The Effects of Changes in the Environmental Temperatures on the Growth Potential of Bone

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ABSTRACT

The growth potential of tails (tail bones) was studied in a group of mice kept at 8°C and 31°C for more than 451 days. Thus the tails of the cold group grew slowly and retained their potential for a long time but when transferred at an earlier age to 33°C, the tails grew rapidly, gradually consumed their potential and ceased growth earlier than those of littermates transferred later in the experiment. The tail growth of mice kept permanently at 8°C was associated with a gradual reduction in the growth potential and finally its loss at the age of 310 days. The hot temperature led to the rapid production of cells at the germinative zone of the growth plate and these differentiated into large cartilage cells along the epiphyseal columns and then rapidly removed towards osteogenesis. On the other hand, the cold environment led to the slow production of cells and these differentiated into a small cartilage cells and then slowly removed.

The potential of outliner mice with short tails was also studied to verify whether the "stimulant" effect of the hot temperature could increase the growth rate of these tails so as to attain the length of the "standard" littermates kept at the same temperature. Like the above hot group, the tails of the runts born at 21°C and then transferred to 33°C, also grew faster and completed their potential earlier than those of the "standard" mice. But the rates of body growth were similar between the 2 groups. This indicates that the genetically programmed potential in the tails of the runts is less than that of the "standard" group. It also indicates that bone growth is controlled by local factors independent from those of controlling body growth. All mice born at 8°C and 33°C were outliners and although the tail lengths were equal at birth, the tails of the hot group grew faster and longer than those of the cold group, which retained their potential until the age of 240 days.

The rates of body growth of mice born at 33°C were similar to those of "standard" mice born at 21°C and then transferred to 33°C. But the rates for mice born at 8°C were below their control group. These changes were explained as changes in the nutrition of the pregnant mice and variability of the maternal environment.

We have previously shown that groups of mice born into a temperature of 21°C but later kept at higher or lower environmental temperature have growth patterns that differ from the control group kept at 21°C[1,2,3]. Thus those kept at 8°C are bigger, heavier, and have shorter tails; whereas those kept at 33°C are smaller, lighter, and have longer tails. Furthermore the tail bones of the 33°C group grew faster and longer than those of the 21°C or 8°C group. The bones of the latter groups continued to grow slowly and over a long period but failed to catch up with the growth of the 33°C group. We concluded that the 54-60 growth plates of the murine tail bones had a growth potential, defined as the capacity of the cells of the growth plate to divide and differentiate, which are affected by the environmental temperature during the period of growth, and that varying the environments could be used to investigate the growth potential. The aim of the present study was to investigate and assess the growth potential of the murine tail bone growth plates in groups of mice kept at 8°C and 33°C.

METHODS

Strain A albino mice of both sexes inbred (brother-sister mating) at King's College Hospital, London maintained at 3 experimental temperatures

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of 8°C, 21°C and 33°C were used. The details of these environments have been described previously[4]. The mice were weighed daily to the nearest 0.1 gm and the tail lengths were measured to within 0.5 mm by a graduated glass tube[5].

A. *Effects on tail growth potential of mice with standard tail length :* 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: *Group 1* (6 mice) were transferred to the hot room (33°C) at the age of 32 days. *Group 2* (2 mice) were transferred to the hot room at the age of 77 days. *Group 3* (one mouse) were transferred to the hot room at the age of 119 days. *Group 4* (one mouse) were transferred to the hot room at the age of 208 days. *Group 5* (2 female mice) remained in the cold room but at the age of 310 days one of these mice was taken to the hot room and returned to the cold room after one week. The second mouse was taken to the hot room at the age of 352 days and returned to the cold room after one week. In addition 2 male mice maintained separately in the cold room from the age of 25 days were also taken to the hot room at the age of 441 days and returned to the cold room after 10 days.

B. *Effect on tail growth potential of mice born in the environmental conditions:* (1) *Hot group.* Earlier in this work when the optimum experimental environment was being explored, 4 pregnant female mice were transferred at 15 days of gestation to 33°C. When these littered, the young mice were underweight and undersized and died shortly after birth, except for 2 which were followed for 132 days. These also died in an accident. (2) *Cold group.* When male and female mice were left together in the cold room at 8°C, one of the females became pregnant. Three of the litter of 5 died shortly after birth but 2 were followed until the age of 320 days. At the age of 172 days, one of the these mice was taken to the hot room (33°C).

To provide a comparative study of body weight for the above groups a control hot group (16 mice) and a control cold group (18 mice) were used. These were weaned at 23 days of age and housed in the cold room from 25 days of age onwards.

C. *Effects on tail growth potential of short tailed mice born in the temperate environment of 21°C:* During the randomisation procedure[1] undersized and underweight mice with short tails are always discarded because they superfluously inflate the statistical variation. On one occasion however, 2 of these runts (group 1) were followed and together with a control group (group 2) of 8 mice they were weaned at 23 days old and transferred to the hot room (33°C) at 25 days old.

RESULTS

A. *Effect on tail growth potential of mice with standard tail length, body weight and size:* Figure 1 and Table I show the tail growth of the 5 groups. In

Figure 1

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 "parallellograms" were produced.

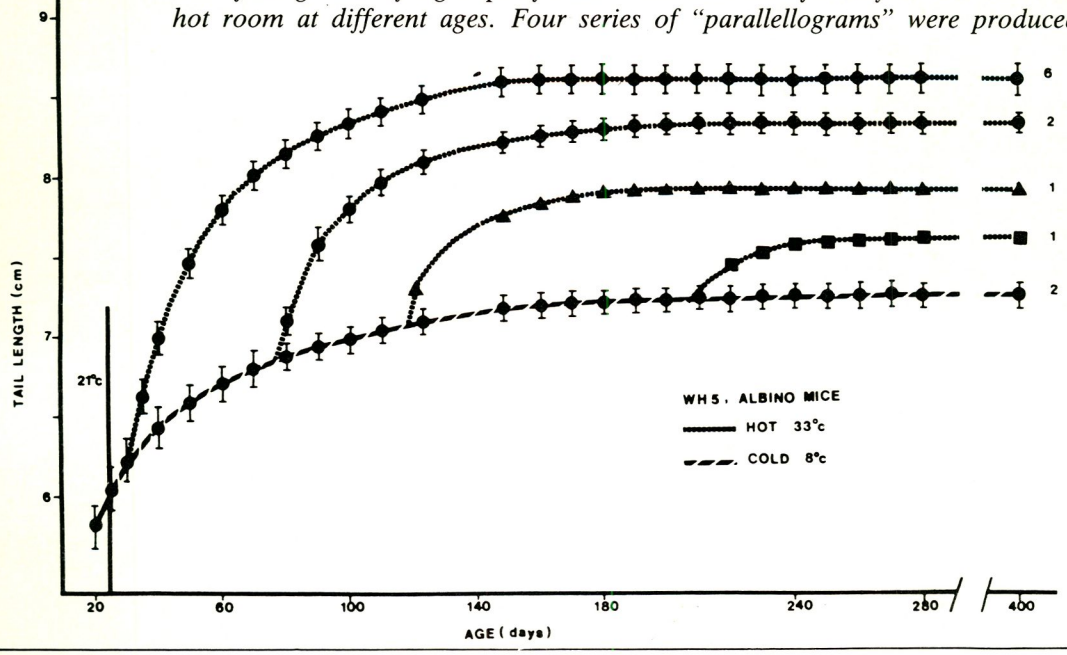


Table I

Group	No. of mice	Period (months) 1	2	3	4	5	6	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	6	0.570	0.147	0.100	0.047	0.000	0.000				
2	2	0.363	0.183	0.065	0.013	0.000	0.000	32	7	157	125
3	1	0.197	0.043	0.013	0.000	0.000	0.000	77	52	191	114
4	1	0.107	0.013	0.000	0.000	0.000	0.000	119	94	196	77
5	2	0.200	0.083	0.057	0.037	0.010	0.005	208	183	258	50

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) room. In group 5 the mice were kept permanently in the cold (8°C).

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 2 female mice of group 5 were followed for a long time in the cold room, and when one mouse was transferred at the age of 310 days (285 days in the cold room) and kept in the hot room for one week, no tail growth was detected. 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 female mice, but when 2 male mice were housed in the hot room at the age of 441 days (416 days in the cold room), no increase in tail growth was detected. The tails of these female and male mice were accordingly considered as the group permanently kept in the cold.

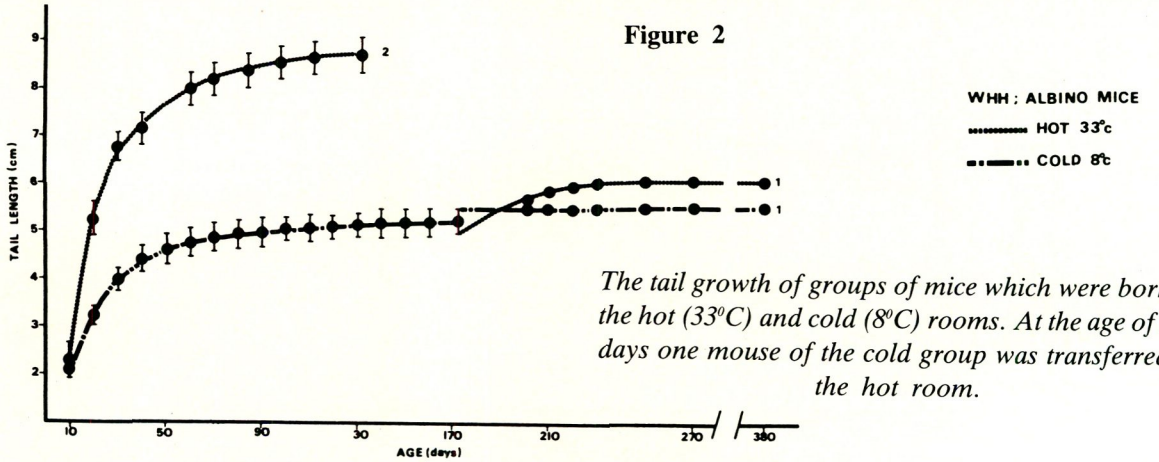
The tails of the hot groups grew rapidly and attained greater length but stopped growing earlier than those of the cold groups. The growth rate decreased gradually in all groups as the mice became older. The later the mice were transferred from the cold room to the hot room, the earlier was the cessation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

The mean tail lengths of the mice in the cold room were more or less equal at any age (maximum SD 0.24, minimum SD 0.08). 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 groups. They were also longer than the tails of the other groups which were transferred to the hot room later in the experiment. Thus the mean tail length of group 1 was 0.1 cm longer than that 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 tails lengths of the hot groups (group 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 did not catch up with the tail lengths of groups 1 and 2.

B. *Effect on tail growth potential of mice born in the experimental environmental temperature:* Figure 2 and Table II show the mean body weight and tail

Figure 2



The tail growth of groups of mice 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.

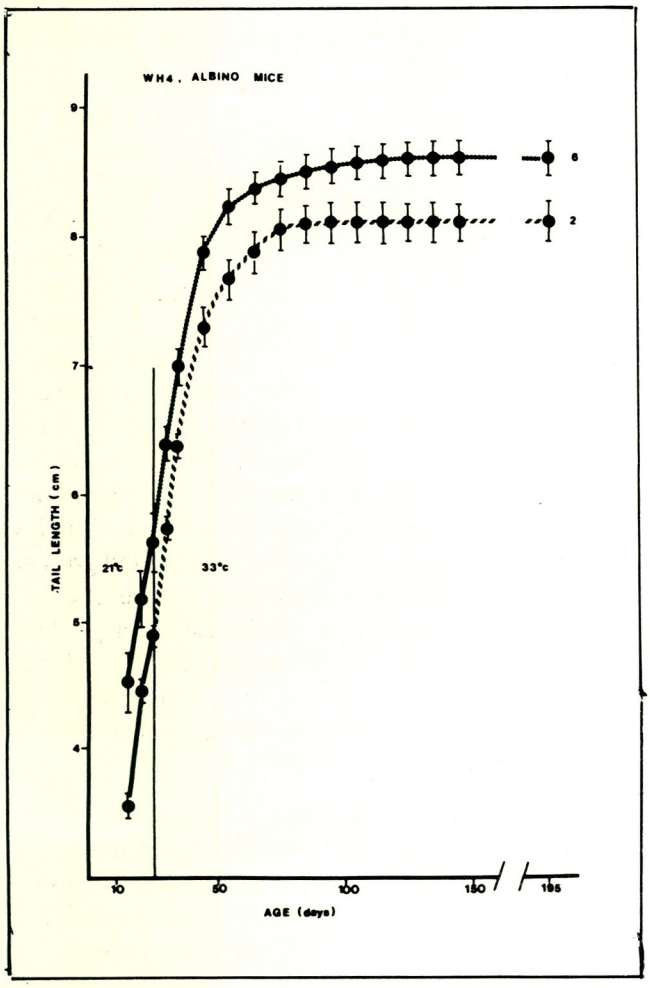
Table II

Group	HOT (33°C)				COLD (8°C)			
	Body Weight (gm)		Tail Length (cm)		Body Weight (gm)		Tail Length (cm)	
No. of mice	2				2			
Age (days)	10	132	10	132	10	172	10	172
Range	2.2 - 2.7	14.4 - 19.4	2.2 - 2.4	8.25 - 9.05	2.3 - 2.4	12.2 - 13.7	1.92 - 2.22	4.9 - 5.45
Mean	2.45	16.9	2.3	8.65	2.35	12.95	2.06	5.18
S.D.	0.35	3.54	0.14	0.57	0.07	1.06	0.23	0.39
Growth rate:								
First 4 weeks	1.65 (1.73) gm/wk		0.171 cm/d		1.26 (1.80) gm/wk		0.081 cm/d	
Later weeks	0.54 (0.2) gm/wk		0.017 cm/d		0.29 (0.85) gm/wk		0.006 cm/d	

Body weights (gm) and tail lengths (cm) of 2 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 group and control cold groups are also shown.

Figure 3

The tail growth of 2 mice (group 1) with short tails and 8 control mice (group 2). Both groups were born at the animal house temperature of 21°C and transferred to the hot room (33°C) at the age of 25 days.



length of mice born in 33°C (hot group) and 8°C (cold group). In the hot room the growth in body weight was consistent with that of control animals maintained at the same temperature, and in the cold room the growth rates in body weight were below those of their control group.

Although the mean tail length of the hot and cold 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, 3.51 cm longer than the tails of the cold group. The maximum growth of 1.2 mm/day was seen in the first 4 weeks and thereafter the rate gradually slowed to about 0.13 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 0.7 mm/day was also seen in the first 4 weeks and thereafter the rate was maintained at about 0.06 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 172 days the tail increased in length by about 1.1 cm and stopped growing after 68 days in the hot room.

C. *Effect on tail growth potential of outliner mice born in 21°C and transferred later in life to 33°C:* Table III shows the body weights and Figure 3 shows the curves of tail growth of groups 1 and 2. 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.

Table III

	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

Body weight of 2 mice with short tails (group 1) and 8 control mice (group 2). Both groups were kept at 33°C from the age of 25 days.

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 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).

DISCUSSION

The growth in length of long bones, as exemplified here by the tail bones, consists of 3 inter-dependent processes[6,7]; (a) The division of cartilage cells in the germinative zone of the growth plate, (b) the palisade differentiation of the cartilage cells in the epiphyseal column, and (c) the removal of the cartilage cells and calcification of the matrix. Thus, one may postulate 3 possible groups of factors which may control the growth of long bones.

The first group of factors concerns the genetically programmed ability of the germinative cartilage cells for a definite potential of growth [3,6,8,9,10,11] . This may simply mean the total number of cell divisions occurring in the germinative zone during the growth period. It also means that the basic shape and final length of bone is inherited in the bone itself and thus controlled by "local control factors". Therefore, 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 preordained number of cell divisions and differentiation are completed more quickly. This early completion of the cellular programme would be responsible for the early cessation of tail growth in the hot groups while those of the cold group retain their potential for a long time. The final length of bone therefore, represents changes in the growth period of the cellular programme[1]. The hot environment leads to the achievement of the full capacity of the growth plate in a very short time.

The concept of growth potential may also explain the failure of the tails of the lower temperature to catch up with those of the higher temperature. Likewise, it can also account for the failure of the tails of the "parallelograms" to catch up with the tail lengths of one another[1]. It appears that the prolonged maintenance of 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 was used and this resulted in some increase in tail length which remain 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 became older. Thus one would normally expect a gradual reduction of the growth potential with age, and this may be related to the degree of vascularity of the growth plate and the impending fusion of the metaphyses to the epiphysis[12].

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 and closure of the epiphysis[2]. Thus when mice 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 is the influence on the linear dimensions of differentiated cartilage cells in the epiphyseal column[3,7,9]. This factor may be termed the "epigenetic control factor". 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 these hypertrophic differentiated cartilage cells just before these cells are removed. The linear dimensions may be determined or influenced by metabolic[1,3] and hormonal factors but the number of hypertrophic cells actually produced may be determined genetically. Of course, if the number of cells in the germinative zone of the epiphyseal column remain constant and the number of generations of any cell line also remain constant then the number of the cartilage cells produced will depend simply on the number of cell divisions carried out by the "stem cells".

The environmental temperature may not only stimulate cell divisions at the germinative cartilage zone of the growth plate but also the size of the differentiated cartilage cells[1]. Accordingly, the growth plate of the tail bones produce, at the lower temperature, smaller differentiated cartilage cells along the epiphyseal column than at the higher temperature. It follows that the slow production of cartilage cells at the germinative zone which differentiate into small cartilage cells may account for the shorter tail bones in the lower temperature groups than those of the higher temperature groups[3].

The third group of factors which may determine the final length of long bones is related to the rate of removal of cartilage cells, the calcification of the matrix and its replacement by bone. These processes are largely determined by the degree of vascularity of the growth plate[6] which is increased in the hot and decreased in the cold environment[13,14]. For example, in the hot environment 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 calcified matrix and bone formation. However, the degree of vascularity of the growth plate indicates the metabolic activity of this structure[1]. Therefore, the "vascular factors" could not be regarded as "primary growth factors" directly related to changes in the environmental temperature, but as a "secondary growth factors" related to the metabolic activity of the growth plate.

The response of undersized and underweight mice with short tails to changes in the environmental temperature present 3 problems; the body size, the body weight and tail growth. Regrettably, the animals maintained in the hot room died in an accident and an analytical study of body size similar to that of Dixon[1,5] on outliners was not possible.

The effect of undernourishment and malnourishment on the growth of mammalian body is well known and it seems 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[12,16,17]. This has resulted in permanently undersized and underweight animals. The failure of these animals to gain weight was examined by Widdowson and McCance[18,19] who concluded that, the earlier in the life of the animals the undernourishment was imposed, the more serious and permanent the effect would be. In addition, the brain (possibly the hypothalamus) probably controls the growth of mammalian body[19,20]. 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 the 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[21] and variability in the maternal temperature[22]. The genetic influence is also equally important[12,16,22].

The results in the runts confirm the view that the hot environment is a favorable condition for the small and light animals to show a positive peak in tail growth after the change of environmental temperature[12,16]. Thus the tails of these animals reached a considerable length in a relatively short time. This form of "favourable growth potential" towards the accomplishment of tail length greater than the adult length in underweight animals is mainly related to the earlier onset of the "growth stimulus" i.e. the hot environment. The tails of these animals also stopped growing earlier than those of the control group maintained at the same temperature. In this way the hot environment brought the sum of the definite number of programmed cell 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 is also 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 length. 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 (8°C) since the age of 25 days.

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 animals maintained in the cold room (8°C) from the age of 25 days[1]. 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 is 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.

CONCLUSION

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

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