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THE EFFECTS OF CHANGES IN THE ENVIRONMENTAL TEMPERATURE ON THE GROWTH OF TAIL BONES IN THE MOUSE

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Summary.—The tails of baby mice grow rapidly and independently of environmental temperature because they are kept warm by their mothers.

After weaning, at approximately 3 weeks of age, tail growth is strictly related to environmental temperatures. During the first 2 weeks after weaning growth rates of 1·2–1·4 mm/day/tail were seen at 33° and a maximum of 2·43 mm/day/tail was observed in one group kept at 36°. Animals kept at 8° or 4° showed tail growth rates of 0·4 mm/day or less. However, the tails of animals transferred from either hot to cold or cold to hot during their first 2 weeks after weaning immediately grew at the same rate as those of animals kept in these conditions continuously, thus indicating that heat was acting directly on bone growth.

The tails of animals kept continuously in the hot environment at 33° completed their growth early so that their growth rate fell below that of controls after about 3 weeks of treatment (when they were 6–7 weeks old) and below that of "cold" animals after about 4 weeks (7–8 weeks old). The tails of the "control" and "cold" animals grew slowly for a very long time, 150–195 days. Even so, because of the very rapid early growth of tails in the hot environment, their final length was always greater than either the "control" or "cold" tails.

IN A GEOGRAPHIC SURVEY of mammals in the North American continent, Allen (1905) noted wide variation within any one species in the relationship of the body size of the animals to their peripheral parts. As one progressed from the colder northern parts to the warmer southern regions several species developed longer peripheral parts, which included the tail, ears, paws, antlers and skull. Many other investigators confirmed Allen's rule (1905). Sumner (1909, 1913, 1915) reported smaller ears and short tails in mice reared at a lower temperature and this was supported by Sundstroem (1922). Przibram (1931) interbred mice in a wide range of temperatures 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.

The direct relationship between tail length and temperature has since been observed by several workers in mice and rats (Ashoub, 1958; Harrison, Hiorns and Weiner, 1959; Harrison and Clegg, 1969; Chevillard, Portet and Cadot, 1963; Noel and Wright, 1972), but the reasons for this are debatable. Does it represent a specific thermoregulatory adaptative response to environmental temperature (Harrison, 1958; Harrison *et al.*, 1959; Harrison and Clegg, 1969; Chevillard *et al.*, 1963), or that of a direct and local effect of the temperature on the tail bones (Barnett, 1965; Noel and Wright, 1970, 1972)? Most authors studying the effects of environmental temperature on the mammalian body and its peripheral parts were either concerned with the physiology of the adaptation mechanism (Brody, 1945;

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Allee *et al.*, 1949; Scholander, 1955; Yousef, Horvath and Bullard, 1972), or the responses of the different animal genotypes to changes in the environmental temperature (Barnett and Scott, 1963; Barnett, 1965; Harrison *et al.*, 1963; Harrison and Clegg, 1969). Most of these studies have been relatively short-term and therefore it is the aim of this work to investigate the long-term effects of different environmental temperatures on the growth of tail bones in mice.

MATERIALS AND METHODS

Strain A, albino mice of both sexes inbred (brother–sister mated) at King's College Hospital Medical School, London, SE5 were used. The mice in the experimental group were arranged using a table of random numbers based on body weight and tail length. The tails were measured to within less than 0·5 mm by a graduated glass tube (Howard-Flanders and Wright, 1957). The details of the experimental environmental conditions have been described previously (Al-Hilli and Wright, 1979).

The temperature inside the cotton-wool nest (see below) was measured using an electric thermometer with a 1mm exposed sensor (Ellab, Copenhagen. Supplied by Sierex Ltd, 15–18 Clipson Street, London W1).

Selection of age and temperature.—Normally tail growth is highest during the first 3 weeks after birth. Thus changes in the growth rate of tail would be most easily demonstrated during this period. But when 1 week old mice and their mothers were given ample cotton-wool for nesting and housed at 4° the young mice died after 2 days. Likewise the 10–12-day-old mice were also unable to survive temperatures of 36° and 38°. Even the adult mice failed to survive a temperature of 38°.

In another attempt to find the extremes of temperature that these animals can survive, 4 1-week-old litters and their mothers were used. Two litters (11 mice) were kept at 8° (cold group), 1 litter (5 mice) at 33° (hot group) and 1 litter (5 mice) at the Animal House temperature of 21° (control group). The cold group were provided with a nest made by wrapping several thin layers of cotton-wool around wood shavings. All groups survived.

From the results of this study the standard procedure adopted thereafter was to wean the mice at 23 days of age and transfer them to the experimental conditions at 25 days of age.

Long-term effects of 3 temperatures.—Four weaned litters (19 mice) were divided at the age of 25 days into: hot (8 mice); control (5 mice) and; cold (6 mice) groups—maintained at 33°, 21° and 8° respectively.

Effects of short exposure of different temperatures.—Three weaned litters (21 mice) were divided at the age of 25 days into 4 groups: (i) *Continuous hot group* (8 mice) housed at 33°; (ii) *Continuous cold group* (5 mice) housed at 8°; (iii) *Hot-cold-hot group* (4 mice) kept at 33° for 4 days, transferred to 8° at the age of 29 days and back to the 33° at the age of 39 days: and (iv) *Cold-hot-cold group* (4 mice) kept at 8° for 4 days transferred to 33° at the age of 29 days and back to 8° at the age of 39 days.

Comparison of five temperatures.—Three weaned litters (18 mice) were housed in the cold room (8°) at 25 days of age. At the age of 35 days the animals were divided into 5 groups and housed at 36° (4 mice), 33° (3 mice), 21° (3 mice), 4° (4 mice) and at 8° (4 mice).

RESULTS

Selection of age and temperature

Figure 1 shows the curves of tail growth of the experimental groups which were housed at different environmental temperatures with their mothers shortly after birth. When 1 week old the mean tail lengths of these groups were closely similar (2·42 cm in the hot, 2·19 cm in the control and 2·05 cm in the cold group). 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 at about 1·2 mm/day during the same period.

The tails of the cold group also grew rapidly until they were 21–25 days old (1·6 mm/day) but after this the growth rate was only 0·03 mm/day. This unexpected early rapid growth was assumed to be due to the mothers' successful efforts to keep the young mice warm. By the age of 75 days the tails of the cold group were 2 cm shorter than those of the hot group.

In another 1-week-old litter (5 young mice) housed with their mother at 8° the temperature inside the cotton-wool nest was found to be 34·1°.

Long-term effects of 3 temperatures

Figure 2 shows the curves of tail growth of 3 groups of mice exposed to the 3 experimental environmental temperatures

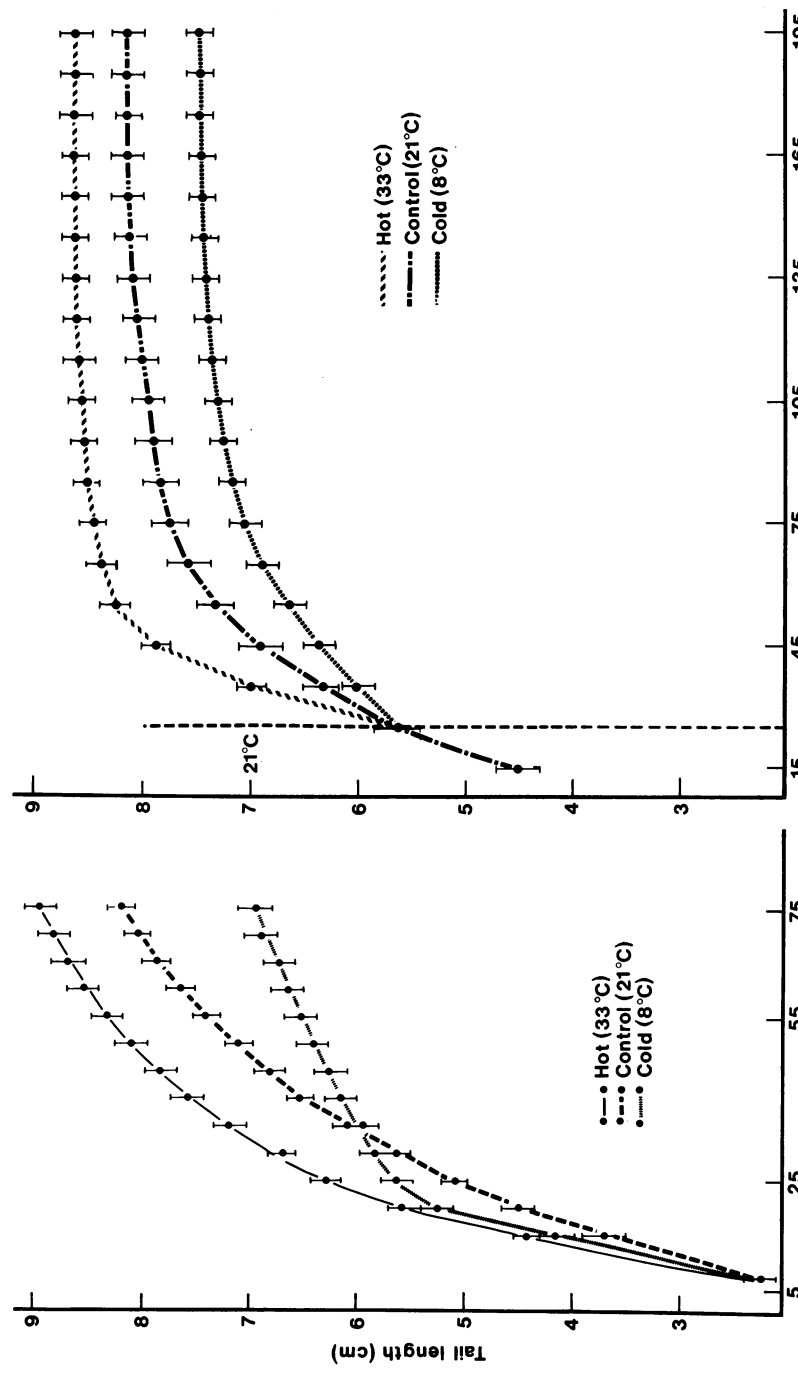
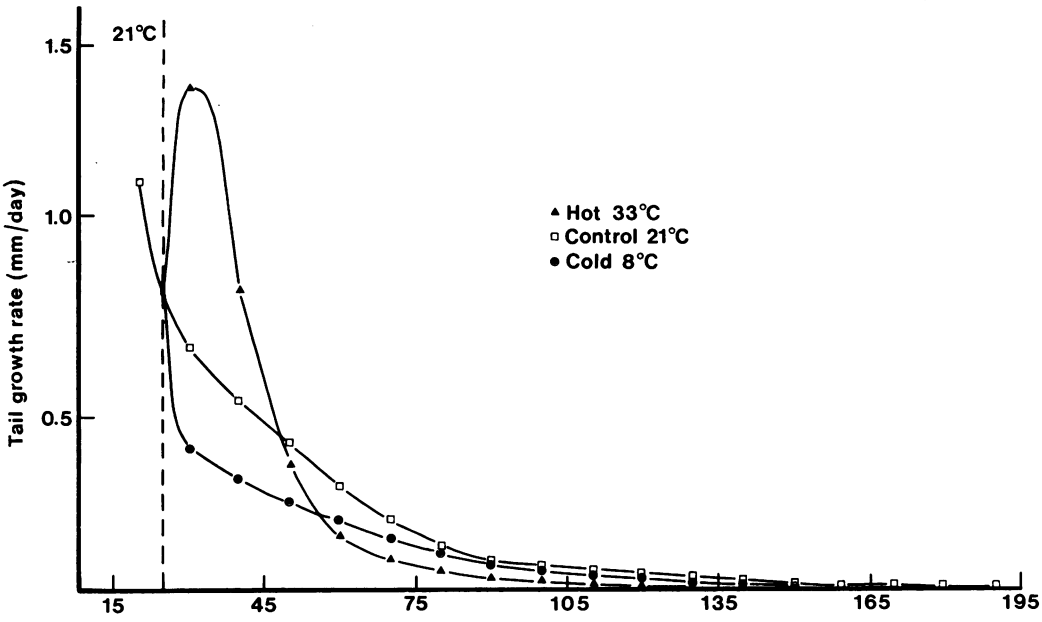


FIG. 1.—The curves of tail growth of week-old mice housed with their mothers at different environmental temperatures. The tails of the cold group grew much slower after 20–23 days.

FIG. 2.—The curves of tail growth of groups of mice transferred to different environmental temperatures when 25 days old.



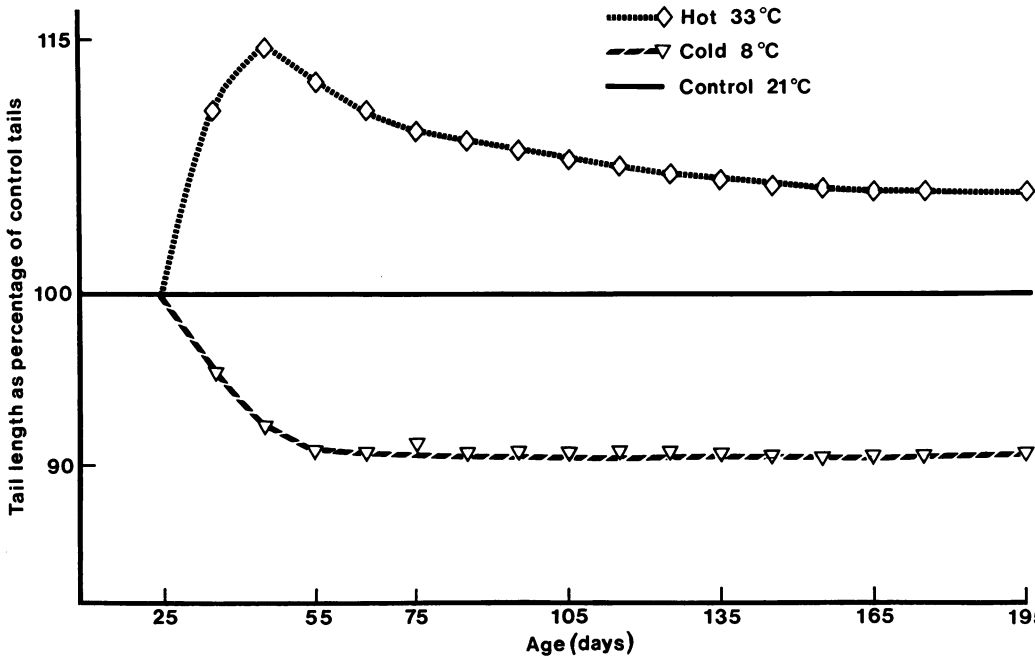


FIG. 3.—The curves of growth rate of the tails of the groups of animals shown in Fig. 2. Soon after transferring the 25-day-old mice to the experimental temperature, the growth rate of the tails increased in the hot and decreased in the cold.

FIG. 4.—The mean tail lengths of the groups of animals shown in Figs 2 & 3 expressed as percentages of the mean tail lengths of the control group.

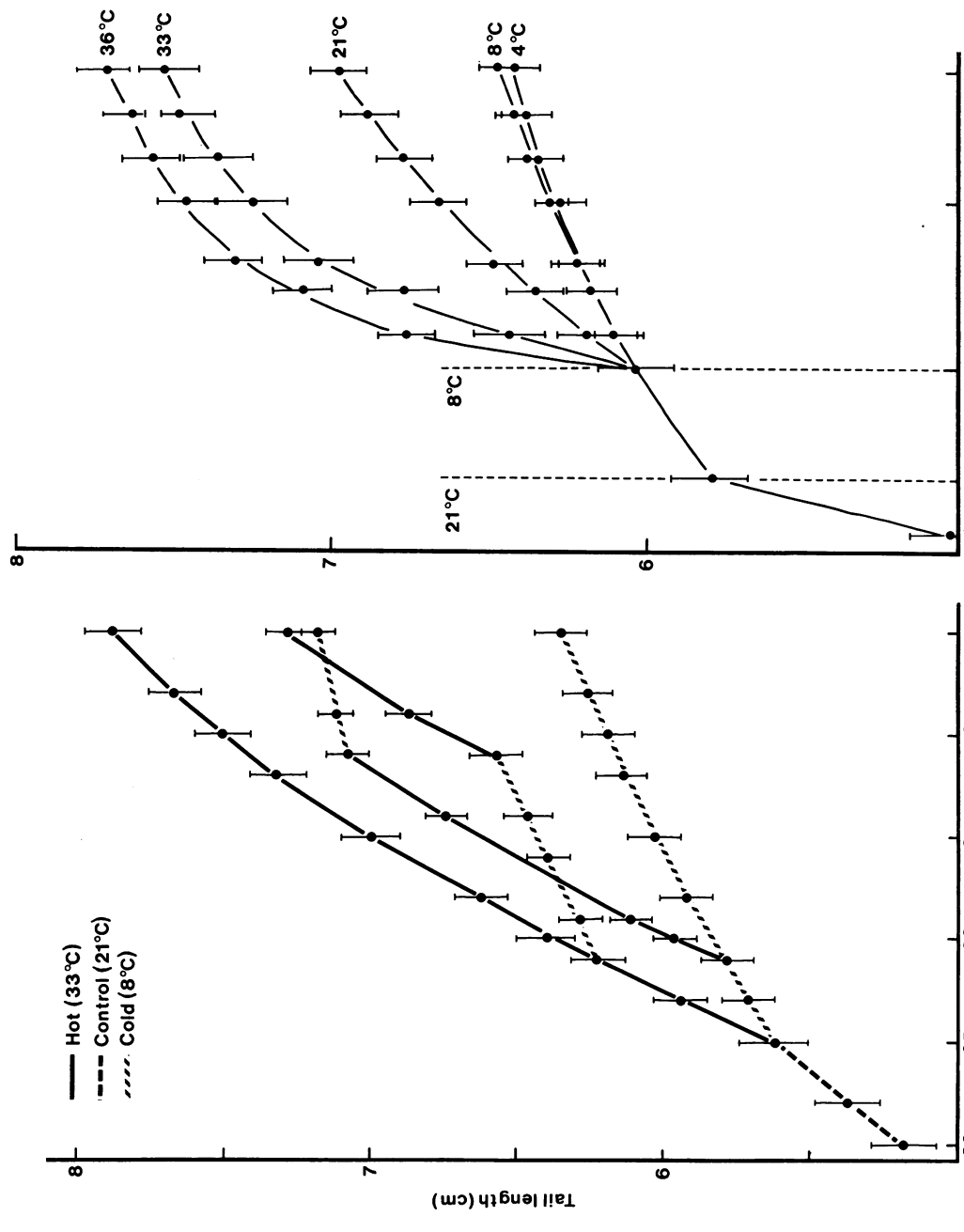


FIG. 5.—The tail lengths of groups of mice exposed to different temperatures continuously and changed from one temperature to another. This resulted in the appearance of graphical "parallelograms" between the tails of the hot and cold groups.

FIG. 6.—The curves of tail growth of groups of mice maintained at different environmental temperatures (36°, 33°, 21°, 8° and 4°). All animals were kept at 21° until 25 days old and at 8° between 25 and 35 days of age.

at the age of 25 days. The mean tail lengths of the groups were equal at the age of 25 days (s.d. 0·15 mm). The tails of the mice permanently kept in the hot room grew very rapidly at first but slowed and stopped earlier than the other groups. At 195 days the tail lengths were 8·63 cm, 8·16 cm and 7·48 cm in the hot, control and cold environments respectively.

In addition to being shorter at 195 days the tails of the cold group grew slowly over a long period. The growth of the control group was midway between the tails of the hot and cold groups.

Figure 3 shows the curves of growth rate of the tails in the 3 groups. After going into the hot room, the tails grew rapidly during the first week (1·4 mm/day) then the growth rate fell rapidly between the 4th and 5th week to below the rates of the other groups (0·3 mm/day). The growth rate of the tails of the control group fell steadily and was close to zero at 160 days. The cold group showed an immediate rapid fall in growth rate followed by a steady decline. After about 80 days the growth rate was very similar to that of the control group (less than 0·1 mm/day).

Figure 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 lengths of the hot group lies above the 100% line while that of the cold group lies consistently below it. Soon after exposing the mice to the hot environment there was a rapid increase in the tail length reaching a maximum of 115% in the first 4 weeks. Thereafter the ratio gradually diminished to a minimum of 106%. On the other hand exposing the mice to the cold environment was accompanied by a gradual decreasing ratio which reached a minimum of 91% during the first 4 weeks.

Effects of short exposure to different temperatures

Figure 5 and Table I shows the tail growth in the 4 experimental groups. Soon after housing the 25-day-old animals there was a 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 15·3 mm longer than the tails of the continuous cold group.

In Groups (iii) and (iv) there were also marked changes in the tail growth rates associated with the changes of mice from 1 environmental temperature to another (Table I). The environmental changes produced a rapid increase (in the hot) or decrease (in the cold) in the growth rate of the tails which were reversed by housing the mice at the different temperatures. By changing the environmental temperature and plotting the tail growth with time a series of graphical "parallelograms" were obtained between the tails of the hot and cold groups (Fig. 5).

Comparison of five temperatures

Figure 6 and Table II shows the tail growth of the 5 groups of mice exposed to 36°, 33°, 21°, 8° and 4°. 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

TABLE I.—*The effects of short exposure to different environmental temperatures on the growth rate of tails. The rates, which were greater in the hot (H) than the cold (C) environment, were reversed by changing the temperature*

Group	No. of mice	Growth rate (mm/day) Period (days) 4	10	6	
1 (H)	8	1·5	1·2	0·8	Mean 1·17
2 (C)	5	0·4	0·4	0·3	Mean 0·37
3 (H-C-H)	4	1·5	0·3	1·2	
4 (C-H-C)	4	0·4	1·3	0·2	

TABLE II.—*The effect of several environmental temperatures on the growth rate of tails*

		Growth rate (mm/d) Age (days)					
		20–25	25–35	25–38	38–42	42–50	50–62
		Days (of exposure)					
Group	No. of mice	5	10	3	4	8	12
36°	4			2·43	0·88	0·48	0·21
33°	3			1·33	0·85	0·46	0·23
21°	3	1·50		0·53	0·40	0·38	0·26
8°	4		0·24	0·23	0·19	0·17	0·13
4°	4			0·23	0·19	0·15	0·11

while the animals were in the control environment (21°) to 0·3 mm/day during the following 10 days in the cold room (8°). The latter rate was also changed by transferring the mice to 36°, 33°, 21° and 4°. The difference in the growth rate (1·1 mm/day) between the 2 hot groups (36° and 33°) was seen only in the first 3 days and later on the rates were very similar. Finally, by the age of 62 days, the tails of the 36° group were 1·8 mm longer than those of the 33° group.

No difference in the growth rate between the 8° and 4° groups was seen during the first 8–9 weeks, but later on a small difference was observed. Finally by the age of 62 days the tails of the 8° group were 0·5 mm longer than those of the 4° group.

The tail length of the mice maintained at 21° lay midway between the hot (36° and 33°) and the cold (8° and 4°) groups.

DISCUSSION

By trial and error it was found that 7-day-old mice survive satisfactorily when housed with their mothers at 8° and 33° but younger litters did not survive. The difference 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 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 in the cotton-wool nests. However, at about the age of weaning young mice depend on themselves for nutrition and leave the nest. This exposes them to the cold temperature, hence the reduction of the growth rate of their tails after the age of weaning. Therefore although about 3 cm of the tail growth remained to be completed after this age, the standard procedure was to wean the mice at 23 days of age and transfer them to the hot and cold room at 25 days of age. At this age the mice were found to survive temperatures as low as 4° and as high as 36°, but at 38° they failed to gain weight and died.

The results of this work confirms the influence of environmental temperature on the growth of tails (Sumner, 1909, 1913, 1915; Sundstroem, 1922; Przibram, 1931; Ogle, 1934; Harrison *et al.*, 1959; Harrison and Clegg 1969; Chevillard *et al.*, 1963; Barnett, 1965; Noel and Wright, 1970). Thus all adult mice reared at the higher temperatures had tails 1–2 cm longer than all litter-mates reared at the control environment and as much as 2–3 cm longer than all those reared at the lower temperatures. In this way the growth of tails confirmed to Allen's rule (1905).

The effect of environmental temperature on the growth of tails was particularly marked during the first 3–4 weeks of exposure to the environmental conditions especially at the higher temperatures. The final length of the tail (and tail bones) is determined by changes in the growth rate and the period of growth. Thus in the case of the mice in the hot environment the tails grew rapidly, attained greater final length, matured and stopped growing earlier than the tails of the control and

cold groups. On the other hand, exposure to the cold markedly slowed the growth rate of the tails but prolonged the growth period. This may account for the late cessation of growth of these tails. However, the tails of the cold groups were permanently shorter than those of the control and hot groups. The standardized percentage curves also illustrate the changes in the growth rate and growth period.

The strict dependence of growth rate on environmental temperature is shown by repeatedly changing the mice between the hot and cold conditions and thereby producing graphical "parallelograms". The changes appear to be immediate and shows that in the short term the growth rate in one condition is not influenced by the previous exposure.

The mouse tail contains between 27 and 30 caudal vertebrae and 54–60 epiphyseal cartilage plates (Fekete, 1941) of which only 23–26 bones (46–52 growth plates) lie outside the body and are affected by changes in the environmental temperature (Noel and Wright, 1970). For practical purposes increases in tail length depend on epiphyseal growth although a minute contribution may be made by growth of the intervertebral discs.

Different temperatures may influence the growth rate of tail bones in 2 ways: (i) Changes in the rate and number of cell divisions occurring in the germinative zone of the growth plate. (ii) Changes in the rate of differentiation, as well as the size of the differentiated cartilage cells of the plate. These factors may in turn be influenced by the direct effect of temperature on the metabolic activities of these cells.

The relationship of temperature to cell division is well known (Swann, 1957, 1958; Troshin, 1967). The duration of mitosis in the epidermal cells of the tails of rats was estimated at 476 h in acclimatized animals maintained at 6° and 6 h in those kept at 30° (Heroux, 1959, 1960). However, under the same environmental temperatures the mitotic activity of the duodenal mucosa was not affected because in the latter case the duodenal mucosa is kept under the control of deep body temperature. A cold environment of 4° was also found to reduce the mitotic activity of the epidermal cells in the ears of the mice and to delay radiation damage caused by 9000 rad to the tails (Shewell and Wright, 1967).

The size of the differentiated cartilage cells is known to determine normal endochondral bone growth as well as the length of long bones (Sissons, 1971; Kember, 1978) and it seems probable that the growth plates of the tail bones may, at the lower temperature, produce smaller differentiated cartilage cells than at the higher temperature. Again, this may be due to changes in the metabolic activities of these cells. 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 compared to those of the higher temperature.

The very striking initial increase in growth rate of tails of mice kept at 36° (2·43 mm/day) compared with those kept at 33° (1·33 mm/day) during the first 3 days of exposure can be explained as due to the effects on the differentiated cells already in the cartilage column because of the transitory nature of the phenomenon.

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