THE ADAPTABILITY OF MICE TO HIGH ENVIRONMENTAL TEMPERATURES

By G. AINSWORTH HARRISON

Anthropology Laboratory, Department of Anatomy, University of Liverpool and The M.R.C. Unit, Department of Anatomy, University of Oxford

The adult morphology of some mammals and birds has been shown to be determined by the temperature at which they are reared (Przibram, 1925; Sundstroem, 1930; Allee & Lutherman, 1940). In particular, mice raised at high environmental temperatures have much longer tails, larger feet, less fur and are usually smaller *when mature* than mice kept under temperate conditions (Sumner, 1909; Sundstroem, 1922; Ogle, 1934; Sakharov, 1949; Harrison, Morton & Weiner, unpublished). Harrison, Morton & Weiner have shown that the magnitudes of some of these heat responses, i.e. the differences between the environmental forms, are dependent upon the genotype of the animals, but apart from body weight, no exceptions to the above generalizations were found by them.

The aim of this investigation is to ascertain whether these morphological characters, and the physiological ones which are also known to be determined by environmental temperature, facilitate survival in the environment that produced them, i.e. are adaptive, or whether they have no effect on, or reduce, somatic fitness, i.e. are 'passive' (Harrison, 1958a). One method of determining the appropriateness of the high-temperature phenotype is to compare the heat tolerance of heat-reared animals with those reared at a lower temperature. Although there is no absolute distinction between the morphological and physiological components of the heat response, a particular structure, once formed, usually has a biological significance which is independent of the way it was formed; and whereas environmentally induced physiological changes are typically rapidly reversible, morphological changes, which are strictly a function of growth, are irreversible once the structure concerned has stopped growing and are usually only slowly reversible if still growing. To distinguish between morphological and physiological components in the heat response seems therefore justifiable, and they are to some extent experimentally separable. Some attempt is also made to separate the effects of single characters within the morphological response and to determine the way in which an effect is produced.

MATERIAL AND METHODS

C57BL × RIII F_1 hybrid mice, bred at a dry-bulb temperature of 21° C. and an Assman wet-bulb temperature of 16° C. and weaned at 3 weeks of age, were reared either at the temperature at which they were bred (control-reared) or at 32° C. D.B.,

29° C. w.b. (heat-reared). A split-litter experimental design was employed, using body weight as the criterion for allocating animals into one or other of the environments, the two sexes being treated separately. Full details of this design are given in Harrison (1958*b*). The heat tolerance of the animals at an age of 8 weeks was determined by measuring survival time and death order at 41·7° C. D.B. (± 0·3° C.) 29·4° C. W.B. (± 0·5° C.). This temperature, when combined with a moderate air speed, is not too high to conceal small differences in fitness nor too low to make the experiment so protracted as to be unmanageable.

The exposures were performed in a well-insulated room, heated by the input of circulating hot air and steam, and controlled by electronic thermostats. Permanent records of the room temperatures throughout an exposure were made on a Honeywell Brown potentiometer. The animals, in shallow open-topped cages with holes bored in two of the sides, were placed at a standard height in a current of hot air maintained by a series of fans which reduced both horizontal and vertical temperature stratification to a minimum. The cages were arranged with their holed sides at right angles to the current. In this way it was possible to keep thermal conditions inside the cage identical with those outside. All the males in any one exposure were kept in a single cage and all the females in another, the two cages being placed side by side. The conditions were controlled as rigorously as possible because a great deal of evidence has been obtained that when an animal is living at the limit of its temperature regulatory capacity very small variations in temperature have profound effects.

The attempt to partition the causation of any difference in the survival time of control-reared and heat-reared animals into strictly morphological and physiological effects was undertaken by transferring animals from one environment to the other, 48 hr. before the heat-shock exposures. This procedure provided two further types of treatment 'transferred to control' and 'transferred to heat'. The choice of animals for transference was based on a method similar to that used at weaning. There is no reason to suppose that by 48 hr. 'transferred to heat' animals were as physiologically acclimatized to the conditions as those reared in the high-temperature room, nor that the 'transferred to control' animals had completely lost their high-temperature acclimatization, but by this time the rectal temperatures of the animals transferred to the heat were very similar to those of litter-mates reared in the heat. Although a longer period between transference and heat shock might have produced a closer similarity in the physiology of the transferred and indigenous animals, it would also have tended to effect a morphological similarity which was unwanted.

The effects of a tail on survival were investigated by amputating at 3 weeks of age the tail of a few C57 × RIII hybrids, and comparing the performance of these animals with normal litter-mates in a heat-tolerance test, after both had been reared in the hot environment until 8 weeks old. The tails were removed with a sterilized scalpel at their junction with the body and the wound cauterized. Even immediately after recovery from the anaesthesia the animals show no concern about the wound and there can be little doubt that 5 weeks later any operative shock has been overcome.

Before a heat-shock exposure the animals were weighed. At precisely the same time the animals from the control and hot rooms were taken into the lethal temperature environment and animals of the same sex were immediately put in the same cage, so that all were subject to the same temperature fluctuations. The transference unavoidably involved the exposure of the animals from the hot environment to control conditions for 4–5 sec. The animals were provided with both food and water, but on only two occasions were individuals observed to drink. At death each animal was weighed, and in two of the exposures they were also weighed at 1, 5 and 7 hr. after the beginning of the tolerance test.

RESULTS

An analysis of survival time is complicated by the facts that (1) there is some evidence that the survival time of control-reared animals is bimodally distributed; (2) an analysis of variance, ignoring the effects of this distribution, reveals a significant difference in the survival time of at least the control-reared animals in different exposures; this is in part, but not entirely, due to the better control of the environment in some of the later exposures, following an unavoidable reconstruction of the lethal temperature room; (3) in some of the exposures there is also a significant difference in the survival times of identically treated animals in the two cages, but this cannot be interpreted as a sex difference in resistance since either of the sexes may be the longest lived; (4) some of the animals, particularly in the later exposures when the temperature fluctuations were smaller, survived for so long that the experiment had to be concluded before their death (e.g. > 15 hr.).

As a consequence, the actual survival times of heat-shocked animals in different exposures and in different cages cannot be combined in a comparison of the effects of differences of treatment. It is possible, however, to make within-exposure and within-sex comparisons of the order of death and to combine the frequencies with which a particular event occurs in different exposures and in different cages. Since it is also possible that there are litter differences, the comparison is made a within-litter one as well. The death order is established from the mean survival time of similarly treated animals, and the two sexes are treated as if in different litters. The probability of obtaining any particular frequency is determined by combining the appropriate terms of the binomial expansion

$$\frac{\lfloor n}{\lfloor x . \lfloor y} p^x . p^y .^{*}$$

In those comparisons, where the frequency of one type of event is not significantly different from the frequency of the complementary type, rank numbers are given to the differences in survival time of the means compared, according to their magnitude

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* When only one type of event occurs on a number of occasions, the total probability of this occurring by chance is the ultimate term of the expansion, i.e. p^n. When both types of event occur, the probability of the particular frequency is added to the probability of the less probable frequencies.

(Wilcoxon, 1945). The observed magnitude of the difference is a function of the severity of the exposure, so to offset the bias that could be introduced from this source the observed magnitude is scaled as a percentage of the mean survival time of the control-reared animals in the exposure. Admittedly this assumes that survival time is linearly related to the severity of an exposure; this may not be the case, but over a small range of conditions it is probably very nearly true. The effects of the four different treatments on survival are compared by this method in Table 1.

Table 1. *Comparison of the death order of differently treated* $C\,57 \times RIII$
hybrid mice at $41\cdot7°$ *C.* D.B., $29\cdot4°$ *C.* W.B.

(H = heat-reared, C = control-reared. Arrows indicate transference.)

Comparison		No.	No. in which A lives longer than B	Probability of no. diff.	Mean B % of A survival times
A	B				
H	H → C	9	5	$\simeq 1/2$	$\simeq 108$
H	C → H	9	9	1/512	< 76
H	C	11	11	1/2048	< 68
H → C	C → H	12	8	$\simeq 1/5$*	< 86
H → C	C	9	9	1/512	< 78
C → H	C	10	9	1/93	76

* But < 1/20 by the ranking method explained in the text.

There is very strong evidence that the untransferred heat-reared animals survive in the high lethal temperature environment much better than either the untransferred control-reared ones or those that were transferred to the hot environment 48 hr. before the heat shock. There appears, however, to be no significant difference* in the survival times of heat-reared and 'transferred to control' (H → C) animals. On the other hand animals transferred to the heat (C → H) survive significantly longer than litter-mates kept in the control conditions until the heat-tolerance test. The one case in which the present experimental results are not clear cut is in the comparison of the death order of animals transferred to the control and those transferred to the heat. Since there is no significant difference in the survival of the heat-reared and the 'transferred to control' animals, and since the former live very significantly longer than animals transferred to the heat, one would have expected that the latter would not live as long as the 'transferred to control' group. Yet in four litters out of the twelve tested the mean survival time of the animals transferred to the heat was greater than that of those 'transferred to control'. However, if the ranking method of Wilcoxon is applied to the scaled differences in survival time, the 'transferred to control' animals are found to survive significantly longer than the 'transferred to heat' animals. (It is impossible to allot rank numbers to the differences in survival of heat-reared and 'transferred to control' animals since in many cases one or more animals did not die during the exposure. But even if

* Throughout 'significant' refers to < 5 % probability level.

rank numbers could have been allotted, whatever their distribution, no significant difference in the effects of these two treatments would have been found with the particular frequencies observed.)

The survival of the tailed and tailless litter-mates are compared in Table 2.

Table 2. *Within-litter comparison of death order of heat-reared tailless and tailed C 57 × RIII F$_1$ Hybrids*

Litter	No. of tailless animals	Mean body weight (g.)	No. of tailed animals	Mean body weight (g.)	No. of tailless animals dead before death of first tailed	Probability
1	2	18·0	2	18·9	2	1/6
2	1	19·1	1	22·1	1	1/2
3	1	16·4	1	17·3	1	1/2
4	1	17·0	1	18·0	1	1/2
5	1	22·5	1	21·8	1	1/2
6	2	14·4	3	15·3	2	1/10
Total	—	—	—	—	—	1/960

In every within-litter comparison the tailless animals die before the normal ones. Since the presence or absence of a tail is apparently the only distinguishing character, this experiment clearly demonstrates the survival value of a tail in withstanding high temperatures.

During a heat-tolerance test animals lose a lot of weight before death occurs. The absolute total loss is a function of initial size, so to facilitate a comparison of animals of different weight the loss per gramme of body weight has been calculated. The analysis of variance of the weight loss of control hybrids reveals that there are significant differences in the different exposures. It will be recalled that a significant difference was also found in the survival time of these animals in different exposures. There is a significant positive correlation between the mean survival times of males and the mean losses in weight per unit body weight in the different exposures ($r = +0.77$ (7 D.F.)); the correlation coefficient of the females is also positive ($r = +0.57$ (6 D.F.)) though not significant at the 5 % level.

The losses in weight of similarly treated males and females are compared in Table 3. This has been done by calculating the mean loss in each exposure and then obtaining from these an over-all mean for the different exposures. Because of the differences in weight loss in the different exposures the means are balanced so that comparisons are based on results from the same exposures.

The comparisons reveal that there is no significant difference in the weight loss of similarly treated males and females when allowance is made for differences in original weight. The results for the two sexes have therefore been combined in the comparison of the effects of treatment, which shows that the weight loss of control-reared animals is not significantly different from that of heat-reared ones. Since the control-reared animals typically have a shorter survival time than heat-reared litter-mates, it follows that they lose weight more rapidly.

Table 3. *Comparison of the mean loss in weight (g./g. of original body weight) of
similarly treated male and female C57 × RIII hybrids and of differently treated
C57 × RIII hybrids in heat-tolerance tests*

Comparison						D.	S.E.	't'
Mean	No.	S.E.	Mean	No.	S.E.			
Control♂♂			Control♀♀					
0·177	7	0·0168	0·212	7	0·0190	0·035	0·0255	N.S.
Heat♂♂			Heat♀♀					
0·175	8	0·0156	0·177	8	0·0134	0·002	0·0206	N.S.
Control♂♀			Heat♂♀					
0·187	9	0·0158	0·176	9	0·0145	0·011	0·0215	N.S.

The rate of loss was studied in two exposures by weighing animals at 1, 5 and 7 hr. after they were put in the lethal temperature environment. Since there is no significant difference in the total loss of control-reared and heat-reared animals, the results have been expressed as the percentage lost, at each of these times, of the total loss at death, and the mean litter means are recorded in Table 4.

Table 4. *Mean percentage loss of total loss in weight at 1, 5 and 7 hr.
after the onset of two heat-tolerance tests*

Exposure	Control-reared				Heat-reared			
	1 hr.	5 hr.	7 hr.	Survival time (min.)	1 hr.	5 hr.	7 hr.	Survival time (min.)
1	52·5	85·6	90·2	431	41·0	70·9	82·4	554
2	48·6	72·0	88·2	557	39·9	55·5	66·7	699

As one would expect from these figures there is a negative correlation between the amount of weight lost by an animal in the first hour of an exposure and its survival time. Using all the animals, irrespective of treatment, in these two exposures, the correlation coefficient in one of them is $-0·41$, which on 33 degrees of freedom is significant at the 5 % level, and in the other is $-0·35$, which on 23 degrees of freedom is approaching significance at this level. Unfortunately, the correlations are not sufficiently good to allow one to use loss in weight in the first hour as a reliable measure of the heat tolerance of an animal, but it does indicate that the animals which lose weight most slowly have the greatest chance of a long survival.

DISCUSSION

The results demonstrate very clearly that the changes which occur when mice of the genotype C57 × RIII are reared at 32° C. D.B. 29° C. W.B. instead of at 21° C. D.B. 16° C. W.B., facilitate survival at a yet higher temperature. It may be concluded,

therefore, that the over-all environmentally determined response of the heat-reared animals is adaptive as tested in this way and that heat acclimatization is a very real phenomenon. Although the very nature of the test makes it impossible to state categorically that the forms produced by the two environments are the forms best fitted to these environments, the evidence suggests that this is probably the case.

The fact that the 'transferred to heat' animals survive significantly longer than their control-reared litter-mates confirms the reality of a physiological component in the acclimatization, since little or no morphological change occurred during the 48 hr. which these animals spent in the hot room. It is probable, indeed, that the bimodal distribution of the survival time of control-reared animals is due to the acquisition of physiological acclimatization during the heat-tolerance test itself. The nature of the physiological changes has not been investigated in the present work, but has been rigorously studied in other comparably treated mammals (Robinson, 1952; Findlay & Beakley, 1954). The 'transferred to heat' animals may not be as physiologically acclimatized to high temperatures as the heat-reared ones; nor can it be assumed that the 'transferred to control' animals have lost all their physiological acclimatization to the heat. However, the fact that not only the heat-reared but also the 'transferred to control' animals survive longer in a heat-tolerance test than the animals transferred to the heat is a strong indication that the morphological component in the phenotype is itself adaptive. Indeed, if the transferred animals have become as physiologically acclimatized to their new environments as the 'native' animals, it must be concluded that the morphological heat responses are more important in survival than the physiological ones. This of course does not mean that within the morphological category itself all the components are adaptive.

A few mice of other genotypes, both inbred and F_1 hybrid, have been tested in a similar way to the $C_{57} \times RIII$ hybrids. In each case control-reared animals survived less well than heat-reared ones. Too few animals were, however, transferred from one environment to the other to permit a systematic partition of the adaptability into physiological and morphological components, but in all genotypes save one the results were consistent with the above conclusions.

Where the responses to the environment are adaptive the lability of the phenotype will presumably have its own particular genetic basis, determining the extent and direction of the environmental modification of development, and if all the environmentally caused differences in phenotype of different genotypes in the same environment are adaptive, then these differences will represent the diverse capacities or requirements of the various genotypes for adaptation.

The most striking morphological difference that distinguishes control-reared and heat-reared animals is tail length, and since the morphological component of the heat-response has been shown to be adaptive and the possession of a tail has such a profound effect on heat tolerance, it seems likely that variations in tail length will change an animal's thermoregulatory capacity. (It is somewhat surprising that the body weights of the tailed and tailless animals are so similar, after allowance is made for the weight of the tail. This suggests that at least up to temperatures

of 32° C. other mechanisms can compensate for the absence of a tail when the rest of the environment is near optimal.) Whereas one might expect a tail of the appropriate length to become genetically fixed in some climates, in a fluctuating one the lability itself may have considerable value. Thus, for instance, although a long tail may be of adaptive value in the summer, it cannot easily become genetically fixed in the population if the fittest winter animal is a short-tailed one. The dependence of tail growth on temperature, however, is likely to provide successive generations with the tail length appropriate to the conditions prevailing when they begin breeding.

At an environmental temperature of 32° C., 4° or 5° C. below that of the body, the value of a long richly vascular tail functioning as a heat radiator is self-evident, but since there are no sweat glands in the mouse its value at an environmental temperature above that of the body is not so obvious, and it might in fact have been expected that under these conditions a long tail would increase heat gain rather than heat loss. It must be concluded that there is effective insensible perspiration, although no convenient way of demonstrating it has so far been devised. Such a conclusion is confirmed by the findings of Njaa, Utne & Braekkar (1957) who have discovered indirect evidence that there is a considerable passage of water across the tail of the rat in a dry environment, and it has been shown on innumerable occasions that insensible perspiration is responsible for much of the water-loss in man and his domestic animals (Du Bois, 1927; Kuno, 1934; Findlay, 1950).

The role of the other extremities in heat regulation has not been studied but one would expect them to function in a similar way to the tail. The effects of environmentally caused differences in body size on temperature adaptation are impossible to determine experimentally, since it is not only difficult to isolate weight differences from the physiological and other morphological changes that also occur, but, being a character which reflects vigour, one cannot distinguish between its own biological function and the multitude of other functions which determine it. Even, for instance, were it shown that genetically small animals were better adapted to the heat than genetically large ones, it would not necessarily mean that a reduced growth rate at high temperatures is an adaptive response. Indeed, there is indirect evidence (Harrison, Morton & Weiner, unpublished) that it is not, in which case genotype differences will not represent different capacities to adapt, but different abilities to buffer development against environmental effects.

Loss of water must be mainly responsible for the loss in weight during a heat-tolerance test. That there is a significant difference in the weight loss in different exposures suggests that animals may die when there is still water available for evaporation. The positive correlation between the severity of an exposure and the total water-loss indicates, in fact, that animals die when they can no longer lose water rapidly enough to maintain their body temperature. The final cause of death would appear then to be heat stroke, but this is a consequence of progressive dehydration.

Since the total water available for evaporation is the same in control-reared and heat-reared animals, the better survival of the latter is likely to be mainly due to

their slower loss of this water. They may lose water more slowly either because they have less metabolic heat to lose or because such water as is lost is used more efficiently. The physiological components of high-temperature acclimatization appear to function principally in the reduction of metabolic heat production, but the morphological adaptations can only influence heat loss. One might expect that the loss of water by insensible perspiration across the skin, particularly across the naked skin of the tail, feet and ears, is a more efficient way of using water for cooling than by panting and rubbing saliva in the fur. If this is so then the longer tail and feet, more richly vascular ears, and scantily covered body skin of the heat-reared animals makes their thermoregulatory capacity more efficient than that of the control-reared animals. Certainly their better survival in a heat-tolerance test favours such a conclusion.

SUMMARY

1. $C57 \times RIII\ F_1$ hybrid mice reared at 32° C. D.B., 29° C. W.B., from 3 to 8 weeks of age, survive longer at 41·7° C. D.B., 29·4° C. W.B., than litter-mates reared at 20° C. D.B., 16° C. W.B.

2. The transference of animals from the hot environment to the cooler one 48 hr. before they are exposed to the lethal temperature has little or no effect on their heat tolerance; but transference in the opposite direction greatly increases survival time.

3. Mice whose tails have been amputated 5 weeks before they are exposed to the lethal temperature have a lower heat tolerance than normal animals.

4. The total loss in weight of an animal exposed to the lethal temperature is independent of the environmental temperature at which it has been reared; but heat-acclimatized animals lose weight less rapidly than control ones.

5. It is concluded that at least some of the changes, both physiological and morphological, which occur when mice are reared at high temperatures, are in their over-all effect adaptive.

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