Journal of Gerontology
1985, Vol. 40, No. 1, 8–14

A Longitudinal Study of Tolerance to Cold Stress Among C57BL/6J Mice[1]

Mark I. Talan, Bernard T. Engel, and John R. Whitaker[2]

C57BL/6J male mice of different ages were movement-restricted and exposed to 10 °C for 3-hr periods every other week while colonic temperature was measured. A longitudinal trend in cold tolerance related to age and to initial colonic temperature was demonstrated. Adaptative thermoregulatory changes during cold exposure occurred during the first two tests. These were similar for all age groups except 30-month-old mice. There was no adaptation of colonic temperature during cold exposure among aged mice with repeated testing; however, their baseline colonic temperatures prior to testing increased after the first two tests. This finding suggests that old animals adjust to repeated cold stress differently than do younger mice. Specifically, younger animals are capable of adjusting their thermoregulatory responses *during* cold stress with no change in baseline (pre-stress) temperature. Old animals do not modify the responses emitted during the stress; however, they do adapt by raising their baseline temperatures. Repeated cold exposure started later in life increased mortality among old animals but did not affect maximum lifespan.

Key Words: Cold exposure, Cold stress, Cold adaptation, Age, Mouse

IN a previous report (Talan & Engel, 1984) we confirmed earlier findings (Estler, 1971; Finch et al., 1969; Grad & Kral, 1957; Trujillo et al., 1962) that the ability of old mice to tolerate cold exposure was significantly poorer than that of young adult animals. Our findings were based on a 3-hr cold stress test (10 °C) using restrained mice. Neither our findings nor any others were based on cross-sectional samples representative of the life span; all involved comparisons of two age groups only. Thus, it is unclear whether the age differences observed indicate age trends or merely differences in tolerance to cold. Furthermore, there are no data based on a longitudinal study with repeated cold exposure that could reveal age-related differences in adaptation to cold because it is already known that cold-acclimated animals can increase heat production during cold exposure (see Hart, 1963, 1971; Jansky, 1966). Moreover, there is no indication in the literature of how repeated cold exposure might affect chronic body temperature (i.e., temperature between exposures). It would be reasonable to expect that animals of different ages respond differently on repeated cold exposure with respect to both changes in cold tolerance and chronic changes in body temperature.

The objective of the present study was to test the following hypotheses: (a) Repeated exposure to cold differentially alters the ability of C57BL/6J mice of different ages to tolerate cold. (b) There are longitudinal trends in cold tolerance that are related to the age of the animals at the time of first testing. (c) Repeated cold exposure alters body temperature under ambient conditions, and this alteration is age related. (d) Repeated exposure to cold affects survival of aged mice.

Material and Methods

Sample. — C57BL/6J mice kept in Gerontology Research Center (GRC) animal colony originally were obtained from the Jackson Laboratory at the age of 5 weeks. Longevity of this strain of mice in this Institute was evaluated previously (Goodrick, 1975). Mean longevity (and standard error) was determined for males as 827 ± 34.2 days; median lifespan was about 28 months and longest-lived 10% was about 32 months. Animals were housed in standard plastic cages (5 to 6 per cage) with sawdust bedding. Filtered water and food (NIH-07 formula) were provided ad libitum and ambient temperature in the vivarium was maintained at 22 ° ± 0.5 °C with a light:dark cycle of 12 hr.

Every 6 months the colony was monitored for viroprofile, testing for mycoplasma, and gross end-

[1]The authors acknowledge the valuable comments of Dr. Donald K. Ingram to an earlier draft of this manuscript. The Gerontology Research Center is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

[2]Gerontology Research Center (Baltimore), National Institute on Aging, National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, and the Baltimore City Hospital, Baltimore, MD 21224.

and exoparasites. Tests were routinely performed by Microbiological Associates (unit of Whittaker Corporation) and included detection of respiratory enteric organism, K-virus, polyoma, minute virus of mice, mouse adeno, lymphocytic chorio meningitis, pneumonia virus of mice, sendai, mouse hepatitis, ectromelia, poliovirus and mycoplasma. Parasites were checked by the staff of the animal resource facility. The procedure for viromonitoring was: about 20 animals were purchased from the Jackson laboratory; 20% of them were sacrificed, and sera was obtained for analysis immediately at arriving; the rest of the animals were placed for 30 days in the vivarium prior to testing.

A viroprofile was conducted in the GRC colony before animals were selected for the experiment (October, 1982). This profile revealed no health problems except mouse hepatitis virus which was detected in one sample of young animals (6%). Animals with the following characteristics were not used: inactive, visible tumors, skin ulcerations, lack of vibrissae, hairless spots, or weight outside the range of 24 to 44 g.

A total of 80 male C57BL/6J mice of different ages were obtained from the GRC animal colony and housed in a separate room in standard plastic cages (5 per cage) with sawdust bedding. Conditions in this room and treatment of the animal were comparable to those in the main vivarium. The ages and group sizes at the beginning of the observation were: 8 months (n = 10); 13 months (n = 10); 15 months (n = 30); 22 months (n = 20); 30 months (n = 10). More animals in 15- and 22-month-old groups were included in anticipation of the probable increase in mortality in these groups related to repeated testing. In addition to the experimental animals, 19 animals, initially 22 months old, chosen according to the same standards, were maintained in the same room but were never subjected to the cold stress test.

Procedure. — Core body temperatures and body weights were measured in all animals every 2 weeks. Briefly, the cold stress test (Talan & Engel, 1984) consisted of placing the mouse into a Plexiglas® tube with an internal diameter of 30-mm and with 10-mm holes drilled at about 30-mm spacing. The tube containing an animal was placed into a rack that held 12 tubes, and the entire apparatus was placed into a cold room maintained at 10 °C. The tube did not prevent the mouse from shivering but did restrict its movement. Throughout the 3-hr cold stress test, colonic temperature was measured every 30 min by inserting a thermoprobe 2 to 2.5 cm into the rectum. Because colonic temperature (Tco) was measured, we will use this term rather than body or rectal temperature. If Tco dropped below 24 °C in fewer than 3 hrs, the test was terminated, and the animal was removed. Animals were retested at 2-week intervals between 10 a.m. and 1 p.m.

Statistical procedures. — The linear regression of Tco during each test for each animal (temperature slope) and the mean slope of temperature for each age group was calculated. The temperature slope (temperature/time) is our definition of cold tolerance, and the change in slope across tests is our definition of adaptation in cold tolerance.

Analyses of variance with repeated measures were used to determine age differences and effects of repeated tests (Dixon & Brown, 1979). Results of the first eight tests were analyzed. Cases with missing data were removed from these calculations.

A multiple correlation coefficient was calculated for each test; the coefficient estimated the extent to which age and Tco predicted the slope of the temperature during the test (Dixon & Brown, 1979).

RESULTS

Age differences and adaptation in cold tolerance. — Prior to the testing, Tco for the different age groups were ($M \pm SD$): 8-months, 38.2 °C ± 0.5; 13-months, 38.6 °C ± 0.2; 15-months, 38.2 °C ± 0.4; 22-months, 36.6 °C ± 0.4; 30-months, 36.3 °C ± 0.8. The decline in body temperature among old animals is similar to that reported by Talan (1984).

The results of the first eight cold stress tests are presented in Figure 1. Mean slope of the temperature during each cold exposure is given for each age group (Figure 1A). The results of the analysis of variance of individual slopes revealed a significant main effect of age, $F(4, 63) = 14.89$, $p < .001$, which demonstrated that the age groups differed across all tests. There was a significant main effect of tests, $F(7, 44) = 6.91$, $p < .001$, showing that the slope across all age groups differed from one test to the next. Finally, there was a significant interaction between age and test, $F(28, 441) = 2.29$; $p < .001$, which indicated that the age groups responded differentially to repeated stress.

Visual inspection of the date in Figure 1A suggested that for each age group, except the 30-month old animals, tolerance to cold stress im-

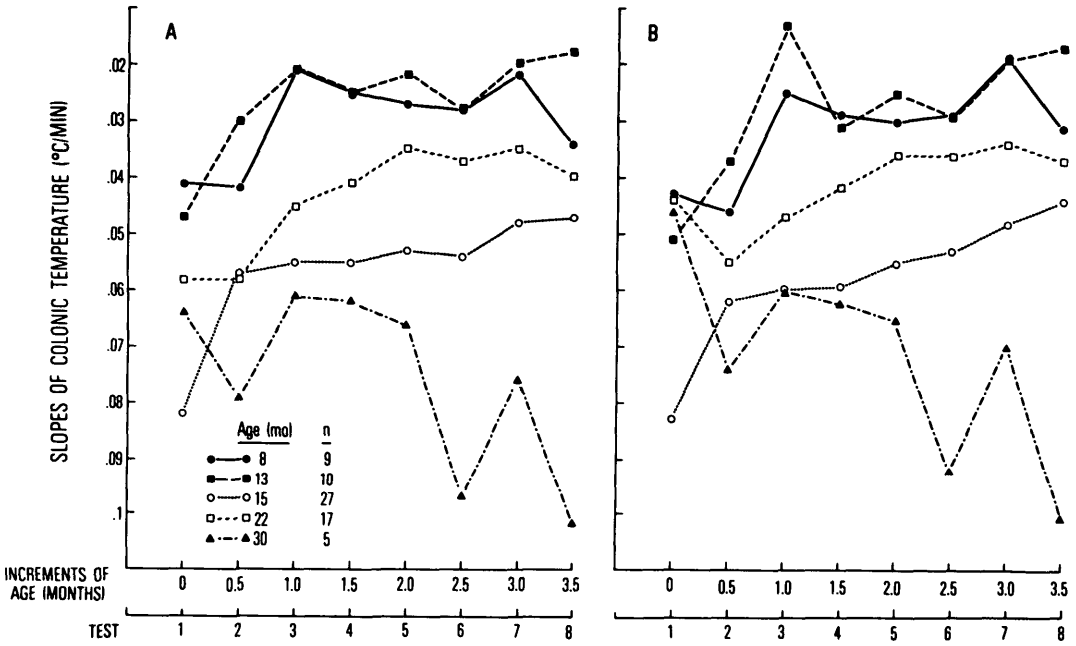

Figure 1. Results of the first eight cold stress tests for different age groups. Panel A: Mean slopes of colonic temperature during tests. Panel B: Mean slopes of colonic temperature during tests adjusted to colonic temperature before test calculated as a covariate.

proved primarily from the first to the second or third test. To verify this impression, repeated measures analyses of variance were carried out iteratively; the data from each of the age groups in combination with the data from the first test and the first two tests subsequently were excluded from the calculations (Table 1). Results indicated that the Age × Test interaction was significant as long as the 30-month old animals remained in the analyses, regardless of whether all tests were included or the first test, or the first two tests were excluded. When 30-month old mice were excluded from the analyses, however, the Age × Test interaction always was nonsignificant, $p > .05$ (Table 1). Furthermore, elimination of the first two tests together with 30-month-old group also rendered the main effect of test nonsignificant, $p > .05$ (Table 1) suggesting that there was no further adaptation to testing after the first two tests in the other age groups.

We also assessed whether baseline Tco affected cold tolerance. In order to test cold tolerance independently from initial Tco, all the calculations were repeated using the initial Tco as a covariate (Table 2). Under this condition the Age × Test interaction was always nonsignificant, $p > .05$, after excluding the 30-month-old group (Table 2). Elimination of the first two tests tended to reduce the significance of the differences among the tests:

Table 1. The Effect of Removal of Various Age Groups and the First or First Two Tests on the Slope of the Temperature Response to Cold Stress

Age group excluded	Number of tests excluded: 0		1		1 and 2	
Age × Test interaction						
none	2.79	(28,441)**	2.44	(24,378)**	2.74	(20,315)**
8 month	3.21	(21,385)**	2.78	(18,330)**	3.14	(15,275)**
13 month	3.27	(21,378)**	2.78	(18,324)**	3.10	(15,270)**
15 month	2.69	(21,259)**	2.43	(18,222)*	2.51	(15,185)*
22 month	3.49	(21,329)**	3.08	(18,282)**	3.90	(15,235)**
30 month	1.14	(21,413)	1.01	(18,354)	0.83	(15,295)
Test main effect						
none	6.91	(7,441)**	4.96	(6,378)**	4.06	(5,315)*
8 month	5.03	(7,385)**	3.48	(6,330)*	3.31	(5,275)*
13 month	5.35	(7,378)**	4.87	(6,324)**	4.51	(5,270)**
15 month	5.18	(7,259)**	5.02	(6,222)**	4.12	(5,185)*
22 month	5.56	(7,329)**	4.81	(6,282)**	5.40	(5,235)**
30 month	12.73	(7,413)**	4.61	(6,354)**	0.95	(5,295)

Note: Entries are F ratios; degrees of freedom are shown in parentheses.

*$p < .01$; **$p < .001$.

when the 8-month-old, or 13-month-old, or 30-month-old animals and the first two tests were excluded, the differences were nonsignificant, $p > .05$ (Table 2).

Table 2. The Effect of Removal of Various Age Groups and the First or First Two Tests on the Slope of the Temperature Response to Cold Stress

Age group excluded	Number of tests excluded: 0		1		1 and 2	
Age × Test interaction						
none	3.14	(28,440)***	2.31	(24,377)***	2.69	(20,314)***
8 month	3.69	(21,384)***	2.62	(18,329)***	3.07	(15,274)***
13 month	3.61	(21,377)***	2.38	(18,323)***	2.77	(15,269)***
15 month	3.72	(21,258)***	2.71	(18,221)***	3.24	(15,184)***
22 month	3.88	(21,328)***	3.03	(18,281)***	3.73	(15,234)***
30 month	1.16	(21,412)	1.00	(18,353)	0.81	(15,294)
Test main effect						
none	4.94	(7,440)***	4.47	(6,377)***	2.42	(5,314)*
8 month	2.99	(7,384)**	2.84	(6,329)*	1.90	(5,274)
13 month	2.83	(7,377)**	3.27	(6,323)**	2.11	(5,269)
15 month	3.79	(7,258)***	4.86	(6,221)***	3.03	(5,184)*
22 month	4.58	(7,328)***	4.23	(6,281)***	3.60	(5,234)**
30 month	12.63	(7,412)***	4.43	(6,353)***	0.91	(5,294)

Note: Entries are *F* ratios; degree of freedom are shown in parentheses. All analyses used initial colonic temperature as a covariate.

*$p < .05$; **$p < .01$; ***$p < .001$.

Table 3. Mean Slopes of Colonic Temperature for Different Age Groups during Cold Stress Tests 1 and 3

Age (months)	Test 1	Test 3	Changes between tests[a]
8	0.041	0.021	0.021 ± 0.023
13	0.047	0.021	0.026 ± 0.025
15	0.082	0.055	0.022 ± 0.040
22	0.058	0.045	0.017 ± 0.026
30	0.064	0.061	0.002 ± 0.018

[a]Mean plus or minus standard deviation

Table 4. Multiple Correlation Coefficients between Age, Tco Before Testing, and Slope of the Temperature during Cold Stress and Partial Correlation between Age and Slope of the Temperature during Cold Stress Controlled for Initial Tco

Test number	*R*	Partial *R*
1	.13	.11
2	.47**	.13
3	.31*	.31
4	.59**	.16
5	.46**	.06
6	.44**	.44
7	.49**	.36
8	.65**	.30

*$p < .05$; **$p < .001$.

The results of these analyses suggest that the 30-month-old animals failed to adapt to repeated testing; in fact, their tolerance declined. This effect can be seen readily in Figure 1B which is similar to Figure 1A except that the slopes of Tco have been adjusted statistically to account for initial temperature for each group at each test. Figures 1A and 1B show that the pattern of adaptation across tests is similar for all age groups except the 30-month-old animals. Cold tolerance improves across tests except for 30-month-old animals, where it falls.

The results shown in Tables 1 and 2 and Figure 1 also suggest that the adaptation effect could be seen reliably by the third test. Tukey *t* tests were calculated to check the test differences for every age group (Winer, 1971). It was shown that for 13- and 15-month-old animals, results of Test 3 were significantly different from results of Test 1. There were no significant test effects for 8- and 22-month-old groups. Even among the 8- and 22-month-old animals, however, there was a tendency for the cold tolerance to improve by Test 3 (i.e., to demonstrate less decline in Tco than in Test 1). Table 3 presents the mean slopes of Tco for Test 1 and Test 3 for all age groups. Adaptation to cold stress between Test 1 and Test 3 was similar for the 8- to 22-month old groups, $F(3, 65) = 0.21$, $p = .9$, whereas adaptation for the 30-month-old group was only about 10% of that for the other four groups. Thus, although extending testing beyond the third test does provide some additional information, especially when old animals are being studied, for practical purposes, three tests appear to reveal the major extent of the adaptational process.

Table 4 presents the results of a multiple regression analysis using age and initial Tco to predict the slope of Tco during each of the eight tests. During the first test there was no significant correlation between these factors, but beginning with the second test there always was a significant positive correlation between age and Tco before cold exposure, and slope during exposure. The results of the partial correlation analyses also shown in Table 4, however, revealed that when pretest Tco is taken into account, the correlation between age and slope is decreased substantially for most tests. These data, taken in conjunction with the data presented already, show that there are age-related differences in cold tolerance, and that these differences are, in part, related to the fact that old animals have lower body temperatures.

Adaptational response of initial Tco. — As seen in Figure 1 and Table 4, Tco prior to cold exposure

influenced cold tolerance and contributed to the results that revealed age-related differences in cold tolerance. Initial Tco itself, however, could be changed as a function of repeated cold exposure. An analysis of variance of Tco showed a significant main effect of age, $F(4, 63) = 27.51, p < .001$, that could be accounted for by the lower temperature among old mice. There was also a significant main effect of tests, $F(7, 441) = 27.33, p < .001$, which indicates that mean Tco across all age groups differs from one test to the next. Finally, there was a significant interaction between age and test, $F(28, 441) = 11.4, p < .001$, which indicates that different age groups responded differentially to the repeated measures.

Figure 2 presents the mean Tco for every age group prior to each cold exposure. Visual inspection of these data suggests that Tco of animals in the 8-, 13-, and 15-month-old groups were very similar before the first cold exposure and remained similar in spite of an overall slight decline with repeated measures. The exception is the Tco of 13-month-old group measured before the third test. At that time Tco fell almost to the level of the old animals, but beginning with next test it returned to the previous level. The Tco of old animals (22- and 30-month-old) was quite different. It was lower at the first test, rose to the average level of the younger groups, and then declined steadily for the 30-month-old mice, whereas Tco of the 22-month-old group remained at an elevated level. With exclusion of the data from the 30-month-old group and exclusion of the results of the first two tests, an analysis of variance revealed a nonsignificant age effect, $F(3, 59) = 2.51, p < .05$, whereas the main effect of test remained significant, $F(5, 295) = 18.9, p < .001$ (Table 5). These data are consistent with previous findings that Tco of aged mice is lower than that of younger animals. In conjunction with the results described in the first part of this Results section, these data show that even though aged animals failed to demonstrate any adaptation during cold exposure with repeated testing, they still were able to respond to repeated testing by raising their baseline temperature after the first and second tests.

Mortality as a function of repeated cold exposure. — Figure 3 describes the survival rates for each age group. Among the groups of 8- and 13-month-old animals, there was almost no mortality during 18 tests (8.5 months of observation), whereas mortality among 30-month-old mice was about 60% for 4 months of observation. After 6

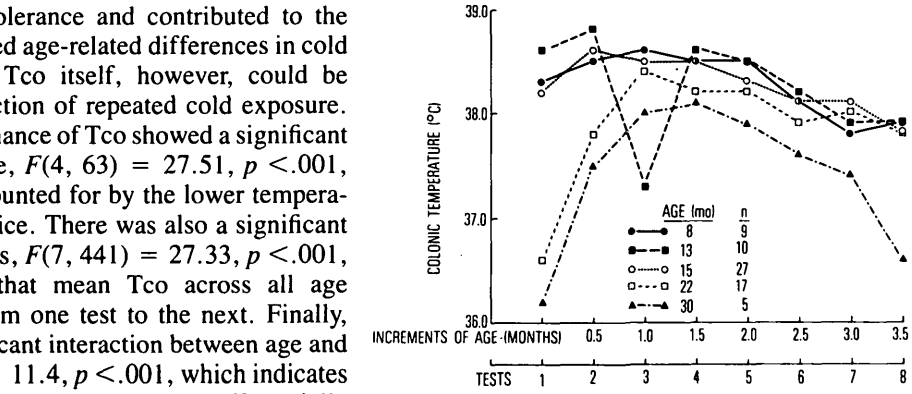

Figure 2. Mean colonic temperature for each age group measured prior to each of the first eight cold stress tests.

Table 5. The Effect of Removal of 30-Month-Old Group and the First or First Two Tests on Initial Colonic Temperature

Age group excluded	Number of tests excluded: 0		1		1 and 2	
Age main effect						
none	27.51	(4,63)**	13.65	(4,63)**	9.21	(4,63)**
30 month	24.43	(3,59)**	5.90	(3,59)*	2.51	(3,59)
Test main effect						
none	27.33	(7,441)**	22.7	(6,378)**	24.95	(5,315)**
30 month	19.43	(7,413)**	19.45	(6,354)**	18.90	(5,295)**

Note: Entries are *F* ratios; degrees of freedom are shown in parentheses.

*$p < .01$; **$p < .001$.

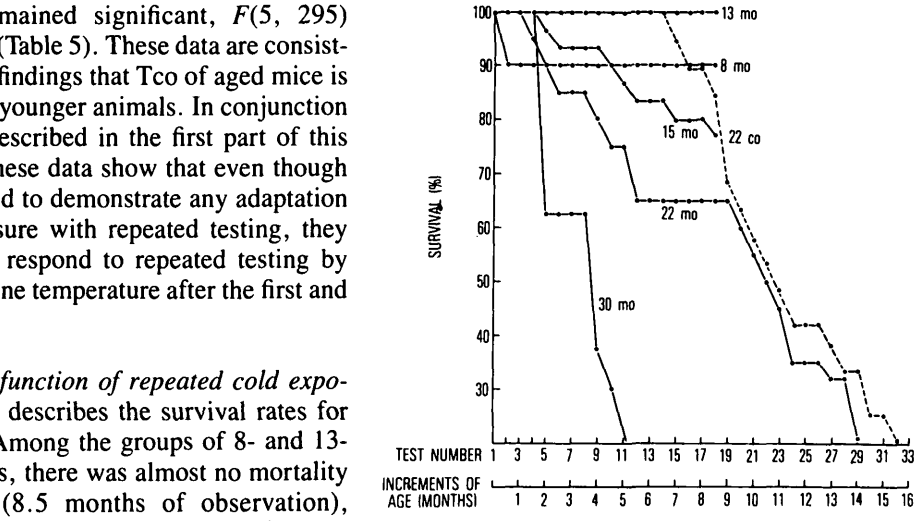

Figure 3. Survival rate among C57BL/6J mice participating in repeated cold stress tests starting at different ages.

months of observation, the survival rate among the age groups was as follows: 8-months, 90%; 13-months, 100%; 15-months, 84%; 22-months, 65%; there were no survivors among 30-month-old animals.

The survival curve for 22-month-old mice fell rapidly beginning with the third test; 35% of the animals died during the first 5 months of the experiment. During the next 3.5 months, however, there was no further mortality. It is of interest to compare the mortality among the 22-month-old animals to a control group that was observed over the same period but that was never subjected to cold-stress testing. Among the control animals, during the first 6.5 months of observation there was no mortality; however, 60% of the mice in this group died during the next 5 months. Therefore, under conditions of cold stress testing every 2 weeks, 35% of the 22-month-old animals that experienced cold exposure died approximately 4.5 months earlier than their control counterparts. Toward the end of the 6th month of experiments, survival among the experimental, 22-month-old group was significantly lower than among 22-month-old control group (Fisher exact probability = .005). By the end of the 16th month, however, only one animal survived in the experimental group and two animals in the control group (Fisher exact probability = .32). Therefore, repeated cold exposure started later in life increases mortality among old animals but does not affect maximum lifespan and median survival. This conclusion was supported by survival analysis (Dixon & Brown, 1979). Overall survival curves were similar for both experimental and control groups. Generalized Wilcoxon coefficient yielded 1.62 and generalized Savage 1.41, $p = .2$ for both. Similarity, however, was mainly for 50% and 25% survival. For 75% survival, estimated time elapsed since the beginning of observation to death among 22-month-old animals was 4.8 months for cold-exposed mice (26.8 months of age) and 8.7 months for control animals (30.7 months of age). Median survival was 9.9 and 10.2 months, respectively.

Discussion

Our results support four general ideas: (a) Mice are capable of adapting to repeated cold stresses in that the slope of the decline of Tco during the test decreases dramatically after the first two tests. (b) Aged animals are impaired in this capacity and demonstrate an increased slope of heat loss. (c) Aged animals respond to repeated cold exposure by a different adaptive mechanism (i.e., raising of basal Tco). (d) Repeated cold exposure, started later in life, appears to increase mortality among older animals.

Our findings confirm and extend previous reports: Cold tolerance improves with repeated testing (Hart, 1963, 1971; Jansky, 1966). Furthermore, our findings indicate that this effect is present after the first two tests and that cold tolerance does not change significantly in subsequent testing. The exception is among 30-month-old animals, whose cold tolerance does not improve but gradually worsens. These last data support the notion that one of the most prominent features of old age is a reduced ability to adapt to environmental stress (Finch, 1971).

On the basis of our results, however, it is possible to suggest that the adaptational ability does not disappear with advanced age, rather the mechanism of adaptation changes. The colonic temperature of old animals (22 and 30 months of age) increased after the first and second tests, a definite response to repeated cold exposure (Figure 2). The improvement in cold tolerance occurred mainly during the first two tests, and the differences in mean slopes (i.e., the adaptation to repeated testing) for different age groups between Test 1 and Test 3 are almost identical (with the exception of 30-month-old animals). Previous literature indicates that cold tolerance is mediated by an increase in heat production (Estler & Ammon, 1969) and that there is a decrease in heat production among old animals rather than increase in heat loss (Estler, 1971). On this basis we suggest that, although there is a significant decline in heat production with age, the mechanism of adaptation (i.e. the improvement of the responses during repeated cold exposure) does not change until later in life when it is replaced by another type of adaptation, an increase in baseline body temperature. This increase in basal Tco is sufficient to offset the impaired adaptive responses to the cold stressor itself as can be seen in Figure 1. For instance the mean Tco of 30-month-old animals at the end of the first test, calculated on the basis of mean intercept and mean slope was 25.1 °C. During the next four tests, in spite of steeper slopes, final temperatures were higher than those after the first test. The 22-month-old animals were the only group that demonstrated both types of adaptation. Their Tco taken prior to the tests rose, and their cold tolerance improved with repeated testing.

It is possible that the well known increase in number of cases of hypothermia among elderly people is related to their inability to respond immediately to cold exposure by increasing heat produc-

tion. On the other hand, the altered mechanism of adaptation to cold exposure — an increase in chronic body temperature — may reflect a cold-induced increase in metabolic rate without any improvement in heat production in response to cold, per se, because the multiple regression between age, initial Tco, and cold tolerance during test (slope) were significant for every test (except Test 1) indicating that there is an age-related decline in cold tolerance (Table 4).

The data on survival rate show that repeated cold exposure started later in life does not change the maximum life span but does increase mortality among old mice. This observation might reflect an increasing risk among older animals with other health problems (e.g., pulmonary or kidney disease). At this point we do not have any data on the change of mortality rate of old animals experiencing repeated exposure to cold starting early in the life.

Some findings in this study suggest that thermoregulatory ability among middle-aged mice (in our study 15-month-old group) is impaired. It was shown (Figure 1A) that during the first test the cold tolerance of 15-month-old animals was worse than that of the 22- or 30-month-old groups. This observation probably explains why the multiple regression between age, initial Tco, and slope was not significant during the first test (Table 4). In spite of the fact that cold tolerance of this group of animals during the first test was worse than that observed among older groups, they still had sufficient adaptative ability to improve their performance significantly during subsequent tests. The present data also replicate our previous observation, performed on a separate group of C57BL/6J mice from the GRC colony (Talan, 1984), that initial Tco of 15-month-old male C57BL/6J mice is significantly less than that of animals ranging from 3 to 20 months of age. It is also noteworthy that when 13-month-old mice were tested in present study for the third time when their age was 14 to 14.5 months, the mean Tco was significantly lower than during all other testing. One hypothesis suggested by these findings is that after a certain age (13 to 14 months) neuroendocrine changes that affect the thermoregulatory mechanisms appear. It is not clear, however, why these capacities should return in older animals. In any case, this finding deserves further study.

Our findings indicate that if investigators wish to use cold stress as an experimental intervention with C57BL/6J mice, the experimental cold-stress test should be preceded by two preliminary cold exposures at 2-week intervals in order to eliminate the confounding influence of cold adaptation from the results of the experiment.

References

Dixon, W. J., & Brown, M. B. (Eds.). (1979). *Biomedical computer programs P-series*, University of California Press, Berkeley, CA.

Estler, C.-J. (1971). Efficiency of thermoregulation in acutely cold exposed young and adult mice. *Life Science, 10*, Part 1, 1291–1298.

Estler, C.-J., & Ammon, H. P. (1969). The importance of the adrenergic beta-receptors for thermogenesis and survival of acutely cold exposed mice. *Canadian Journal of Physiology and Pharmacology, 47*, 427–434.

Finch, C. E. (1971). Comparative biology of senescence: Some evolutionary and developmental questions. In *Animal models for biomedical research, VI*. Washington, DC, National Academy of Science.

Finch, C. E., Foster, J. R., & Mirsky, A. E. (1969). Aging and regulation of cell activities during exposure to cold. *Journal of General Physiology, 54*, 690–712.

Goodrick, C. L. (1975). Life-span and the inheritance of longevity of inbred mice. *Journal of Gerontology, 30*, 257–263.

Grad, B., & Kral, V. A. (1957). The effect of senescence on resistence to stress. 1. Response of young and old mice to cold. *Journal of Gerontology, 12*, 172–181.

Hart, J. S. (1963). Physiological responses to cold in nonhibernating homeotherms. In C. Herzfeld (Ed.), *Temperature: Its measurement and control in science and industry* (Vol. 3). Reinhold, New York.

Hart, J. S. (1971). Rodents. In G. C. Whittow (Ed.). *Comparative physiology of thermoregulation* (Vol. 2). Academic Press, New York.

Jansky, L. (1966). Body organ thermogenesis of the rat during exposure to cold and at maximum metabolic rate. *Federal Proceedings, 25*, 1297–1305.

Talan, M. (1984). Body temperature of C57BL/6J mice with age. *Experimental Gerontology, 19*, 25–29.

Talan, M., & Engel, B. T. (1984). An improved cold stress test for aging mice. *Experimental Gerontology, 19*, 79–86.

Trujillo, T. T., Spalding, J. F., & Langham, W. N. (1962). A study of radiation-induced aging: Response of irradiated and nonirradiated mice to cold stress. *Radiation Research, 16*, 144–150.

Winer, B. J. (1971). *Principles in experimental design*. McGraw-Hill, New York.