, but as Thompson (1917) has elegantly shown, almost all mammalian bones of all sizes and shapes can be shown to have similar shape if they are transformed by altering the rectangular coordinates by relatively simple mathematical transformations. The conclusion must therefore be that the tail vertebrae of the mouse are indistinguishable from any human long bones. On the other hand, although the size and shape of a particular bone may vary enormously from species to species, the changes in the final length of the bone are brought about by different amounts of growth in several different directions.

Another inference deriving from Thompson's (1917) assumptions and Medawar's (1945) analysis would be that the basic shape of the bone is inherited in the bone itself and not determined by factors outside the bone such as the nutritional status and the generally circulating hormones. It could be argued that the different parts of the bone may respond differently to the same concentration of growth hormone but that the difference in growth would still be specific to that part of the bone. For example, a known titre of the hormone may affect the cells of the active and growing growth plate of any bone but would have no effect on bones with already closed epiphyses.

Among other factors which control skeletal growth (Siffert, 1966; Rang, 1969; Sissons, 1971; Moss, 1972; Little, 1973; Sinclair, 1973; Potts and Deftos, 1974 and others) environmental temperature is important

(Allen,1905; Sumner,1909,1915,1915; Sundstroem,1922; Przibram,1931; Ogle,1934; Harrison and co-worker 1959,1963,1969; Chevillard et al, 1963; Barnet,1965; Noel and Wright,1970b). Therefore, mice reared at a higher temperature have longer tail bones than litter mates reared at a lower temperature. This effect was brought about by changes in both the growth rate and the growth period of the tail bones. Thus the tail bones of the hot-reared mice grew rapidly, attained greater adult size and ceased bone growth earlier than the tail bones of the cold-reared mice which grew slowly and over a long time. Although the "cold tail bones" retain some growth potential for a very long time, they fail to catch up with the final length of the "hot tail bones". The prolonged maintenance of the mice at lower temperature is associated with gradual reduction of the growth potential of the tail bones until it is finally lost after about one year.

In the series of experiments described in this work, the simplest hypothesis to explain the facts is that the growing bone develops from cells which are programmed for a definite potential for growth (Siffert, 1966; Rang,1969; Owen,1970; Hall,1970,1971; Little,1973; Noel and Wright,1972). This could simply mean the total number of cell divisions occurring in the germinative cartilage zone of the growth plate. Therefore when an animal is transferred from a lower to a higher temperature, the process of cell production is greatly accelerated. Accordingly, the total definite number of the programmed cell divisions and differentiations are brought to an end at an earlier stage, hence the early cessation of the growth of tail bones in the mice maintained at high temperature.

On the other hand, when mice with short tails were left to grow in the hot room, the tail bones grew faster than a control hot group but they also finished their "programme" earlier, and matured and ceased growth earlier.

However, in the case of endochondral bone growth, one other factor could be equally important. This is the influence on the linear dimensions of the last hypertrophic cartilage cells in the epiphyseal column (Pritchard, 1974). Thus one can look at the final length of a long bone as determined exclusively by the sum of the linear dimensions of each of the hypertrophic cells just before these cells are removed. The linear dimensions may be determined or influenced by metabolic and hormonal influences but the number of hypertrophic cells actually produced may also be determined genetically. Of course if the number of cells in the generative zone of an epiphyseal column remains constant and the number of generations of any cell line also remains constant then the number of cartilage cells produced will depend simply on the number of cell divisions carried out by the "stem cells".

The importance of the last hypertrophic cartilage cells in the determination of the linear growth of bone was established when a supra-lethal dose of X-irradiation (5000 r) was applied to the tail bones before housing the animals in the hot room at 33°C. The growth of tail bones was found to be immediate and in less than 2 hours, but it only lasted for 12 hours. This is because the supply of new cells at the top of the epiphyseal columns was blocked permanently, and the linear bone growth was entirely dependent on the differentiated cartilage cells which survived the irradiation and continued their programmed cellular differentiation towards bone formation.

Microscopically, the linear dimensions of the last hypertrophic cartilage cells were almost doubled in 12 hours. The hot environment seems to have altered the metabolic activities of the differentiated cartilage cells and made them undergo immediate enlargement beyond the usual limits of cartilage cell hypertrophy.

The concept of the linear dimensions of the last hypertrophic

cartilage cells may also explain the failure of the tail bones of the lower temperature to catch up with the growth and length of the tail bones of the higher temperature. In this case, the differentiated cartilage cells produced by the growth plates of the lower temperature may be comparatively smaller than those produced at higher temperature.

A. The Local Growth Control of Tail Bones

It may be debated whether the factors which determine the growth of tail bones in the mouse and rat represent specific adaptations to environmental temperature (Harrison and co-workers, 1959, 1964, 1969; Chevillard et al, 1963) or local effects of the environmental temperature on the growth of tail bones.

In the cold environment the shortening of the tail length in relation to the body size (Ogle, 1934; Barnett, 1965) indicated that the effect of temperature on the growth of tail bones was due to the local effect of the temperature rather than to an adaptive mechanism. It has also been shown from the transplantation studies (Huggins and Blocksom, 1936; Felts, 1961; Noel and Wright, 1972; Noel, 1973) that when the tail bones were grafted to a site of higher temperature, such as into the kidney capsule of the same animal, 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 environmental condition and dependent on such "local growth factors" as temperature, vascularity and oxygen tension.

In the mouse, the importance of the tail as a thermoregulatory organ is well established (Sundstroem, 1922; Brody, 1945; Scholander et al, 1950a, 1950b, 1950c, 1955; Barnett, 1965; Rand et al, 1965; Whittow, 1971; Yousef et al, 1972). The temperature of the tail is not controlled by homeothermic mechanisms (Harrison and Clegg, 1969) and in fact is usually several degrees lower than that of the body (Sumner, 1913). 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 similar "local growth factors" as those found in the capsular space of the kidney.

A further indication of the local effect of environmental temperature on the growth of bone is evident from the study of the internal tail vertebrae and the long bones of the mouse. These bones are little affected by changes in the temperature (Noel and Wright, 1970a) because they lie inside the body and presumably are under the control of the body temperature. On the other hand, the bones which lie outside the body such as the rest of the tail vertebrae and the bones of the hands and feet (Allen, 1905; Sumner, 1913) are markedly affected by changes in the environmental temperature.

B. Mechanisms of the Local Growth Control of Tail Bones

The mechanisms by which the environmental temperature determines the growth rate of the growth plate are not clear but may be through:

(1) Direct action on the metabolic processes of the cells of the cartilage plate; (2) Direct action on the length of the mitotic cycle and/or the differentiation of the cartilage cells in the epiphyseal column; (3) Alteration of the blood flow to the growth plate particularly the dividing cartilage cells. In any case, the mechanism must be that of direct and local effect of the temperature on the growth plate.

1. Changes in the metabolic activity of the growth plate

Generally speaking, most chemical reactions are influenced by temperature (for review and details see Fisher and Solomon, 1965; Baldwin, 1967; Troshin, 1967). Thus the rate of any metabolic function of the cells such as their oxygen consumption, may be increased (or decreased) with raising (or falling) temperature. The enzyme reactions are no exception to this rule, but because enzymes are susceptible to thermal

inactivation, at high temperature they are inactivated and destroyed. Therefore, for any set of experimental conditions, it is possible to determine the "optimum temperature", i.e. the temperature at which the enzyme-chemical reactions would produce the highest rate of change. At "sub-optimum temperatures" these reactions become more stable and their activities may last longer and proceed more slowly. On the other hand, at temperature levels above the optimum, the chemical reactions may occur rapidly but the enzymatic properties may be destroyed at the same time.

In the present work, the optimum temperature was found to be between 33°C and 36°C . This is because at 38°C , the tails of the mice grew only for 24-36 hours indicating the contribution of the differentiated cartilage cells in the growth of tail bones. It also seems that at this temperature, the enzyme-chemical reactions occurring in the cells of the growth plate were rapidly inactivated; thus normal cell division and differentiation was not possible. On the other hand, a temperature level of as low as 4°C was found "sub-optimal", because tail growth was still possible at this level but at a very slow rate. There are, however, some reports of breeding mice at -3°C (Barnett, 1965) and it seems that a cold environment of -10°C (Scow, 1949) may freeze the cell organelles and inactivate the enzymes of the growth plates.

According to van't Hoff's law, the rate of any enzyme-chemical reaction may increase 2 to 3 times for each rise of 10°C . This rise of 10°C is known as the temperature coefficient and represented by Q_{10} . Therefore, one may expect these reactions to increase many-fold in the growth plates of the hot (33°C) reared mice compared with those of the cold (8°C) groups. However, the exact enzyme-chemical pathway (s) at which the temperature may influence the growth plate is not clear. This is not only because the biochemistry of the growth plate is still obscure (Siffert, 1966; Rang, 1969) but also because the normal endo-

chondral bone growth involves many reactions at different levels of the epiphyseal column.

In any case, the metabolic changes may influence the growth of tail bones in two possible ways: (1) The environmental temperature may change the metabolic activity of the dividing cartilage cells. In this way the hot environment may stimulate the production of new cells while the cold environment slows cell division. (2) The process of cell division and mitotic potential may remain unchanged, but the environmental temperature may alter the metabolic activities of the differentiated cartilage cells as well as the removal of these cells to the zone of osteogenesis, i.e. transit time. In this way the proliferation of cartilage cells may proceed rapidly in the hot groups and slowly in the cold groups. In addition, these cells were seen to enlarge beyond the usual limits of cartilage cell hypertrophy. These mechanisms are, however, inseparable. For example, the hot environment may stimulate cell division, reduce the transit time and cause an increase in the size of the differentiated cartilage cells.

2. Changes in the mitotic activities of the cells of the growth plate

The nucleus of any cell is the essential component for cell division. The DNA of the nucleus not only transmits the genetic growth potentials to the daughter cells but also determines the specific morphological and chemical characteristics of each cell (Bloom and Fawcett, 1975). Although itself a nucleoprotein, the DNA directs and controls the chemical and enzymatic activities of the cell and by inference the synthesis of proteins in general (Baldwin, 1967). Therefore one may postulate that a change in the metabolic activity of any cell is primarily due to changes in the DNA. This is supported by the findings of Dayson and Pond (1967) who reported an increase in the DNA synthesis of the epidermal cells of the ears of rabbits due to the local application of heat generated by

ultrasonic waves.

The relation of temperature as a trigger mechanism towards the initiation of cell division is also found in the works of Swann (1957, 1958, 1961), Troshin (1967), Jenkin (1970) and others. Thus the reproduction of many pathogenic bacteria of mammals occurs maximally at an optimum temperature of $35-37^{\circ}\text{C}$, which is in general the body temperature of the host. In plants, a drop of temperature below a threshold level of 0°C is associated with a failure to initiate mitosis (Grif, 1967). In cestodes, the mitotic rate (of *Diphyllbothrium osmeri*) was also found to increase with rising temperature (Wikgren, 1967).

In addition, the completion of cell division in the epidermal cells of the ears and tails of mice was estimated at 105 and 476 hours in the acclimatised animals maintained at 6°C and 7 and 6 hours in those kept at 30°C (Heroux, 1959, 1960). However, under the same environmental temperatures the mitotic activity of the duodenal mucosa was not affected. This is because in the latter case the duodenal mucosa is kept under the control of deep body temperature. A cold environment of 4°C was also found to reduce the mitotic activity of the epidermal cells of the ears of the mice and to delay the radiation damage of 9000 rad applied to the tails (Shewell and Wright, 1967).

In the case of the growth plate the rate of cell division occurring in the germinative zone was estimated in this study as 0.7 cells/epiphyseal column/day in the tails of the mice maintained in the hot room (33°C) and 0.2 cells/day in those kept in the cold (8°C) room. These rates may also be due to changes in the DNA of the cells of this zone.

In the "hot tail bones", the rapid production of new cells was also associated with rapid proliferation and differentiation of the cartilage cells along the epiphyseal column and increased removal of the calcified matrix. Again this is because the cells in the epiphyseal columns are

produced at the same rate as they are removed (Kember,1967). Conversely, the cold environment does not stop mitosis and/or differentiation of the cartilage cells, but slows these processes.

3. Alteration of the blood flow

It is generally agreed that the changes brought about in the bone and soft tissue under conditions of altered vascularity due to the local application of temperature are secondary to changes in the metabolic, chemical and biophysical properties of the bone (for review and details see Fisher and Solomon,1965; Brookes,1971). The vascularity of the growth plate is increased in the hot and decreased in the cold environment (Trueta et al,1953,1950a,1950b,1950c,1963a,1963b,1964; Sunden,1967; Rang,1969; Brookes,1971; Little,1973). The increased vascularity indicates that the growth plate is metabolically an active structure because the increased blood flow is required to wash away the metabolites (Stillwell,1965) as well as to supply the necessary metabolic requirements such as oxygen (Weiss,1959).

The vascular supply to the growth plate has been reviewed by many authors (Trueta et al,1960a,1960b,1960c,1963a,1963b,1964; Siffert,1966; Brookes,1971; Little,1973 and others). It consists of vessels which supply the germinative cell layer of the plate, and metaphyseal vessels which supply the zone of osteogenesis. The increased blood flow through these sets of vessels which accompanies exposure to the hot environment may result in: (1) Rapid cell division in the germinative zone leading to increased production of cartilage cells. (2) Increased removal of calcified matrix in the zone of osteogenesis. Despite the speed of these factors, the number of cells in the epiphyseal column remain constant because the cells pass from one zone to another as fast as they are removed (Kember,1960). Therefore, raising the temperature on either side of the growth plate leads to acceleration of the cellular activities of

the epiphyseal column.

On the other hand the reduced vascularity of the tail bones of the cold reared mice may lead to: (1) Reduced vascular flow to the germinative layer of the plate and hence a reduced rate of cell division. (2) Reduced metaphyseal flow so that even if chondrogenesis proceeds normally, the rate of removal of the calcified matrix may be reduced.

In conclusion, the effect of environmental temperature on the growth plate of tail bones may operate at two levels. The first on the germinative zone, through changes in the DNA synthesis, and the second on the dividing cells after they finish their division. The latter cells are unable to divide. However, during their proliferation-differentiation pathway, these cells undergo immediate and rapid changes in their linear dimensions which are possibly due to changes in their metabolic activities rather than to any alteration of DNA synthesis.

CHAPTER VI. OTHER EFFECTS OF CHANGES IN THE ENVIRONMENTAL TEMPERATURE ON THE MOUSE

Higher animals have evolved efficient mechanisms for maintaining a constant temperature for their constituent cells. The adaptive responses of the body to the changes in the environmental temperature are reflected at the cellular level (Troshin, 1967) and in the form of changes in the size and shape of the different parts of the body (Harrison and Clegg, 1969).

During the course of this study several changes, other than those of the tail bones, were observed. These changes are recorded here.

A. EFFECTS OF ENVIRONMENTAL TEMPERATURE ON THE BODY WEIGHT

The effects of environmental temperature on body growth as reflected by body weight have been reported by many authors (Sumner, 1909, 1915; Sundstroem, 1922; Ogle, 1934; Biggers et al, 1958; Hale et al, 1959; Harrison et al, 1959, 1963, 1964, 1969; Barnett et al, 1963, 1965; Chevillard et al, 1963 and others). The results of these authors conflict because they are either concerned with adaptation mechanisms to extreme temperature levels or with testing the responses of the different animal genotypes under such levels. Studies on the biological effects of changes in the environmental temperature on the body growth are lacking.

Materials, Methods and Results. A total of 56 animals maintained at 33°C, 21°C and 8°C (16, 22 and 18 mice) were used. These animals were weighed daily to the nearest 0.5 gm and followed for a period of 9 months.

Figure 6.1 shows the curves of growth in body weight of the 3 groups. During the first 16 weeks the control group grew faster (0.86 gm/week) than the cold (0.79 gm/week) and the hot (0.62 gm/week) groups. During the later weeks, the growth rates were equal (0.06 gm/week) in the 3 groups. The body weights of the control and cold groups fall very close

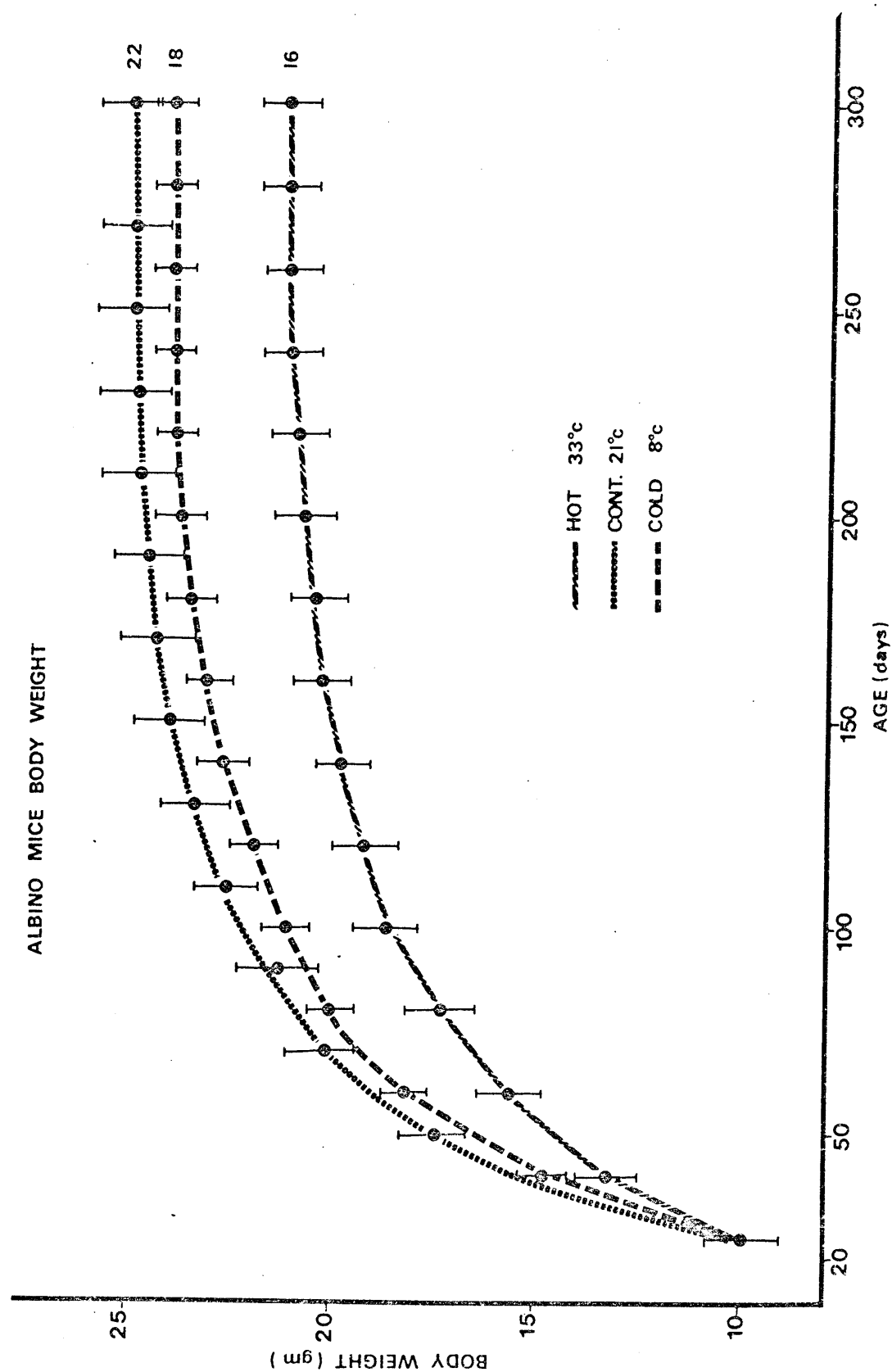
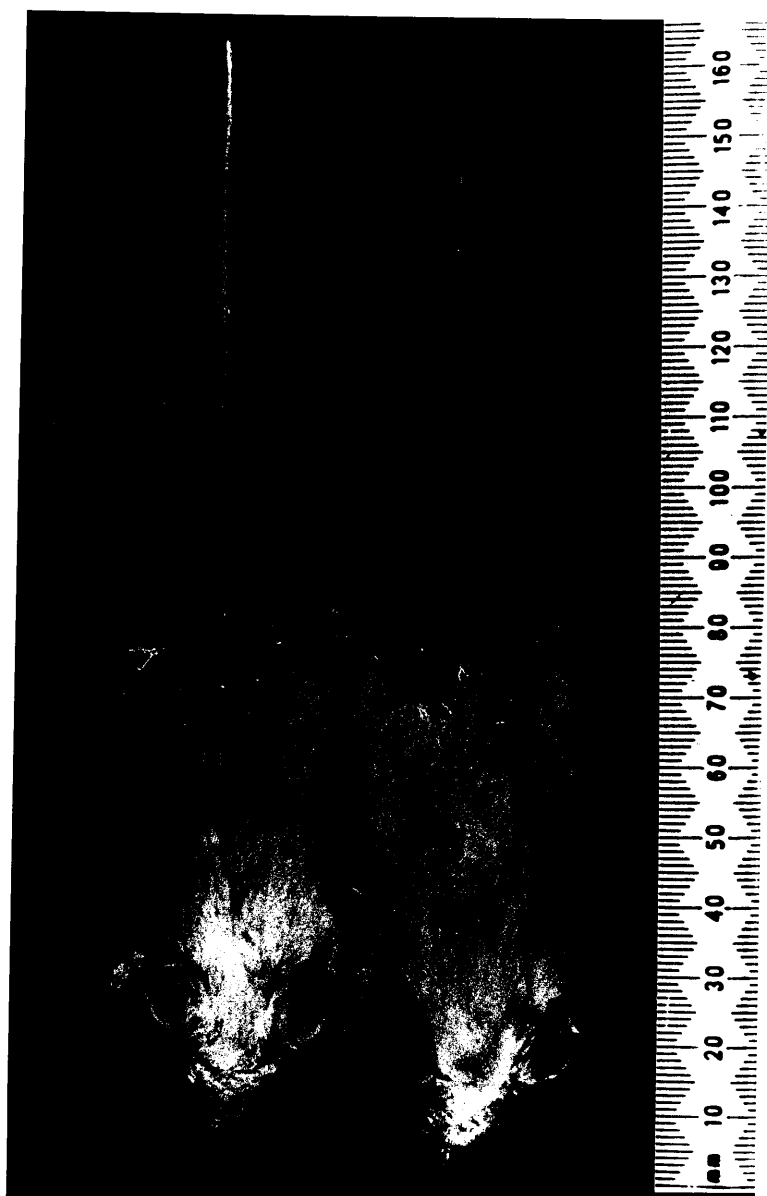


Fig.6.1 The curves of body weight of 3 groups of mice maintained at 33°C, 21°C and 8°C

Fig. 6.4 A photograph of 2 mice at the age of 40 days. The upper (from the hot group) has a long tail and small body while the lower (from the cold group) has a short tail and larger body



together and by the age of 300 days the controls were 1.0 gm and 3.8 gm heavier than the cold and hot groups.

(p.131)

Figure 6.3 shows the mean body weight of the hot and cold group expressed as a percentage of the mean body weight of the control group.

Figure 6.4 shows a photograph of 2 mice at the age of 40 days. The upper (from the hot group) has a long tail and a small body while the lower (from the cold group) has a shorter tail and larger body.

Discussion

As to whether or not the growth of body weight is determined by the temperature at which the organism is maintained is controversial. The continuous exposure of experimental animals to low or high temperatures has been said to result in reduced adult size (Sumner, 1909, 1915; Sundstroem, 1922; Ogle, 1934; Biggers et al, 1958; Harrison et al, 1959, 1963, 1964, 1969; Barnett and Scott, 1963), but there are some exceptions. For example, it has been shown earlier in this study (p. 87) as well as in the works of Harrison et al (1959, 1963, 1964, 1969) that small animals achieve their greatest adult weight under conditions of heat.

The present results, which support the relationship of body growth to environmental temperature, were consistent with most authors (Sumner, 1909, 1951; Sundstroem, 1922; Ogle, 1934; Hale et al, 1959), and any divergence from other reports (Harrison et al, 1959, 1963, 1964, 1969; Chevillard et al, 1963; Barnett et al, 1963, 1965) may be due to the use of different animal genotypes in which body growth may possibly be genetically determined.

It has been shown earlier that enzyme-chemical activity is greatly influenced by temperature and that the rates of such activities may be increased (or decreased) with rising (or falling) temperature (for details see Troshin, 1967; Hart, 1971). One may therefore conclude that changes in environmental temperature on body growth may be reflected by the changes in metabolic activities.

In the hot room the mice consume small amounts of food and water (see later). They also perform minimal physical activities and lie motionless in their cages. But their metabolic activities are raised ^{than} by 2-3 times/those of the cold and control group (van t's Hoff's law). However, these activities are principally directed towards heat loss rather than body growth. In addition the small bodies of the hot room mice mean that the animals have a relatively larger surface area in relation to its volume and this may facilitate thermoregulation (Scholander et al,1950a,1950b,1950c,1955).

In the cold room several factors may influence the final adult body growth. It is now well established that under conditions of constant and continuous cold exposure, as maintained in the cold room, homeothermy is maintained through greater metabolic rate (Hart,1971), an increase in food consumption (Cottle and Carlson,1954; Barnett and Little,1965) and a high rate of heat production for long periods (Depocas et al,1957). Because of this heat production a decrease in shivering thermogenesis and an increase in non-shivering thermogenesis becomes evident (Hart et al, 1956,1971; Bruck,1970). As will be shown later, some of these factors were confirmed in this study. For example, food and water consumption was increased, the mice were physically active, non-shivering thermogenesis was provided by the brown adipose tissue and shivering was not seen. In general, homeothermy was adapted and adjusted and the metabolic demands were fulfilled and were directed towards body growth which provided the animals with better adaptive responses to the cold environment.

The heavier body weights in the cold group compared with the hot group may in part be due to the heavier body organs (see later) but also to the different chemical body composition. The cold group were reported to have more water and less fat, collagen, nitrogen, calcium and phosphorus than the hot group (Barnett et al,1959,1963,1965; Himms-Hagen,1965).

Although the body weights of the control and cold groups fall within the same range, the heavier weights of these groups than those of the hot group may be due to changes in thyroid activity. Increased thyroid activity in cold-acclimatised animals is well known (Heroux, 1969). The increased thyroid activity may lead to (1) increase in body weight, (2) increase in basal metabolism (Hart, 1971), (3) increased tissue oxygenation of such organs as the liver, kidneys and muscles (Barker, 1955; Weiss, 1954, 1959), (4) increase of coenzyme A level in the liver (Campbell et al, 1960; Tabachnik and Bonnycastle, 1954).

Finally, the growth pattern of the control group maintained at 21°C indicates that this temperature is better than 8°C or 33°C.

B. EFFECTS OF ENVIRONMENTAL TEMPERATURE ON THE BODY ORGANS

The effects of environmental variables, particularly temperature, on body organs and a number of metabolic indices have been studied by various authors (Emery et al, 1940; Hale et al, 1958, 1959; Heroux and Gridgeman, 1958; Mefferd et al, 1958a, 1958b; Barnett et al, 1959, 1963, 1965; Harrington and Nardone, 1959; Weiss, 1959; Himms-Hagen, 1965 and many others). Thus almost all body organs and systems were found to be heavier in cold-reared animals than those reared at a higher temperature. However, most of these studies were concerned with the effects of extreme temperature. On the other hand, the long-term effects of temperature on the weight and histological morphology of these organs have received little attention. These effects are analysed in this chapter. The body organs may be divided into 6 groups: (1) Those primarily concerned with metabolism (e.g. liver) and excretion of metabolites (e.g. kidneys). (2) Those responding to functional and physiological demands, e.g. the heart. (3) Organs which are not directly concerned with the body metabolism, e.g. spleen, thymus, bone marrow. (4) Insulating organs (discussed separately, p.144). (5) Organs concerned with heat generation, e.g. brown adipose tissue (discussed separately, p.149). (6) Organs which

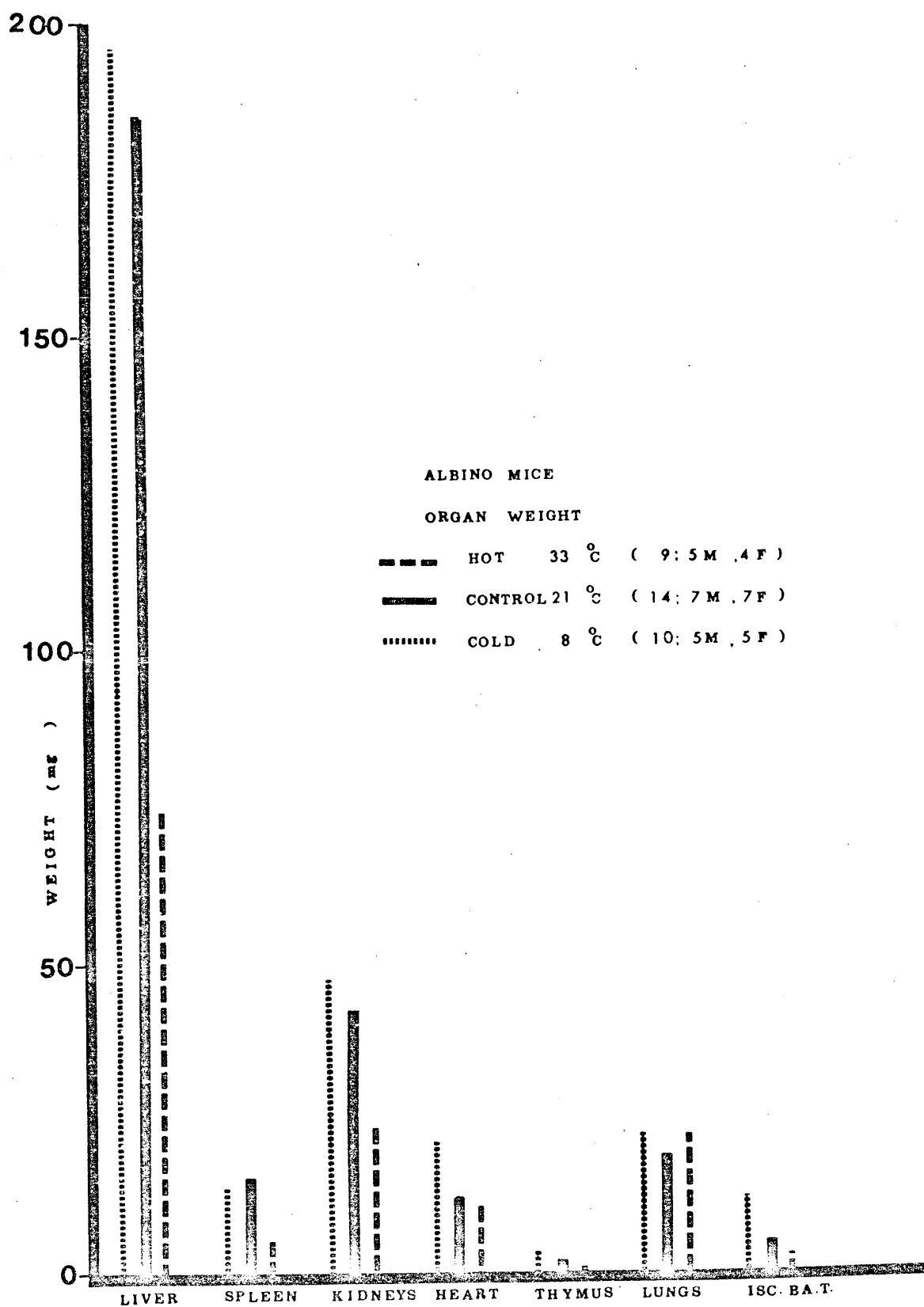


Fig.6.2 A histogram of the mean organ weights of the 3 experimental groups

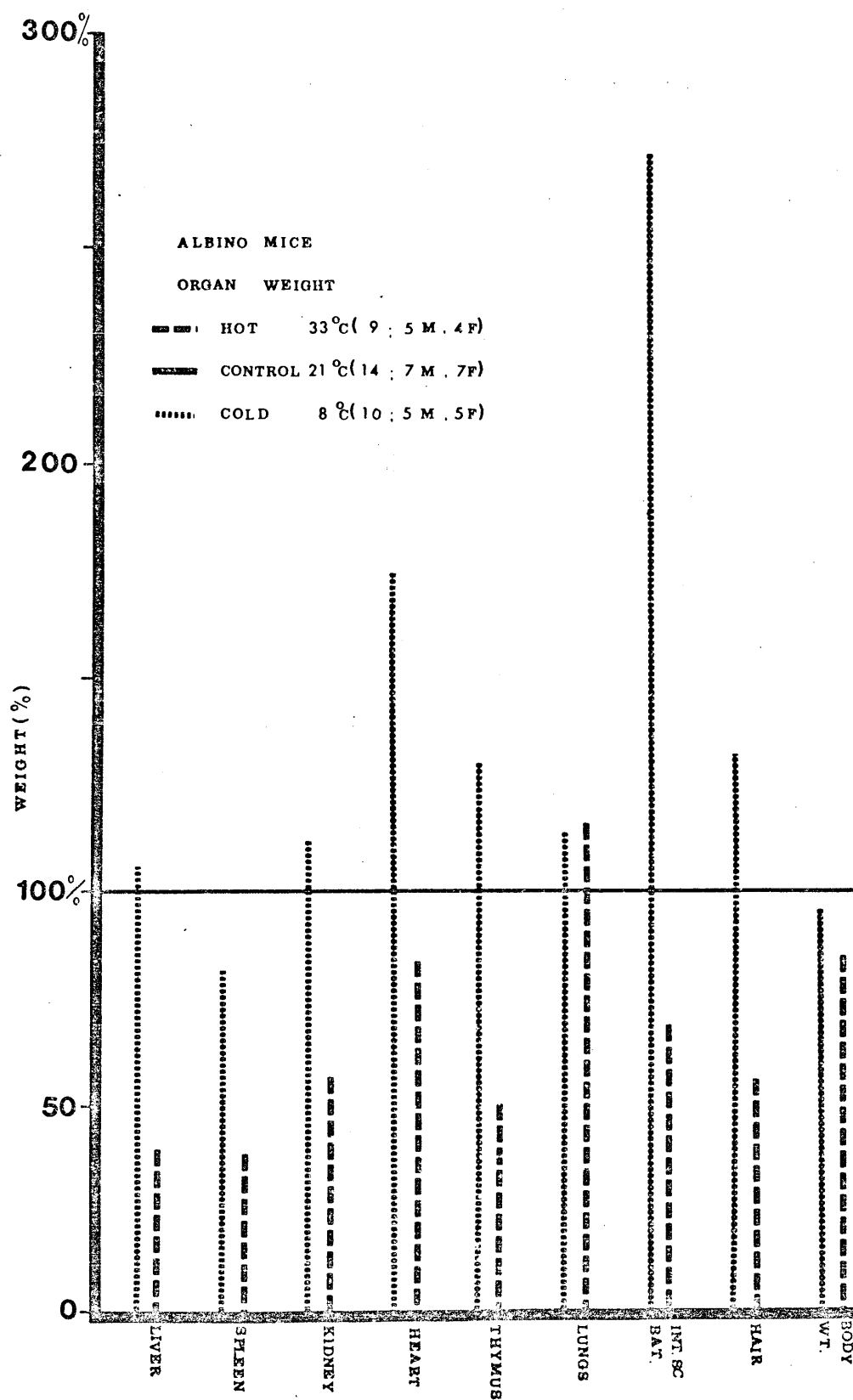


Fig.6.3 The mean weights of the body organs, interscapular BAT, hair and body of the cold (8°C) and hot (33°C) groups expressed as percentage of the mean weights of the control group (21°C)

are little affected by changes in the environmental temperature, e.g. the lungs.

Materials and Methods. A total of 33 animals of both sexes which were maintained at 33°C, 21°C and 8°C (9, 14 and 10 mice respectively) for more than 6 months were used. These mice were killed by decapitation. The liver, spleen, kidneys, heart, thymus and lungs were carefully dissected and weighed to the nearest 0.0001 mg. The organs were then sliced, fixed in formol saline, processed for histological examination and stained with H & E.

To study the cellularity of the bone marrow, the tail bones of the three experimental groups at the age of 40 days were examined.

Results

1. Organ weights

Figure 6.2 shows the mean organ weights of the 3 groups and Figure 6.3 shows the mean organ weights of the cold and hot groups expressed as a percentage of the mean organ weights of the control group. Except for the lungs, the organ weights (liver, spleen, kidneys, heart and thymus) of the hot group were lighter than those of the cold and control groups. The organ weights of the latter groups were similar or fell within the same range.

2. Organ histology

i. Liver. The general histological pattern of the liver was similar in the 3 experimental groups. However, those of the cold group were generally more vascular than those of the other groups. The main histological differences were seen in the liver cells. Thus in the cold, these cells appeared larger than the other groups with large nuclei and abundant darkly-stained slightly vacuolar eosinophilic cytoplasm. The chromatin condensations of the nuclei were also more than the other groups. Binucleated parenchymal cells were seen in abundance. The liver cells near the periphery of the hepatic lobules were more deeply

stained than those around the central veins.

In the hot group, the liver cells appeared small with small rounded nuclei and scanty lightly stained basophilic and finely granular cytoplasm. Binucleated cells were infrequent.

In most respects the liver cells of the control group lay midway between the cold and hot groups.

In the hot and control groups the liver sinusoids were dilated and empty but in the cold group the sinusoids were filled with blood.

In the 3 experimental groups occasional megakaryocytes and areas of mild non-specific inflammatory activity were seen.

ii. Spleen. Again the general histological appearance of the spleen was similar in the 3 groups, but with more vascularity in the cold group than the other groups. Therefore the amount of blood pigments was also greater. Active haematopoiesis was also more evident in the cold group.

In the spleen of the mouse, several multinucleated giant macrophages and megakaryocytes were seen, especially near the splenic capsule. The number of these cells was greater in the cold, less in the control and infrequent in the hot group. In the cold group these macrophages appeared larger than the other groups and were made of abundant faintly stained basophilic cytoplasm and between 4 to 8 rounded nuclei which were also lightly stained. The number of nuclei was less in the other groups.

In the cold the megakaryocyte appeared typically as a giant - although smaller than the multinucleated macrophages - irregular shaped cell with a single darkly stained nucleus and eosinophilic cytoplasm.

iii. Kidney. In most sections of the kidney, including those of the control group, diffuse areas of hyaline degeneration and angiomatous stroma were seen at the corticomedullary junction. Occasional mild non-specific inflammatory activity was also evident, but in none of the groups was there sufficient damage to indicate serious functional impairment.

However, the glomeruli of the cold group were smaller than the controls but larger than those of the hot group. In the cold group, the cells of the renal tubules were large, lightly stained and contained uniform vesicular nuclei. In contrast, the cells of the renal tubules of the hot and control groups were small, darkly stained and contained darkly stained nuclei. Albuminous degeneration and dilatation of the convoluted tubules were found to be more frequent in the hot and cold groups than in the controls.

iv. Heart. The general histological pattern of the heart was similar in the 3 environmental groups, but the vascularity was greater in the cold group than in the other groups. In addition, the width of the myocardial muscle fibres appeared greater in the cold and less in the hot than the control group. In the cold and control groups these fibres appeared darkly stained and contained large nuclei. In contrast, the muscle fibres of the hot group were lightly stained and the nuclei were small.

v. Thymus. The histological appearance of the thymus was similar in the 3 groups with the cold more cellular and vascular than the other groups. There were also more reticulum cells in the cold than the other groups. These cells typically had pale spherical nuclei with fine chromatin granules and ill-defined cell outlines.

The distinction between medulla and cortex in the thymus gland of the hot group was inconspicuous. However, occasional multinucleated giant macrophages, similar to those found in the spleen section, were also seen.

Hassall's corpuscles were seen in the histological sections of the 3 groups, but were more frequent in the cold and control groups than in the hot group.

vi. Bone marrow. In the tail bones of the control and cold groups

(Figure 3.19) the bone marrow was almost completely yellow and fatty with a few clusters of haemopoietic cells which were seen mainly near the growth plate on the metaphyseal side.

The picture was entirely different in the tail bones of the hot group. The marrow cavity was filled with highly cellular red marrow. The predominant cells were granular leucocytes. A typical field showed stem cells, many eosinophils, myelocytes, reticular and endothelial cells. Erythrocyte precursors were rather scanty but megakaryocytes were frequent.

vii. Lungs. The histological appearance of the lung tissue was similar in the 3 experimental groups. Aggregates of lymphocytic infiltration and mild non-specific inflammatory activity were seen in the 3 groups.

Discussion

The relationship between the organ weights and the environmental conditions is only one aspect of the processes by which an animal adapts to its environment. These processes are the end products of many feedback mechanisms which regulate the rates of body metabolism. For example, on exposure to cold, food may be diverted from growth to heat production (McCance, 1959; Barnett and Widdowson, 1965) and the growth of certain body organs may be favoured at the expense of others. Moreover, the morphological changes seen in any organ only imperfectly reflects its activity.

The weights of body organs may vary greatly according to the tissue structure, differences in blood content and the amount of extracellular or intracellular water and fat. It has been shown that mice inbred at -3°C (Barnett et al, 1959, 1963, 1965) and at $2-4^{\circ}\text{C}$ (Himms-Hagen, 1965) were lighter than control mice maintained at 21°C . They also had less fat, collagen, nitrogen, calcium and phosphorus but contained more water. It would appear that the increased body metabolism which the cold environment demands is so high that despite the increased food consumption (Barnett

and Little,1965), the mice in the cold room were unable to meet their fat requirements. One may therefore assume that the main cause for the heavier body organs in the cold than the other groups was their high content of water.

The liver weights in the cold and control groups fall within the same range, thus it is not plausible to assume that the liver was enlarged in these groups. In contrast, the livers of the hot group had undergone a considerable reduction in weight. This may also be the result of reduced water content. A further possible cause for the reduced liver weight in the hot group may be that the body metabolism was directed from growth and storage to heat loss. Consequently, the heavier kidney weights of the cold and control groups than those of the hot group may indicate kidneys actively secreting the waste products of increased body metabolism.

It is often said that a small body tends to have smaller organs with smaller cells compared with an animal with a large body. In this case all the organ weights (except for the lungs) were smaller in the hot group than in the other groups. In addition, some histological evidence may confirm this assertion. In the cold group the parenchymal liver cells, cells of the renal tubules and even the glomeruli were found to be large with large vesicular nuclei, while in the hot group the cells were smaller and had nuclei of variable size. These findings agree with those of Hale et al (1959).

The metabolic pathways which may be responsible for the differences in weight (and cell size) of the liver and kidneys are not clear. However, the blood flow and oxygen uptake of these organs have been found to increase in cold and to decrease in hot conditions (Weiss,1954,1959; Barker,1955; Barnett et al,1959). Certain liver enzymes were also reported to be equally involved (for review and details see Hart,1971).

The adaptive capability of the heart in response to functional and

physiological demands is well known. The heavier hearts in the cold group reflect higher metabolic activity and possibly the increased peripheral vascular resistance associated with cold exposure. As a result the cardiac output and respiratory rate are reportedly increased (Barnett et al,1959,1965). The enlargement in the cold group was shown by the increased width of the myocardial muscle fibres in the cold group compared with the other groups. These findings are also consistent with those of Hale et al (1959).

In the normal rat (and possibly the mouse as well), the vertebrae of the trunk which lie inside the body contain red marrow and yellow bone marrow appears abruptly in the tail vertebrae outside the body and extends to the tip of the tail (Huggins and Blocksom,1936). This sharp transition suggests that a physical factor may be responsible. One of the main differences between the trunk and tail vertebrae is the higher temperature of the former. In this study, when the tail temperature was raised to the level of the body temperature the yellow fatty marrow (of the cold and control groups) changed to red marrow (in the hot group). This may also suggest that the haemopoietic tissue is very sensitive to changes of temperature and found only where the temperature is raised such as the internal bones of mammals (Huggins and Blocksom,1936; Bloom and Fawcett, 1975) and birds (Muller,1927) in addition to the haemopoietic organs, notably the spleen and thymus.

The different environmental temperatures affected the spleen and thymus in the same way as the bone marrow. Thus the lighter weights of these organs in the hot group compared with the other groups may indicate that active haemopoiesis is taken over by the tail bones and possibly by the bones of the feet and hands (Huggins and Blocksom,1936).

The distribution and formation of haemopoietic tissue in man and most mammals is related to temperature (Huggins and Blocksom,1936). Thus in normal mice (and possibly the cold reared mice) it is found in the

peripheral bones (such as the sternum, ribs, pelvic bones, vertebral column and proximal end of femur) where the temperature is a few degrees lower than the internal body temperature. In the hot room, the temperature of the tail bones was raised to that level of the peripheral bones and this was accompanied by the appearance of red marrow in these bones.

Of particular interest are the multinucleated macrophages and megakaryocytes, which are normally seen in the internal organs of the mouse (Gorer, 1946; Fekete, 1941), and were more frequently seen when the temperature was raised. Similarly they were found to be more common in the spleen and thymus of the cold and control groups than in the same organs of the hot group. Was this because in the latter group they appeared to be more localised in the bone marrow of the tail bones?

In the rat and mouse the thymus gland is retained throughout life (Fekete, 1941; Rowett, 1974) and continues to produce the hormones thymosin (which stimulates the growth of the lymphoid tissue) and promine (which stimulates the general body growth). The greater thymus weight and the increased cellularity of the thymic and splenic tissues in the cold groups may reflect the action of thymosin, whereas the increased body and organ weights in the control and cold groups than that of the hot group may reflect the action of the promine. On the other hand, the inconspicuous distinction between the medulla and cortex in the "hot thymus" possibly means that heat has enhanced the involution of the thymus.

Of particular interest is the case of the lung. The gross (e.g. fresh weight) and histological features were found to be similar in the 3 experimental groups despite the dry air in the hot room and the high level of humidity in the cold room (p. 30). Similar findings were also reported by Heroux and Gridgeman (1958) from rats maintained at 30°C and 6°C. It seems, therefore, that environmental temperature has no effect on the structural morphology of the lung tissues.

C. EFFECTS OF ENVIRONMENTAL TEMPERATURE ON THE GROWTH OF BODY HAIR

The relationship of the surface area to the volume in mammals and birds is believed by Scholander (1955) to be of little importance in the climatic adaptation of these species. Whether this is true or not, he and his colleagues (1950a, 1950b, 1950c) report that the differences in fur thickness have a profound effect on thermal insulation. Indeed, it is widely recognised that arctic mammals have thick fur coats whereas many tropical mammals have sparse hair covering. It is therefore of interest to determine whether or not the environmental temperature determines the growth of body hair so that the appropriate form is produced for a particular environmental condition.

Experimental Procedures and Results

1. Weight of Body Hair. A total of 17 mice, aged 5-6 months, maintained at the three environmental conditions of 33°C, 21°C and 8°C (6, 5 and 6 mice) were used. The animals were killed by decapitation, weighed to the nearest 0.0001 mg and then painted with a depilating paste. After 10-15 minutes the whole body hair (including the vibrissae) was scraped off with a spatula. The animals were then washed in running water, dried with absorbent paper and a hair dryer and then re-weighed.

The depilating agent was made (Makie and McCartney, 1950) by mixing 7 equal parts of barium sulphide, white household flour, talcum powder and one part of Castile soap powder. The mixture was then made into a smooth paste with water.

Table 6.1 and Figures 6.2 and 6.3 show the weights of total body hair in the 3 groups. The hair weight in the cold group was 256% and 140% heavier than that of the hot and control groups respectively.

2. Length and Thickness of Body Hair. An additional 34 mice aged 5-6 months maintained at the above climatic conditions (14, 9, 11 mice in 33°C, 21°C and 8°C) were also used. The body hairs of these animals were

	HOT (33°C)	CONTROL (21°C)	COLD (8°C)
No. of cases	6	5	6
Range	0.1810 - 0.2693 mg	0.3912 - 0.4543 mg	0.5165 - 0.6357 mg
Mean	0.2282 mg	0.4186 mg	0.5852 mg
S.D.	0.031	0.024	0.043
P	0.00002	0.00011	

Table 6.1 Weights of total body hair in the 3 experimental groups. P indicates the significance of the differences between the control group and the hot and cold groups. The significance of difference between the hot and cold groups was highly significant ($P < 0.00001$)

No. of hairs/hair follicle	1	2	3	4	5	6	7	8	+8	Total	No. of animals	Average
HOT (33°C)	No.	138	151	26	3	2				320	14	1 - 2
	%	43.1	47.2	8.1	0.9	0.6						
CONTROL (21°C)	No.	57	62	105	36	13	7			280	9	1 - 3
	%	20.4	22.1	37.5	12.9	4.6	2.5					
COLD (8°C)	No.	22	15	17	49	35	60	47	19	332	11	4 - 8
		6.6	4.5	5.1	14.8	10.5	18.1	14.2	20.5	5.7		

Table 6.2 Number of body hairs/hair follicle in the three environmental groups

		HOT (33°C)	CONTROL (21°C)	COLD (8°C)
No. of animals		14	9	11
1. Abdominal hair				
No. of hairs examined		64	60	58
Range		3.70 - 4.18 mm	3.52 - 4.26 mm	6.36 - 7.22 mm
Mean		3.89 mm	3.88 mm	6.75 mm
S.D.		0.17	0.30	0.44
2. Interscapular hair				
No. of hairs examined		66	61	73
Range		4.07 - 6.85 mm	3.70 - 5.99 mm	4.48 - 6.55 mm
Mean		5.89 mm	5.78 mm	5.67 mm
S.D.		0.89	0.91	0.37
3. Dorsocaudal hair				
No. of hairs examined		78	83	96
Range		4.44 - 5.74 mm	3.70 - 4.59 mm	5.92 - 9.99 mm
Mean		5.28 mm	4.06 mm	7.01 mm
S.D.		0.43	0.41	1.45
4. Total Mean Length		4.87 mm	4.57 mm	6.48 mm

Table 6.3 Lengths of body hair of the abdominal, interscapular and dorsocaudal regions

		HOT (33°C)	CONTROL (21°C)	COLD (8°C)
No. of animals		14	9	11
1. Abdominal hair				
No. of hairs examined		64	60	58
Range		28.23 - 37.65 um	25.00 - 38.64 um	35.29 - 49.41 um
Mean		33.65 um	31.82 um	41.31 um
S.D.		3.65	4.55	4.57
2. Interscapular hair				
No. of hairs examined		66	61	73
Range		9.41 - 18.82 um	25.00 - 38.64 um	28.23 - 40.00 um
Mean		14.35 um	32.20 um	32.22 um
S.D.		2.82	5.07	4.80
3. Dorsocaudal hair				
No. of hairs examined		78	83	96
Range		14.12 - 18.82 um	18.18 - 25.00 um	25.88 - 40.00 um
Mean		16.47 um	23.49 um	33.72 um
S.D.		1.66	2.75	4.08
4. Total mean thickness		21.41 um	29.17 um	35.75 um

Table 6.4 Thickness of body hair of the abdominal, interscapular and dorsocaudal regions

examined under a dissecting microscope to determine the number of hairs per hair follicle.

Samples of body hair (monotrich type) were epilated from the abdominal, interscapular and dorsocaudal regions. The hairs were mounted on a microscopic slide and their lengths and thicknesses measured.

Table 6.2 shows the number of body hairs/hair follicles in the 3 groups of mice. In the hot group, 43.1 - 47.2% of the body hair occurred in hair follicles which contained 1-2 hairs. In the control group 20.4 - 37.5% of the hairs were seen in follicles with 1-3 hairs. The cold group had follicles containing a variable number of hairs with 14.8 - 20.5% of these follicles containing 4-8 hairs.

Tables 6.3 and 6.4 show the length and thickness of body hair in the 3 groups. Generally, the body hairs of the cold group were longer and thicker than those of the control and hot groups. However, the length and thickness of the body hair of the control and hot groups fell within the same range.

In the 3 groups the hair lengths of the interscapular region were similar. Likewise, the hair thickness of the same region was also similar in the control and cold groups.

3. External Changes. Throughout this study the external changes of the body hair and its distribution were carefully examined under a dissecting microscope. These changes will be emphasised in the discussion to follow.

Discussion

1. Effects of Environmental Temperature on the Thermal Insulation of Body Hair. Although the method of weighing used in this investigation is suitable for estimating the total amount of body hair, it is not an accurate measure because the results are critically affected by the degree of dryness of the animals when re-weighed. This weighing method, however,

provided a possible crude comparative study of the effects of environmental temperature on the growth of body hair in the mouse.

The results confirm the view of many investigators (Summer, 1909, 1915; Sundstroem, 1922; Scholander et al, 1950a, 1950b, 1950c, 1955) that the most meaningful measure of the fur is the insulation it provides and that its thickness is linearly related to that as well as being determined by the environmental temperature. The subcutaneous fat is a poor insulator compared with fur and does not seem to play the major role in the insulation of terrestrial arctic mammals. Therefore in tropical climates, when demands are made upon the cooling power of the skin, the fur of animals becomes thinner. In his survey of the wild mammals of the North American continent, Allen (1905) reported a thicker fur coat in the northern (cold) animals than the southern (hot) forms. Moreover, it is said that thoroughbred sheep in hot climates yield smaller amounts of wool than those maintained in temperate climates. In addition, LeBlanc (1959) investigated the effects of environmental conditions on the skin and found that the thickness of the Malpighian and horny layers was increased in rats and goats reared at a cold temperature.

The underlying factors responsible for the increased weight of body hair in the cold than the other groups may be related to:

- i. Changes in the number of hairs/hair follicle. In mice, the growth of body hair is known to occur in cycles (Butcher, 1934; Johnson, 1958; Ebling and Johnson, 1964) so that the old hairs fall out and are replaced by new hairs. The fewer number of hairs/hair follicle in the hot group may be due to an acceleration of the cyclic hair moulting so as to provide the thinnest fur covering and thus facilitate body cooling.

In the cold environment, the cyclic hair growth seems to have continued, but the hairs were prevented from falling. The retained hairs thus provided the animals with the maximum number of hairs/hair follicle

to ensure good insulation.

ii. Changes in the length and thickness of body hair. The body hair of the mouse has been divided by Dry (1926) into 4 types: monotrich, awls, auchenes and zig-zag. For convenience only the first type was measured in this study.

Again, the longer and thicker body hair in the cold group compared with the other groups provided the mice in the cold room with a better protective insulating fur. On the other hand, the hair lengths of the control and hot groups were seen to fall within the same range, and therefore the thermal insulation of the fur in these groups was probably the same.

However, some regional differences were seen. The similar lengths of the interscapular hair of the 3 groups and the identical thickness of the hair of the same region in the cold and control groups may be related to the underlying brown adipose tissue. The heat which is generated by this form of fat (see p.159) may have stimulated the interscapular hair of the control and cold groups to grow longer and thicker than the hot group. In the hot environment this form of fat was transformed to the ordinary white adipose tissue which has no thermogenic properties. On the other hand the heat balance in the hot room would favour a shift towards heat loss rather than heat generation.

The similar length and thickness of the dorsocaudal hair in the control and hot groups may be related to heat loss (see below).

2. Effects of Environmental Temperature on the Distribution of Body Hair. When the mice were born or housed at the age of one week in the hot environment, the fur became progressively sparser and thinner. In some cases, the young mice appeared hairless, a condition which may simulate "alopecia totalis", but with the vibrissae usually spared. Similar observations were also reported by Sundstroem (1922) and

Scholander et al (1950a,1950b,1950c,1955). These conditions which occur in the mice for only a few days following their housing in the hot room may correspond to the alopecia associated with febrile disease in man (Kligman,1961).

In some other cases there was patchy loss of body hair especially from the abdominal and cervical regions. Such loss appears grossly somewhat like alopecia areata. The appearance of this condition may be related to the cyclic nature of hair growth which begins in the ventral region and spreads dorsally (Butcher,1934; Johnson,1958; Ebling and Johnson,1964).

The animals may also vary the fur and skin insulation greatly by erecting the hairs and by vasomotor control of the skin and tissue temperature immediately under the fur, and by changes in evaporation. There are also considerable regional differences in heat loss from the body (limbs, tails, ears) and heat generation (interscapular and cervical BAT). The ears, feet and hands were better covered by hair in the cold than the hot groups. In the cold these changes provide the animals with better insulation and thus reduce the loss of body heat (Scholander et al, 1950a,1950b,1950c,1955). The hanging scrotal sacs of the males in the hot room were devoid of hair to aid in heat loss and body cooling. On the other hand, the genitalia of the cold-reared mice were well clothed by hair so that the distinction between the sexes was sometimes difficult.

Another thermal effect on the growth of hair which may also be due to the heat loss changes was seen in the tails. The base of the tail where it joins the body was also found to be devoid of hair in the hot reared mice and well covered in the mice of the cold room.

Under the effect of low environmental temperature the body seems to respond by producing a thick covering of vital organs such as the tail so as to minimise heat loss. It also seems, however, that this result may reflect a direct and local effect of the low temperature on the

growth of tail hairs similar to the effect on the growth of tail bones.

3. The Effects of Environmental Temperature on the Pigmentation of Body Hair. Certain pigmentation changes in the body hair were also seen in the hot reared mice and to a lesser extent in the control mice. In the hot group, a band of light chocolate brown discoloration was noted over the interscapular region which extended to just below the ears. Most of the animals with this pigmented hair were males. In addition, creamy streaks were seen over the abdomen and the region around the external genitalia was found to be yellowish. In contrast, the cold reared mice were clean white. At first these discolorations were explained as due to lack of cleanliness but the colour was not removed by washing. On the other hand, these changes cannot be attributed to the food, because they were not seen in the cold reared mice which were maintained on the same diet and ate more. These pigmentary variations are probably due to the coloration of the hairs rather than the skin.

Similar findings were also reported by Sundstroem (1922) who regarded the pigmentation as due to the presence of melanin in the hair or tyrosine in the skin. But he failed to demonstrate the latter and was in doubt about the former. It seems that both factors (and possibly more) are involved

The selective regional difference in hair pigmentation may also be due to the higher concentration of a pigment-producing factor in the pigmented areas than other parts. The nature of these factors is unknown but their appearance in the hot reared group at 33°C (and to a lesser extent in the control group at 21°C) may indicate certain enzyme-chemical reactions which are activated only when a certain level of temperature was attained.

Finally, it may be concluded that hair growth, distribution and pigmentation are influenced by the external environmental temperature and that fur growth is directed towards accommodating to the appropriate climatic condition.

D. CHANGES IN THE ADIPOSE TISSUE

In most mammals there are 2 distinct types of adipose tissue which are embryologically derived from the primitive connective tissue (Bell, 1909; Inglis, 1927; Wells, 1940), but differ in their colour, distribution, vascularity, structure and functional metabolic activities. One type is the familiar yellow or white adipose tissue (WAT) which forms the bulk of body fat; the other has a characteristic brown colour and a lobular gland-like appearance and is called brown adipose tissue (BAT).

The initial observations in this part of the study was that when the mice were held by the interscapular skin during the measurement of tail lengths certain degree of increased skin laxity was noted in the cold group compared to the hot group. Histologically, however, the subcutaneous fat in the interscapular area proved to be ordinary WAT in the hot group and BAT in the cold group.

Materials and Methods. 26 animals (both sexes) maintained at 33°C (hot group), 21°C (control group) and 8°C (cold group) (9, 9 and 8 mice) for more than 6 months were used. These mice were killed and carefully dissected under the dissecting microscope to determine the distribution of the WAT and BAT. The dissected specimens were weighed (to the nearest 0.0001 mg) and then processed for histological examination (H & E, reticulin).

An additional 2 groups of mice, age 3 months, were also used.

Group 1 (2 mice). These were maintained in the cold room (8°C). They were anaesthetised and the interscapular BAT (see later) was exposed by a dorsal midline incision. A biopsy for histological examination was taken from this tissue. The wound was then closed and the animals were allowed to recover from the anaesthesia and then transferred to the hot room (33°C).

Group 2 (2 mice). These mice, maintained in the hot room (33°C),

underwent the same procedures and were then transferred to the cold room (8°C).

After 4 weeks, the animals in both groups were killed and the remaining interscapular BAT was examined histologically.

Results

A. Gross Anatomy. The morphological organisation and distribution of WAT and BAT was confirmed by histology in all cases. In the 3 experimental groups the distribution of these forms of adipose tissue was similar.

The white or yellow adipose tissue was mainly subcutaneous along the dorsal midline, around the base of the tail and thighs and in the anterior abdominal wall especially at its lateral borders. It also filled the axillary fossae and the ventral side of the neck between the parotid gland and the major vessels of the neck. In the abdomen it was found in the retroperitoneum, mesentery, and in the mesometrium in the females and as testicular fat bodies in the males. WAT was not, however, limited to these areas. It could be demonstrated almost anywhere where there was loose areolar connective tissue. It was thus difficult to provide a rough estimate of this form of fat in the mouse.

BAT occurred in the form of glistening lobules, often in relation to blood vessels and mixed with WAT in the loose connective tissue. Its colour varied according to the environmental condition. Thus it was brown in the cold group, light tan in the control and yellowish-brownish in the hot group.

The presence of BAT was sought in 5 areas: interscapular, axillary, cervical, thoracic and abdominal.

1. Interscapular BAT. This formed the largest deposits of BAT in the mouse. There were two symmetrical elongated masses on either side of the dorsal midline which filled the depression between the

muscles over the scapulae. These masses were clearly separated from each other and secured to the underlying semispinalis capitis muscles by thin vascular connective tissue adhesions. The upper thirds of these masses were covered by the rhomboideus major muscles and the lower two thirds by skin.

(p.130)

Figure 6.2/ shows the weights of the interscapular masses in the 3 experimental groups, and Figure 6.3/ shows the weights of the masses in the hot and cold groups expressed as a percentage of those of the control group. It is clear that the weights of the cold group were heavier than the control and hot groups.

(p.131)

From the interscapular masses, lobules of BAT extended laterally to the axillae. In doing so, the lobules at first ran along the inferior borders of the scapulae on the serratus anterior and covered by the trapezius muscles, and then the lobules extend anterior to the scapulae, between the subscapularis and serratus anterior muscles.

In the cold group lobules of BAT were also seen subcutaneously along the lateral borders of the anterior abdominal wall.

2. Axillary BAT. This occurred in the form of lobulated masses which covered the axillary blood vessels and filled the axillary fossae. Delicate lobules were also seen between these vessels. The axillary lymph nodes were embedded in a mixture of WAT and BAT. In the axilla these masses were covered by the pectoral muscles.

The axillary BAT was also seen connected to the cervical BAT by a delicate lobular prolongation which intersected the axillary muscles and ran under the clavicles and along the axillary vessels to the posterior and anterior triangles of the neck.

3. Cervical BAT. This was seen as a lobulated strip which ran across the ventral side of the neck over the major blood vessels sending delicate prolongations between these vessels and the cervical lymph nodes.

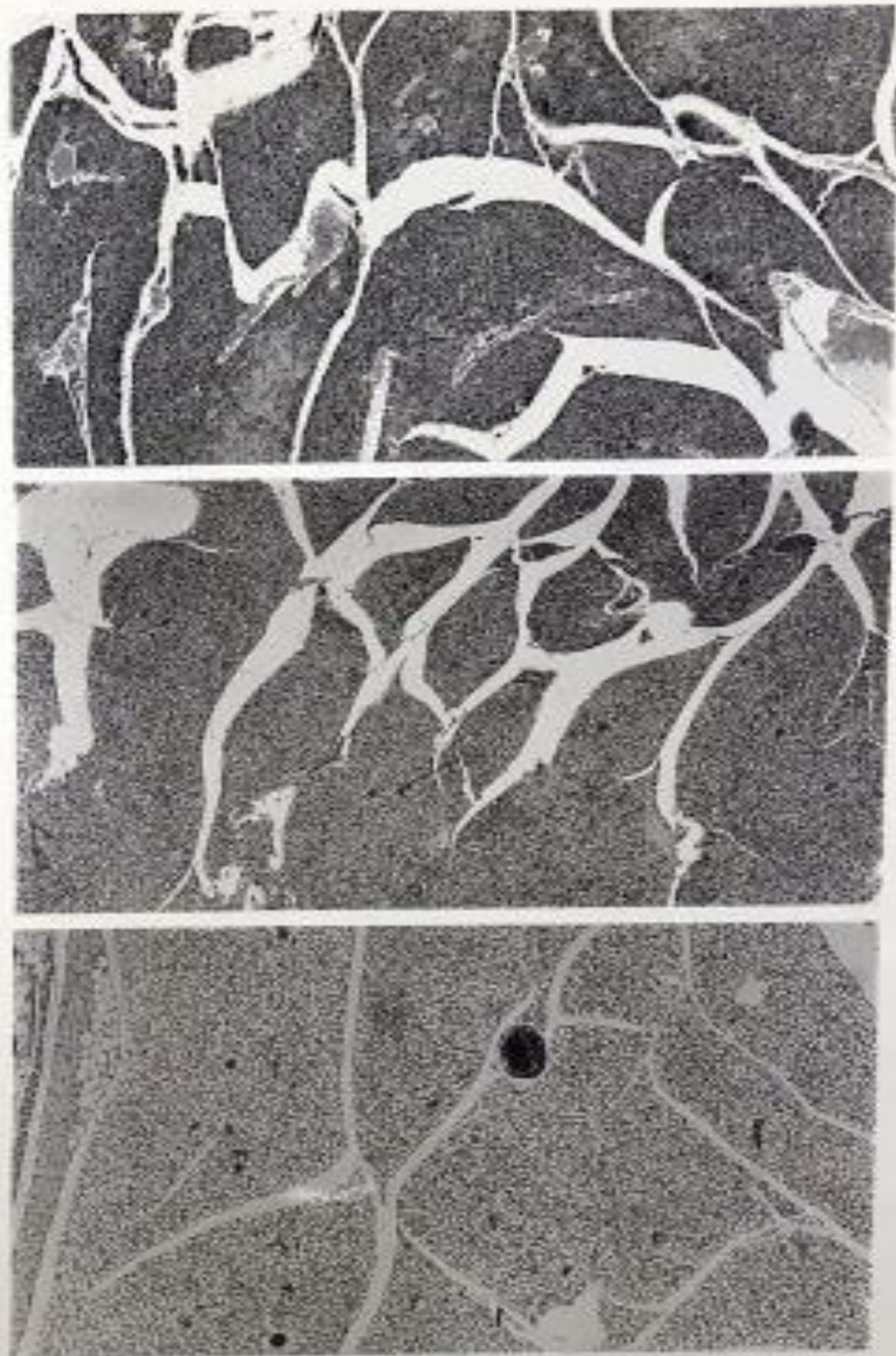


Fig. 6.5 Photomicrograph (H.E) of the interscapular BAT in the cold (upper), control (middle) and hot (lower) groups (x 50)

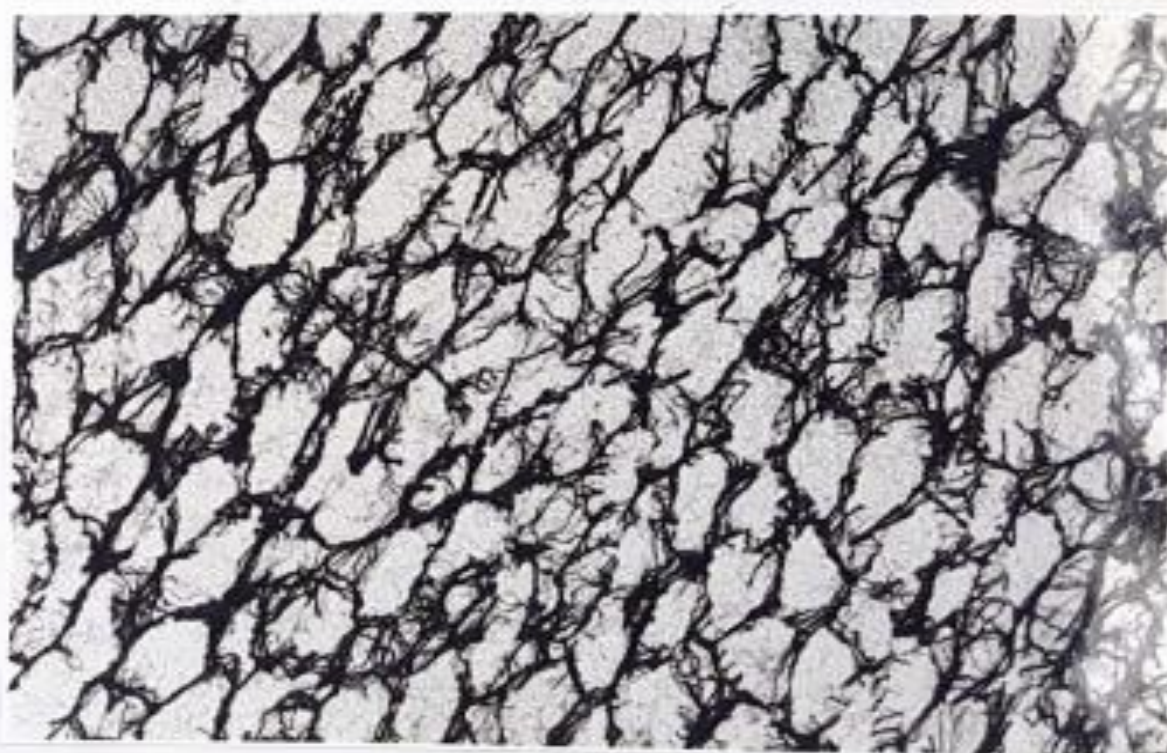


Fig.6.6 Photomicrograph (Reticulin stain) of the interscapular BAT in the cold (upper) and hot (lower) groups(x 515)

It extended over the sternothyroid and sternohyoid muscles of the neck and was covered by the parotid gland.

4. Thoracic BAT. In the thorax, BAT was seen as discrete islands around the thymus, heart and the hilum of the lungs. It also formed slender lobules along and on either side of the thoracic aorta, filling the costo-vertebral angle and extending to the abdomen.

5. Abdominal BAT. In the abdomen, BAT also occurred in the form of discrete islands along the abdominal aorta and its major branches. It was also found at the hila of the spleen and kidneys and around the upper third of the ureters.

B. Microscopic Anatomy. In the cold and control groups the interscapular, axillary and cervical masses had the typical appearance of BAT. It was covered by a capsule of delicate areolar connective tissue which contained blood vessels, nerves and a variable number of adipocytes. From the capsule, septa of loose vascular connective tissue penetrated the whole brown mass dividing it into irregular but well-defined lobes and lobules (Figure 6.5).

The vascular pattern of BAT resembled that found in glands. The interlobular vessels entered the lobules and divided into an intralobular capillary network which surrounded the individual cells. The blood vessels and capillary network were packed with red blood cells especially in the cold group and to a lesser extent in the control group.

The parenchyma of the brown lobules was composed of closely packed brown adipocytes. These are polygonal cells occurring in groups and clumps interlaced by abundant coarse randomly orientated reticulum fibres (Figure 6.6). The average size of these cells was 18 μ m in the interscapular BAT of the cold group and 23 μ m in the control group.

The nuclei of the brown adipocyte was usually central, large and spherical and never peripheral or flattened as in the white adipocyte.

Occasionally, however, some eccentric nuclei were seen. The chromatin material formed a granular network, in the meshes of which 1-3 prominent nucleoli were seen. In all the sections of BAT examined the nuclei were similar and mitotic figures were absent. The average size of the nuclei was 6 μ m in the interscapular BAT of the cold group and 4 μ m in the control group.

Unlike the large single lipid globule of the white adipocyte with its thin peripheral rim of cytoplasm, the cytoplasm in the brown adipocyte was abundant but varied between the cold and control groups. In both, the cytoplasm was laden with drop-like lipid inclusions. These droplets generally had a uniform rounded shape but their size and number varied both between cells in the same animal and group of animals as well as between the cold and control groups. In the cold group the cytoplasm of the brown fat cells was coarsely granular, abundant, deeply stained and contained a variable number of lipid droplets of various sizes. In contrast, the cytoplasm of the cells in the control group was less granular, scanty, lightly stained and contained more lipid droplets than the cold group.

Several other forms of adipocyte were also seen. Occasionally brown adipocytes with one or two relatively larger lipid inclusions and several smaller droplets of various size were seen. In some other cases, the cells were entirely filled with 2-3 large inclusions. White adipocytes were also seen between the closely packed brown adipocytes. These forms were more frequently observed in: (1) the capsule and connective tissue septa of BAT, (2) close to large blood vessels, (3) the BAT of the control group, (4) the thoracic and abdominal BAT of the cold and control groups.

The thoracic and abdominal BAT of the cold and control groups had the same structure, but the vascularity was less than that of the inter-

scapular, axillary or cervical BAT. On the other hand, white adipocytes as well as those forms with 2-3 large lipid droplets were more frequent.

In the hot group, the microscopic anatomy of the 5 above masses simulate that of WAT, but with the lobular pattern preserved and the vascularity greatly reduced. In sections, the interscapular masses were formed of closely packed large polyhedral cells, largely occupied by a large single lipid globule. The cytoplasm was very scanty and the nuclei were flattened and displaced to the thin rim. As in the BAT cells, these cells were also surrounded by abundant coarse reticulin fibres of random orientation (Figure 6.6). The average size of these cells in the interscapular masses was 35 μ m.

The biopsy specimens which were obtained from groups of animals maintained at 8°C and 33°C and then transferred to 33°C and 8°C for 4 weeks showed:

Group 1. The histological appearance of typical BAT.

Group 2. The histological appearance of WAT.

Discussion

In most mammals BAT not only has a similar anatomical distribution but also similar histological appearances (Afzelius, 1970). Rasmussen (1923-1924) listed the presence of BAT in 57 species: 7 insectivora, 9 chiroptera, 33 rodentia, 6 carnivora, ungulata and homo sapiens. It is also present in the Macacca rhesus monkey (Afzelius, 1970).

In this study the distribution of WAT and BAT in the adult mouse under 3 environmental conditions was studied. The anatomical organisation of both types was similar in the 3 groups. The distribution of BAT was divided into 5 parts: interscapular, axillary, cervical, thoracic and abdominal BAT. However, the bulk of the BAT in the mouse was found in the two symmetrical masses forming the interscapular part.

Changes in the gross anatomical mass of BAT have been studied by

several authors (for review and details see Lindberg,1970). Thus in a newborn mouse weighing 1.4 gm it accounted for 1-2% of the total body weight (Afzelius,1970). The weight of BAT is influenced by exposure to a cold environment (Bruck,1970). In the rat, 95% of the BAT was found in the interscapular, cervical, thoracic and inter-renal parts (Smith and Roberts,1964) and fresh weight of BAT reached a maximum after 8 days of cold exposure (Roberts and Smith,1967). The interscapular BAT has been recognised as the most important site of BAT and changes in the fresh weight of these masses to represent the changes in the fresh weight of total BAT in the body (Barnard and Skala,1970; Smalley,1970). Thus, after 6 weeks of cold acclimatisation the fresh weight of these masses was found to increase 4 times (Barnard and Skala,1970). In the present study, the fresh weight of interscapular BAT in the cold reared mice was 272% and 395% heavier than that in the control and hot groups.

These differences in the fresh weight are mainly due to the degree of vascularity. Thus, although the WAT is usually considered to be poorly vascularised, all BAT is richly vascular (Rasmussen,1923-1924; Fawcett,1952; Afzelius,1970; Bloom and Fawcett,1975 and others). In the rat the vascularity of BAT was estimated to be 4-6 times greater than that of WAT (Hausberger and Widelitz,1963). Indeed it is the numerous blood vessels which surround the individual brown adipocytes and the red cells in these vessels that gives BAT its characteristic colour and hence its name (Fain et al,1967).

The distinction between WAT and BAT has been investigated by many authors (Fawcett,1952; Remillard,1958; Napolitano and Fawcett,1958; Napolitano,1963; Angervall et al,1965; Afzelius,1966,1970; Hull and Segall,1966; Ohkawa and Farber,1968; Barnard and Skala,1970; Hirvenen et al,1974; Bloom and Fawcett, 1975 and others). Thus, whereas the poorly vascularised WAT is made of large globules of fat, the highly

vascular BAT is made of smaller cells which contain small droplets of lipid. Because of the presence of these droplets, the BAT is often termed multilocular adipose tissue to distinguish it from the unilocular adipose tissue of the ordinary WAT. In addition, the uniform appearance of brown adipocyte nuclei as well as the absence of the mitotic figures identifies BAT as a fully differentiated tissue.

According to the results of this study, it is possible to divide the BAT of the mice maintained at the 3 environmental temperatures into "cold fat", "hot fat" and "control fat". The first is typical of BAT and occurred in the mice reared at 8°C. The second resembled the WAT and occurred in the mice reared at 33°C. The third occurred in the mice maintained at 21°C and may represent a transitory stage between the first and second forms. Therefore one may postulate that the environmental temperature may determine the form of BAT in the mouse. The main distinctive features of the "control fat" are: (1) Its fresh weight is heavier than the "hot fat" but lighter than the BAT. (2) The vascularity is moderate between the highly vascular BAT and the poorly vascular "hot fat". (3) The adipocytes in the "control fat" were larger than the brown adipocytes but smaller than those of the "hot fat". Generally, the size of the cells depends on the animal species (hibernators-non-hibernators), the developmental stage (newborn-adult) and the degree of lipid depletion or loading (Afzelius,1970). Fat depletion can be brought about by prolonged starvation (Lever,1957), exposure to cold (Sidman and Fawcett,1954) or hibernation (Remillard,1958; Hayward and Ball,1966 and others) in which both factors are combined. The brown adipocyte contains a few small lipid droplets and abundant cytoplasm, thus it may represent a state of fat depletion. On the other hand, the single lipid globule and the scanty cytoplasm of the "hot adipocyte" may indicate fat loading. The adipocytes of the "control fat", however, contained many large lipid droplets but the

degree of fat depletion was greater than that of the "hot fat" and the fat loading was more than the BAT. (4) It contained more white adipocytes as well as many forms of fat cells in different stages of maturation than BAT.

Whether BAT can be converted into WAT is often disputed (Rasmussen 1923-1924). But Dyer's (1968) in vitro study of isolated adipocytes confirmed such conversion. In this study BAT and WAT ("hot fat") were converted to and from each other by transferring the animals between the hot and cold rooms. The transformation of BAT into "hot fat" seems to be a replacement by WAT rather than a change in the structure, i.e. increased lipid loading because (1) the final histological picture of the converted tissue was typical of "hot fat" which in most respects resembles WAT, (2) the multilobular pattern of BAT was preserved in the "hot fat", (3) the arrangement of the reticulin fibres was also preserved in the "hot fat". Normally each WAT fat cell is enclosed in fine delicate reticulin fibres whereas in the BAT the reticulin fibres are coarse and of random orientation, and (4) in the connective tissue septa and in areas where the temperature is high, such as adjacent to blood vessels and in the thorax and abdomen, the predominant BAT cells are those with 1-3 large lipid droplets and a few small lipid inclusions. This picture may be a transitory stage before the droplets coalesce and form one large single fat globules.

Functions of BAT (for review and details see Rasmussen 1923-1924; Johansson, 1959; Lindberg, 1970). At first (1670-1817) BAT was considered as a part of the thymic tissue, then (1817-1863) as an endocrine gland concerned with haemopoiesis, and later (1863-1902) as a reservoir for food substances. Between 1902 and 1961, BAT was once again believed to be an endocrine tissue. Its relative abundance among hibernating species led Joel (1965) to conclude that BAT is important in the generation of heat.

During exposure to cold, the animals need more oxygen and heat (Hayward and Ball, 1966; Drahota, 1970). To provide such requirements, nerve impulses to the BAT were postulated to release norepinephrine at the nerve endings (Cottle, 1970; Schonbaum et al, 1970). This leads to the activation of lipases in the adipocytes and results in the breakdown of triglycerides into glycerol and fatty acids (Himms-Hagen, 1965). The oxidation and re-esterification of these fatty acids which occurs thereafter leads to more oxygen consumption and heat generation. The heat produced by the vascular VAT then warms the blood flowing through the tissue and thus raises the body temperature of the animal as a whole.

One of the main differences between the WAT and BAT is the presence in the latter of large and numerous mitochondria (Napolitano and Fawcett, 1958; Napolitano, 1963; Afzelius, 1966, 1970; Barnard and Skala, 1970; Bloom and Fawcett, 1975). This accounts for the presence of colour in BAT. The brown colour is partly due to the increased vascularity of this tissue but mainly due to the presence in the mitochondria of a high concentration of iron-containing cytochrome oxidase (Hirvonen et al, 1974). This enzyme is also essential for the oxidation of the fatty acids and the generation of heat.

The heat generated by the interscapular BAT was found to account for several thermally-related observations. Thus, the external (skin) temperature of this region (as well as that of the cervical region) in the cold-reared mice (4°C and 8°C) was only a few degrees lower than the hot (33°C) and control (21°C) reared animals. This skin temperature was also as high as the internal temperature of the mice. This is consistent with the findings of Dawkins and Hull (1964) who inserted subcutaneous thermocouples into the interscapular BAT of newborn rabbits and found that its temperature was $2-3^{\circ}\text{C}$ higher than the temperature of the colon. On the other hand, when the bodies of bats were scanned by thermo-

graphic mapping, after the arousal of these animals from hibernation, a delineated "hot area" was demonstrated over the interscapular BAT (Hayward and Ball, 1966).

The heat generated by the interscapular BAT may also be responsible for the hair over this area growing longer and thicker in the cold and control reared mice than in the hot group (p.142,143)

E. THERMOREGULATION IN THE MOUSE

It has been shown earlier that the mouse cannot control the temperature of its tail, and that when exposed to a particular environmental condition the tail assumes the temperature of the surrounding environment. On the other hand, the internal temperature of mice maintained in the cold (4°C) or hot (33°C) temperatures fell within the same range as the control mice maintained at 21°C . Similar findings have also been reported (Alee and Lutherman, 1940; Schplander et al, 1950a, 1950b, 1950c, 1955) in a substantial number of arctic, temperate and tropical mammals and birds. These observations not only indicate the local effect of the temperature on the tail but also the importance of the tail as a thermoregulatory organ (Sundstroem, 1922; Harrison, 1958; Barnett, 1965; Rand et al, 1965).

Thermoregulation in the mouse may be accomplished by two principal adjustments: (1) Changes in heat production through changes on the general metabolism of the body. With the exception of changes in the adipose tissue (p.159), these changes are beyond the scope of this study (for review and details see Whittow, 1971; Yousef et al, 1972). (2) Changes in heat loss. These could be brought about by morphological changes in the size of tails, ears, feet, pelage and scrota (Sumner, 1909; 1915; Sundstroem, 1922; Ogle, 1936; Harrison et al, 1959, 1969).

It is not clear, however, whether these changes are achieved through auxiliary organs of thermoregulation or the direct result of the

environmental temperature. In the present chapter the importance of these changes are evaluated. The changes in the growth of feet and hair have been discussed separate (p.71,139).

Experimental Procedures and Results

1. The Tails. Four 24 day old mice were used. The tails were amputated close to the body and the bleeding stumps were cauterised. Two animals were then taken to the hot (33°C) and two to the cold (8°C) rooms.

The mice in the hot room were found dead after 5 hours but those in the cold room were alive until the following day when they were transferred to the hot room, where they were found dead after $3\frac{1}{2}$ hours.

2. The Ears. Due to the complex shape of the pinna, an accurate measure of ear size was not possible. However, some indirect parameters such as the weight of ear tissue and/or that of its image on photographic paper were used.

A group of mice maintained for more than 6 months in the 3 experimental conditions of 33°C , 21°C and 8°C (12, 7 and 10 mice) were killed and the ears dissected from the bodies and fixed in formol saline. The ears were then laid flat and the ear pinnae were cut (under the dissecting microscope) close to the tragus of the ear from as low as the lower extremity of the incisura intertragica (a point homologous to the human ear) to the tip of the ear pinna. The ears were then weighed by two methods: (1) Wet-weight method - the ears were weighed to the nearest 0.0001 mg; (2) Area-weight method - the ears were laid as flat as possible between two thin layers of glass and their images enlarged on photographic paper. These images were cut out and weighed (also to the nearest 0.0001 mg) and the estimated weights were divided by the magnification factors to calculate the original weight of each ear.

Table 6.5 shows the ear weights as measured by both methods. In the area-weight method the ears of the hot group were heavier than those of the

METHOD	WET WEIGHT (mg.)			"AREA WEIGHT" (mg.)		
Temp. (°C)	8	21	33	8	21	33
No. of cases	10	7	12	10	7	12
Range	0.022 - 0.033	0.026 - 0.034	0.015 - 0.022	0.180 - 0.208	0.195 - 0.224	0.227 - 0.268
Mean	0.027	0.030	0.019	0.196	0.211	0.241
S.D.	0.003	0.003	0.002	0.009	0.010	0.013
P(1)	0.07629	<0.00001		0.00426	0.00015	
P(2)		0.00001			<0.00001	

Table 6.5

The size of the ears of the 3 experimental groups as estimated by the wet weight and area weight methods. P(1) is the significance of the differences between the control and the cold and hot groups. P(2) is the significance of the difference between the cold and hot groups. (see footnote to Table 2.3)

cold and control groups. In contrast, the wet-weight method shows that the ears of the hot group were lighter than other groups.

3. External Changes and Behavioural Reactions. Throughout this study the external changes occurring in the body and tails as well as the behavioural reactions were carefully observed in the mice maintained in the 3 experimental conditions. These changes will be emphasised in the discussion to follow.

Discussion

1. The Tail as a Primary Thermoregulatory Organ

In most homeothermic mammals, the body appendages play a significant part in heat loss and its regulation (Scholander et al, 1950a, 1950b, 1950c, 1955; Whittow, 1971; Yousef et al, 1972). The particular appendage concerned may vary from one species to another. For example, the ears may be the primary organ concerned with the thermoregulation in the elephant and rabbit. The neck and limbs may have the same role in the camel, llama, giraffe, and possibly the fingers and toes in man (Burton, 1939). In the mouse and rat (and possibly other species), the tail seems to be the primary thermoregulatory organ (Sundstroem, 1922; Scholander et al, 1950a, 1950b, 1950c, 1955; Harrison, 1958; Barnett, 1965; Rand et al, 1965; Harrison and Clegg, 1969).

The tail is a richly vascular structure containing many arteriovenous anastomoses (Gordon et al, 1953). By controlling the blood flow to and from the tail, the heat loss may be regulated (Rand et al, 1965). The occlusion of the blood flow of the tail in muskrats caused the internal body temperature to rise in the hot environment (Johanson, 1962). In the rat, sudden vasodilation^{was} seen in the tail when the external temperature rose to between 27-30°C with the blood flow rising from 5 to 40 ml/100 ml tissue/minute (Rand et al, 1965). In the monkey, however, more than 10% of the cardiac output was estimated to flow to the tail when the environmental temperature exceeded 40°C (Hong and Luck, 1953).

On the other hand, the "absence of hair" on the tail and its large surface area in relation to its volume makes it a suitable heat radiator.

Mice and rats have long tails with no sweat glands (Rowett, 1974). Thus the possession of a tail increases the fitness of these animals to survive in a hot environment. Indeed, the evolutionary progress and the wide geographical and climatic ranges (Allen, 1905) of these species may in part be due to the possession of tails.

In this study, mice with amputated tails could tolerate the cold environment of 8°C and adjust their thermoregulatory mechanisms. But in the hot room, they died within a few hours. These observations are consistent with those of Harrison (1958) and Harrison and Clegg (1969) that the removal of the tail decreases the adaptability of these animals to a hot environment.

Immediately after transfer to the hot room the tails of young mice became engorged with blood, clean and moist, but after 3-4 weeks the tails became dry and scaly. The long spindly tails of the hot-reared mice were always held straight out behind the animals. This may be to increase the heat loss from the body to the air which is a bad conductor to heat. The mice of the cold room, however, sat on their tails so that the heat loss was reduced and the tails warmed by the body. This conserved heat. The tails of the cold-reared mice always appeared clean and white (vasoconstriction), but showed immediate reactive hyperaemia when taken to a warmer temperature. Thus, in the hot room, heat loss was increased by opening up the arteriovenous anastomoses to increase blood flow while in the cold room heat loss was reduced by closing these anastomoses. It is not clear why the initially clean, moist and highly vascular "hot tails" after 3-4 weeks in the hot room became dry and scaly tails of low vasculature. This may be related to the growth of the underlying tail bones which nearly completed their growth during that period and thus needed little amount of vascular nutrition.

2. Auxiliary Organs of Thermoregulation in the Mouse

According to Allen (1905), certain peripheral parts of various animals tended to enlarge in the higher temperatures as one moves towards the tropics. In mammals such as the mouse, this is manifested by the size of the ears, feet and in cows by the horns. Sumner (1909,1915) reported smaller ears and shorter tails in mice reared at a low temperature and this has been confirmed by Sundstroem (1922), Scholander et al (1950a,1950b,1950c,1955) and many others. But in most reports the size of the ear in relation to that of the body was not accurately measured. This is probably because of the peculiar complex shape of the ear. In the present work, weighing methods, though crude, were devised as a way of measuring the size of ears and thus of the effects of environmental temperature on the growth of ears. The weight of the photographic reprints gave a measure of the surface area of the pinnae, while the weight of the ear pinna itself gave an indication of the thickness of the ear. Thus the ears of the heat-reared mice were not only larger than the other groups, but also thinner. This was because the larger surface area/volume ratio was needed in these animals, so that the ears could act as auxiliary cooling organs in the hot environment. It is not clear, however, whether these changes in the size and thickness of the ears represented a generally adaptive mechanism or merely a local effect of temperature on the hyaline cartilage of the ears. The differences in the external temperature of the ears, particularly of the animals maintained at 4°C and 8°C (Figure 3.11), point to the possibility of a local effect similar to that on the cartilage cells of the growth plate.

Scrotal sacs have also been reported to be influenced by changes in environmental temperature (Sundstroem,1922; Ogle,1934). The male mice observed throughout this study formed no exception to this rule. The hanging scrotal sacs which are devoid of hair may have acted as auxiliary cooling organs in the hot-reared male mice. The testes of the

cold-reared males remained inside the abdominal cavity as long as the mice were in the cold room, but if transferred to the hot room, the scrota emerged to hang down again. It seems probable that the testes are very sensitive to changes in the temperature and that a cooler temperature than the internal body temperature is necessary for the production of sperm.

In the cold room, the testes are drawn up into the passage which connects the scrotal sac to the abdominal cavity (the mice have no distinct inguinal canal). The temperature at this position is a few degrees lower than that of the body.

3. Behavioural Thermoregulation (for review and details see Hart, 1971)

The control mice were always found in the centre of the wood shavings (p. 28) which they usually took to one end of the cage away from the food and water source. They also kept it clean; their droppings were distributed outside.

The mice took food at night. This was probably related to the darkness and the fall of temperature at night. When one litter was exposed to direct electric light which raised the temperature inside the cage to about 25°C they ate less. On the other hand, most growth in body weight and tail length was found to occur at night.

The water and food consumption was estimated at about 120-165 ml and 125-175 gm/week/litter of 5 mice.

In the cold room, the mice reacted differently. These mice were able to build a nest inside the cotton wool (p. 28) so as to survive the cold temperature. These mice have been interbred for hundreds of generations under Animal House conditions at 21°C, and it seems that this ability was preserved in the genes of these generations and was manifested when their life was endangered.

The behaviour of the mother mice in warming their young (p. 29) was another interesting observation. But as weaning age approached, when

the mice injure the mother with their sharp incisors and interfere with the mother's feeding, the young were rejected into sub-compartments in the cotton wool nest and had to depend on themselves for survival.

In the cold room the mice were found to grind the food cubes and cover the floor of the metal cage with a thick layer of "powdered food particles" which added more insulation to the cage. They also spread the powder to cover the nest. This behaviour served 3 useful functions: (1) Helped the mice to eat while grinding. (2) Performed physical exercise and generated heat. (3) The mice were exposed for a longer time to the cold environment. The powder was usually removed periodically and ad libitum feeding was maintained. This made the estimation of food consumed erroneous, thus between 200-250 ml of water and 445-610 gm of food was consumed/week/litter of 5 mice. However, when the "food powder" was left in the cage and more food cubes were given, the mice did not come out from their nest.

In the cold the nest was always kept clean and the droppings were found outside. The dead mice were also found outside the nest, probably because a cold body tended to cool the rest of the litter.

In the hot room the mice removed the sawdust bedding from one or both ends of the cage and left that part of the cage bare and clean. In doing so they lost some of their body heat to the floor of the metal cage which is a good heat conductor.

During the first few days in the hot room the mice were very active. This was probably due to the stimulation of all metabolic and nervous activities of the body. Later on, when the mice had adjusted their thermoregulation mechanisms, they tended to lie motionless in their cage.

The hot-reared mice consumed little food and water. This was estimated at 90-120 gm and 85-125 ml/week/litter of 5 mice. These findings are consistent with the preliminary report of Harrison and Clegg (1969).

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