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Successful Cold Acclimation Following Bilateral Adrenodemedullation in Rats (40702)

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Although adrenodemedullated rats have been exposed to cold environments (2 to 4°C) for a few hours (1–5) or up to many days (6–9), the contribution of the adrenal medulla in developing cold acclimation remains unclear. Gilgen *et al.* (3) reported that demedullated rats failed to survive in 4°C exposure, dying within 11 hr of exposure. Colonic temperatures were reported to decrease by 4.2°C (1) and 6.1°C (2) after exposure to 4°C environments for 8 or 3 hr, respectively. However, Pouliot (6) found no changes in the colonic temperature of demedullated rats after 6 days in 3°C. Manara *et al.* (4) reported no difference in oxygen uptake between intact and demedullated rats following 1 hr of exposure to 4°C. Conversely, Ring (5) observed a 10% lower oxygen uptake in demedullated than in intact rats acutely exposed to 2–4°C for 3 hr. However, the body temperature of his demedullated rats had fallen below 30°C while the body temperature of intact animals was approximately 33°C. Schönbaum *et al.* (7, 8) have suggested that adrenodemedullated rats given adequate time to permit full regeneration of the adrenal cortex could survive in a cold environment for many weeks and develop the same increased resistance to cold as intact animals. In view of these conflicting results the present study was designed to determine whether adrenodemedullated rats could be successfully cold acclimated (c-a) as indicated by their survival and their calorigenic responses to tyramine and a more intensive cold stimulus of −10°C.

Methods. Twenty-four male Sprague–Dawley rats,[1] each initially weighing 150 g, were randomly divided into two groups. Bilateral adrenodemedullation was performed on one group under sodium pentobarbital (40 mg/kg body wt, ip) anesthesia (10). Three days were allowed for recovery following surgery. Half of the animals in each group were then placed in an environmental chamber at 5°C (±1°C) (c-a), the other rats being maintained at 23°C (±1°C) (non-c-a). All animals were housed in individual cages and Purina Laboratory Chow and water were provided *ad libitum*. A 12-hr light–dark cycle was maintained and food intake and body weight were recorded twice weekly. At the end of the 5-week acclimation period the calorigenic response to an injection of tyramine was determined in all animals. Four or five days later they were exposed to a colder environment (−10°C). Oxygen uptake was measured in a constant-volume, closed system (volume meter, Med Science, model 160) with the rat in a sealed Plexiglas chamber and the expired CO_2 absorbed by soda lime. Two constant-temperature baths were utilized to maintain chamber temperatures of either 28 or −10°C. Colonic and chamber temperatures were continuously recorded on a dual-channel recorder utilizing thermistor probes. Oxygen uptakes and colonic temperatures were first measured at 28°C for 30 min to establish a baseline and to determine the influence of cold acclimation on oxygen uptake and colonic temperature. The animals were then temporarily removed from the chamber, given tyramine–HCl (20 mg/kg body wt, ip) and returned to the chamber for another hour of measurement. Heroux *et al.* (11) had determined that 20 mg/kg of body weight dosage could always elicit a maximum response without apparent toxicity to animals. We were interested in finding a dosage below 20 mg/kg of body weight that might be sufficient in inducing maximal response and determined a tyramine dose–response curve on nine animals using 5, 10, and 20 mg/kg of body weight. However, our finding only served to confirm the observation of Heroux *et al.* Cold tolerance was determined by first

[1] In conducting this research, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee for Laboratory Animal Facilities and Care, of the Institute of Animal Laboratory Resources, National Academy of Sciences–National Research Council.

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measuring oxygen uptakes and colonic temperatures at 28°C and then transferring the animal to the cold bath (−10°C). Measurements of oxygen uptake and colonic temperature were continued for 3 hr or until the animal's colonic temperature reached 17°C. The experimental data were analyzed statistically by a three-factor factorial analysis of variance with repeated measures across time. Where significant interaction effects were found, tests of simple main effects followed. The 0.05 level of significance was selected for rejection of the null hypothesis (12). Since some animals had to be removed before the 3-hr period ended, the analysis of the cold tolerance data was made on the first 100 min of cold exposure. Decreases of colonic temperature in −10°C were analyzed by a series of linear regressions across time to determine the best fitting curve. Significant differences between regression coefficients were determined by the *t*-test.

Results. Removal of the adrenal medulla did not adversely influence growth as indicated by the similarity in mean body weights of the intact and demedullated rats living in either 23 or 5°C. There were no significant differences in either O_2 uptake or colonic temperature measured at 28°C between intact and demedullated rats (Table I). All c-a animals had significantly higher O_2 uptakes ($P < 0.05$) measured at 28°C than the non-c-a animals (Fig. 1, Table I). There was no significant difference in the response of intact or demedullated animals consequent to the cold acclimation period.

All animals had significant increases ($P < 0.01$) in O_2 uptake and colonic temperatures subsequent to the injection of tyramine. In those animals maintained at 23°C, intact rats showed a 55.5% increase in O_2 uptake from 207 to 322 ml/kg 10 min^{-1} while demedullated rats increased 66.8% from 184 to 307 ml/kg 10 min^{-1}. In the c-a groups, intact rats raised their O_2 uptakes by 93.5% (248 to 480 ml/kg 10 min^{-1}) while the demedullated rats showed a 69.7% (234 to 397 ml/kg 10 min^{-1}) increase. Non-c-a rats had 2.1 and 1.7°C increases in colonic temperature while c-a rats exhibited elevations of 3.7 and 3.0°C, respectively, for the intact and demedullated animals. The increases in O_2 uptake and colonic temperature were significantly ($P < 0.01$) greater in c-a animals than in non-c-a animals. There were no significant differences in the increases of O_2 uptake or colonic temperature between the intact and demedullated groups; however, intact c-a rats had significantly ($P < 0.01$) greater increases in O_2 uptake following tyramine than demedullated c-a rats (Fig. 1).

Upon exposure to −10°C there was an immediate increase in oxygen uptake in all rats which attained its highest level some 30 to 40 min after the onset of exposure and then gradually declined. All c-a rats had significantly ($P < 0.05$) higher oxygen uptakes (2720 ml/kg 100 min^{-1} increase over basal uptake in the intact rats and 2863 in the demedullated) than the non-c-a rats (2060 for the intact and 1778 for the demedullated). However, there was no significant difference between the intact and demedullated animals in either the c-a or non-c-a groups (Fig. 2). All animals exhibited a gradual decline in colonic temperature with increased duration of cold exposure. The decline in colonic temperature was significantly ($P < 0.001$) faster non-c-a rats (0.103°C/min for intact and 0.120°C/min for the demedullated) than in the c-a rats (0.054°C/min for the intact and 0.058°C/min for the demedullated). In the non-c-a groups the colonic temperature of demedullated animals decreased at a significantly ($P < 0.05$) faster rate than in intact rats (Fig. 3, Table II).

TABLE I. BODY WEIGHTS, OXYGEN UPTAKES, AND COLONIC TEMPERATURES OF INTACT AND DEMEDULLATED ANIMALS MEASURED AT 28°C FOLLOWING 5 WEEKS OF ACCLIMATION[a]

	Body weight (g)	O_2 Uptake (ml/kg 10 min^{-1})	Colonic temperature (°C)
Non-c-a, intact	405 ± 22.4	207 ± 9.6	36.9 ± 0.2
Non-c-a, demedullated	396 ± 15.6	184 ± 10.2	37.0 ± 0.2
c-a, intact	359 ± 22.8	248 ± 14.7[b]	36.6 ± 0.3
c-a, demedullated	375 ± 11.2	234 ± 7.5[b]	37.1 ± 0.2

[a] Mean ± SE of six animals in each group.
[b] Significantly ($P < 0.05$) higher than corresponding animals maintained at 23°C.

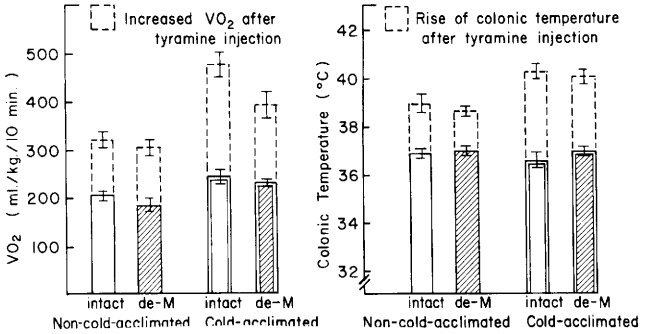

FIG. 1. Oxygen uptakes and colonic temperatures in response to tyramine: Oxygen uptake (left) and colonic temperature (right) measured at 28°C in intact and demedullated animals acclimated at 5°C (c-a) or 23°C (non-c-a) before and after injection of tyramine. Each bar represents the mean ± SE of values from six rats.

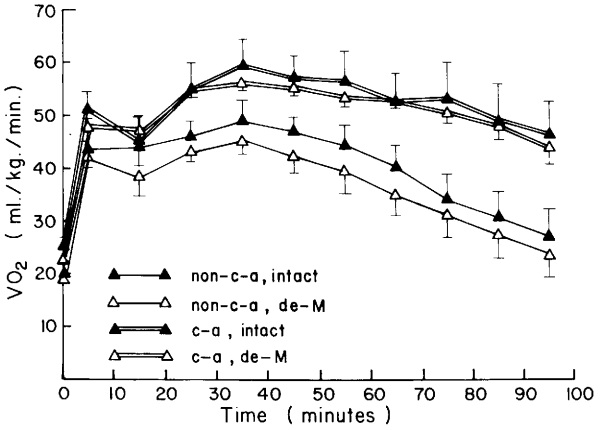

FIG. 2. Oxygen uptakes during first 100 min of exposure to −10°C. Each data point represents average oxygen uptake (ml/kg min^{-1}) in each 10-min period during exposure. Each line represents the mean ± SE of six rats.

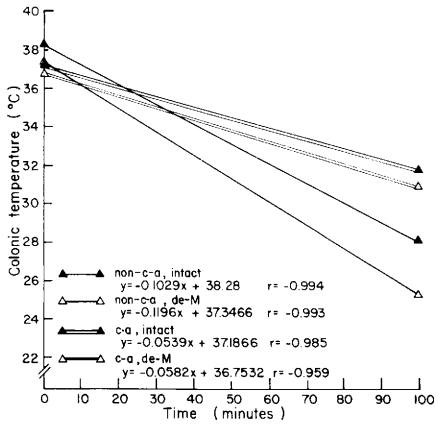

FIG. 3. Regression lines of fall of colonic temperature across initial 100 min time in exposure to cold environment of −10°C. Each line represents a group of six rats. The symbols on each line are for the purpose of identification of groups.

TABLE II. COMPARISON OF REGRESSION COEFFICIENTS (β) FOR PREDICTION OF CHANGES IN COLONIC TEMPERATURE IN −10°C WITH TIME (100 min).

	β	t	df	p
Intact vs demedullated				
Intact, non-c-a	−0.1029			
Demedullated, non-c-a	−0.1196	2.8	8	0.05
Intact, c-a	−0.0539			
Demedullated, c-a	−0.0582	0.96	8	n.s.
Non-cold-acclimated vs cold-acclimated				
Non-c-a, intact	−0.1029			
c-a, intact	−0.0539	11.13	8	0.001
Non-c-a, demedullated	−0.1196			
c-a, demedullated	−0.0582	8.5	8	0.001

Discussion. Despite some conflicting evidence that demedullation could result in a failure to survive a cold stress (3), there is

suggestive information that cold acclimation in demedullated animals could occur (6–9). Our data provide convincing evidence that demedullated rats do undergo typical acclimation processes. Pouliot (6) stated that demedullated rats were all normal and normothermic after 6 days of cold exposure. Schönbaum *et al.* (7, 8) observed that demedullated rats after living 2 weeks in 2°C had decreased electromyographic activity in cold compared to non-cold-acclimated, demedullated rats. Jarratt and Nowell (9) noted that the absence of the adrenal medulla did not prevent the usual fluctuations in the blood sugar level observed in intact rats during chronic cold exposure of 130 days. However, the sustained hyperglycemia recorded in prolonged exposure in intact rats was not observed in the demedullated rats.

Evidence that cold acclimation of demedullated rats was similar to that observed in intact rats was shown by: (i) successful survival through 5 weeks of exposure to 5°C, (ii) oxygen uptake measured at 28°C following cold acclimation was raised to the same level observed in intact cold-acclimated animals, (iii) significantly higher increases in oxygen uptake and colonic temperature in response to tyramine administration following cold acclimation although the increase in demedullated animals was less than the increase in intact rats, and (iv) during −10°C exposure increases in oxygen uptake and rates of decrease of colonic temperature were similar to those observed in intact cold-acclimated rats.

During the −10°C exposure colonic temperature decreased at a significantly faster rate in demedullated non-c-a rats than in intact non-c-a rats. In non-cold-acclimated rats the main mode of heat production in cold exposure is shivering thermogenesis. Demedullated animals shiver more vigorously during cold exposure than intact animals (7, 13). The more intense and vigorous shivering in the demedullated animals probably was responsible for the greater heat loss than that seen in intact animals and therefore resulted in a more rapid fall in colonic temperature. Berti *et al.* (1), Maickel *et al.* (2), and Gilgen *et al.* (3), who exposed their intact and demedullated non-cold-acclimated rats to a moderate cold stress of 4°C, also observed that demedullated rats had a more rapid decrease in colonic temperature than did intact rats.

The enhanced calorigenic response to tyramine in c-a rats, intact or demedullated, further confirmed the development of norepinephrine (N-E) thermogenesis and receptor hypersensitivity in cold acclimation. Tyramine acts by releasing endogenous N-E (14). It is not clear whether the enhanced tyramine response in c-a animals is due to increased N-E release or increased sensitivity of β-receptors on the effector cells. The increased β-receptor responsiveness has been elegantly demonstrated by Le Blanc (15) and most recently by Fregly *et al.* (16) in intact c-a rats. There is no direct evidence that tyramine released more N-E in c-a rats although the urinary N-E excretion has been observed to be elevated in these animals (17). However, the smaller increase in oxygen uptake observed in the demedullated than in the intact c-a rats suggested the development of some other adaptive mechanisms. We calculated the heat production and body heat content of both groups of rats before and after the tyramine administration based on body weights, oxygen uptakes, and colonic temperatures. There was no difference in heat production (0.44 kcal/10 min) of the intact or demedullated rats before tyramine, although the mean colonic temperature of the demedullated rats was 0.5°C higher than that of the intact animals (Fig. 1, Table I). Following the tyramine injection intact animals produced more heat than demedullated rats (0.83 kcal/10 min for intact and 0.72 for demedullated; $P < 0.05$). The mean colonic temperatures of both groups after tyramine were similar (40.3 and 40.1°C for the intact and demedullated, respectively). Thus the demedullated rats were not only able to attain a higher colonic temperature than intact rats at comparable levels of heat production prior to tyramine administration, but maintained a similar colonic temperature with a smaller heat production following administration of tyramine. This suggests the possibility that enhanced peripheral vasoconstriction brought about by N-E in the demedullated animals resulted in a lowered heat loss to the environment.

Our results are at variance with the observations of Cottle and Carlson (18). They reported that rectal temperature fell and that

the increased heat production in demedullated cold-acclimated rats exposed to 5°C was significantly less than that in the intact animals. The response to 5°C of their demedullated 28°C acclimated rats did not differ from that of their intact 28°C animals. In their experimental protocol, however, the rats were first cold acclimated at 5°C, demedullated, and then returned to the cold room for additional cold acclimation. Their animals were also anesthetized, curarized, and artificially ventilated when evaluated at 5°C. These differences in experimental design may well explain the difference in results obtained.

Our demedullated rats living at 5°C apparently survived the handicap of not having epinephrine from adrenal medulla available to them. This may have been a consequence of substitution of other catecholamines in the early stage of cold acclimation since increased excretion of norepinephrine has been reported to occur during the first 2 weeks following demedullation (19). Immediate increase of norepinephrine urinary excretion following cold exposure (2–4°C) of demedullated animals has been observed within the first 24 hr (6, 8, 17, 20). Our demedullated rats may have developed norepinephrine thermogenesis sooner than intact rats; however, some epinephrine sources may have been available. The urinary epinephrine excretion by cold-exposed, demedullated rats has been reported to be either nonexistent (21) or slightly increased in the cold-exposed than the non-cold-exposed animals (6, 8, 19, 20, 22). This slight increase in epinephrine excretion in cold-exposed, demedullated animals may have come from extramedullary chromaffin cells although there has been no experimental evidence to support this.

Our data indicate that adrenal medullary catecholamines are not necessary for successful cold acclimation but they do not denigrate the potential role of epinephrine in the acclimation process. They simply indicate the ability of an organism to meet a stressful situation by utilizing alternative resources.

Summary. Bilaterally adrenodemedullated rats were successfully cold acclimated at 5°C for 5 weeks. This was evidenced by (i) successful survival, (ii) elevated oxygen uptake measured at 28°C, (iii) enhanced calorigenic response to tyramine although demedullated rats had significantly lower oxygen uptakes than the intact rats, and (iv) an increased tolerance to cold (−10°C) similar to that observed in intact rats after 5 weeks of acclimation. Non-cold-acclimated demedullated rats were unable to maintain their colonic temperature to the same extent as the intact non-cold-acclimated rats during exposure to −10°C. All cold-acclimated rats had greater response to tyramine and better tolerance to −10°C than non-cold-acclimated rats.

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1. Berti, F., Lentati, R., and Usardi, M. M., Med. Pharmacol. Exp. **13,** 227 (1965).
2. Maickel, R. P., Matsussek, N., Stern, D. N., and Brodie, B. B., J. Pharmacol. Exp. Ther. **157,** 103 (1967).
3. Gilgen, A., Maickel, R. P., Nikodizevic, O., and Brodie, B. B., Life Sci. **12,** 709 (1962).
4. Manara, L., Costa, E., Stern, D. N., and Maickel, R. P., Int. J. Neuropharmacol. **4,** 301 (1965).
5. Ring, G. C., Amer. J. Physiol. **137,** 582 (1942).
6. Pouliot, M., Acta Physiol. Scand. **68,** 164 (1966).
7. Schönbaum, E., Sellers, E. A., and Johnson, G. E., Canad. J. Biochem. Physiol. **41,** 975 (1963).
8. Schönbaum, E., Johnson, G. E., and Sellers, E. A., Amer. J. Physiol. **211,** 647 (1966).
9. Jarratt, A. M., and Nowell, N. W., Canad. J. Physiol. Pharmacol. **47,** 1 (1969).
10. Evans, G., Amer. J. Physiol. **114,** 297 (1935).
11. Heroux, O., Pagé, E., Le Blanc, J., Leduc, J., Gilbert, R., Villemaire, A., and Rivest, P., J. Appl. Physiol. **38,** 436 (1975).
12. Winer, B. J., "Statistical Principles in Experimental Design," p. 539. McGraw-Hill, New York (1971).
13. Cannon, W. B., Querido, A., Britton, S. W., and Bright, E. M., Amer. J. Physiol. **79,** 465 (1927).
14. Neff, N. H., Tozer, T. N., Hammer, W., and Brodie, B. B., Life Sci. **4,** 1869 (1965).
15. Le Blanc, J., "Catecholamines and Stress," p. 409. Pergamon, Elmsford, N. Y. (1976).
16. Fregly, M. J., Field, F. P., and Nelson, E. L., Jr., J. Appl. Physiol. **42,** 349 (1977).
17. Leduc, J., Acta Physiol. Scand. Suppl. 53, **183,** 5 (1961).
18. Cottle, W. H., and Carlson, L. D., Proc. Soc. Exp. Biol. Med. **92,** 845 (1956).
19. Biscardi, A. M., Carpi, A., and Orsinger, O. A., Brit. J. Pharmacol. **23,** 529 (1964).
20. Johnson, G. E., and Pritzker, K., J. Pharmacol. Exp. Ther. **152,** 432 (1966).
21. Le Blanc, J. A., and Nadeau, G., Canad. J. Biochem. Physiol. **39,** 215 (1961).
22. Johnson, D. G., Acta Physiol. Scand. **68,** 129 (1966).

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