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The Effect of Acclimatization to Cold on the G Tolerance of Rats.

Bureau of Medicine and Surgery
Subtask MR005.15-0002.3 Report No. 6
The Effect of Acclimatization to Cold on the G Tolerance of Rats

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SUMMARY

Two groups of rats were acclimatized to cold (4 to 6°C) for 37 days and then exposed to acceleration of 20 positive G until the heart rate decreased to 2 beats per second. No statistically significant difference in tolerance to acceleration was found between the cold-acclimatized animals and their controls. Exposure to cold caused loss of weight and increase in adrenal gland size.
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INTRODUCTION

Much thought and experimentation has been turned toward various factors which might increase or decrease tolerance to acceleration in humans and animals. Cross-acclimatization, the effect one stress has on another, also has been investigated. Recently, Pregly (1) found a negative cross-acclimatization between cold and lowered barometric pressure. He found that cold-adapted animals lost their righting reflexes more quickly than control animals when brought to 39,000 feet and that animals adapted to lowered barometric pressure showed a negative cross-acclimatization when exposed to cold.

This paper describes the effect of acclimatization to cold on the tolerance of rats exposed to an acceleration of 20 positive G.

METHODS

Animals used in these experiments were male Sprague-Dawley rats obtained through Hormone Laboratories, Chicago, Ill. Mean weight on acceptance was 142.9 grams with an approximate age of six weeks. The animals were allowed to adapt to the conditions of the animal house at the Aviation Medical Acceleration Laboratory (AMAL) for two weeks before being tested at 20 positive G for three minutes to eliminate any animals which were physically unable to withstand this short duration of positive G. Animals subjected to cold (4 to 6°C) were separately caged in a wind-free environment in which the temperature could be held constant. Duration of the cold exposure was 37 days as outlined in Table I. Control animals were maintained in the animal house under similar conditions except that the temperature was regulated to 24°C. All the rats were fed purina chow with water ad libitum. Animals exposed to cold were weighed in the morning five days a week; the control animals were weighed once a week.

For the 20 positive G acceleration, electrodes were attached by wound clips on the chest and back of each animal which was then separately restrained in an individual, loose-fitting, wire-mesh cage (Figure 1) and placed horizontally on the 8-foot centrifuge with the animal's head inboard (Figure 2). Acceleration was stopped when the animal's heart rate decreased to 2 beats per second for ten seconds at which time he was considered dead (2). The average heart beat under resting conditions before acceleration was 6 to 8 beats per second. Heart rate was monitored by means of a transistor amplifier (3), a method developed at AMAL for determining the physiological endpoint of an animal's tolerance to acceleration.
<table>
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<tr>
<th>Type of Conditioning</th>
<th>No. of Animals</th>
<th>Age (days) Start</th>
<th>Age (days) End</th>
<th>Duration of Exp. (days)</th>
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<tbody>
<tr>
<td>Cold (4-6°C)</td>
<td>18</td>
<td>321.7</td>
<td>335.7</td>
<td>37</td>
</tr>
<tr>
<td>Control (animal-house 22°C)</td>
<td>21</td>
<td>317.7</td>
<td>396.6</td>
<td>112</td>
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<td>May and June 1960</td>
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<td></td>
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<td></td>
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<tr>
<td>Total 39</td>
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<td></td>
<td></td>
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<td>GROUP I</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Cold (4-6°C)</td>
<td>16</td>
<td>409.3</td>
<td>381.9</td>
<td>168</td>
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<tr>
<td>Control (animal-house 22°C)</td>
<td>16</td>
<td>409.3</td>
<td>463.8</td>
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<td>July and Aug. 1960</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total 32</td>
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<td></td>
</tr>
<tr>
<td>GROUP II</td>
<td></td>
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Fig. 1. Rat in restraining cage.
Fig. 2. Rat placement in cage and holder on centrifuge.
Since survival time under an acceleration of 20 positive G did not follow a normal distribution, data were analyzed by the method of Litchfield, (4,5) for time-percent effect curves. This method gives a close approximation to the procedure developed by Bliss (6) of using logarithmic-probability transformation for biological studies except that data in their original form may be plotted directly onto logarithmic-probability paper. By means of nomographs, the parameters and confidence limits of the time-percent effect curves may be made directly from original data.

RESULTS

Figures 3, 4, and 5, show plots of percent survival vs. time of exposure to acceleration for several experiments. As may be noted in Table II, no significant difference was shown between the animals exposed to cold and their controls. Median survival time of cold-acclimatized animals, Group I, was 10.5 minutes compared to 11.2 minutes for their controls; in Group II, 9.6 minutes compared to 8.3 minutes for the controls. On the other hand, the increase in adrenal weight of the cold-exposed animals was statistically significant (Table II). Animals exposed to cold showed two weight loss patterns (Figure 6): Those less than three months old which would be gaining weight rapidly under normal conditions, showed a weight loss during the first 7 to 11 days followed by a return to their original experimental weights by the end of the second week, and thereafter an increase, but at a slower rate than their controls. On the other hand, animals more than three months old, which normally would be gaining weight more slowly, continued to lose weight throughout the experiment establishing a new baseline after 8 to 10 days which remained approximately steady for the remainder of the 37-day experiment (Figure 6). Similar results were found in a preliminary study for two groups of animals of different ages (2 and 4.5 months old) maintained in the cold for a 16-day period.

A comparison of the two groups in Figure 5 (Group I, 112 days: Group II, 168 days at time of acceleration) shows a significant difference in the control groups which may be attributed to age, one of the possible factors contributing to a decreased tolerance to acceleration (7). Heroux and Hart (8) made a similar observation testing with more severe cold and found no correlation with weight (possibly age) in the cold-acclimatized animals but they did find a correlation in the control groups acclimatized to 30°C.

In Group II, combined pelt and hair weights were taken and it was found that there was a slight decrease in the cold-acclimatized animals (Table II). This decrease in insulation is probably...
<table>
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<tr>
<th>Group</th>
<th>Measure</th>
<th>X</th>
<th>G</th>
<th>C</th>
<th>est X</th>
<th>Sig. Diff.</th>
<th>P</th>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>Group I</td>
<td></td>
<td>10.5</td>
<td>11.2</td>
<td>3.1</td>
<td>2.9</td>
<td>0.78</td>
<td>0.73</td>
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<td>Body weight (grams) at end of experiment</td>
<td>335.7</td>
<td>396.5</td>
<td>24.3</td>
<td>24.9</td>
<td>5.9</td>
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<td>Weight of left adrenal (mgms)</td>
<td>41.6</td>
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<td>7.1</td>
<td>4.5</td>
<td>1.7</td>
<td>1.0</td>
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<td>9.6</td>
<td>8.3</td>
<td>3.2</td>
<td>2.9</td>
<td>0.83</td>
<td>0.74</td>
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<td>381.9</td>
<td>463.8</td>
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<td>17.9</td>
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<td>Weight of left adrenal (mgms)</td>
<td>35.1</td>
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<td>4.1</td>
<td>3.3</td>
<td>1.06</td>
<td>1.04</td>
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<tr>
<td></td>
<td>Weight of pelt and hair (gms)</td>
<td>57.18</td>
<td>74.9</td>
<td>7.02</td>
<td>23.79</td>
<td>1.8</td>
<td>7.52</td>
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</table>
Fig. 3. Probit plot of percent survival vs. log time of cold-acclimatized vs. control animals — Group I.
Fig. 4. Probit plot of percent survival vs. log time of cold-acclimatized vs. control animals — Group II.
Fig. 5. Comparison of probit plot of Group I and Group II.
Fig. 6. Comparison of weights (Group I and Group II) of cold-acclimatized animals vs. their controls.
due to an improved peripheral circulation. In contrast, rats kept outdoors during the winter months instead of in a regulated cold environment, experience an increase in insulation (9).

DISCUSSION

Many methods of assaying mammalian changes to different types of stress and the resultant increase, decrease, or no change in adaptation have been devised: biochemically—measurements of products of intermediary metabolism and output of hormones from endocrine glands; hematologically—analyses of changes in the blood fractions; histo-pathologically—records of weights, sizes and changes of the tissues of the various organs of the body and the effects produced in them; and physiologically the observations and recordings of noticeable adjustments in vivo. As is more often the case, scientists use some or all of the various fields of investigation available. To test for adaptation, the imposition of a more intense stress of the same or of a different kind is usually applied and a comparison made between those animals and a control group maintained under similar conditions with the exception of the imposed adaptive stress. Thus, cold acclimatization may be tested by more intense cold (8); adaptation to G tested by a higher acceleration (10); adaptation to low barometric pressure measured by higher altitude (1), etc. Cross-acclimatization—acclimatizing or adapting animals to one stress and testing with a different stress—also has been studied (11) to see if such adaptation might be antagonistic, synergistic, or perhaps irrelevant. This investigation in cross-acclimatization was conducted to ascertain the effect acceleration to 20 positive G might have upon animals adapted to cold (4 to 6°C) for 37 days duration.

A survey of the literature showed there was a variety of opinions on the length of time required to adapt an animal to cold of 4 to 6°C. Heroux and Schonbaum (12) measuring the increase in adrenal weight when animals were exposed to cold (5+1°C) found the increase in weight occurred in the first week with an increased activity for three weeks. However, after twelve weeks the in vitro production of steroids was no greater and sometimes less than in the control rats although the hypertrophy persisted. Thus the activity of the adrenal gland is not necessarily proportional to the change in its size (12,13). Fregly (11) exposing rats to the same temperature for 2, 4, 5, and 10 days found that rats acclimatized after 4 to 10 days. Sellers (14) however, feels acclimatization occurs more slowly, a little in the first two weeks, and a maximum after five to six weeks of exposure. Accordingly, since there seems to be a difference of opinion as to the time required for acclimatization to cold to occur, preliminary experiments were done with small groups of animals. The first period of cold
exposure (4 to 6°C) was for 16 days with the same observations in these experiments as were later recorded for the longer cold exposure of 37 days, i.e., no significant difference was noted between the experimental and control groups as to survival time when subjected to acceleration of 20 positive G.

Increase in the adrenal size as well as a weight loss was significant, however, in the acclimatized animals of both the 16- and 37-day cold exposure groups (Table II). In the 16-day, as well as the 37-day groups, two different ages of animals were used. In all the cold-exposed animals, loss of weight occurred after the first or second day; the pattern varied thereafter between the two age groups. The younger groups began to regain weight after 7 to 11 days in the cold and continued to do so for the rest of the experiment but at a slower rate than the control animals of the same age. On the other hand, the older animals continued to lose weight throughout the experiment, never returning to the starting weight (Figure 6). The conclusion may be drawn that younger rats can increase their food intake to meet the increased metabolic requirement while the older rats are much less able to adjust. That growth is slowed by cold even though rats in the cold consume much more food has been noted by several investigators (13,14,15,16,17). Heroux (14) illustrated that the mitotic activity in the ear epidermis was almost completely arrested for the first 21 to 28 days in the cold. He showed, also, that muscle growth was reduced.

A derangement of metabolism seems to occur (16) in the first few days in the cold with many different compensations taking place. Masoro, et al (16) showed the ability of the 1 and 2-day cold rats to synthesize fatty acids from acetate was less than one-tenth that of the controls but, after 5 to 10 days, synthesis returned to normal. During the first 2 - 3 weeks in the cold, oxygen consumption (14) increases 2 - 3 times normal and heat production (5) also increases. Much of the increase occurs in the first five days with a change-over from production of heat by shivering to a non-shivering mechanism (12) which change is usually complete after approximately one month. Cottle and Carlson (15) found heat production dropped gradually and heat loss was not significant between the acclimatized rats and their controls by the end of the fifth week. They interpreted these findings to mean that there is a time when secondary changes occur with a decline of metabolism, all of the regulatory mechanisms acting to return the animal to a state of homeostasis.

The question arises as to why cold-acclimatized rats with an increased metabolism show no significant difference when exposed to an acceleration of 20 positive G. Speculation might be made that the advantages of an increased metabolism are offset by other physical and physiological factors. However, from these experiments, it appears that there is no cross-adaptation between acceleration and cold stress.
REFERENCES


REFERENCES con't.


U. S. NAVAL AIR DEVELOPMENT CENTER, JOHNsville, PA. AVIATION MEDICAL ACCELERATION LABORATORY

The Effect of Acclimatization to Cold on the G Tolerance of Rats: by Elizabeth Reeves, LCDR, MSC, USN, 19 pp., 9 June 1961.

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