Acclimation of Adrenalectomized Rats to Low Environmental Temperature

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Several authors have interpreted the adrenal hyperactivity of normal animals exposed to low temperatures (1, 2) and the reduced cold resistance of adrenalectomized animals (3-5), as indications of the importance of the adrenal glands in the acquisition of resistance to low temperatures. However, in studies on acclimation to other types of stressing conditions (6, 7) it has been found that adrenalectomized animals, not treated with adrenal hormones, would develop a certain degree of resistance if maintained on a saline drinking solution and exposed only gradually to the stressor.

The possibility that cold resistance might be developed in adrenalectomized animals under similar conditions has been considered in the present investigations. In a first series of experiments, cold resistance of normal and adrenalectomized rats with or without implanted DCA pellets was compared after warm or cold acclimation. In a second series, cold resistance was determined in adrenalectomized warm and cold acclimated rats that were examined, by three different methods, for evidence of accessory or regenerated adrenal tissue.

EXPERIMENTAL PROCEDURES

Series I. One hundred and six male Sprague Dawley white rats (mean weight 242 gm range 200-284 gm) were assigned to four groups. Treatment received by each group is indicated below: group I, 40 intact controls; group II, 20 sham operated; group III, 32 bilaterally adrenalectomized (6) and each implanted with a pellet (15 ± 0.3 mg) of desoxycorticosterone acetate (DCA); group IV, 14 bilaterally adrenalectomized not supplied with DCA.

Each of the above groups was then divided into two subgroups, one of which was acclimated at 30°C for 44 days and the other maintained at 30°C with the following exposure to cold: a) days 6-9 exposed daily for 4 hours (9:00 A.M. to 1:00 P.M.) to 15°C; b) days 10-12 exposed daily for 4 hours (9:00 A.M. to 1:00 P.M.) to 6°C; c) days 13-44 exposed daily for 6 hours (9:00 A.M. to 3:00 P.M.) to 6°C.

During acclimation, the animals were weighed at 4-day intervals. Control and sham-operated animals were supplied 'ad libitum' with Master Fox Chow and tap water while all the adrenalectomized rats received Master Fox Chow and 0.9% saline drinking solution.

Series II. After adrenalectomy, 20 Sprague Dawley rats (mean weight 202 gm range 183-226) were allotted at random to two groups of 10 rats that were warm or cold acclimated with temperature conditions, food and saline as previously described in series I. To detect any regenerated adrenal tissue, the adrenalectomized rats were subjected at various intervals to the epinephrine test (9) and the water tolerance test (10). For the epinephrine test, the number of circulating eosinophils before and after a subcutaneous injection in the neck of 200 µg (in 1 ml) of epinephrine was estimated by Speirs and Meyer's method (11), using essentially the technique described previously (12). Cold exposures were not made on days when water tolerance tests were carried out. The technique used was assessed by repeating the tolerance test on a group of five intact rats maintained at room temperature (mean weight: 357 gm range 290-347 gm).

After acclimation, all animals in series I and II were subjected to 12-hour survival tests with food but no water at -18°C following procedures previously described (13). After death, final weight of pellets of DCA was determined, animals of series II were autopsied and histological examination was made of all pieces of adrenal-like tissue found on the dorsal peritoneal wall between the kidney and the diaphragm.

RESULTS

Series I. Gain in weight. Difference in acclimation temperatures had no significant effect on the amount of weight gained by the test animals (table I). Within each acclimation group, however, normal and sham-operated rats, as well as adrenalectomized DCA implanted rats had equivalent weight gain whereas those not given the pellet had a much lower gain in weight (table I).

DCA absorption. There was no apparent...
Table 1. Initial and final average weights of normal and adrenalectomized rats after warm and cold acclimation

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<td>gm</td>
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<tr>
<td>Warm acclimation</td>
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<tr>
<td>I. Intact controls</td>
<td>233.6</td>
<td>364.0</td>
<td>130.4</td>
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<td></td>
<td>(20)</td>
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<tr>
<td>II. Sham-operated</td>
<td>253.7</td>
<td>376.3</td>
<td>122.6</td>
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<td></td>
<td>(10)</td>
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<tr>
<td>III. Adrenalecto-</td>
<td>234.0</td>
<td>358.7</td>
<td>124.7</td>
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<td>mized + DCA</td>
<td>(7)</td>
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<td>IV. Adrenalecto-</td>
<td>246.7</td>
<td>334.8</td>
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<td>mized, no DCA</td>
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<td>Cold acclimation</td>
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<td>I. Intact controls</td>
<td>237.7</td>
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<td>II. Sham-operated</td>
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<td>III. Adrenalecto-</td>
<td>239.1</td>
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<td>mized, no DCA</td>
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* No. of rats. † Standard error.

The difference in the DCA absorption of warm acclimated and cold acclimated adrenalectomized rats. The average final DCA weight adjusted* for common initial weight (15.1 mg) was 6.9 ± 0.5 for the former group and 7.3 ± 0.5 mg for the latter group.

Survival. Under each acclimation condition, intact and sham-operated rats survived appreciably longer on the average than the adrenalectomized ones, the difference achieving the 1% level of statistical significance (table 2). After cold acclimation, however, adrenalectomized rats, DCA-treated or not, survived longer than any of the warm acclimated animals. Survival of adrenalectomized rats whether warm or cold acclimated was not prolonged by DCA treatment.

Series II. Before adrenalectomy, epinephrine produced a normal decrease in eosinophils.

* On the average, each 10 mg difference in initial weight was associated with a difference of 11.4 mg in final DCA weight, at both acclimation temperatures. Therefore, it was necessary to adjust mean final weights to common mean initial weights in order to make comparisons among the treatments.
in all animals (table 3) and 3 or 4 days after adrenalectomy, as expected, a rise in eosinophils was observed in each animal. This eosinophilia was maintained in all but two or three animals at both temperatures. However, even when temporary decreases were observed, the actual eosinophil levels remained higher than those of normal rats.

With the water tolerance test, a slight positive response (water return >42%) was obtained in one out of five warm acclimated rats and in two out of six acclimated ones, 25-26 days after adrenalectomy. But after 43-44 days, the test was negative in all animals used in survival tests. In our hands, the test appeared reliable, since on the five intact rats, the following water returns were obtained: +59%, +68%, +73%, +78%, +82%.

Four rats in series II died during the cold acclimation period. Analysis of log survival time of the remaining animals showed that, on the average, the cold acclimated rats survived longer than the warm-acclimated ones. This difference achieved the 1% level of statistical significance. No accessory or regenerated adrenal tissue was found around the kidneys of any of these animals.

**DISCUSSION**

Adrenalectomized rats, DCA-treated or not, exposed to 6°C a few hours every day for 39-42 days had a longer survival time at -18°C than normal or adrenalectomized animals at 30°C. Therefore, the adrenal does not appear to be necessary for development of a certain degree of cold acclimation. Although the presence or absence of a DCA pellet in the adrenalectomized groups did not lead to any appreciable differences in survival times, the pellets appeared to be the main factor affecting weight gain in these tests. Therefore, gain in weight and resistance to low temperature were not equivalent criteria of acclimation to cold.

After cold acclimation, the adrenalectomized rats had a much shorter survival time than the sham-operated or the intact animals. Full development of cold acclimation may therefore be impossible without a normally active gland. Alternatively, full acclimation to a low environmental temperature might have been achieved but its complete expression under the severe conditions used, could not be realized in the adrenalectomized rats.

The increased cold resistance of cold acclimated adrenalectomized rats does not seem to be due to active accessory or regenerated adrenal tissue. Tests for adrenal function were negative in all animals used in survival tests. Transitory positive responses which were not consistently observed in rats, were below the values obtained on normal rats and were not correlated at all with cold resistance. Actually, two of the rats which showed a positive response died before the end of the experiment. The histological examination, although of limited value provides further evidence for the absence of adrenal tissue.

Results of the present investigation show that in adrenalectomized rats most probably devoid of any regenerated glands, survival and even some adaptation to low temperature is possible when the change is gradual and sustained exposure to low temperature is avoided. This suggests that adrenal hyperactivity previously observed in cold-exposed animals (1, 2, 14) occurs apparently only in the initial stage of drastic and prolonged metabolic readjustment. This conclusion is also supported by previous observations of normal adrenal activity in fully cold acclimated rats (12, 15, 16).

**SUMMARY**

The cold resistance of adrenalectomized rats supplied with 0.9% NaCl drinking solution, with or without supplementary DCA, was increased by short daily exposures of the animals to 6°C for 39-42 days. This increase in cold resistance was demonstrated by the ability of the animals to survive at -18°C for a longer time than adrenalectomized and non-adrenalectomized warm acclimated animals.

In six cold-acclimated adrenalectomized rats, no evidence of regenerated adrenal tissue could be found: response to epinephrine test or to the water tolerance test was negative and histological examination of the tissue surrounding the kidneys did not reveal any adrenal tissue.

Treatment with a small amount of DCA appeared to be essential for normal gain in
weight of adrenalectomized animals. Although DCA-treated and untreated animals gained weight at different rates, their survival times at $-18^\circ C$ were similar. Thus, gain in weight and survival time were not equivalent criteria of acclimation in those tests.

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