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Effects of Post-Weaning Differential Housing on Serum Testosterone Levels in Male Mice throughout Aging

SATOSHI KOIKE[1] and TETSUO NOUMURA

Department of Regulation Biology, Faculty of Science, Saitama University, Urawa, Saitama 338, Japan

ABSTRACT—The influence of differential housing (1, 2, 5 or 10 per cage) on serum testosterone (T) levels was studied in male ICR mice from 21 days to 550 days of age. In socially-housed individuals, the circulating T levels were lowered with increasing group size from 60 to 200 days of age. Peak T levels in groups of 2, 5 and 10 males were 5.55, 4.09 and 2.65 ng/ml, respectively. However, individual T levels showed great variations ranging from 1.67 to 20.49 ng/ml within a cage. Suppressive effect was exclusively focused on a few members in each cage, and intensified with increasing group size throughout 200 days of age. But the difference of serum T levels among different group sizes and the individual variations within a cage were gradually decreased with age since 300 days of age. On the other hand, singly-housed individuals showed different age-associated changes in serum T levels from those of socially-housed ones. These results suggest that (1) testicular endocrine function may be greatly influenced by grouping size from sexually maturing period through adult life in male mice, (2) this effect is concentrated on a few members within a cage, and (3) singly-housing condition may be qualitatively different from socially-housing condition.

INTRODUCTION

There are many reports for age-associated changes in endocrine functions in mice [1–5]. Most of them reported the increase in reproductive functions and in circulating levels of sex steroids during sexually maturing period, and the decrease in the senescent and old age. Machida *et al.* [6] determined plasma testosterone levels over almost the whole range of the life span of male mice of their ICR colony.

On the other hand, it is known that the size of group in gregarious species plays very important roles on their body growth, physiological and psychological conditions [7–13]. In laboratory mice, body weight gain was decreased with increasing population density [7–9] and the onset of puberty was delayed [14, 15]. For relationship between group sizes and endocrine functions in male mice, many investigators have reported that the functions of hypothalamic-pituitary-gonadal axis were decreased with increasing population size, but those of hypothalamic-pituitary-adrenocortical axis were increased [12, 13, 16].

In the present paper, we examined the influence of housing conditions with different group sizes (1, 2, 5 and 10 per cage) on serum testosterone levels in male mice from juvenile to senescent period.

MATERIALS AND METHODS

Animals

Mice were raised from a randomly bred ICR strain colony maintained in the animal room of the Department of Regulation Biology in the Saitama University. The day when litters were found was designated as day 1 of life for the pups. At 21 days of age, male mice were weaned and randomly divided into groups of 1, 2, 5 and 10 per cage (15 × 22 × 12 cm plastic cage with a wire cover). All animals were maintained in a temperature (21 ± 1°C)—and humidity (50% relative humidity)—controlled animal room with a light cycle of 14 hr of light/10 hr of dark (lights on at 0600). Mouse chow (Charles River CRF-1) and water were pro-

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[1] Present address: Upjohn Phamaceuticals Limited, Tsukuba, Ibaraki 300-42, Japan.

vided *ad libitum*. In this colony of the ICR mouse, the mean life span was around 450 days for male animals maintained in group-housing, two to ten per cage. On socially-housing groups, the members of each group were shared to two subgroups, social dominance (D) or subordinance (S) in each cage, according to observation of wounding and coat conditions.

Testosterone determination

Blood samples were collected into non-heparinized syringes by cardiac puncture under ether anesthesia at the appropriate age (21, 30, 60, 90, 120, 150, 200, 300 and 550 days of age, respectively) between the hours of 1100 and 1300 in order to control for diurnal fluctuations in hormone levels, immediately centrifuged twice at 3,000 rmp for 10 min, and then the serum was separated and stored at -20°C until assayed.

Serum testosterone (T) levels were determined by a radioimmunoassay. The antiserum (against testosterone-11a-succ-BSA, Teikoku Hormone Mfg, Co., Ltd.) used reacted with testosterone, 5a-dihydrotestosterone, androstenedione, androstenediol and other steroids to the extent of 100, 13.5, 2.24, 1.39 and less than 0.2%, respectively. Therefore, chromatographic separation of DHT from T after extraction was not carried out. In order to examine the accuracy of the method, various quantities of nonradioactive T were added to serum obtained from female mice and assayed as references. This gave the recovery of 94%. Precision of the method was ascertained by calculating a coefficient of variation. The interassay variation was 5.26% and the intrassay variation was 5.39%. The least detectable T dose in the assay was 4.0 pg/tube. Individual T value was the mean of duplicate determinations and was expressed as nanograms per mililiter of serum.

The mean T values were calculated in each group size and each subgroup, respectively. Statistical comparisons were performed either by Student's t-test or by Aspin Welch's *t*-test. Significant levels were $P<0.05$.

RESULTS

Serum T values of each male from 30 to 550 days of age were shown in Figure 1. In both singly-housed and socially-housed mice, considerable individual variation in serum T levels was observed at most ages. In particular, a wide range of individual variation was 0.20–18.07 at 150 days for 1 per cage, 2.03–11.22 at 60 days for 2 per cage, 1.67–20.49 at 150 days for 5 per cage, and 0.78–8.85 ng/ml at 90 days of age for 10 mice per cage.

The mean values of serum T concentration were shown in Table 1 and Figure 2. In all groups, the mean T values raised linearly from 21 to 60 days of age, but the rate of increase varied with group sizes. Singly-housed males took two peaks on the mean T level; the first peak value was 3.22 ± 0.73 ng/ml at 60 days and the second, a higher peak level was 6.70 ± 1.66 ng/ml at 120 days of age. Age-associated changes of T levels in singly-housed males were different from socially-housed ones, which took one peak, from 21 through 120 days of age. But age-associated changes in singly-housed ones were similar to those in 2-males group after 150 days old. In socially-housed males, the circulating T levels tended to be lowered with increasing group size from 30 through 90 days of age and the peak level did in a similar manner (5.55 ng/ml for 2-males at 60 days, 4.09 ng/ml for 5-males at 60 days, 2.65 ng/ml for 10-males at 90 days of age). So, age-associated changes in serum T levels showed characteristic of each group size throughout 200 days of age, but at 300 and 550 days of age there were not so much differences among different group sizes except for a lower level in 10-males group at 550 days old.

In socially-housed mice, a rough correlation was shown between social structure and serum T level in male mice within a cage. The mean T level of dominant male was higher than the average T concentration of the subordinates. Then, it was examined whether age-associated patterns of serum T levels differ between subgroups. Age-associated changes of mean T levels of every subgroups in each group size were shown in Figure 3. In all groups, the mean T values of the dominants were remarkedly raised between 30 and 60 days of age, and then held at high levels. Age-associated patterns were characteristic in each group size. In 2-males, serum T achieved to peak levels 7.43 ± 1.36 ng/ml at 60 days and gradually

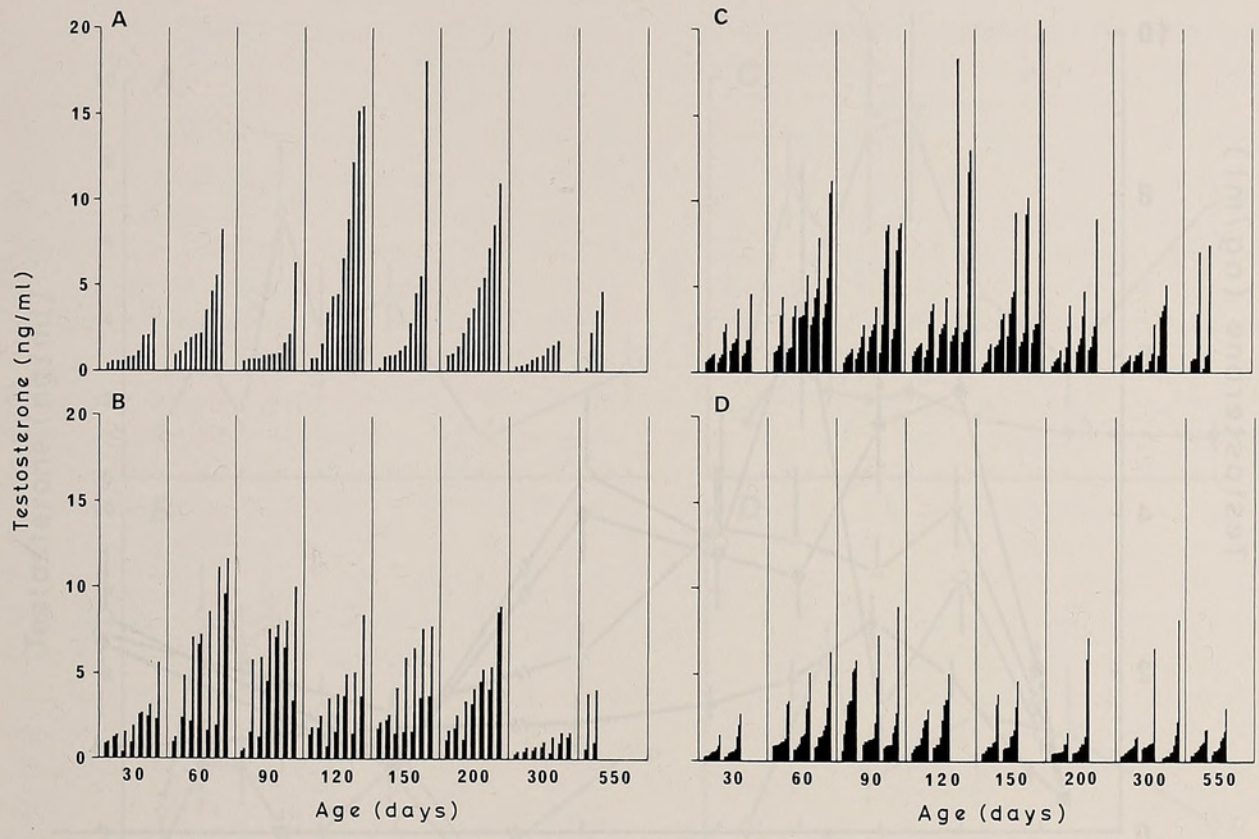

FIG. 1. Individual serum testosterone levels in males mice maintained in differently grouping sizes. A: One-male. B: 2-males. C: 5-males. D: 10-males. One column showed one male. In 2-, 5- and 10-males group, each cage was showed as one block consisted of two, five and ten columns, respectively. Some blocks consisted of 4 and 9 columns in 5- and 10-males group at 300 and 550 days of age, respectively, because one male died just before the sacrificed day.

TABLE 1. Serum testosterone levels of male mice housed in different group sizes from 21 days of age. Serum testosterone values at 21 days of age represented the result for assays of serum in a litter unit of male pups obtained from 4 mothers.

Mice per cage	Age in days								
	21	30	60	90	120	150	200	300	550
1	0.41[b]	1.21	3.22	1.53	6.70	3.66	4.52	0.91	2.69
(11)[a]	±0.02	±0.28	±0.73	±0.50	±1.66	±1.69	±0.99	±0.18	±0.95
		(10)[c]	(10)	(11)	(11)	(10)	(11)	(9)	(4)
2	0.41	2.04	5.55	5.11**	3.20	3.50	4.00	0.85	2.39
(7)	±0.02	±0.36	±1.02	±0.85	±0.54	±0.68	±0.65	±0.12	±0.92
		(14)	(14)	(14)	(14)	(14)	(14)	(14)	(4)
5	0.41	1.66	4.09	3.26	3.70	3.82	2.21	1.51	2.47
(5)	±0.02	±0.23	±0.52	±0.56	±0.88	±0.88	±0.46	±0.32	±0.94
		(20)	(25)	(25)	(24)	(25)	(19)	(20)	(9)
10	0.41	0.78††	1.96††	2.65††	1.93*	1.59††	1.38**	1.30	1.07
(3)	±0.02	±0.15§§	±0.27§§	±0.41	±0.28†	±0.29§	±0.41††	±0.34	±0.16
		(20)	(30)	(28)	(20)	(19)	(20)	(28)	(19)

a Number of cages
b Mean ± SE (ng/ml)
c Number of mice
*,** Significantly different from 1 mouse per cage (* $p<0.05$, ** $p<0.01$)
†,†† Significantly different from 2 mice per cage († $p<0.05$, †† $p<0.01$)
§,§§ Significantly different from 5 mice per cage (§ $p<0.05$, §§ $p<0.01$)

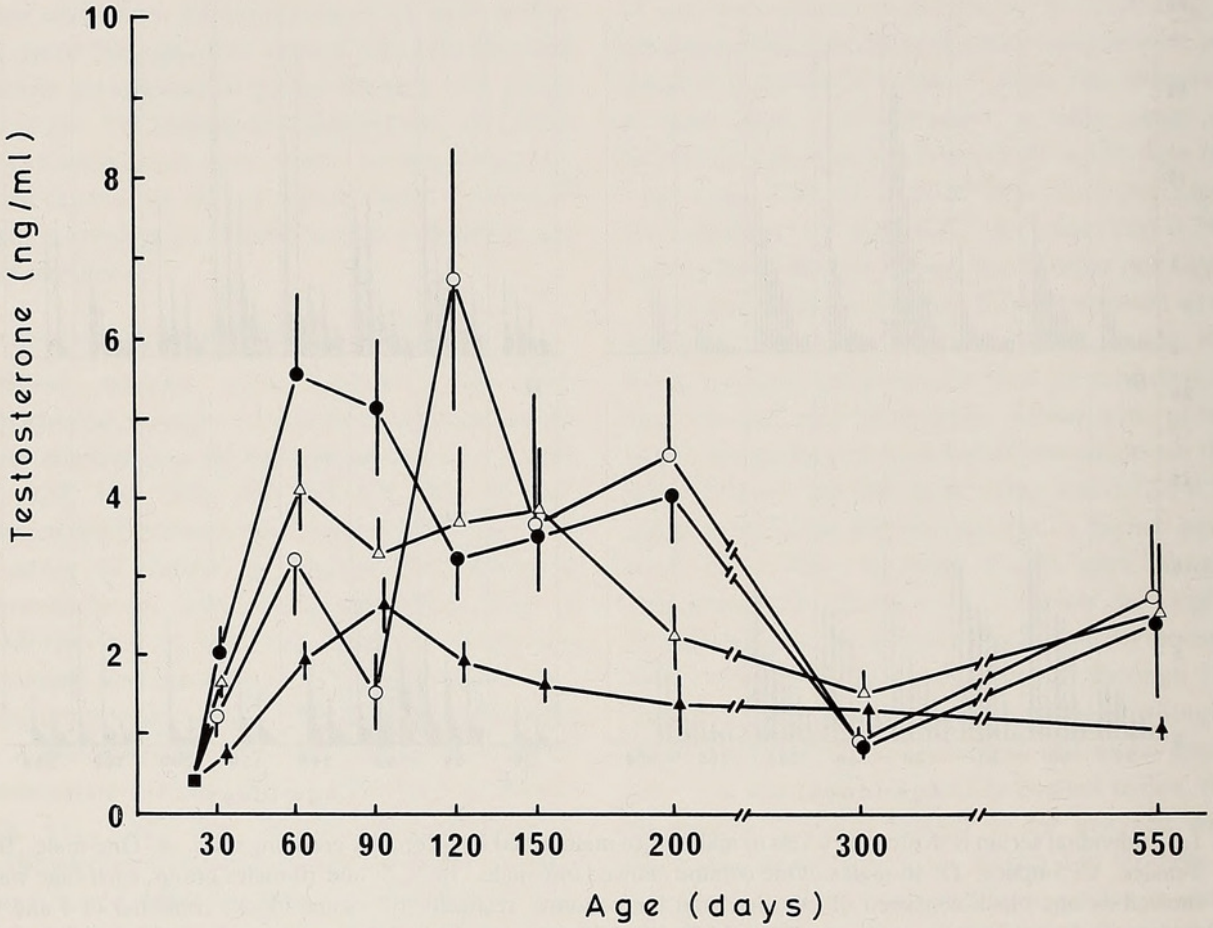

FIG. 2. Age-associated changes in serum testosterone levels of male mice from weaning to senescent ages when maintained in differently housing conditions. Each point showed mean testosterone levels and vertical bar showed standard error of the mean. Statistical significance represented Table 1. Open circle (○), closed circle (●), open triangle (△) and closed triangle (▲) expressed one-, two-, five- and ten-males group, respectively.

decreased through 300 days but increased again at 550 days. In 5-males, serum T levels showed a similar pattern to those in 2-males but the second, higher peak level appeared at 150 days (9.04 ± 2.70 ng/ml). In 10-males, serum T increased linearly through 90 days to peak levels of 6.43 ± 0.72 ng/ml, and continued second high levels of about 4 ng/ml between 200 and 300 days, but decreased again to 2.24 ± 0.43 ng/ml at 550 days.

T levels of the subordinates took much lower values compared with those of the dominants since 30 days of age. In the subordinates in 2-males serum T levels showed age-associated changes through 200 days and then decreased to less than 1 ng/ml since 300 days; peak levels taken at 60 and 200 days were 3.68 ± 1.22 and 3.53 ± 1.00 ng/ml, respectively. In 5-males, T levels of the subordinates gradually decreased from 60 days through 550 days with the peak levels of 3.03 ± 0.30 ng/ml at 60 days of age. The subordinates in 10-males had peak T levels of 1.83 ± 0.24 ng/ml at 90 days and had low levels as 1 ng/ml since 200 days of age.

DISCUSSION

In this study, it is suggested that the post-weaning housing conditions may influence serum T levels in male mice through 200 days of age. From puberty to adult, the circulating T levels in both the average and the peak values were lowered with increasing group size. In 10-males group, the age when serum T reached the first peak values delayed. These results are partially consistent with the previous findings. Jean-Faucher *et al.* [15]

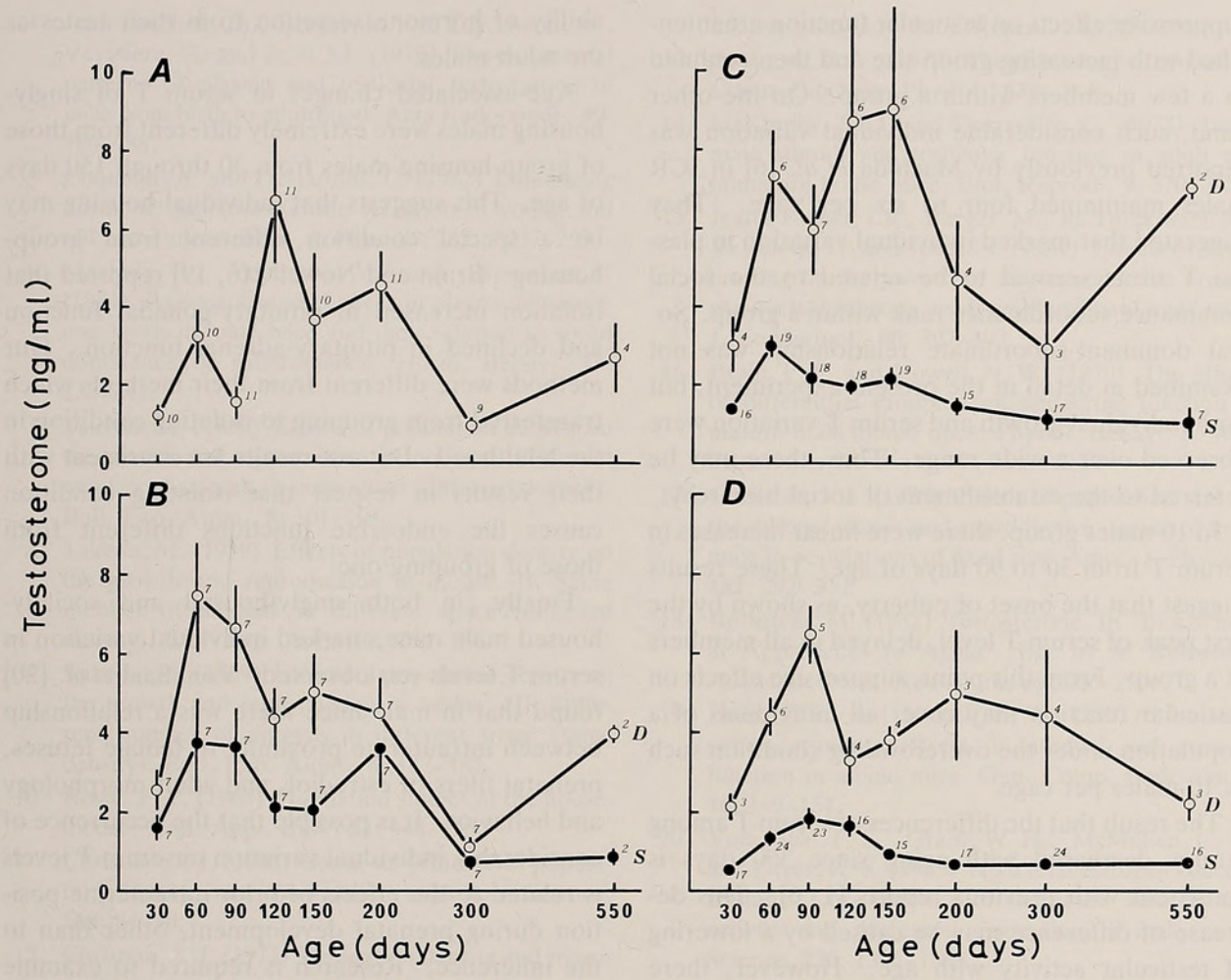

FIG. 3. Age-associated changes in serum testosterone levels between dominants (D) and subordinates (S) in differently housing male mice. A: One-male. B: 2-males. C: 5-males. D: 10-males. Each point showed mean testosterone levels and vertical bar showed standard error of the mean. The number on each point showed number of mice classified into dorminance and subordinance.
Statistically significant difference between D and S represented 120, 150 and 550 days in 2-males, from 60 to 120 and 550 days in 5-males, and from 30 to 150 and 550 days of age in 10-males.

reported that plasma T levels were lowered with increasing group size from weaning to 50 days but increased at 60 and 90 days in female-containing male groups, and Christian [12, 17] suggested that functions of hypothalamic-pituitary-testicular axis were decreased with increasing population size and population density in the small mammals. On the other hand, there is a suggestion that reduction of circulating T level might be partially related to increases in plasma ACTH and adrenocortical hormones which would be caused by the social stress within a group [16]. From this point, group size might influence the degree of stress within a group to affect serum T levels.

The reduction of mean serum T level with increasing group size was caused largely by the decrease in serum T level of the subordinates to which belonged the most part of group, and there was the extreme decrease in a few members within subordinates. The degree of decrease in serum T levels of the subordinates was enhanced with increasing grouping size, because serum T level was higher in 2-males than in 5- and 10-males, respectively and because the subordinates of 5-males were higher T levels than those of 10-males, respectively. However, testicular functions in members of the dominance would be stimulated by an increase in groups size because they kept greatly higher T level compared with those of the subordinates in all ages. These results suggest that

suppressive effects on testicular function are intensified with increasing group size and then confined to a few members within a group. On the other hand, such considerable individual variation was reported previously by Machida *et al.* [6] in ICR males maintained four to six per cage. They suggested that marked individual variation in plasma T titers seemed to be related to the social dominance/subordinance rank within a group. Social dominant-subordinate relationship was not examined in detail in the present experiment, but both individual growth and serum T variation were observed over a wide range. Thus, these may be referred to the establishment of social hierarchy.

In 10-males group, there were linear increases in serum T from 30 to 90 days of age. These results suggest that the onset of puberty, as shown by the first peak of serum T level, delayed in all members of a group. From this point, suppressive effects on testicular function may cover all individuals of a population under the overcrowding condition such as 10-males per cage.

The result that the differences of serum T among groups decreased with aging since 300 days is consistent with previous reports [1, 6]. This decrease of difference may be caused by a lowering of testicular activity with age. However, there were still many differences within cages in all socially-housing groups after 300 days of age, much the same as before 200 days. This suggests that the suppressive effect due to housing condition is unevenly distributed among individuals within a cage through life span and that some males may keep high testicular activities, even at the highest density of 10 per cage, and some males may be consistently suppressed.

Mean T level at 550 days was higher than that at 300 days of age in all grouping sizes. In the colony of the ICR mouse used in this experiment, the last tenth survival day was 380, 520, 540 and 550 for 1-, 2-, 5- and 10-males, respectively. Therefore, testosterone secretion in male mice that survived until 550 days of age might not reduce. Rothstein [18] reviewed that there were no changes in plasma levels of T between the adult (8–11 months) males and the healthy old (29–31 months) males in C57BL/6J mice. These results suggest that the healthy senescent males may maintain the same ability of hormone secretion from their testes as the adult males.

Age-associated changes in serum T of singly-housing males were extremely different from those of group-housing males from 30 through 150 days of age. This suggests that individual-housing may be a special condition different from group-housing. Brain and Nowell [16, 19] reported that isolation increased in pituitary-gonadal function and declined in pituitary-adrenal function. Our methods were different from their methods which transferred from grouping to isolating condition in the adulthood. But our results are consistent with their results in respect that isolating condition causes the endocrine functions different from those of grouping one.

Finally, in both singly-housed and socially-housed male mice, marked individual variation in serum T levels was observed. Vom Saal *et al.* [20] found that in male mice there was a relationship between intrauterine proximity to female fetuses, prenatal titers of estradiol, and adult morphology and behavior. It is possible that the occurrence of considerable individual variation in serum T levels is related to the effects of prior intrauterine position during prenatal development, other than to the inherence. Research is required to examine this relationship.

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