NOR-ADRENALINE AND COLD ACCLIMATION

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The possibility of increasing the degree of cold adaptation by day-to-day administration of I.M. nor-adrenaline to albino rats during cold acclimation has been investigated. Such treatment, as compared to the non-injected ones, did not show any improvement in the tolerance time, rectal temperature, or the swimming capacity of the experimental animals subjected to (i) a constant environmental temperature of +8°C, or (ii) a daily exposure of five hours to −5°C. The total duration of such treatment was two and half months. Analysis of blood, drawn after two hours of cold exposure at the expiry of two and half months, for the group of animals exposed to the latter condition did not show any change either in blood urea or lactic acid level. The blood sugar level, however, was found to be lowered in nor-adrenaline injected acclimated group, whereas no such change was observed in the non-injected acclimated group; thus indicating probably a better acclimation for the latter group towards cold. The overall result indicated that nor-adrenaline could not improve or potentiate the degree of adaptation in animals when administered during the development of cold acclimation.

Nor-adrenaline infusion in cold acclimated man and animal causes increase in metabolic rate\(^1_{,2,3}\). HSIEH and CARLSON\(^4\) have demonstrated a marked calorigenic effect when nor-adrenaline is intramuscularly injected in cold acclimated albino rats and have suggested nor-adrenaline as the mediator or non-shivering thermogenesis. More recent work by LE-BLANC and POULIOT\(^5\) has shown that repeated subcutaneous injection of nor-adrenaline at room temperature in albino rats maintains a higher rectal temperature and allowed longer survival when exposed to −20°C as compared to the controls. The indications that even intramuscular or subcutaneous administration of nor-adrenaline could help in cold acclimation led to the possibility of its general use as a promotor to the process of cold acclimation. It is also known that daily exposure to cold induces partial acclimatization to cold stress\(^6\). With these in view, the present study has been initiated to explore the possibility of improving the degree of cold adaptation in albino rats, subjected daily to intramuscular nor-adrenaline injection during the development of cold acclimation. Cold acclim-
ation was induced either by subjecting the animals to a continuous exposure at +8°C for two and half months, or by a daily exposure of 5 hours at -5°C for the same stipulated period.

**EXPERIMENT AND METHOD**

Male albino rats from laboratory stock, body weight varying from 160-180 gm, were used as experimental animals. Animals were fed ad libitum with standard laboratory diet. The experiment was carried out in two sets, A and B, each consisting of three groups including the control.

**SET A:** Two groups of animals (IIA and IIIA) were maintained at +8°C (approx.) for a period of two and half months. Of these, animals from Group IIIA were given a daily I.M. injection of nor-adrenaline (100 µg/rat) during this period of exposure. The third group (IA) was used as control and was kept at room temperature (31°C approx.). At the end of the stipulated period, the representative animals from all the different groups, were examined for their tolerance time and rectal temperature maintenance in acute cold exposure (-20°C) and swimming time in moderately cold water (+18°C).

**SET B:** In this set, all the three groups (IIB, IIIB and IIIB) of animals were kept at room temperature (31°C approx.). Animals belonging to Groups IIIB and IIIB received a daily exposure (excepting on Sundays) of 5 hours at -5°C in a current of air for a period of two and half months. Lesser time of exposure was given to animals during the first three days of the experiment. During the entire period of exposure, animals belonging to Group IIIB were daily subjected to intramuscular nor-adrenaline (100 µg/rat) injection. At the end of two and half months, the representative animals from all the groups were examined for rectal temperature maintenance and swimming time; their blood was also analysed for glucose, urea and lactic acid levels.

During the study of tolerance time, the nor-adrenaline treated animals of Group IIIA received their usual dosage of drug before the exposure. Tolerance time was taken as the time needed by the animals to lie prostrate. Animals under such condition, did not respond to pricking or show any other sign of life except for a very feeble respiration. When brought back to room temperature such prostrated animals took about an hour to come back to normal consciousness. The animals were brought back to normal condition after studying their tolerance time on first exposure at -20°C and were kept in their respective environmental temperatures. On the following fourth day, animals of Groups IIA and IIIA received a second exposure at the same acute cold temperature (-20°C) for eleven hours; animals were then brought back to normal condition and kept in their respective environmental temperatures. On the eighth day, they were again exposed to -20°C for the third time and their tolerance time was noted.

Rectal temperature records for all the groups in Set A were taken with thermocouples at hourly intervals while exposing the animals to -20°C for a period of 8 hrs. Similar record were made for animals in Set B by noting the rectal temperature after 2 hours and 4 hours of exposure at -5°C (in a current of air). The nor-adrenaline injected groups (IIIA and IIIB) in both sets were given their usual injection at the start of exposure. All animals were fasted for 24 hrs. before subjecting them to rectal temperature study.

Swimming time for animals in Set A was recorded by transferring them from their normal environmental temperature to a water bath, maintained at +18°C±0.5°C. Animals belonging to Set B, however, were first exposed to -5°C (in a current of air) for 1 hour, and then placed in a water-bath kept at 9°C±0.5°C. The nor-adrenaline injected groups IIIA and IIIB received their usual dosage, 1 hour prior to swimming. Drowning of the
animals for 30 secs. was taken as the end point for swimming time. The swimming
time for seven random cases was found to be very high as compared to that of other
members of the same group. Such cases were not taken into consideration. It was
noted that these animals did not move their legs as frequently as others.

Blood was drawn from the heart of animals, fasted overnight, under ether anaesthesia
and analysed. In Set B, blood was taken from representative animals of all groups at
room temperature and after two hours of cold exposure at \(-5^\circ\mathrm{C}\) (in a current of air).
Group III\(_B\) animals received their usual nor-adrenaline injection just before cold exposure.
Another set of blood sugar analysis was carried out for Group III\(_B\) animals subjected
to nor-adrenaline injection at room temperature, the blood was drawn 15 mins. after
injection.

Karr's method was used for serum urea estimation, Nelson and Samogy's method
was employed for the estimation of blood sugar and Barker and Summerson's method
for the determination of lactic acid\(^7\). Folin and Ciocalteu's method was used for
serum protein estimation\(^8\).

RESULTS AND DISCUSSION

It can be seen from Table 1, that the tolerance times for Groups II\(_A\) and
III\(_A\) were very much longer than Group I\(_A\) (control), when all the groups in
Set A were exposed to \(-20^\circ\mathrm{C}\). The tolerance time of Group II\(_A\), however,
was 12 hrs. 52 mins. \(\pm 25\) mins. as compared to 13 hrs. 10 mins. \(\pm 21\) mins. for
Group III\(_A\). This difference in the tolerance time is not statistically significant.
Hence it is clear that long term intramuscular nor-adrenaline injection (approx.
500\(\mu\)g/Kg B. W.) to animals maintained at \(+8^\circ\mathrm{C}\) does not help them to tolerate
acute cold exposure \((-20^\circ\mathrm{C})\) longer. It may be argued that by cold acclimation
at \(+8^\circ\mathrm{C}\) these animals reached their maximum tolerance capacity at \(-20^\circ\mathrm{C},\)
so that the time of tolerance could not be further improved by any physiological means. This conclusion is not so, as shown by the fact that repetition
of exposure at \(-20^\circ\mathrm{C}\) helped both Groups II\(_A\) and III\(_A\) to increase their tolerance
at \(-20^\circ\mathrm{C}\) (Table 1). The present study thus clearly indicates that mild cold
exposure helps in the process of cold acclimation, whereas long term nor-adrenaline injection to animals during the process of acclimation to mild cold
does not help in further improving their cold acclimation capacity.

Rectal temperatures (Table 2) were recorded for Set A up to 8 hours when
all the groups were exposed to \(-20^\circ\mathrm{C}\). In the control group (Group I\(_A\)) rectal
temperature started to decline after 1 hour of exposure and within 4 hours
the mean value was as low as 68.5\(^{\circ}\mathrm{F}\). Animals belonging to the control group
started lying prostrate at this stage and in about 5 hours none was in the
conscious state. Animals of Groups II\(_A\) and III\(_A\) maintained normal body
temperature up to 6th hour of exposure, on the 7th hour their rectal temperature
started declining and at the 8th hour their body temperature did decrease
substantially. Both the groups behaved similarly. Thus long term nor-adrenaline administration during the process of cold acclimation at \(+8^\circ\mathrm{C}\) did not
help the animals (Group III\(_A\)) to maintain body temperature in acute cold.
### Table 1. Tolerance time at −20°C

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Group**</th>
<th>Pre-treatment</th>
<th>Tolerance time at −20°C mean S.D.</th>
<th>Comparison with respect of Group I_A on 1st exposure</th>
<th>Comparison with respect to the corresponding groups on 1st and 3rd exposure</th>
<th>Comparison among groups II_A and III_A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I_A</td>
<td>Room Temp.</td>
<td>4 hrs, 53 mins. ±31 mins.</td>
<td></td>
<td>&lt;.001</td>
<td>0.29</td>
</tr>
<tr>
<td>Set A</td>
<td>II_A</td>
<td>+8°C</td>
<td>12 hrs, 52 mins. ±25 mins.</td>
<td></td>
<td>&lt;.001</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>III_A</td>
<td>+8°C and N.A.*</td>
<td>13 hrs, 10 mins. ±21 mins.</td>
<td></td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

* N.A.—Nor-adrenaline. ** Six animals in each group. For other details see text.

### Table 2. Rectal temperature during cold exposure

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Group</th>
<th>Pre-treatment</th>
<th>Temp. of Cold exposure</th>
<th>Rectal Temperature (°F) &amp; hrs. of cold exposure Mean±S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 hr.</td>
<td>2 hrs.</td>
</tr>
<tr>
<td>Set A</td>
<td>I_A</td>
<td>Room Temp.</td>
<td>-20°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II_A</td>
<td>+8°C</td>
<td></td>
<td>86.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>±10.6</td>
<td>±1.3</td>
</tr>
<tr>
<td></td>
<td>III_A</td>
<td>+8°C and N.A.</td>
<td></td>
<td>99.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>±1.8</td>
<td>±1.2</td>
</tr>
<tr>
<td>Set B</td>
<td>I_B</td>
<td>Room Temp.</td>
<td>-5°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II_B</td>
<td>-5°C 5 hr./day</td>
<td></td>
<td>92.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>±1.8</td>
<td>±1.8</td>
</tr>
<tr>
<td></td>
<td>III_B</td>
<td>-5°C 5 hr./day and N.A.</td>
<td></td>
<td>93.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>±1.5</td>
<td>±1.5</td>
</tr>
</tbody>
</table>

Six animals in each group. N.B.—In set B cold exposure was always accompanied with rapid air circulation.
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TABLE 3.
Swimming time.

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Group*</th>
<th>Immediate pre-treatment</th>
<th>Bath temperature</th>
<th>Swimming time Mean—S. D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set A</td>
<td>IA</td>
<td>Room temperature</td>
<td>+18°C±0.5°C</td>
<td>14 min. 26 sec. ± 1 min. 5 sec.</td>
</tr>
<tr>
<td></td>
<td>II A</td>
<td>+8°C</td>
<td>&quot;</td>
<td>13 min. 30 sec. ± 1 min. 33 sec.</td>
</tr>
<tr>
<td></td>
<td>III A</td>
<td>+8°C</td>
<td>&quot;</td>
<td>13 min. 45 sec. ± 1 min. 20 sec.</td>
</tr>
<tr>
<td>Set B</td>
<td>IB</td>
<td>-5°C (in a current of air)</td>
<td>+9°C±0.5°C</td>
<td>8 min. 20 sec. ± 1 min. 9 sec.</td>
</tr>
<tr>
<td></td>
<td>II B</td>
<td>&quot;</td>
<td>&quot;</td>
<td>9 min. 0 sec. ± 20 sec.</td>
</tr>
<tr>
<td></td>
<td>III B</td>
<td>&quot; and N. A.</td>
<td>&quot;</td>
<td>8 min. 23 sec. ± 32 sec.</td>
</tr>
</tbody>
</table>

* Ten animals in each group.

exposure, any better than the non-injected ones (Group II A). On the other hand animals acclimated to mild cold, injected or otherwise, were able to maintain their body temperature much better than the non-acclimated control animals.

Data on swimming time did not reveal any change in the swimming capacity at +18°C of the different groups pertaining to Set A (Table 3). No difference in swimming capacity of the control (Group IA) and the experimental groups (Groups II A and III A) was rather unexpected. It might be possible that cold acclimated groups would show better swimming capability in a more acute cold bath as survival time and rectal temperature were improved under acute cold stress. In any case, swimming time data indicated that nor-adrenaline treatment did not help the cold acclimated animals to have a better swimming capability.

It may be observed from Table 2, that all the three groups in Set B became hypothermic within 2 hours of cold exposure at -5°C (in a current of air) and there was no further significant dropping of body temperature in any of them within 4 hours.

The swimming time of the different groups in Set B at +9°C also did not show any significant difference (Table 3). Thus, daily exposure to animals at -5°C (in a current of air) improved neither the rectal temperature nor the swimming time as compared to the control. Long term nor-adrenaline injection in such cold exposed animals also failed to make any improvement.

There was no difference of serum urea and blood lactic acid levels between the three groups of Set B at the basal level or after 2 hours of cold exposure.

From the results obtained so far with Set A and Set B animals; it seems unlikely that nor-adrenaline release is the main physiological factor in cold
acclimatization. An analysis of blood sugar level of the different groups in Set B further substantiates this conclusion. Basal blood sugar level for groups IB, IIB and IIIB animals, fasted overnight, were 118±6.6 mg%, 113±5.8 mg% and 117±6.3 mg% respectively. After 2 hours of cold exposure at −5°C (in a current of air), the respective values were 95±18.4 mg%, 117±6.2 mg% and 95±16.6 mg%. The control (Group IB) and the nor-adrenaline injected partially adapted (Group IIIB) did not indicate any change. Blood sugar of Group IIIB animals increased markedly (154±5.2 mg%) after 15 mins. of nor-adrenaline injection, and yet its value fell below the basal level, after 2 hours of cold exposure at −5°C. The maintenance of blood sugar at the basal level even after 2 hours of cold exposure as in Group IIB (non-injected partially acclimated) is a possible indication of better acclimation of this group as compared to the completely unadapted control group (Group IB). In contrast, Group IIIB shows a close similarity in blood sugar values to Group IB after 2 hours of cold exposure. GHOSE et al. have reported that single injection of nor-adrenaline given to unadapted albino rats markedly increase after 15 minutes of injection their blood sugar level both at room temperature and at −5°C. On continuing the exposure at −5°C (in a current of air) for 2 hours, however, the blood sugar level falls below the basal value. The close similarity in the blood sugar picture between groups of animals, daily injected and partially acclimated to cold (Group IIIB), non-injected unacclimated (Group IB) and singly injected unacclimated, also their dissimilarity to non-injected partially acclimated group (Group IIIB), raise a suspicion that daily administration of nor-adrenaline to albino rats during the development of cold acclimation may act as a hindrance to the development of certain biochemical processes normally found in cold adaptation.

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