1978

The effect of developmental temperature on morphology, energy metabolism, growth hormone and thyroid stimulating hormone in Long-Evans rats

Dana Elizabeth Quinn

Portland State University

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AN ABSTRACT OF THE THESIS OF Dana Elizabeth Quinn for the Master of Science in Biology presented November 22, 1978.

Title: The Effect of Developmental Temperature On Morphology, Energy Metabolism, Growth Hormone and Thyroid Stimulating Hormone in Long-Evans Rats.

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

John Wirtz, Chairman

Leonard Simpson

Stan Hillman

Long-Evans rats were raised from birth to eight weeks of age at 5°C, 20°C and 30°C. Blood was taken from animals two to eight weeks of age and assayed for growth hormone and thyroid stimulating hormone. The 30°C reared rats were found to have the longest ear, tail and hind limbs, followed by the 20°C reared rats. The 5°C rats were found to have the shortest ear, tail and hind limb. The 30°C and 5°C reared rats were found to have similar masses at the termination of the experiment. The 20°C reared rats had the smallest mass. Differences in size between the three groups when compared on a weekly basis were not
found to be related to weekly obtained serum levels of growth hormone and thyroid stimulating hormone. Food consumption was greatest for the 5°C reared rats followed by the 20°C reared rats. The 5°C reared rats were found to have the lowest routine metabolic rate in the 5°C chamber. The 20°C reared rats were found to have the lowest routine metabolic rate in both the 20°C and 30°C chamber.

When 30°C and 20°C reared rats were put in the 5°C chamber with the 5°C reared rats, they initially lost mass, however after three weeks in the 5°C chamber they began to gain mass. The 30°C reared rats gained mass at a greater rate than did the 20°C reared rats. The 5°C reared rats continued to gain mass throughout this period. While in the 5°C chamber the total food consumption of the 20°C reared rats was the greatest, whereas the 5°C and 30°C reared rats consumed similar amounts of food.
THE EFFECT OF DEVELOPMENTAL TEMPERATURE ON MORPHOLOGY, ENERGY
METABOLISM, GROWTH HORMONE AND THYROID
STIMULATING HORMONE IN
LONG-EVANS RATS

by

DANA ELIZABETH QUINN

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE
in
BIOLOGY

Portland State University
1978
TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the Committee approve the thesis of Dana Elizabeth Quinn presented November 22, 1978.

John Wirtz, Chairman

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CHAPTER I

INTRODUCTION

Morphologic measurements in endotherms have been shown to be related to temperature, (1, 4, 5, 15, 34, 38, 39, 63, 69, 71, 86). Bergmann's rule states that a species of endotherm increases in size as the ambient temperature decreases. Allen's rule states that appendage size (ears, tail and limbs) decreases as ambient temperature decreases. Both of these rules indicate that endotherms will show minimized surface area in a cold environment and maximized surface area in a hot environment. Both rules are broad generalizations with many other factors involved in determining morphology and thermoregulatory capabilities of endotherms. These rules have been applied to the heat dissipatory and conservational mechanisms of the animal.

Growth hormone and thyroid hormone are of major importance with respect to mammalian growth, (10, 44, 53, 54, 56, 57, 75, 76, 77). In addition to growth, thyroid hormone had been found to be important in the thermoregulatory capabilities of mammals, (20, 25, 26, 51, 70). This has not been shown for growth hormone, though both hormones have been found to be greatly effected by ambient temperature, (20, 25, 26, 27, 51, 64, 66, 80).

The purpose of this study was an attempt to determine whether ambient temperatures govern levels of growth hormone and thyroid hormone which then govern resultant morphology. In addition, the effect of the resultant morphology on thermoregulatory capabilities of rats was studied.
CHAPTER II

REVIEW OF THE LITERATURE

Thermoregulation in rats is a composite of morphologic, physiological and behavioral factors. Important thermoregulatory behavior includes nest building, huddling and selection of appropriate thermal microenvironments, (73, 79). The temperature selected is affected by the ambient temperature to which they have been previously acclimated, (79).

Physiologic thermoregulation at low ambient temperatures may be accomplished in rodents through elevation of basal metabolic rate, (31, 50, 73, 80), however other researchers are not in agreement, (12, 36, 87). At high ambient temperatures oxygen consumption may be depressed in an effort to thermoregulate, (12, 31, 42, 50, 87), again there is disagreement. Vascular phenomena associated with rats born and raised at different ambient temperatures also aids in the thermoregulatory capabilities of the animal, (17, 29, 37, 42, 43, 68, 86). Mammals born and raised in a 5°C environment appear to demonstrate more vasoconstriction than mammals born and raised in a 30°C environment, (17). The peripheral tissues of the hot acclimated rodents are better supplied with blood hence greater growth is a possible result. The stunting of growth in a cold environment is more severe in the distal segments of the appendages. This also suggest a vascular phenomenon as the blood vessels are smaller in diameter toward the distal ends of the tail and limbs and therefore are more severely affected by lower environmental temperature, (17).
Morphologic adaptations to temperature are generally concerned with body and appendage size as well as insulation. It is argued, however, whether body and appendage size are the result of temperature or adaptations to temperature. Bergmann in 1847 stated cold temperatures tend to delay sexual maturity thereby producing a longer growth period before reaching physical maturity. Allen said that in cold climates natural selection tends to favor those that have the advantage in surface area/volume ratio for the conservation of heat,(87). Metabolic rate can not be related to mass or a function of surface area alone but it is an important factor as the rate of heat transfer is proportional to surface area. The intensity of oxygen and nutrient flow is a function of the sum of the internal surface which is proportional to body surface also,(29). Other factors of importance influencing basal metabolic rate are central nervous system control, tissue capillary density and genetic fixing,(29).

There are two very different effects of temperature on growth, as determined in the laboratory. When mice grown at 31-33°C are compared to mice raised at a cold temperature (10°C) the mice reared in the lower temperature were heavier and longer than those reared in the higher temperature,(1,33,63). In experiments using different orders of mammals or different species of mice and rats, the cold reared animals,(3-7°C), were lighter and smaller than animals kept at 21-30°C,(5,17,38,42,54,83). It must be kept in mind however that these experimental procedures varied widely, for example some had a restricted food intake and some did not. The age of the animals subjected to the hot or cold environment and the age of the animal at the termination of the experiment also varied as did the length of time the animal remained in the rather severe environ-
ment. All reports do agree, however, on the increased tail and appendage length in the hot environment, decreased length in the cold environment and intermediate length of the thermoneutral controls, (1,5,17,33,38,63,83).

Generally speaking, a structure once formed usually has a biological significance which is independent of the way it was formed. Environmentally induced physiological changes are typically rapid reversible morphological changes which are strictly a function of growth and are irreversible once the structure has stopped growing, (30). The problem with body size is that it is most difficult to distinguish between its biological function and the magnitude of other functions which determine it. For example if it were that genetically small animals were better adapted to the heat than larger ones, one would not necessarily conclude that reduced growth at a high temperature is an adaptive response, (30).

Generally tails of animals in cold environments are shorter than those of animals living in warmer temperatures. This is believed due to its role in the regulation of body temperature of the animal. A short tail with its smaller surface area may allow for heat conservation and a long tail with its relatively larger surface area may allow for heat dissipation, (1,2,5,15,17,25,30,33,43,50,63,68,83). Of course the circulatory changes previously mentioned will greatly influence this, i.e. a more vasoconstricted tail will promote heat conservation, whereas a more vasodilated tail will promote heat dissipation. Another factor not to be overlooked is the greater cutaneous circulation that accompanies a larger tail with its larger surface area.

Should the tail of the mouse or rat be amputated five weeks before exposure to the lethally high temperature, the survival time will be
much lower than for rats and mice raised under the same conditions with their tails, (30, 33, 34).

The length of the distal caudal vertebrae are more affected by temperature than that of the proximal caudal vertebrae which suggests a direct effect of temperature on tail growth. This is true of most murid and cricetid rodents that have been studied, (1, 15, 30, 33). Pigs show essentially the same results as the rodents, (86). The temperature induced differences in the tail are generally due to a change in the lengths of the individual vertebrae being most extreme in the distal segments. In some cases however the cold exposed animals had an actual decrease in the number of vertebrae, (5, 30, 33, 34).

The temperature of the tails of the rats studied were found to be nearly equal to the temperature of the environment i.e. poikilothermic, except when the animal is overheated or sitting on its tail, which they often do in a cold environment, (68). In rats not acclimatized to a hot environment vasodilation did occur, elevating their tail temperature. Rats not reared in a cold environment had a problem keeping their long tail warm and necrosis was often noted, (68).

In the fluctuating environment the time spent at the high temperature appears to be more influential in determining tail length than time spent at the low temperature, however the low temperature used was only 16°C, (33).

If mice spend their pregnancy in a cold environment, (10°C), and at weaning time half of their offspring are put in a hot environment, (33°C), the offspring will grow long tails and the cold reared offspring will develop short tails. The general body length and weight of the mice put in the hot environment was retarded. A similar effect occurs when
mice are born and reared in a hot environment and half of the offspring are put in the cold environment i.e. the mice in the hot environment will develop long tails and the mice in the cold environment will develop short tails. Therefore the length of the tail appears to be a factor for increasing or decreasing the body surface area to facilitate heat loss or heat conservation.

Temperature studies on young pigs revealed that the lengths of the nasoocciput portion of the skull, lengths of the femur, tibia, first metatarsal, humerus, radius, ulna and metacarpal were invariably less in the cold exposed pigs, the heat reared were the longest and the controls intermediate, (86). The reason for this increase in length and not so much width may be due to the proliferation of the chondrocytes in the epiphyseal plate being more affected than the proliferation of the osteoblasts under the periostium. Since cartilage itself has no blood vessels and relies on nutrients that diffuse through the matrix from the outside, such a general decrease in blood flow in response to the cold will greatly reduce this supply and hence the proliferative activity of the chondrocytes. The more highly vascularized deeper periosteum would be less affected by a change of vasomotor tone in a cold environment, (15,52).

In male laboratory albino rats the growth curves of the cold rats gradually diverged from those of the controls in the first few weeks. During the later weeks of the experiment the two curves ascended more or less in parallel. In the control rats the rates of gain in body length and tail length were greatest at the start and then followed a straight line decline during the first few weeks of the experiment, (15).
The growth rate of the body length in the cold rats averaged about $\frac{3}{4}$ the amount of the controls during the first week in the cold and this rate was maintained for three weeks until its curve met the declining growth curve of the controls and then the two temperature groups grew at the same rate until the termination of the experiment. From this it appears that growth retardation by the cold is the most severe during the first few weeks of exposure to the cold, (the rats were already weaned upon exposure to the cold). The period of growth retardation by the cold ends at about six weeks of age in mice, (2). Susceptibility of a mouse's growth to cold begins before weaning, (69).

Generally speaking there appears to be three growth cycles in endotherms; first there is a rapid growth rate which tapers off up to maturity and this is followed by a much slower increase and actually decreases during senility, (87).

Several investigators have suggested that growth of rats and mice should be greatest under optimum conditions, that is, the most favorable temperature ranges and it should be retarded under less favorable conditions such as too hot or too cold, (15). This has not been the case, however. The differences in growth are partly related to the metabolic effort that the young mouse or rat must make to compensate for the heat loss from its relatively large surface area when subjected to the cold. (however if the animal were born and raised in the cold perhaps the surface area would be comparatively smaller).

Other factors affecting growth rates include seasonal effects, (17), number of rats per cage, (17, 36), and genetic factors, (33). Weight gain and photoperiod appear not to be related, (17, 36).

Thyroid hormones play a physiologically important role in the
regulation of metabolic rate. Elevated thyroidal function results in increased oxygen consumption and depressed thyroidal function results in decreased oxygen consumption.

Thyroid hormones are also important in the development of the nervous system, reflex responses, mental activities and reproductive system functions. Not all tissues exhibiting strong responses to thyroid hormones show associated increases in oxygen uptake.

When rats are exposed to the cold there is an increase in the basal metabolic rate which is due in part to augmented activity of the thyroid gland,(18,19,26,39,48,51,70,80). The maintenance of the higher metabolic rate also requires adrenal gland secretions,(13,18,45). The elevation of basal metabolic rate in cold temperatures can be abolished by thyroidectomy,(80). The accelerated thyroid hormone release is temporary reaching its maximum 26 days after exposure to the cold and being virtually absent 40 days after exposure to the cold (0-2°C), (5).

Thyroidal cold responses involve the pituitary stalk. Without the pituitary stalk there is only enough thyroid stimulating hormone produced to keep the thyroid activities normal under normal conditions, however, it will not exhibit the cold response, i.e. elevated thyroidal function. The hypothalamus receives its thermal information from the central nervous system and increases the thyroid stimulating hormone through impulses transmitted through the stalk. The basic secretory rhythm must be independent of the pituitary stalk under normal conditions and under humoral influences. The hypothalamus and pituitary stalk must participate in regulation of the anterior pituitary secretions in specific adjustments to certain environmental situations, (5,14,19,20,21,48).
Experiments have shown that thyroid hormone production is counterbalanced by peripheral degradation of extrathyroidal hormone which increases as ambient temperature decreases and vice versa, (7, 8, 9, 16, 24, 25, 26, 70). Thus the thyroid gland behaves as though its secretion were essential to the internal environment through a compensatory homeostatic increase in the production of thyroid stimulating hormone when thyroid utilization increases, (16, 70). Since the pituitary production of thyroid stimulating hormone stimulates the thyroid's production and secretion of thyroid hormone and since thyroid stimulating hormone is not maintained at nearly normal levels, it should be a more accurate measure of thyroid activity than a measure of thyroid hormone would be.

The initial elevation of plasma levels of thyroid hormone in response to lowered temperature may be in part due to a change in the distribution of the hormone as well as increased secretion, (8, 24). Thyroid hormone may be released from sites in the liver for example. The rate of thyroid loss to the feces increases after exposure to the cold which may account for the elevated secretion of thyroid hormone. However, this is also in part dependent on elevated food consumption and increased passage of material along the intestine. The extent of which the rapid passage might impair thyroid hormone reabsorption is not clear, (7, 8, 11, 24, 25, 39).

Growth hormone levels are affected by plasma glucose levels, stress, bacterial pyrogens, neurotransmitters, hypothalmic stimulatory and inhibitory hormones and short feedback control. Growth hormone also appears to be affected by temperature, however little work has been done with rats in this area. Ultimate body size which is greatly affected by growth hormone may play a role in thermoregulation, for example a larger surface
area to mass ratio may allow for increased heat dissipation. If temperature does indeed have an effect on growth hormone, it should be apparent in ultimate body size.

In rats exposed to cold temperatures there is a marked depletion of pituitary growth hormone, within one hour of exposure, (25). These depletions are interpreted to mean that a release of the hormone has occurred resulting in elevated plasma growth hormone. This depletion returns to normal or slightly elevated values within two hours after the acute depletion, (25). This does not appear to be due to stress as in the rat a stressful situation will cause a decrease in the plasma growth hormone levels unlike most other mammals, (24).

Growth hormone levels do not increase in cold exposed humans, but upon rewarming they greatly increase, (13,18,19,20). Shivering may be related to human growth hormone release but data is lacking, (19).

In heat exposed humans growth hormone levels will gradually increase, (13). A sharp increase will occur if exercise is performed. Since there are not significant increases in people given prior administration of glucose, it may be that the increased human growth hormone response is induced by diminished energy substrate, (20). Obese people invariably had a smaller human growth hormone response, (20). It should be concluded that exercise is an important variable and that although the size of the animal is affected by growth hormone, growth hormone apparently is also affected by the size of the animal.

Both thyroid hormone and growth hormone act on a large number of cell types. Thyroid hormones synergize with growth hormone in supporting
skeletal growth, although thyroid hormone unlike growth hormone pro-
motes bone maturation,(9,10,23,54,58,76,77). Thyroid hormones also act
on the hypothalamus and pituitary gland to increase growth hormone sec-
retion. Reproductive and nervous system maturation are other important
functions of thyroid hormones in animal development. Both growth and
thyroid hormones play an important role in the overall weight of the
animal,(58,76).

Thyroid hormone and growth hormone administered to both normal and
hypophysectomized animals causes a greater increase in weight than either
of the two alone. Growth hormone exhibits the plateauing effect in that
the initial dosage becomes ineffective after approximately 100 days for
Long-Evans rats,(21,55,57). Thyroid hormone, unlike growth hormone,
does not exhibit the plateauing effect,(57).

When a normal Long-Evans rat is given growth hormone injections,
all organs appear to increase in mass to the proportion as skeletal
mass,(53). The fibers of the skeletal and cardiac musculature were
hypertrophied as was the connective tissue fiber,(55).
CHAPTER III

MATERIALS AND METHODS

Three sets of four male rats of the Long-Evans strain were used. One set was put into a 5°C environmental chamber and the second set was put into a 30°C environmental chamber. The third set (the controls) were kept at room temperature (20°C) in the animal room. The mothers of these rats gave birth not less than 30 days after they had been put into the environmental chambers, indicating mating and pregnancy occurred in the environmental chambers. Photoperiod was set at 14 hours to coincide with that of the animal room. Bedding in the form of newspaper strips was supplied for the purpose of nest formation. An excess of food and water were supplied. The offspring (approximately 10 per female) were not in any way handled until weaning.

At weaning all males were taken from each of the three groups. These males were put into individual wire bottom cages without bedding to prevent nest building and huddling. Four of the males from each group were weighed once weekly and amounts of Purina Lab Chow consumed was recorded. Body, tail, hind foot and ear lengths were also recorded on these four males. Blood was obtained once weekly from the four males via tail vein puncture. The blood was then centrifuged in test tubes coated with EDTA. The serum was collected and stored at -40°C, to be assayed at a later time for growth hormone and thyroid stimulating hormone.
After weaning the mothers were given a seven day rest period, after which the males (same as initially used) were reintroduced into the cage. After the second parturition of the three mothers, four male offspring from each of the three groups were killed in order to obtain a two week sample of blood. Weights and measurements of the body length, tail, hind limb and ear were also obtained. This procedure was repeated with offspring taken at the three week period.

Total length was taken as the length from the tip of the snout to the tip of the tail. Body length was considered as the total length minus the tail length. The tail length was considered as the base of the tail (end of the well haired portion of the body) to the tip of the tail. Lengths of the hind limbs began at the heel and ran to the tip of the longest claw. The ear was measured from notch to the apex.

At the termination of the experiment routine metabolic rates were taken on the four males from each of the three groups. The routine metabolic rates were studied at 5°C, 20°C and 30°C for all three groups in random order. The rats resided in gallon jars used for the routine metabolic rate measurements 24 hours prior to measurement. Samples of air from inside the jar were taken at the beginning and end of a five minute period and run through the Beckman Oxygen Analyzer model OM-14. This was done five times for each rat. The highest and lowest samples were omitted from the calculations.

Wet weights of the liver, heart and kidneys were recorded at nine weeks of age.

The serum was assayed via radioimmunoassay for thyroid stimulating hormone and growth hormone.

The remaining male rats from each of the environmental chambers
were all put in the cold (5°C) chamber. At this point the cold reared rats were nine weeks of age, the heat reared were 10 weeks of age and the control reared were 11 weeks of age. Amounts of food consumed were recorded. The mass of the rats were recorded at three, five and six weeks after exposure to the cold.
### TABLE I

SUMMARY OF MORPHOLOGIC FIGURES FOR RATS RAISED AT 5°C, 20°C AND 30°C EXPRESSED IN STANDARD DIVIATION

<table>
<thead>
<tr>
<th>week</th>
<th>set</th>
<th>weight (g)</th>
<th>hind foot (cm)</th>
<th>ear (cm)</th>
<th>tail (cm)</th>
<th>body (cm)</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>5 C</td>
<td>20.0±.70</td>
<td>1.9±.07</td>
<td>.8±.07</td>
<td>4.5±.11</td>
<td>8.2±.22</td>
</tr>
<tr>
<td></td>
<td>20 C</td>
<td>20.0±.70</td>
<td>1.9±.08</td>
<td>1.0±.02</td>
<td>4.8±.18</td>
<td>8.1±.35</td>
</tr>
<tr>
<td></td>
<td>30 C</td>
<td>20.7±.90</td>
<td>1.9±.05</td>
<td>.7±.07</td>
<td>4.7±.26</td>
<td>8.6±.30</td>
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<tr>
<td>3</td>
<td>5 C</td>
<td>22.4±1.21</td>
<td>2.4±.04</td>
<td>1.4±.04</td>
<td>6.1±.15</td>
<td>8.8±.44</td>
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<tr>
<td></td>
<td>20 C</td>
<td>26.8±3.74</td>
<td>2.3±.07</td>
<td>1.3±.09</td>
<td>6.1±.38</td>
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<td>30 C</td>
<td>31.3±1.00</td>
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<td>1.6±.04</td>
<td>8.3±.19</td>
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<td>7.3±.43</td>
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<td>7.8±.50</td>
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<td>1.7±.05</td>
<td>8.2±.60</td>
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<td>20 C</td>
<td>102.1±7.87</td>
<td>3.3±.18</td>
<td>1.7±.12</td>
<td>9.7±.60</td>
<td>14.2±.43</td>
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<td></td>
<td>30 C</td>
<td>131.6±9.08</td>
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<td>1.9±.10</td>
<td>14.9±.49</td>
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<td>1.7±.05</td>
<td>9.5±.86</td>
<td>15.8±1.06</td>
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<td>20 C</td>
<td>128.9±15.20</td>
<td>3.3±.11</td>
<td>1.8±.05</td>
<td>10.7±.21</td>
<td>15.3±1.63</td>
</tr>
<tr>
<td></td>
<td>30 C</td>
<td>171.6±11.60</td>
<td>4.0±.15</td>
<td>2.1±.12</td>
<td>16.3±.56</td>
<td>17.7±1.01</td>
</tr>
<tr>
<td>8</td>
<td>5 C</td>
<td>190.1±14.02</td>
<td>3.5±.04</td>
<td>1.7±.04</td>
<td>9.6±.92</td>
<td>17.0±1.04</td>
</tr>
<tr>
<td></td>
<td>20 C</td>
<td>159.7±26.08</td>
<td>3.6±.99</td>
<td>1.8±.00</td>
<td>12.7±.68</td>
<td>17.0±1.05</td>
</tr>
<tr>
<td></td>
<td>30 C</td>
<td>211.9±3.97</td>
<td>4.3±.08</td>
<td>2.2±.08</td>
<td>19.6±1.18</td>
<td>19.6±1.00</td>
</tr>
</tbody>
</table>
TABLE II

SUMMARY OF THE SIGNIFICANCE OF F IN THE MORPHOLOGICAL MEASUREMENTS FROM TABLE I

Control VS Cold

<table>
<thead>
<tr>
<th>week</th>
<th>mass</th>
<th>hind foot</th>
<th>ear</th>
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</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.000</td>
<td>0.705</td>
<td>0.011</td>
<td>0.154</td>
<td>0.630</td>
</tr>
<tr>
<td>3</td>
<td>0.014</td>
<td>0.168</td>
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<td>0.291</td>
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<tr>
<td>4</td>
<td>0.000</td>
<td>0.020</td>
<td>0.134</td>
<td>0.886</td>
<td>0.043</td>
</tr>
<tr>
<td>5</td>
<td>0.001</td>
<td>0.024</td>
<td>0.094</td>
<td>0.267</td>
<td>0.005</td>
</tr>
<tr>
<td>6</td>
<td>0.137</td>
<td>0.670</td>
<td>0.537</td>
<td>0.016</td>
<td>0.011</td>
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<td>7</td>
<td>0.104</td>
<td>0.488</td>
<td>0.050</td>
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<td>0.642</td>
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<tr>
<td>8</td>
<td>0.125</td>
<td>0.746</td>
<td>0.002</td>
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<td>0.995</td>
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</tbody>
</table>

Control VS Hot

<table>
<thead>
<tr>
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<th>hind foot</th>
<th>ear</th>
<th>tail</th>
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</tr>
</thead>
<tbody>
<tr>
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<td>0.670</td>
<td>0.001</td>
<td>0.895</td>
<td>0.102</td>
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<tr>
<td>3</td>
<td>0.010</td>
<td>0.000</td>
<td>0.001</td>
<td>0.000</td>
<td>0.007</td>
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<tr>
<td>4</td>
<td>0.008</td>
<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
<td>0.253</td>
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<tr>
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<td>0.001</td>
<td>0.000</td>
<td>0.705</td>
<td>0.000</td>
<td>0.025</td>
</tr>
<tr>
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<td>0.005</td>
<td>0.001</td>
<td>0.871</td>
<td>0.000</td>
<td>0.029</td>
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<td>0.001</td>
<td>0.004</td>
<td>0.000</td>
<td>0.048</td>
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<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
<td>0.005</td>
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</tbody>
</table>

Hot VS Cold

<table>
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<tr>
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<th>mass</th>
<th>hind foot</th>
<th>ear</th>
<th>tail</th>
<th>body</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.056</td>
<td>1.34</td>
<td>0.323</td>
<td>0.114</td>
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<td>0.005</td>
<td>0.000</td>
<td>0.126</td>
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<tr>
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<td>0.039</td>
<td>0.000</td>
<td>0.008</td>
<td>0.000</td>
<td>0.193</td>
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<td>0.001</td>
<td>0.001</td>
<td>0.000</td>
<td>0.033</td>
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<tr>
<td>8</td>
<td>0.048</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.005</td>
</tr>
</tbody>
</table>
TABLE III

SUMMARY OF ROUTINE METABOLIC RATES (10²/g·h) AT 5°C, 20°C AND 30°C
OF RATS RAISED AT 5°C, 20°C AND 30°C EXPRESSED IN MEAN ± STANDARD DIVIATION

<table>
<thead>
<tr>
<th>Conditions Under Which Metabolic Rates Were Taken</th>
<th>5°C</th>
<th>20°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5°C</td>
<td>.00249±.00003</td>
<td>.00210±.00009</td>
<td>.00173±.00008</td>
</tr>
<tr>
<td>20°C</td>
<td>.00289±.000045</td>
<td>.00156±.00022</td>
<td>.00142±.00016</td>
</tr>
<tr>
<td>30°C</td>
<td>.00335±.00017</td>
<td>.00276±.00019</td>
<td>.00206±.00005</td>
</tr>
</tbody>
</table>

TABLE IV

SUMMARY OF THE SIGNIFICANCE OF F IN THE ROUTINE METABOLIC RATES
(10²/g·h) OBTAINED AT 5°C, 20°C AND 30°C
USING RATS RAISED AT 5°C, 20°C AND 30°C

<table>
<thead>
<tr>
<th>Conditions Under Which Metabolic Rates Were Taken</th>
<th>5°C</th>
<th>20°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5°C VS 20°C reared</td>
<td>.331</td>
<td>.041</td>
<td>.082</td>
</tr>
<tr>
<td>20°C VS 30°C reared</td>
<td>.287</td>
<td>.005</td>
<td>.007</td>
</tr>
<tr>
<td>30°C VS 5°C reared</td>
<td>.002</td>
<td>.011</td>
<td>.014</td>
</tr>
</tbody>
</table>

TABLE V

SUMMARY OF ORGAN MASS (% BODY MASS) FOR RATS RAISED
AT 5°C, 20°C AND 30°C, EXPRESSED IN MEAN ± STANDARD DIVIATION

<table>
<thead>
<tr>
<th>Conditions</th>
<th>5°C</th>
<th>20°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>heart</td>
<td>.0045±.0002</td>
<td>.0077±.0003</td>
<td>.0450±.0017</td>
</tr>
<tr>
<td>kidney</td>
<td>.0036±.0007</td>
<td>.0086±.0010</td>
<td>.0348±.0046</td>
</tr>
<tr>
<td>liver</td>
<td>.0036±.0007</td>
<td>.0063±.0005</td>
<td>.0369±.0042</td>
</tr>
</tbody>
</table>

TABLE VI

SUMMARY OF THE SIGNIFICANCE OF F IN ORGAN MASS
(% BODY MASS) FOR RATS RAISED AT 5°C, 20°C AND 30°C

<table>
<thead>
<tr>
<th>Conditions</th>
<th>5°C VS 20°C reared</th>
<th>20°C VS 30°C reared</th>
<th>30°C VS 5°C reared</th>
</tr>
</thead>
<tbody>
<tr>
<td>heart</td>
<td>.059</td>
<td>.847</td>
<td>.084</td>
</tr>
<tr>
<td>kidney</td>
<td>.615</td>
<td>.037</td>
<td>.009</td>
</tr>
<tr>
<td>liver</td>
<td>.011</td>
<td>.661</td>
<td>.088</td>
</tr>
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</table>
TABLE VII

SUMMARY OF THE MASS (g) OF RATS RAISED AT 5°C, 20°C AND 30°C AT 5°C FOR 37 DAYS, EXPRESSED IN MEAN ± STANDARD DIVIATION

<table>
<thead>
<tr>
<th>Weeks at 5°C</th>
<th>Temp. Reared</th>
<th>5°C</th>
<th>20°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>237.0 ± 2.3</td>
<td>203.6 ± 6.4</td>
<td>291.6 ± 5.5</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>268.3 ± 6.5</td>
<td>185.6 ± 3.5</td>
<td>255.7 ± 27.6</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>285.0 ± 19.2</td>
<td>181.3 ± 5.3</td>
<td>263.0 ± 33.7</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>295.3 ± 15.4</td>
<td>207.8 ± 3.8</td>
<td>276.0 ± 38.2</td>
</tr>
</tbody>
</table>
FIGURE 1

THYROID STIMULATING HORMONE LEVELS OBTAINED WEEKLY OF RATS RAISED AT 5°C, 20°C AND 30°C, FROM TWO TO EIGHT WEEKS OF AGE
FIGURE 2

GROWTH HORMONE LEVELS OBTAINED WEEKLY OF RATS RAISED AT 5°C, 20°C AND 30°C, FROM TWO TO EIGHT WEEKS OF AGE.
CHAPTER IV

RESULTS

Tables I and II give the morphologic figures and their significance obtained from two to eight weeks of age. The 5°C and 30°C reared rats showed the greatest difference in hind foot, ear and tail size, the 30°C being the largest from the third week on. The greatest difference in hind foot, tail and ear length occurred during the eighth week (P < .000). A comparison of the 20°C and 30°C reared rats hind foot and ear was not nearly as striking however the tail lengths were significantly different (P < .000) from three to eight weeks of age. The differences in lengths of the hind foot, ear and tail in the 20°C and 5°C reared rats were generally nonsignificant. The ear and tail lengths at eight weeks of age were however, significantly different (P < .005). The mass and body length were only consistently different (P < .050) in the 20°C and 30°C reared rats.

The routine metabolic rates and their significance are given in tables III and IV. The 5°C reared rats followed closely by the 20°C reared rats had the lowest routine metabolic rate in the 5°C chamber. The 30°C reared rats had higher routine metabolic rates (P < .050) than the 5°C and 20°C reared rats had in both the 20°C and 30°C chambers.

Tables V and VI give the organ weights and their significance obtained from eight week old male rats reared at 5°C, 20°C and 30°C. The differences in heart, liver and kidney sizes were generally not striking.
The liver of the 5°C reared rats compared to the 20°C reared rats differed significantly (P<.011). The kidneys of the 30°C reared rats were significantly lighter (P<.040) than either the 5°C or 20°C reared rats.

The amounts of food consumed from four to eight weeks of age were greatest for the rats in the 5°C chamber (828.7g±69.5) followed by the rats in the 20°C chamber (642.9±71.7). The least amount of food was consumed by the rats reared in the 30°C chamber, (504.8g±37.6).

Of the five 30°C reared rats that were put into the 5°C chamber, three died, however death was not immediate. One of the 30°C reared rats died after three days and the other after 13 days in the 5°C chamber. Both the 30°C and 20°C reared rats had a reduction in mass during the first three weeks in the 5°C chamber. The 30°C reared rats were all gaining mass after three weeks in the 5°C chamber (see table VII). The food consumption by the 20°C reared rats in the 5°C chamber was the greatest of the three groups. This amounted to 1332.5g±40.7 compared to 1095.6g±4.4 consumed by the 30°C reared and 1176g±55.6 consumed by the 5°C reared rats.

Bleeding and necrosis of the tails of the 30°C reared rats occurred after three to four weeks in the 5°C chamber. The tips of the tails of the 20°C reared rats appeared flushed, however they never bled or necrosed. The tails of the 5°C reared rats in the 5°C chamber were vasoconstricted throughout the entire experiment.

Serum thyroid stimulating hormone and growth hormone levels obtained weekly from two to eight weeks of age from the three temperature groups are given in figures 1 and 2. The results indicate both hormones are affected by temperature, however it can not be determined from the data presented exactly how they are affected. Increases in mass and
lengths of the hind limb, ear, tail and body generally did not correlate with changes in the concentration of growth hormone or thyroid stimulating hormone.
CHAPTER V

DISCUSSION

The results indicate that morphologically Allen's rule holds, however, Bergmann's rule does not hold during the first eight weeks of life. The morphologic changes that occur when in the cold do not appear to be a thermoregulatory advantage as the heat reared rats, with their longer extremities, gained weight when put in the cold chamber at a rate similar to the cold reared rats. However, during the first three weeks of cold exposure the heat reared rats, as well as the control reared rats, lost mass. This may represent a period of physiologic adjustment. Were the experiment to terminate at this point it would appear that the morphologic adjustments were of adaptative importance. In addition to gaining weight at a similar rate as the cold reared rats, the heat reared rats consumed similar amounts of food as did the cold reared rats over the entire five week period in the 5°C chamber. It cannot be concluded from this experiment whether or not there is an adaptative advantage to having a long tail, ear and hind limb in a hot environment as the cold reared rats were not put in the 30°C chamber with the heat reared rats.

The smaller mass of the control reared rats may be due to a lack of stressful conditions to stimulate growth. When the controls were put in the 5°C chamber they ate more than the heat and cold reared and had more difficulty in adjusting to the cold environment. Perhaps a constant
exposure to thermostress enhances biochemical, neural and cardiovascular systems in a manner similar to training effects.

The shortness of extremities in the cold reared rats may be due to a lack of advantageous conditions to stimulate their growth. Since body temperature is kept relatively constant, the mass and body length of the rat would be less affected than the more exposed tail, ear and hind limb. This would be reversed in a hot environment.

Bleeding and necrosis did occur after the heat reared rats had been in the 50°C chamber for three to four weeks. This resulted in a loss of up to eight centimeters of tail. It appears these rats were vasodilating to keep their long tails warm, this would certainly have ramifications in heat loss though the magnitude of this is unknown. The point at which they began to gain weight did coincide with the onset of necrosis, however this may be mere coincidence. The control reared rats when put in the 50°C chamber did not show bleeding and necrosis of their tails. Their tails were flushed however, throughout the five week period in the cold chamber indicating vasodilation did occur.

It is interesting to note that when 30 week old rats of the same litter were put into the environmental chambers, the rats in the 50°C chamber gained weight and eventually grew much larger than the rats in the 30°C chamber. The amount of food consumed by the cold rats was also much greater.

Amounts of food consumed between four to eight weeks of life were greatest for the cold reared rats indicating a greater energy requirement. The heat reared rats ate less but attained a mass that was
significantly greater, at the .05 level of significance, than the control and cold reared rats. This indicates a smaller expenditure of energy was required to maintain body temperature. The activities of the animals should of course be taken into consideration, however they were not observed in this experiment.

The routine metabolic rates recorded were not a true resting conditions as the jar lids had to be removed between each routine metabolic measurement causing some disturbance to the rats. All measurements were obtained under the same conditions. The routine metabolic rates of the heat reared rats in the 5°C chamber were quite high compared to the cold reared rats, indicating the cold reared rats were better acclimated to the 5°C temperature. The heat reared rats had the highest routine metabolic rates under control conditions showing they were also least efficient under control conditions compared to the control and cold reared. The heat reared rats did not have the lowest routine metabolic rates of the three groups in the 30°C chamber. This could possible mean that they were physiologically equipped to deal with the 30°C temperature and could carry out normal functions, whereas the cold and control reared could not. It is interesting to note that the morphologic measurements and routine metabolic measurements of the control and cold reared were generally quite similar compared to the control and the heat reared or the cold and the heat reared.

It should be noted that the cold reared rats could not be put into separate cages after 21 days of age as the other two groups were. When this was first tried all the rats died, presumably due to an inability to thermoregulate. Therefore the cold rats were not put into individual cages until they were 25 days old. The mother was, however, removed at
21 days of age.

Serum thyroid stimulating hormone levels were not found to be related to the mass of the rat when rats of the same age were compared. There did appear to be a relation to temperature of the chamber in which the rat was raised as by the eighth week of life the control and heat reared rats had attained much higher levels of thyroid stimulating hormone than did the cold reared rats. This difference was very small for the heat reared rats, however blood was only taken once weekly starting at two weeks of age. The rather abrupt rise in serum thyroid stimulating hormone that occurred in the four week old cold reared rats corresponded to the removal of the mother from the nest resulting in a more thorough exposure to the 5°C environment in addition to a certain amount of stress from the mother's absence (this is shortly after the rats had acquired the ability to thermoregulate). The heat reared rats would certainly have less metabolic adjustments to make compared to the cold reared rats once the mother is removed, although the placement of these rats into individual cages should cause some stress. This may be why the abrupt increase of thyroid stimulating hormone during the fourth week of the heat reared rats was considerably smaller in the cold reared rats. The control reared rats did not show this abrupt increase at four weeks of age, however it must be remembered that blood was only taken once a week and there may have been a similar increase during the interval of the third and fourth weeks or fourth and fifth weeks of life. The lower levels of serum thyroid stimulating hormone seen in the cold reared rats from the fifth week through the eighth week of life compared to the fourth week may represent metabolic adjustments made by the rats to the cold environment. Of course the different sensitivities of the
rats to thyroid stimulating hormone should be taken into consideration.

Serum growth hormone levels were only found to correlate with size at eight weeks of age, the heat reared rats having the highest levels. Prior to this no correlation was seen. The serum growth hormone levels of the hot, cold and control reared groups were different when compared on a week by week basis, indicating a temperature effect, although it is difficult to determine the significance of this difference. Of course the different sensitivities the rats may have to growth hormone, also the pulses of growth hormone should be taken into consideration. It should also be remembered that blood was only taken once weekly.
CHAPTER VI

SUMMARY AND CONCLUSIONS

Long-Evans rats from the same litter were placed into one of two environmental chambers, one male and one female per cage. One environmental chamber was set at 50°C and the other at 300°C. A third set was kept under control conditions i.e. 200°C. Measurements of the hind foot, ear, tail and mass were taken from four of the male offspring from each group, as was blood to be assayed for thyroid stimulating hormone and growth hormone. This was done from two to eight weeks of age. The heat reared males were found to have a significantly longer tail, ears and hind foot when compared to the control reared and least pronounced when the cold reared were compared to the control reared. The control reared rats were found to have a significantly less mass than either the heat reared or the cold reared, possibly due to a lack of stressful conditions. When 10 week old heat reared rats and 11 week old control reared rats were put into the cold chamber, they initially lost weight though they consumed more food than the cold reared rats. After approximately three weeks the heat reared rats began to gain mass at a rate similar to the cold reared rats, while consuming similar amounts of food, indicating the shorter tail, ears and hind foot of the cold reared rats was of little adaptive advantage, if any with respect to thermoregulation. The control rats had the most difficulty in acclimating themselves to the cold chamber. The inability to acclimate to
a severe environment may be linked to the nonstressful conditions under which they were reared.

Though not significantly different than the controls, the cold reared rats had the lowest routine metabolic rate in the cold environment. The controls had the lowest routine metabolic rate in the control and hot environment, followed by the cold reared rats. The heat reared rats did not have the lowest routine metabolic rate in the hot environment possibly because they were acclimated to this temperature and could carry out normal functions.

The kidneys of the control and cold reared rats were significantly heavier, per kilogram of body weight, than the heat reared. The liver of the control reared was significantly heavier, per kilogram of body weight, than the cold reared.

Weekly determined serum thyroid stimulating hormone levels of the control, cold and heat reared rats did not correspond to morphologic measurements. Peaks seen in the cold reared rats corresponded to removal of the mother resulting in a more thorough exposure to the 5°C environment. A smaller peak was seen in the heat reared rats also corresponding to the removal of the mother.

Weekly determined serum growth hormone levels of the control, cold and heat reared rats generally did not correspond to morphologic measurements, however at eight weeks of age the comparatively high levels of the heat reared rats did correspond to their larger morphologic measurements.
REFERENCES


