ENDOTHERMY AND ECTOTHERMY IN MICE AT $-3^\circ$ C.

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A study is being made of the effects on laboratory mice (*Mus musculus* L.) of breeding at an environmental temperature of $-2$ to $-4^\circ$ C. It has been found that both A (white) and C57 (black) mice, rigorously inbred, can be maintained indefinitely at this temperature, given cotton-wool for nesting (Barnett & Manly, 1954).

In general, adjustment to a low temperature may be of two kinds: (a) internal changes (endothermic), such as raised heat production or improved thermal insulation; (b) alteration of the environment of the animal (ectothermic), for instance nest-building or moving to a warmer place. This paper describes some observations on the combination of endothermic and ectothermic adjustments by which mice maintain themselves at $-3^\circ$ C.

MICE AND METHODS

The mice referred to in this paper were of the A strain, from two sources: the Imperial Cancer Research Fund (Mill Hill, London); and Glaxo Laboratories (Greenford, Middlesex). In one instance reference is made to C57 mice from Mill Hill. All breeding pairs were litter-mates, and were kept together, without other mice except their young up to 3 weeks old, from 5 weeks until they died or were killed. The two strains undoubtedly differ genetically (information on this point will be published separately); but for the purposes of this study they are treated together (with one exception mentioned later), since the differences between them did not influence the results described here.

The cold room was maintained at approximately $-3^\circ$ C., with a differential of $2^\circ$ C; the temperature occasionally rose above $0^\circ$ C., when work was going on. Control mice were kept in a warm room at approximately $21^\circ$ C., with a differential of about $5^\circ$ C. The relative humidity in both rooms was between 60 and 90%. Artificial light was on for 12 hr. each day; for the other 12 hr. the mice were in darkness.

The cages were rectangular metal boxes, about $36 \times 15 \times 11$ cm. high, with wire lids carrying a water bottle and food basket. The floor was covered with sawdust, since the humidity was too high for peat moss. Except where mentioned, cotton-wool was supplied for nesting. The food was diet 41 (Bruce & Parkes, 1949). Water was supplied in bottles in the warm room, and in open jars, placed on the floor of the cage, in the cold room. When the water froze, the mice licked the ice.
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Cages were cleaned twice a week, except that litters of less than 11 days were left undisturbed.

Body and nest temperatures were taken with copper-constantan thermocouples, with a reference couple at $0^\circ$ C. For nest temperatures, the couple was enclosed in thin glass, to protect it from being gnawed by the mice. It was normally placed among the nestlings, as indicated for the highest temperatures shown in Figs. 3 and 4. Internal body temperatures were taken in the colon; for adults, the couple was always inserted to a depth of 3 cm.; for young, as far as it would go without causing injury (generally, 1.0-2.5 cm.). Insertion of a couple is helped if the mouse first discharges the formed faeces from the anus: this is especially important with males, since their faeces are harder than those of females. Skin and surface temperatures were taken with a thread thermocouple. For skin temperatures, the couple was placed on the skin surface in the middle of the back, and the hair flattened over it before the reading was recorded. For surface temperatures the couple was placed lightly on the hair, again in the middle of the back. All the temperatures recorded for adults (but not for young) were from mice which had previously experienced temperature taking three times; after the second time the mice were quite docile, even when the couple was inserted into the anus. Colon, skin and surface temperatures were all taken at between 15:00 and 17:00 hr. G.M.T.

BODY TEMPERATURES

Table 1 shows the results of measurements of temperatures. Mean group values for adult colonic temperatures vary from 37.5 to 38.1$^\circ$ C. Temperatures in the cold were slightly lower than in the warm; the difference is statistically significant ($P=0.05$) for the females but not for the males. Skin and surface temperatures were markedly lower in the cold, and these differences are significant. Differences between the sexes are not significant.

Table 1 also summarizes the body temperatures found for nestlings. No significant difference was found between the sexes, and so their temperatures

| Table 1. Mean colonic, skin and surface temperatures |
|---|---|---|---|
| | Males | | Females |
| | $21^\circ$ C. | $-3^\circ$ C. | $21^\circ$ C. | $-3^\circ$ C. |
| Adults | | | | |
| No. of mice | 15 | 15 | 15 | 15 |
| Colon | 37.7 (1.12) | 37.4 (1.10) | 38.1 (1.14) | 37.5 (1.54) |
| Skin | 36.3 (1.28) | 34.6 (1.54) | 36.8 (0.79) | 34.0 (2.00) |
| Surface | 34.2 (1.68) | 31.3 (0.55) | 34.3 (0.82) | 30.0 (0.61) |
| Young (colon only) | | | | |
| 11-13 days | no. of mice | mean temp. | | no. of mice | mean temp. |
| 18-20 days | (both sexes) | | | (both sexes) | |
| | 18 | 33.9 (0.51) | | 30.0 (2.32) |
| | 26 | 34.3 (2.10) | | 23 |
| | 31.4 (3.87) | | | |
are grouped. Both in the cold and the warm the temperatures of the young were lower and more variable than those of adults. The variability was greater in the cold.

NESTS AND NEST TEMPERATURES

The form of a well-constructed nest is illustrated in Figs. 1 and 2. All mice, except those newly introduced to the cold room, build nests of this sort in the cold. In the warm room similar nests are built, but they are often open at the top, and in general they are more casually constructed. Nests of breeding pairs in both temperatures were assessed for quality of construction. 0, 1 or 2 marks were given for each of three characteristics: (a) degree of closure; (b) domed formation (cf. Fig. 2); (c) fluffing of cotton-wool. The maximum score was therefore six. Table 2 gives results, including some from C57 mice. The effect of the cold environment is clearly shown.

<table>
<thead>
<tr>
<th>No. of nests</th>
<th>A 21° C.</th>
<th>21° C.</th>
<th>21° C.</th>
<th>21° C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean score (with s.d.)</td>
<td>36 (17)</td>
<td>5.4 (1.0)</td>
<td>5.2 (1.0)</td>
<td>3.5 (1.2)</td>
</tr>
<tr>
<td>C57</td>
<td>19</td>
<td>5.2 (1.0)</td>
<td>5.2 (1.0)</td>
<td>3.5 (1.2)</td>
</tr>
</tbody>
</table>

The importance of experience of the cold is also illustrated by the behaviour of mice taken from their parents at 21 days. Those brought up in the cold immediately burrow into the cotton-wool in their new cage; while those reared in the warm, and transferred at this age to the cold room, often remain on the surface of the cotton-wool for some time.

The typical domed nest is made by manipulation of the cotton-wool with the mouth and fore-feet. The cotton-wool, initially in its usual matted state, is fluffed out: it thus comes to occupy more space, and the volume of air in its interstices is increased. The occupied nest is, however, not a stable structure: it is always liable to alteration, and disturbance may lead to the building of a new nest; this applies especially when young nestlings are present, and makes it impossible to record temperatures continuously over long periods.

Systematic study was made of nest temperatures where nestlings of 1–7 days were present. The highest nest temperature recorded was the same for each room, namely, 34.5° C. The lowest temperature, with the thermocouple among nestlings, recorded from 14 nests in the warm room was 27.5° C., that from twenty-six nests in the cold room was 20.2° C. Nest temperatures in the cold were much more variable than those in the warm. A statement of 'average temperature', either for a single nest over a period, or for a group of nests, would, however, give only a spurious impression of precision. Owing to differences within the nest at a given moment, rapid changes of temperature with time and changes in the position of nestlings, it is not possible to make numerically precise statements of high generality about nest
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Fig. 1. Cage with lid removed, showing typical well-made nest.

Fig. 2. Diagram of section of typical well-made nest.
temperatures. Figs. 3–6 give typical examples of the conditions in individual nests. Fig. 5, which should be compared with Fig. 6, shows how the maintenance of a high nest temperature in the cold depends on the presence of at least one adult, and

\[ \text{Air} = -3^\circ C \]

\[ 22.5^\circ C \]

\[ 25.5^\circ C \]

\[ 32.5^\circ C \]

Fig. 3. Diagram of nest in cold room, showing temperature gradient.

Fig. 4. Diagram of nest in warm room, showing relatively uniform temperature within nest. This nest, characteristically, was less well-made than that shown in Fig. 3.

the steep decline in temperature which takes place when both adults are feeding. Fig. 5 also illustrates the fact that nestlings make some contribution to the nest temperature, but not much. Once the young develop hair and are able to move more actively, it becomes impracticable to record anything that can be called 'the' nest temperature. This is
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due partly to the disturbance resulting from the presence of the thermocouple: if a thermocouple is left in place for a time, in the hope that the mice will settle down around it, they move elsewhere.

![Graph](image)

Fig. 5. Temperature fluctuations in a nest, containing a 3-day litter of five (wt. approx. 15 g.), in the cold room. All temperatures were recorded with the thermocouple on or among the litter. The effect of absence of both parents is shown. The broken line represents the decline in temperature of approximately the same weight and volume of water in a similar nest. The difference between the two curves illustrates the contribution made by the nestlings to the maintenance of nest temperature.

![Graph](image)

Fig. 6. Temperature fluctuations in a nest, containing a 2-day litter of eight, in the warm room. Contrast Fig. 5.

EFFECT OF NEST INSULATION ON ADULTS

To test the importance of nest material for adults, mice aged 4–12 months were subjected to the cold environment without cotton-wool. They still had sawdust on the floor of the cage, but there was not enough of this to form more than a very slight hollow where the mouse slept. Each mouse was alone in a cage. This
experiment was done only with mice of the Mill Hill strain, and it is uncertain whether the Glaxo strain A mice would respond in the same way.

The results are shown in Table 3. Eleven mice which had been reared in the cold all survived exposure without cotton-wool for at least 28 days; on the 28th day one death occurred, and five more between 8 and 10 weeks. Each of these deaths was preceded by a sudden decline in weight. Apart from this decline before death, body weights remained stationary in these conditions. But, of the sixteen mice from the warm room, twelve died in the first 48 hr. Such mice, left in a warm room without cotton-wool, not only survive to an age of more than 2 years, but also rear litters.

Table 3. Effect of exposure to cold without nesting material

<table>
<thead>
<tr>
<th>Bred at -3°C.</th>
<th>No. exposed</th>
<th>Mean wt. g. initially (with range).</th>
<th>No. dead in 48 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ø</td>
<td>Ø</td>
<td>Both sexes</td>
</tr>
<tr>
<td>Bred at 21°C.</td>
<td>6</td>
<td>5</td>
<td>24.8 (19.5-28.0)</td>
</tr>
</tbody>
</table>

DISCUSSION

Sumner (1911, 1915) studied the effects of temperatures of around 4°C on the breeding and growth of mice. His work was never completed, and he published no physiological observations. He also had the disadvantage of having to work with a heterogeneous mouse stock. More recent studies of mammals at low temperatures have been either on the effects of acute cold stress over short periods, or on the cold resisting characteristics of animals which normally live in cold climates (reviewed by Burton & Edholm, 1955). An important exception is the work of Laurie (1946) on wild house mice living in meat cold stores at around -10°C. The present study, of which a small part is reported here, is concerned with the physiological adaptations of laboratory mice breeding for several generations at low temperatures, and with genetical variation in the response to cold. The results presented in this paper define the environmental stress to which the mice are subjected, and indicate some of the mechanisms by which the stress is withstood.

The effects of exposure to cold may include the following, among others: (i) increased mortality; (ii) altered growth of the whole body, or of relative growth of parts; (iii) changes leading to increased heat production; (iv) altered behaviour; (v) changes in later generations, e.g. through the maternal environment. All these, except the last, can be conveniently considered here.

(i) Exposure to cold undoubtedly increases mortality in mice, especially among the nestlings (cf. Barnett & Manly, 1954, 1956). Further information on the responses of young mice to cold will be published elsewhere, but it can be said that the critical period for strain A mice is the first 4-5 weeks, and that the maintenance of a relatively high nest temperature during this time is of especial importance.
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This is true even though, as mentioned above, when the young mice become active nest temperatures are very unstable. Presumably, when parents and young are all asleep there is a high nest temperature, but this has not been directly observed. From the point of view of keeping up the temperature of the young, the high heat output of adults, reflected in the high surface temperature, is an advantage, since it makes possible a rapid restoration of nest temperature after the absence of the adults. High nest temperatures are, however, not the whole story. Hill (1947) has shown that infant rats are poikilothermous up to 18 days, and Fairfield (1948) found that they can survive exposure to temperatures as low as $2^\circ$ C. The body temperatures reported in the present paper show that suckling mice too are thermally unstable. No doubt their ability to withstand fluctuations of body temperature is important in view of the inconstancy of the nest temperature in the cold environment.

(ii) Mice are lighter, both at weaning (Barnett & Manly, 1954) and as adults (Retzlaff, 1939), in the cold. This is probably due, in the adult, largely to the presence of less fat (unpublished), and must tend to increase the difficulty of maintaining body temperature: catabolic adaptation must cope, not only with reduced environmental temperature, but also with a body subject to a relatively higher rate of cooling. Evidently, increased food intake does not fully compensate for the additional expenditure of energy imposed by the high rate of heat loss. Changes in fur insulation might compensate for reduction in body weight. Scholander, Walters, Hock & Irving (1950) have shown that in arctic mammals insulation provided by skin and hair is an important aspect of adaptation to low temperatures. Another type of adaptive growth change, according to 'Allen's rule', might be an alteration in bodily proportions (Allen, 1877). Sumner (1915) reported that rearing mice in the cold led to a reduction in the weights and lengths of feet, ears and tails. Changes of this kind, in the mice used in the present experiments, will be reported elsewhere, but it can be said now that there is no evidence of any thermally important allometric change: relative tail lengths are slightly reduced in the cold; but, more important, the relative weight of the skin (including the hair and subcutaneous fat) is reduced; this again is probably due to reduction in the amount of fat in mice in the cold. It serves to emphasize that either the chemical regulation of temperature, or ectothermic control, are the important factors.

(iii) In the present study, the role of chemical regulation is most clearly shown in the different response of acclimatized and non-acclimatized mice to exposure without nest material: most of the latter failed to survive for 2 days, while those reared in the cold lived for some weeks unprotected. This is in accordance with the observations of Hart (1953a, b) on the importance of acclimatization in mice. Catabolic changes alone are, however, not enough to ensure indefinite survival, in the strain studied: some, at least, of the acclimatized mice showed a slow decline, culminating in death after some weeks.

The character of the chemical regulation has not been studied in the present experiments. Hart & Heroux (1954) have shown that, in short-term acclimatization of deer mice to cold, muscular activity, whether in the form of exercise, shivering or increased tension, must be supplemented by a passive increase in metabolic rates.
(iv) It is only when chemical regulation can be supplemented by ectothermic adjustment that normal survival is possible: that is, when a nest can be built. There is considerable scope for behavioural study of the building and use of nests in different environmental conditions. Kinder (1927) showed that albino rats make better nests if the environmental temperature is lowered, and that they improve with practice. In mice there is undoubtedly a complex interaction between innate and learned components, in the building of a nest in the cold; these remain to be studied.

In general, the present study shows that the two modes of adaptation to low temperature, the endothermic and ectothermic, are both important in the lives of mice in an environment at $-3^\circ$C. It is commonly said that the success of mammals, in a wider range of niches than was previously occupied by reptiles, is due to their combination of two qualities: (a) a high degree of homeostatic regulation through internal changes, and (b) individual adaptability of behaviour in varying conditions (cf. Young, 1950). The results reported here provide a specific example supporting this generalization.

**SUMMARY**

Laboratory mice (*Mus musculus* L.) have been bred at an environmental temperature of $-3^\circ$C., with controls at $21^\circ$C. The mice were given cotton-wool for nesting.

Body temperatures, taken in the colon, of adult control mice were about $38^\circ$C.; those of mice reared in the cold were slightly lower. Skin temperatures, taken below the hair, and temperatures on the surface of the hair, were lower in the cold than in the warm. Surface temperatures were, nevertheless, high ($30^\circ$C. for females in the cold), indicating a high rate of heat loss.

Mice less than 3 weeks old have lower and more variable body temperatures than adults.

Better nests are built in the cold than in the warm. Nest temperatures, taken among nestlings less than 7 days old, are high (up to $34.5^\circ$C.) in the cold as well as in the warm; but nests and nestlings in the cold are subject to sharp declines in temperature when both parents leave the nest to feed.

Adults reared in the warm were transferred to cages in the cold without cotton-wool; most of these mice died within 48 hr. Similar mice, reared in the cold, and transferred to cages without cotton-wool, survived for some weeks, though not indefinitely. The difference reflects the state of partial metabolic (endothermic) adaptation achieved by mice reared in the cold.

The results in general show that both endothermic and ectothermic adjustments play a part in the maintenance of mice at $-3^\circ$C. The ectothermic or behavioural adaptations are those involved in the efficient building and utilization of nests.

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