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Lasting Consistency of Cold Adaptability in Rats Reared in Cold for Many Generations

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Abstract 1) Wistar rats were successively reared in cold at 5°C from 1969 to 1984. The historical changes observed in these rats were reported. 2) The cold-adapted rats reared in cold for 8 to 11 successive generations (C8–11G) were examined on their cold tolerance and nonshivering thermogenesis. C8–11G rats showed greater nonshivering thermogenesis than that of the warm-adapted control group (W), and rats exposed to cold for periods of 2 to 8 weeks (C). The nonshivering thermogenesis of C8–11G rats was diminished to a similar level to that of W and C rats by administration of a ganglion blocker, of reserpine, or of β-adrenoceptor blocker. 3) The de-adapted rats reared in warm at 25°C for 3 generations after being reared for many generations in cold (DA-3G) showed much more nonshivering thermogenesis as compared to W rats. Cold tolerance of DA-3G rats was at a level of intermediate between that of W and C rats. 4) Brown adipose tissue (BAT) weight of DA-3G rats was similar to that of C rats, while chemical composition of BAT in DA-3G rats differed from that of C and W rats.

Key words: cold-adaptation, many generations in cold, de-adaptation, nonshivering thermogenesis, cold tolerance.

A great deal of information on the mechanisms of potent cold adaptability of homeotherms has been obtained and extensively reviewed (CHAFFEE and ROBERTS, 1971). When homeotherms are exposed to chronic variations of an ambient temperature, they develop adaptive modifications of already existing regulatory processes. However, most experiments have been performed for only 1 generation of the animals, lasting several hours, days, or weeks. We are interested in whether physiological adaptation of the animals to change in living environment could induce any genetic changes when environmental stress is experienced continuously for many generations. To investigate this problem, we have reared rats in cold

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at 5°C for many generations since 1969, and examined their cold adaptability at intervals in the course of this period from the viewpoint of metabolic modifications. Moreover, cold tolerance as well as metabolic capacity were studied in the rats transferred to warm environment of 25°C for 3 generations after having been reared at 5°C for many generations.

MATERIALS AND METHODS

1) Preparation of the animals. Wistar brother and sister rats about 2 months of age were exposed to a cold temperature of 5°C for 4 weeks, in May, 1969, and thereafter mated (C0G). Pregnant female rats were transferred to a warm temperature of 24±1°C a few days prior to delivery in order to increase the survival possibilities of the expected newborn rats. Mother and neonate rats were again returned to the cold temperature 6 weeks after delivery and the cold exposure was continued. These newborn rats are referred to as "the first generation in the cold" (C1G). From C1G to the 15th generation in the cold (C15G), the rats were reared and mated between brother and sister in the above manner, but the period of being reared at a warm temperature after birth was shortened to 2 weeks instead of 6 weeks. As the female rats of C15G could not be fertilized when they were mated with their brothers, male rats derived from another Wistar group which had been reared in cold of 5°C for 4 weeks were used to mate with the C15G females. The newborns thus obtained were used as the parents for further brother-sister submating, and are referred to as the parent generation of another series in the cold (C'0G). From the first generation (C'1G) to the 7th generation (C'7G), the period of exposure to warmth after the birth was shortened to 1 week. C'7G pregnant female rats were not transferred to warm temperatures prior to or after giving birth, and the birth and breeding of their newborn rats proceeded normally. However, the number of newborn rats of the 8th generation (C'8G) which reached maturity amounted to only 12 out of 86 (2 out of 13 liters). The survival percentage of newborn rats of the 9th and 10th generation (C'9G and C'10G) was similarly small. Because of reduced number of the pregnant female rats of C'10G, they were again transferred to warmth a few days prior to birth. The 11th generation rats (C'11G) were transferred to cold 3 weeks after birth together with their mothers and C'11G pregnant female rats were able to bear and breed their offspring in the cold. Between the 12th and the 16th generation rats (C'12G and C'16G), the survival rate of neonate rats amounted to almost 90%, and many offspring of C'16G were born and bred in the cold.

Some of the C'12G brother and sister rats were transferred to a temperature of 25°C and mated 4 weeks later in October, 1982 (DA-1G). Their offspring were bred in warmth as the second generation of de-adapted rats (DA-2G), and the de-adapted rats raised in warmth for 3 successive generations (DA-3G) were used to examine cold adaptability.

2) Experiments. Male and female rats aged 2 to 3 months and DA-1G rats aged 6 months were used. Cold adaptability of the rats was assessed by the following parameters:

(1) Changes in the colonic and interscapular brown adipose tissue (BAT) temperatures in curarized rats under cold exposure: The rats were injected with curare (360 μg/rat) in the jugular vein in an experimental room of 26±1°C under light ether anesthesia. Thirty minutes after the first curarization, additional curare (180 μg/rat) was administered i.v. just after exposure to cold at 4°C. The curarized rats were put under the respirator throughout the experiment. Cold exposure of curarized rats was continued for 60 min, being administered i.v. with a second additional dose (180 μg/rat) 30 min after the start of cold exposure. Following cold exposure, curarized rats were taken to warmth of 26±1°C for 30 min. Colonic temperatures (T_C) were measured with a vinyl-sheathed thermister tip inserted about 5 cm into the rectum and BAT temperature (T_{BAT}) with a needle thermister tip inserted under the interscapular BAT every 15 min for 120 min.

In order to assess the role of sympathetic nervous system, a ganglion blocker, hexamethonium bromide (75 mg/kg body weight) was injected into the other jugular vein just before cold exposure. Thirty minutes after cold exposure, additional hexamethonium (36 mg/kg) was injected into the animals.

Reserpine (2.5 mg/kg body weight) was administered s.c. 18–20 hr prior to the experiment in order to see the effect of depletion of norepinephrine (NE).

In order to know the effect of hibernectomy, interscapular BAT was removed surgically just before the experiment.

Propranolol hydrochloride was injected into the jugular vein with doses of 0.75 (0.5 and 0.25) or 1.5 (1.0 and 0.5) mg/kg body weight at the time of the first and second curarization to assess the effect of β-adrenoceptor blockade.

(2) Changes in T_C of unanesthetized rats under severe cold exposure: T_C of C8G, C2W rats exposed to cold for 2 weeks, and W fed male rats (250–400 g) was measured every 20 min during exposure to −20°C for 100 min. After 18 hr of fasting, male W, C5W (rats exposed to cold for 5 weeks) and DA-3G rats (151–388 g) were exposed to −5°C for 120 min, and T_C was measured every 30 min.

(3) NE-induced heat production: Increase in heat production induced by *l*-NE administration (40 μg/100 g body weight, i.p.) was assessed by measuring O_2 consumption ($\dot{V}_{O_2}$) and CO_2 output of unanesthetized rats in an open system at an air flow rate of 500 ml/min with a gas monitor (1H21S, NEC-San-ei, Tokyo) at 25°C.

Changes in T_C induced by NE infusion at a rate of 2 μg/min for 30 min were also measured in the rat anesthetized with hexobarbital (20 mg/100 g body weight, i.p.).

(4) BAT weight and its composition: Fat, fat-free dry matter, and water content of the interscapular BAT were measured by the modified method of FOLCH *et al.* (1951). Fatty acid composition of this fat extract was measured using

Table 1. Influence of cold exposure on colonic temperatures (°C) of curarized warm-adapted, 2 week and 2 month cold-exposed and cold-adapted rats (reared at 5°C for 9 generations).

	No. of rats	Body weight (g)	27°C		4°C			27°C	
			0 min	30 min	0 min	30 min	60 min	0 min	30 min
Female rats									(°C)
W	5	230	35.19±0.61	−1.39±0.33	33.73±0.56	−4.58±0.09	−8.93±0.42	24.74±0.87	+0.50±0.13
C2W	5	245	35.64±0.43	+0.68±0.39	36.34±0.41	−3.60±0.43	−6.74±0.76	29.45±0.96	+1.89±0.38
P vs. W			NS	<0.05	<0.01	NS	<0.05	<0.01	<0.01
C2M	5	225	36.85±0.31	−0.37±0.48	36.42±0.45	−3.99±0.34	−7.30±0.46	29.29±0.91	+1.68±0.27
P vs. W			<0.05	NS	<0.01	NS	<0.05	<0.01	<0.01
C9G	5	265	35.84±0.16	−0.17±0.19	35.73±0.21	−2.22±0.39	−4.25±0.48	31.44±0.42	+2.72±0.31
P vs. W			NS	<0.02	<0.02	<0.001	<0.001	<0.001	<0.001
P vs. C2W			NS	NS	NS	<0.05	<0.05	NS	NS
P vs. C2M			<0.02	NS	NS	<0.01	<0.01	NS	<0.05
Male rats									(°C)
W	14	330	35.29±0.30	−0.52±0.18	34.75±0.31	−3.84±0.19	−7.40±0.33	27.51±0.60	+0.59±0.14
C2W	5	290	36.39±0.39	+0.06±0.36	36.48±0.31	−2.73±0.23	−5.55±0.45	30.85±0.48	+1.13±0.41
P vs. W			NS	NS	<0.05	<0.01	<0.001	<0.01	NS
C2M	5	315	36.91±0.27	−0.67±0.45	36.24±0.51	−2.65±0.58	−4.50±0.82	31.71±1.33	+1.68±0.18
P vs. W			NS	NS	NS	<0.02	<0.001	<0.01	<0.001
C9G	4	380	35.95±0.36	−0.10±0.36	35.85±0.61	−1.84±0.22	−3.28±0.40	32.55±0.52	+1.59±0.21
P vs .W			NS	NS	NS	<0.001	<0.001	<0.001	<0.01
P vs. C2W			NS	NS	NS	NS	<0.01	<0.05	NS
P vs. C2M			NS	NS	NS	NS	NS	NS	NS

Mean±S.E. NS, not significant; W, warm-adapted control rats; C2W and C2M, 2 week and 2 month cold-exposed rats; C9G, cold-adapted rats reared for nine successive generations.

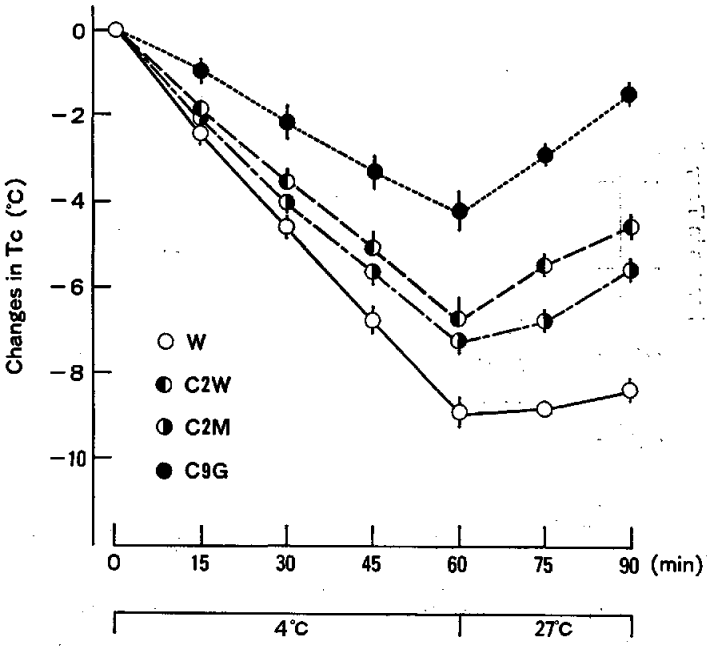

Fig. 1. Changes in colonic temperature (*Tc*) of curarized female rats during and after exposure to 4°C. C9G, cold-adapted rats reared at 4°C for 9 successive generations; C2M, cold-exposed rats for 2 months; C2W, cold-exposed rats for 2 weeks; W, warm-adapted control rats. Vertical line represents S.E.

gaschromatography under the same conditions as our previous study (MORIYA and ITOH, 1969). Protein content was measured by the method of LOWRY *et al.* (1951).

(5) Body and tail lengths: Under anesthesia with ether, body length between the nosal head and the tip of tail and tail length between the base and the tip of tail were measured in the prone rats.

Drugs used in the experiments were NE (Sankyo Pharm. Co.), curare (Yoshitomi Pharm. Co.), hexamethonium bromide (Yamanouchi Pharm. Co.) propranolol hydrochloride (Sumitomo Chem. Industries Co.), reserpine (Sigma), and hexobarbital (Teikoku Chem. Co.).

RESULTS

1) Cold tolerance and nonshivering thermogenesis in cold-adapted rats for 8 to 11 successive generation (C8–11G)

(1) Changes in Tc and TBAT in curarized rats caused by cold exposure. Changes in *Tc* and *TBAT* in curarized male (250–400 g) and female (180–280 g) rats exposed to cold at 4°C are shown in Table 1 and illustrated in Figs. 1 and 2

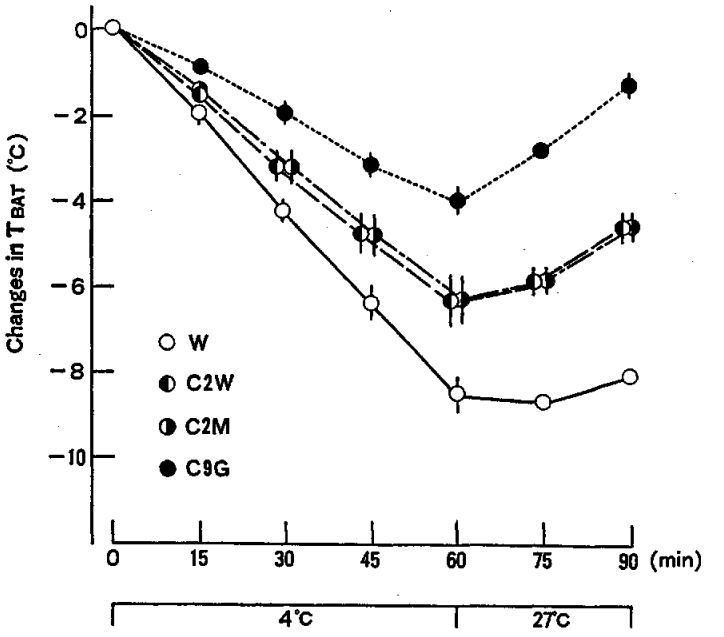

Fig. 2. Changes in interscapular brown adipose tissue temperature (T_{BAT}) of curarized female rats during and after exposure to 4°C. Abbreviations as shown in Fig. 1.

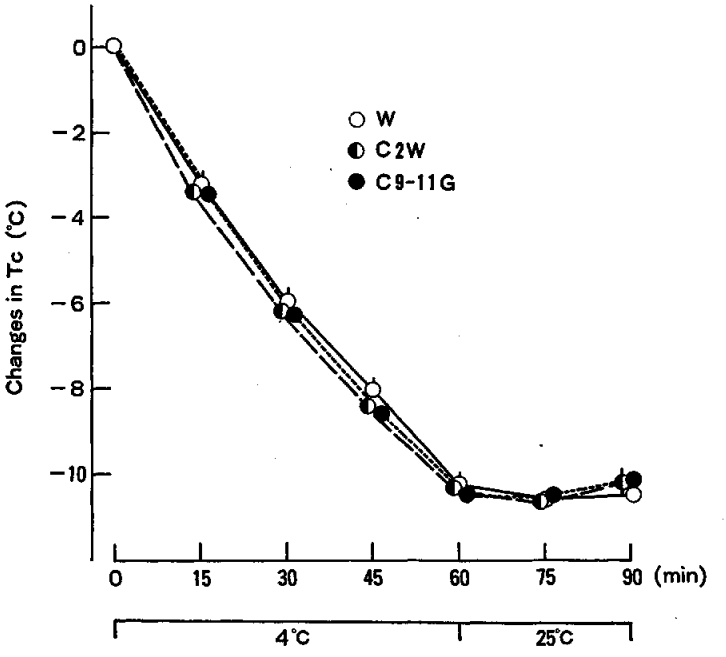

Fig. 3. Changes in colonic temperature of curarized, hexamethonium-treated female rats during and after exposure to 4°C. C9–11G, cold-adapted rats reared at 5°C for 9–11 successive generations. Other abbreviations as shown in Fig. 1.

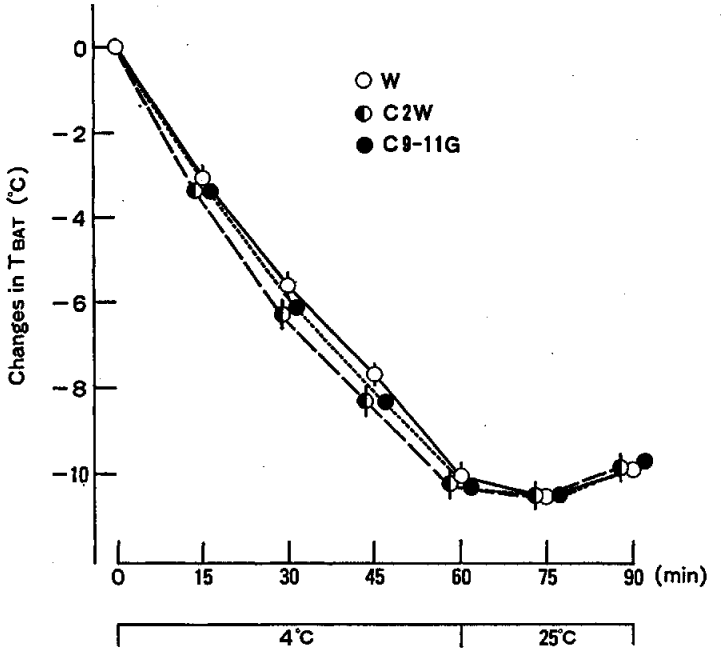

Fig. 4. Changes in interscapular brown adipose tissue temperature (T_{BAT}) of curarized, hexamethonium-treated female rats during and after exposure to 4°C. Abbreviations as shown in Figs. 1 and 3.

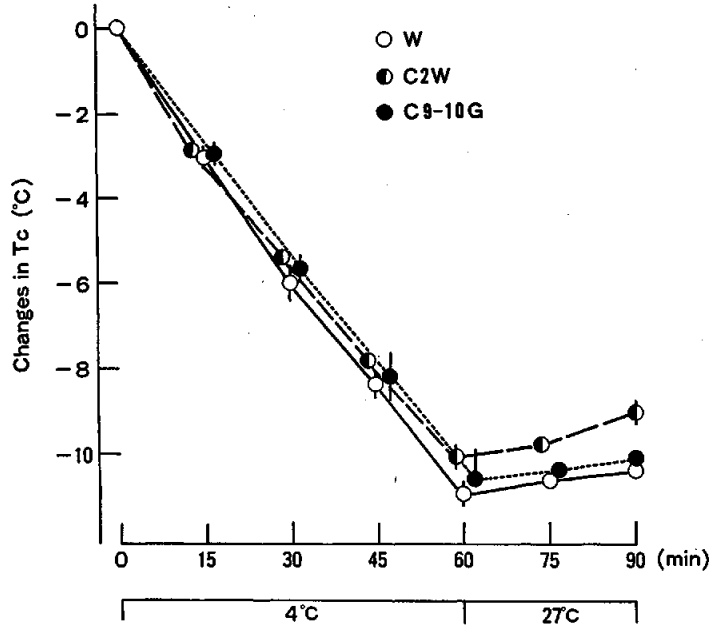

Fig. 5. Changes in colonic temperature (T_C) of curarized reserpine-treated female rats during and after exposure to 4°C. C9–10G, cold-adapted rats reared at 5°C for 9 to 10 generations. Abbreviations as shown in Fig. 1.

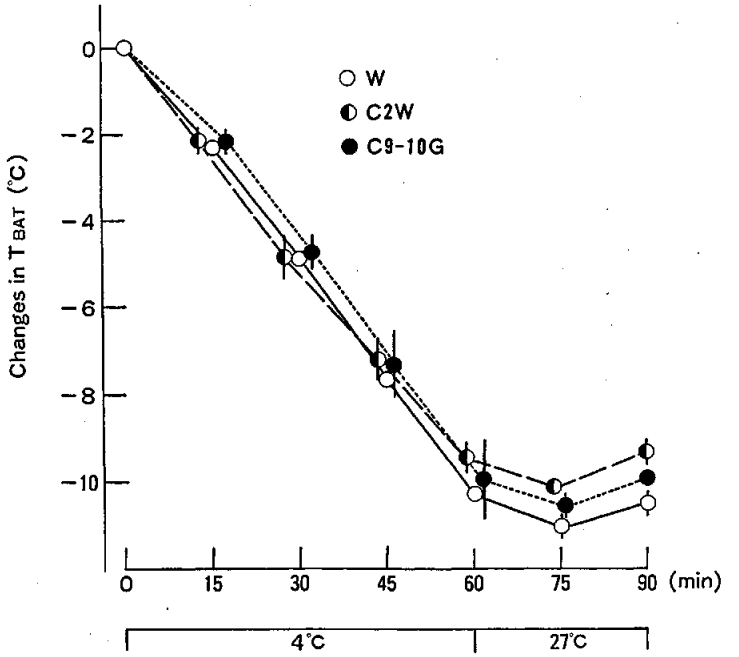

Fig. 6. Changes in interscapular brown adipose tissue temperature (T_{BAT}) of curarized, reserpine-treated female rats during and after exposure to 4°C. Abbreviations as shown in Figs. 1 and 5.

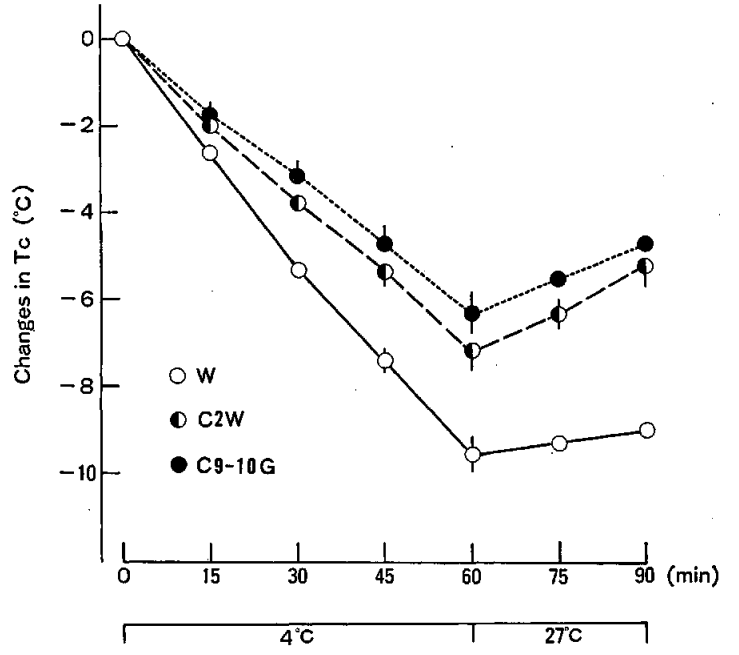

Fig. 7. Changes in colonic temperature (T_C) of curarized, hibernectomized female rats during and after exposure to 4°C. Abbreviations as shown in Figs. 1 and 5.

Table 2. Effect of propranolol on the lowering of colonic temperatures (°C) in curarized warm-adapted, 2 week cold-exposed and cold-adapted female rats (reared at 5°C for 9–11 generations).

		No. of rats	Body weight (g)	4°C			25°C	
				0 min	30 min	60 min	0 min	30 min
Warm-adapted rats								(°C)
I	Control	5	230	33.73±0.56	−4.68±0.19	−8.93±0.42	24.74±0.87	+0.50±0.13
II	Propranolol 1.5 mg/kg	4	240	34.88±0.21	−4.75±0.17	−8.98±0.17	25.78±0.31	−0.35±0.30
	P vs. I			NS	NS	NS	NS	NS
III	Propranolol 0.75 mg/kg	6	230	35.51±0.50	−4.60±0.35	−8.87±0.52	26.52±1.03	−0.20±0.31
	P vs. I			NS	NS	NS	NS	NS
Two week cold-exposed rats								(°C)
IV	Control	5	245	36.34±0.41	−3.60±0.43	−6.74±0.76	29.45±0.96	+1.89±0.38
V	Propranolol 1.5 mg/kg	4	240	35.08±0.59	−4.25±0.23	−8.16±0.25	26.70±0.79	−0.95±0.13
	P vs. IV			NS	NS	NS	NS	<0.001
	P vs. II			NS	NS	<0.05	NS	NS
VI	Propranolol 0.75 mg/kg	5	230	36.64±0.22	−4.34±0.25	−8.22±0.47	28.36±0.53	+0.09±0.67
	P vs. IV			NS	NS	NS	NS	<0.05
	P vs. III			NS	NS	NS	NS	NS
Cold-adapted rats for 9–11 generations								(°C)
VII	Control	5	265	35.73±0.21	−2.22±0.39	−4.25±0.48	31.44±0.42	+2.72±0.31
VIII	Propranolol 1.5 mg/kg	3	260	35.78±0.06	−4.65±0.20	−7.95±0.35	27.73±0.41	+0.02±0.51
	P vs. VII			NS	NS	<0.01	<0.01	<0.01
	P vs. II			<0.02	NS	<0.05	NS	NS
IX	Propranolol 0.75 mg/kg	5	230	37.39±0.32	−3.49±0.26	−6.30±0.42	31.08±0.63	+1.27±0.38
	P vs. VII			<0.01	<0.05	<0.02	NS	<0.02
	P vs. III			<0.02	<0.05	<0.01	<0.01	<0.02

Legends same as in Table 1.

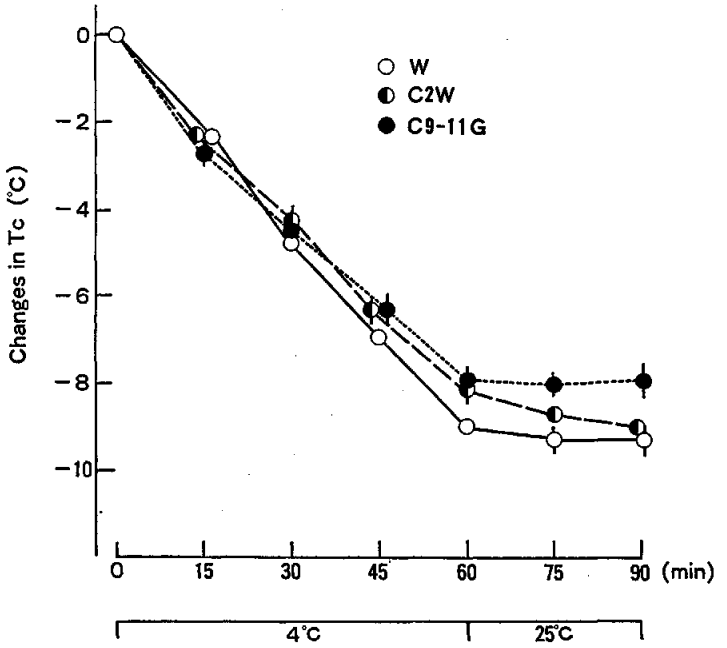

Fig. 8. Changes in colonic temperature (T_C) of curarized, propranolol-administered (1.5 mg/kg body weight) female rats during and after exposure to 4°C. Abbreviations as shown in Figs. 1 and 3.

for females. The decrease in T_C was the greatest in warm-adapted control rats (W)) Table 1 and Fig. 1). The greatest decrease in T_{BAT} was also observed in W rats (Fig. 2). The decrease in T_C and T_{BAT} was least in C9G, and the decreases in cold-exposed rats for 2 weeks (C2W) and 2 months (C2M) were intermediate between C9G and W rats. The recovery of T_C and T_{BAT} in warmth after exposure to cold is also shown in Table 1 and Figs. 1 and 2. The recovery was smallest in W, and greatest in C9G, and these in both C2W and C2M were intermediate between W and C9G. The results of male rats were essentially similar to those of female rats as described above (Table 1).

The curarized, hexamethonium-injected female W, C2W, and C9–11G rats were exposed to cold, and T_C and T_{BAT} were measured (Figs. 3 and 4). The decreases in T_C and T_{BAT} after hexamethonium injection were greater as compared to the decreases in T_C and T_{BAT} in the curarized rats as shown in Figs. 1 and 2. Hexamethonium resulted in no difference in the temperatures between C9–11G and W rats when they were exposed to cold for 60 min. Recovery of the decreased temperatures in a warm room was also smaller in the curarized, hexamethonium-treated rats as compared with that in the curarized rats as shown in Figs. 1 and 2.

T_C and T_{BAT} in W, C2W, and C9–10G rats when injected with curare and reserpine and exposed to cold decreased more (Figs. 5 and 6) than T_C and T_{BAT} of

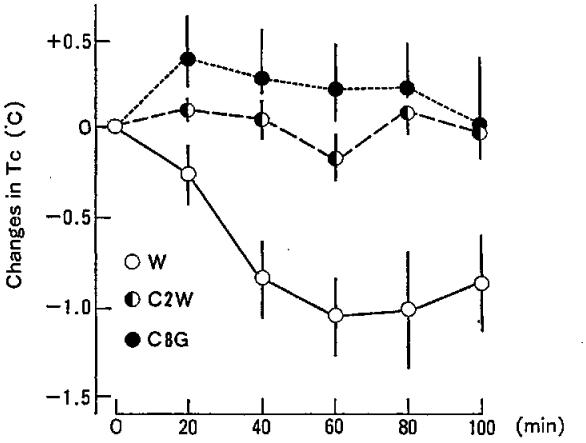

Fig. 9. Changes in colonic temperature (*Tc*) of male rats exposed to −20°C. C8G, cold-adapted rats reared at 5°C for 8 successive generations. Abbreviations as shown in Fig. 1.

curarized, but not reserpinized C9G rats (Figs. 1 and 2). The decreases in the respective temperatures did not differ among C9–10G, C2W, and W rats when administered with curare and reserpine. After reserpinization, recovery of the temperatures in a warm room became similarly delayed in all groups.

Tc of curarized, hibernectomized rats decreased more in W than in C9–10G or C2W under cold exposure (Fig. 7). The influence of hibernectomy appeared most markedly in C9–10G. The decrease in the hibernectomized C9–10G rats after 60 min of cold exposure was significantly greater ($p<0.05$) than that of the control non-treated C9G rats (Fig. 1). On the contrary, the influence of hibernectomy in the decrease of *Tc* was not significant in W and C2W rats.

Decreases in the *Tc* and *T*BAT of curarized, propranolol-administered W, C2W, and C9–11G rats are shown during and after cold exposure in Table 2 and Fig. 8. The effect of propranolol (0.75 or 1.5 mg/kg body weight) on the decrease in *Tc* was not observed in W rats, while in C2W and C9–11G rats *Tc* fall was enhanced by propranolol administration. The decrease in *Tc* during and after cold exposure did not significantly differ among W, C2W, and C9–11G rats which were curarized and administered with propranolol (1.5 mg/kg).

(*2*) *Changes in Tc of unanesthetized rats under cold exposure.* As shown in Fig. 9, *Tc* did not decrease in C2W and C8G rats, while in W rats *Tc* decreased significantly within 40 min (−0.84±0.23°C, mean±S.E.) and remained at almost the same low level during cold exposure for 100 min thereafter.

2) *Cold tolerance and nonshivering thermogenesis in de-adapted rats reared at 25°C for 3 successive generations after being reared at 5°C for many generations (DA-3G)*

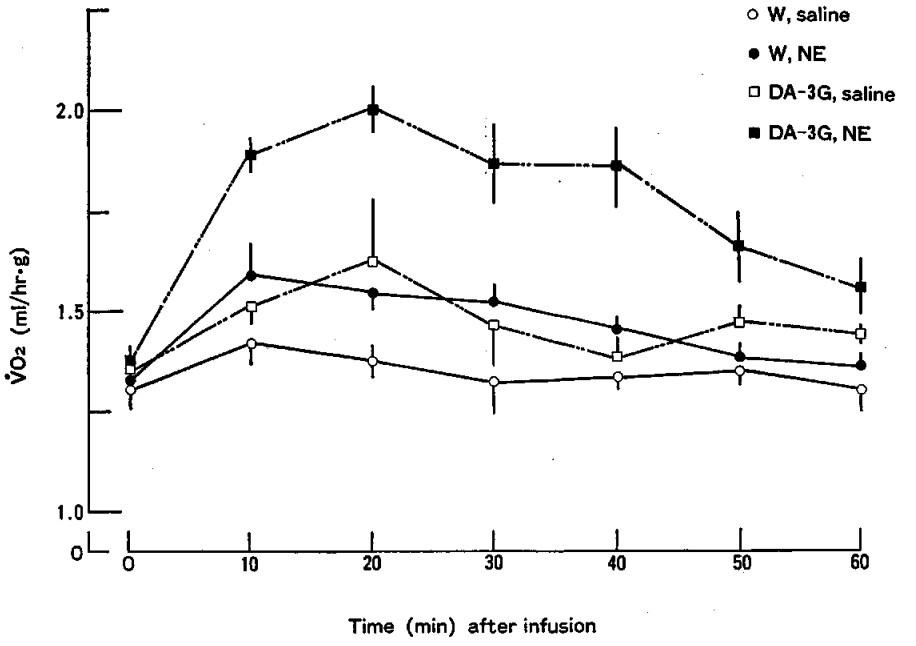

Fig. 10. Changes in O_2 consumption ($\dot{V}_{O_2}$) induced by norepinephrine (NE) administration with a dose of 40 μg/100 g body weight, i.p. W, warm-adapted control rats; DA-3G, de-adapted rats in a warm environment for 3 generations after rearing in cold for many successive generations.

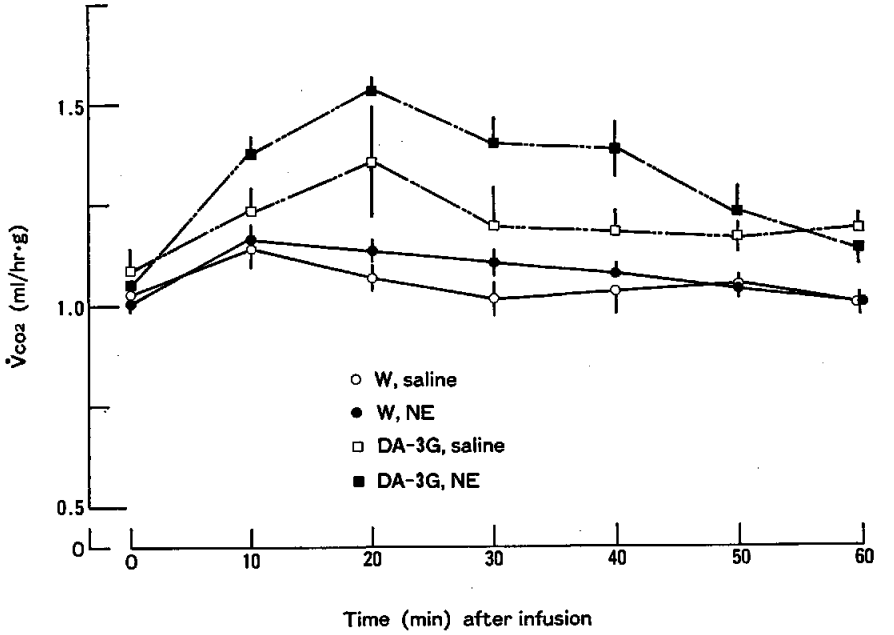

Fig. 11. Changes in CO_2 output induced by norepinephrine (NE) administration with a dose of 40 μg/100 g body weight, i.p. Abbreviations as shown in Fig. 10.

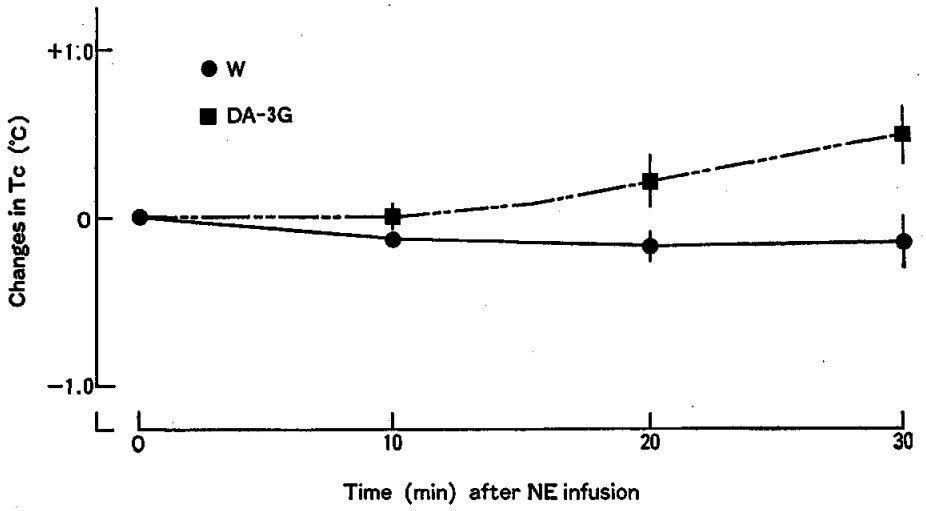

Fig. 12. Changes in colonic temperature (*Tc*) induced by norepinephrine (NE) infusion at a rate of 2 μg/min under hexobarbital anesthesia. Abbreviations as shown in Fig. 10.

Table 3. Influence of cold exposure on colonic temperatures (°C) of fasted, warm-adapted, 5 week cold-exposed, and cold-deadapted male rats.

Groups	Exposure to −5°C			
	0 min	30 min	60 min	120 min
Warm-adapted rats (W)				
(6) 300 g	37.5±0.19	−0.12±0.15	−0.57±0.22	−0.97±0.22
Five week cold-exposed rats (C5W)				
(9) 293 g	38.0±0.18	+0.83±0.12	+0.73±0.18	+0.66±0.15
P vs. W	NS	<0.001	<0.001	<0.001
Cold-deadapted rats (DA-3G)				
(11) 348 g	37.9±0.10	+0.36±0.11	+0.24±0.12	+0.06±0.13
P vs. W	NS	<0.05	<0.01	<0.001
P vs. C5W	NS	<0.02	<0.05	<0.001

Numbers in parentheses represent number of rats. C5W, 5 week cold-exposed rats; DA-3G, de-adapted rats in warm environments for 3 generations after many generations of rearing in cold. Legends same as in Table 1.

(*1*) *Changes in heat production induced by NE in DA-3G rats.* $\dot{V}_{O_2}$ and CO_2 output in the rats injected with physiological saline did not differ between DA-3G and W rats (267–359 g). The increase in $\dot{V}_{O_2}$ induced by NE injection was significantly greater in DA-3G rats than in W rats at 10 ($p<0.01$), 20 ($p<0.001$), 30 ($p<0.02$), and 40 ($p<0.02$) min after injection (Fig. 10). The increase in CO_2 output induced by NE was also significantly greater in DA-3G than in W rats at 10 ($p<0.01$), 20 ($p<0.001$), 30 ($p<0.01$), and 40 ($p<0.01$) min after NE

Table 4. Brown adipose tissue (BAT) weight in warm-adapted, 5 week cold-exposed, and cold-deadapted rats.

Groups	Body weight (g)	Interscapular BAT (mg/100 g body weight)	Cervical BAT (mg/100 g body weight)
Warm-adapted male rats (W) (6)	300±12	40.0±5.86	10.1±0.67
C5W male rats (9)	293±3	111.4±5.48	21.8±1.25
P vs. W		<0.001	<0.001
DA-3G male rats (11)	348±5	98.4±8.40	21.5±1.07
P vs. W		<0.001	<0.001
P vs. C5W		NS	NS

Legends same as in Tables 1 and 3.

Table 5. Dry matter, fat, and water content of interscapular brown adipose tissue (BAT).

Groups	Interscapular BAT (%)		
	Dry matter	Fat	Water
Warm-adapted male rats (W) (5)	14.1±1.45	43.4±4.26	42.4±3.65
C5W male rats (9)	20.4±1.19	26.2±3.79	53.4±3.54
P vs. W	<0.01	<0.02	NS
DA-3G male rats (11)	8.5±0.44	54.2±1.81	37.3±1.64
P vs. W	<0.001	<0.02	NS
P vs. C5W	<0.001	<0.001	<0.001

Legends same as in Tables 1 and 3.

injection (Fig. 11).

Though changes in *Tc* after saline infusion did not differ between DA-3G and W groups (280–383 g), NE infusion at a rate of 2 μg/min for 30 min resulted in a significantly greater rise in *Tc* ($p<0.05$) in DA-3G than in W rats (Fig. 12).

(2) Changes in Tc of unanesthetized, fasted DA-3G rats caused by cold exposure. The change in *Tc* after 120 min of cold exposure was −0.97±0.22°C (mean±S.E.) in W, +0.06±0.13°C in DA-3G, and +0.66±0.15°C in C5W group (Table 3). The change in DA-3G was intermediate between W and C5W rats, and it significantly differed from the changes in W and C5W groups.

3) BAT weights and its chemical compositions

BAT weights of DA-3G rats were significantly heavier than those of W rats and were almost similar to those of C5W rats (Table 4). However, tissue color of BAT in DA-3G rats looked more whitish than that of C5W rats.

The percentage of fat-free dry matter of interscapular BAT was 8.5±0.44

Table 6. Protein content of interscapular brown adipose tissue (BAT).

Groups	Interscapular BAT protein content (mg/g tissue)
Warm-adapted male rats (W) (5)	3.5±0.52
C5W male rats (9)	4.8±0.42
P vs. W	NS
DA-3G male rats	1.9±0.21
P vs. W	<0.01
P vs. C5W	<0.001

Legends same as in Tables 1 and 3.

(mean±S.E.) in DA-3G, being significantly smaller than 20.4±1.19 in C5W rats and 14.1±1.45 in W rats. BAT in DA-3G rats contained a higher percentage of fat in contrast with a lower percentage of dry matter as shown in Table 5. Protein content of BAT in DA-3G rats was also smaller than that in W and C5W rats (Table 6).

Fatty acid composition of interscapular BAT was determined in these groups (Table 7). BAT in DA-3G rats contained a lower percentage of palmitic acid (C16:0) and stearic acid (C18:0) and a higher percentage of linoleic acid (C18:2) as compared with W rats. BAT in C5W rats contained a higher percentage of palmitoleic acid (C16:1) and oleic acid (C18:1) than that in W rats. These results indicated a higher percentage of total poly-unsaturated fatty acids, and a lower percentage of saturated fatty acids in DA-3G than in W rats. A higher percentage of total poly-unsaturated fatty acids and a lower percentage of mono-unsaturated fatty acids in C5W rats was also noted as compared to W rats.

4) Body and tail lengths

The ratio of tail length to body length was smaller in C11–12G than in W rats. This ratio in DA-1G, 2G, and 3G rats was also smaller than that in W rats, being similar to the ratio in C11–12G rats (Table 8).

DISCUSSION

The results on cold tolerance and nonshivering thermogenic capacity in C8–11G rats clearly indicate that the rats reared for many generations under a cold environment developed greater cold adaptability than warm-adapted controls (W) and even animals adapted to cold for one generation (C) (Table 1 and Figs. 1, 2, 9). The augmented cold adaptability in C8–11G rats was decreased to the same level as W or C2W rats by administration of ganglion blocker (Figs. 3, 4), reserpine (Figs. 5, 6), and β-adrenoceptor blocker (Table 2 and Fig. 8) or surgical hibernectomy (Fig. 7). In C rats, NE is well known as a main mediator of non-

Table 7. Fatty acid composition of interscapular brown adipose tissue (BAT). (%)

Groups	Warm-adapted (W)	C5W rats	DA-3G rats
No. of rats	6	9	11
C14: 0	2.3±0.23	1.8±0.08	2.3±0.11
P vs. W		<0.05	NS
P vs. C5W			<0.001
C14: 1	0.1±0.03	0.1±0.02	0.2±0.02
P vs. W		NS	<0.02
P vs. C5W			<0.01
C16: 0	29.6±0.81	28.2±1.02	24.9±0.55
P vs. W		NS	<0.001
P vs. C5W			<0.01
C16: 1	2.0±0.22	1.3±0.12	2.3±0.24
P vs. W		<0.02	NS
P vs. C5W			<0.01
C18: 0	7.9±0.97	10.4±0.34	7.1±0.22
P vs. W		<0.01	NS
P vs. C5W			<0.001
C18: 1	29.0±0.69	25.7±0.52	29.4±0.41
P vs. W		<0.05	NS
P vs. C5W			<0.001
C18: 2	26.4±0.58	28.1±0.82	30.9±0.92
P vs. W		NS	NS
P vs. C5W			<0.05
C18: 3	2.3±0.17	2.1±0.17	2.6±0.11
P vs. W		NS	NS
P vs. C5W			<0.02
C20: 4	0.5±0.20	2.5±0.23	0.5±0.11
P vs. W		<0.001	NS
P vs. C5W			<0.001
Total			
Saturated	39.8±0.75	40.4±0.95	34.2±0.49
P vs. W		NS	<0.001
P vs. C5W			<0.001
Mono-unsaturated	31.1±0.89	27.0±0.57	31.9±0.59
P vs. W		<0.01	NS
P vs. C5W			<0.001
Poly-unsaturated	29.1±0.79	32.7±1.14	33.9±0.99
P vs. W		<0.05	<0.01
P vs. C5W			NS

Legends same as in Table 1.

shivering thermogenesis, since administration of ganglion blocker, reserpine, or β-adrenoceptor blocker could suppress augmented heat production through non-shivering thermogenesis in the cold (HSIEH and CARLSON, 1957; JANSKÝ, 1973). The present findings, together with those previous ones, suggest that essentially

Table 8. Body and tail length of warm-adapted, cold-adapted (reared for 11–12 generations in cold), and cold-deadapted male rats.

Groups	Body weight (g)	Body length (cm)	Tail length (cm)	Total length (cm)	Tail length / Body length
Warm-adapted rats (W) (6)	306±6	23.4±0.23	18.9±0.21	42.3	0.81±0.01
C11–12G rats (15)	373±10	23.3±0.21	17.3±0.37	40.6	0.74±0.01
P vs. W					<0.001
Cold-deadapted rats					
DA-1G (7)	541±16	25.4±0.25	17.6±0.32	42.9	0.69±0.01
P vs. W					<0.001
DA-2G (7)	421±8	24.1±0.22	17.1±0.02	41.1	0.71±0.01
P vs. W					<0.001
DA-3G (10)	329±8	22.5±0.16	16.2±0.14	38.7	0.72±0.01
P vs. W					<0.001

C11–12G, rats reared in cold for 11–12 successive generations; DA-1G to 3G, de-adapted rats reared in warmth for 1 to 3 generations after many successive generations in cold. Legends same as in Table 1.

the same mechanism as that in C rats lies under the higher cold adaptability of C8–11G rats. In addition, it was noted that the rats reared in the cold for many generations showed greater nonshivering thermogenic capacity as assessed by the markedly small decrease of T_C in the curarized animals in the cold, indicating further development of improved cold adaptability due to successive rearing for many generations under cold conditions. The cause of this phenomenon observed in the present study remains unexplained. In this context, the results from the de-adapted rats which derived from a rat strain reared for many generations in the cold would be very suggestive.

The improved cold tolerance and nonshivering thermogenesis observed in C or C8–11G rats were also found in DA-3G, as compared with cold tolerance and nonshivering thermogenesis in W rats. These parameters were assessed from the changes in $\dot{V}_{O_2}$ (Fig. 10), CO_2 output (Fig. 11), and body temperature induced by NE (Fig. 12), or by cold exposure (Table 3). Since the increase in heat production induced by NE is known to depend on the capacity of nonshivering thermogenesis (JANSKÝ, 1973), DA-3G rats may possess more augmented nonshivering thermogenesis than W rats. However, judging from the fact that decrease of T_C in DA-3G rats in the cold was intermediate between decreases of T_C in W and C5W rats, cold tolerance of DA-3G rats is inferred to be smaller than that of C2W or C8G rats (Fig. 9). Nevertheless, it should be noted that DA-3G rats possess greater cold adaptability than W rats though they were reared at 25°C since their birth.

Besides thermoregulatory capacity, some morphological characteristics in relation to thermoregulation, including a shorter tail (Table 8) and larger BAT mass (Table 4), were observed in DA-3G rats as compared with tail length and BAT mass in W rats; DA-3G were similar in tail length and BAT mass to the rats reared

in the cold for many generations (Tables 4, 8). However, the chemical composition of BAT in DA-3G rats differed strikingly from that in rats reared in the cold for many generations and W rats (Tables 5–7). Presently, it is difficult to exdlain these findings in DA-3G rats in association with their cold adaptability. However, it is likely that such persistently improved thermoregulatory functions of DA-3G rats in the cold reflect a genetical change in the cold-adapted rats reared in cold for many generations. DA-3G rats used in the present study were not exposed to cold throughout their lives, including their neonate period. It is known that adult C rats lose their enhanced nonshivering thermogenesis several weeks after they are transferred from cold to warm environments (BARTŮŇKOVÁ *et al.*, 1971). Therefore, an improved cold adaptability observed in DA-3G rats may well come from a genetic change transferred from mothers which have been adapted to cold for many generations. However, it was reported that the young rats reared in warm environments from a mother exposed chronically to cold exhibited an improved ability to tolerate cold through an enhanced nonshivering thermogenesis, and that this ability is possibly mediated by some neurohumoral factors responsible for the enhancement of cold adaptation developed in the mother (DOI and KUROSHIMA, 1982). Moreover, cold adaptability obtained in the neonatal period was reported to last for 18 weeks after the termination of cold exposure (DOI and KUROSHIMA, 1979). Thus, the possibility may not be ruled out that cold adaptability acquired during foetal or neonatal period could persist for a certain period of time. But, it seems difficult to explain an improved cold tolerance in DA-3G rats since the animals were born from parents reared in a warm environment and not exposed to cold at all throughout their lives. In this study, DA-3G rats still exhibited higher cold tolerance and thermogenic capacity than those of W rats. It may be possible that a genetic factor is involved in the higher cold tolerance of DA-3G rats. However, this presumption raises a question as to whether the Wistar rats bred in cold for many generations possess a potential which leads to a genetic change suitable to a cold environment. Recently, BARNETT and DICKSON (1984) found that wild mice after breeding at 3°C without any manipulation for ten generations exhibited changes in maternal milk production and its composition which appeared to reflect a genetical change. Although some problems remain in comparing the results of the present study with theirs, for example, in species differences between rats and mice, a difference in degree of genetic homogeneity between our inbred rats and their outbred mice, and other factors, these results suggest a possibility that physiological adaptation to a living environment could lead to a genetic change (GORCZYNSKI and STEELE, 1980).

The possibility may also exist that selection of cold-tolerant individuals occurs during breeding of the rats for many generations in the cold, as occurred in a study of heat-tolerant rats (FURUYAMA and OHARA, 1978). As written in MATERIALS AND METHODS, the newborn individuals were naturally selected by their cold tolerance and/or maternal ability of breeding in the process of cold

adaptation for many successive generations. Not a small portion of the newborn rats died in each generation until the C'12G was reached, presumably because of their low cold tolerance. Therefore, mating might have proceeded between surviving brothers and sisters considered to have superior cold tolerance. Furthermore, there is the possibility that the newborn individuals which could develop more cold tolerance in utero and exhibit it immediately after birth could survive in cold, since the experience of cold exposure in utero could enhance cold tolerance in neonatal stage of rats (DOI and KUROSHIMA, 1982).

It is likely that poly-gene is related to the quality and quantity of cold adaptability of mammals, and it is believed that phenomena controlled by poly-gene can be affected by various factors of animal's ontogenic environments (WADDINGTON, 1966). Although we could not define the affecting factor(s) or genotropic substances, the genetic activity or composition of rats reared in the cold for many generations might be changed to secure their survival in the cold. Further study is needed to answer the questions raised in this study.

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