— Note —

Reduced Fertility in Gracile Axonal Dystrophy (*gad*) Mice

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Mean litter size in *gad/gad* females was significantly lower than in normal females (+/+ and *gad*/+) in intra- and inter-strain crosses. The reduction in litter size was not dependent on the genotypes of the males, but could be attributed to the *gad/gad* females themselves. The numbers of corpora lutea and implants in *gad/gad* females were slightly reduced as compared with those in the controls, but the number of live fetuses was significantly lower than that in normal females 14 days after copulation ($P<0.02$). Hence, reduced litter size in *gad/gad* females was accounted for mostly by embryonic and fetal death after implantation, which was inferred to be due to impaired uterine function.

Gracile axonal dystrophy (*gad*) is an autosomal recessive mutation in mice which causes degeneration in the gracile nucleus of the medulla oblongata and the gracile fasciculus of the spinal cord. It is characterized by neuroaxonal dystrophy or focally swollen axons [12]. The mutant could be a useful animal model for several human diseases, such as infantile neuroaxonal dystrophy and Hallevorden-Spatz disease. Furthermore, it could be used to investigate the aging process. The homozygotes show ataxia at about 80 days of age due to spinal degeneration. Thereafter, the neurological symptoms become progressively severe and reproductive ability is lost. Because the homozygotes can be distinguished from normals at about 1 month of age by a behavioral test [12], it is possible to use the homozygotes for breeding until the onset of the symptoms. The homozygous females can give birth to two litters. However, it has frequently been observed that litter size in the

homozygous females is smaller than in normal females.

The purpose of the present study was (1) to confirm the reduction in litter size in *gad/gad* females, (2) to identify the stage when embryonic or fetal loss occurs and (3) to present efficient breeding methods to maintain the *gad* gene and establish a congenic strain.

Mice strains used in this study were Gad/Nga (Gad) [12] and C57BL/6Nga (B6). The mice were housed in plastic cages, and water and a diet were available *ad libitum*. Matings were set up about 60 days of age.

Litter sizes in the following matings were examined: (1) matings between normals from the Gad strain, (2) reciprocal crosses between homozygotes (*gad/gad*) and normals within the Gad strain, (3) B6 females $\times$ B6 males, (4) reciprocal crosses between B6 individuals and homozygotes (*gad/gad*) from the Gad strain, (5) matings between F_1 (*gad*/+) individuals from the above crosses, and (6)

reciprocal crosses between the F_1 individuals and homozygotes (gad/gad) from the Gad strain. It should be noted that normal individuals from the Gad strain included both heterozygotes ($gad/+$) and homozygotes ($+/+$). Next, comparison was made between litter sizes at the first and second parturitions in homozygous (gad/gad) females to determine whether litter size at the second parturition is smaller due to the progress of neurological symptoms. The females that produced only one litter were excepted from this examination. Distinction between normal and affected mice was made before mating by the behavioral test as described previously [12] and was confirmed after 80 days of age.

To examine the stage when embryonic or fetal loss occurs, two kinds of crosses, homozygous (gad/gad) females $\times$ normal males and normal females $\times$ normal males, were conducted within the Gad strain. One female and one male were caged at about 60 days of age and kept together until the female showed a vaginal plug. Females with plugs were isolated and the day when the plug was found was scored as Day 0 of gestation. Pregnant females were killed by cervical dislocation 14 days after coitus and the numbers of corpora lutea, im-

plants and live fetuses were determined [6].

Table 1 shows mean litter sizes in the experimental and control crosses of the Gad and B6 strains and their F_1 hybrids. Litter size in the experimental cross (homozygous females $\times$ normal males) was significantly lower than that in its reciprocal cross ($P < 0.05$) and the control cross (normal females $\times$ normal males) ($P < 0.001$). It was about half that in the reciprocal cross.

Genotypes of normal mice from the Gad strain could not be determined in some cases. Therefore, the matings were conducted using B6 ($+/+$) and F_1 ($gad/+$) hybrids between the Gad and B6 strains to determine whether male genotypes have any effect on reduction in litter size in gad/gad females. Significant difference in mean litter size was seen between the reciprocal crosses, homozygous females $\times$ B6 males and B6 females $\times$ homozygous males ($P < 0.001$). Similar results were obtained in the crosses involving the F_1 hybrids, i. e. significant reduction in litter size was observed in the cross of homozygous females $\times$ F_1 ($gad/+$) males. In both crosses litter size was about half that in the reciprocal or control cross. However, litter size was almost the same in both types of crosses, homozygous females $\times$

Table 1. Mean litter sizes in nine types of crosses involving Gad/Nga (Gad) and C57BL/6 (B6) strains, and their F_1 hybrids.

Types of crosses Strains (Genotypes)		No. of pairs	No. of litters	Litter size (mean $\pm$ S.E.M.)
Females	Males			
Normal Gad $\times$ Normal Gad ($+/+$ or $gad/+$)	($+/+$ or $gad/+$)	6	25	7.6 $\pm$ 0.5 ^a
Normal Gad $\times$ Affected Gad ($+/+$ or $gad/+$)	(gad/gad)	8	9	6.8 $\pm$ 1.0 ^b
Affected Gad $\times$ Normal Gad (gad/gad)	($+/+$ or $gad/+$)	7	7	3.1 $\pm$ 1.0 ^{ab}
B6 ($+/+$) $\times$ B6 ($+/+$)		5	15	6.9 $\pm$ 0.4 ^c
B6 ($+/+$) $\times$ Gad (gad/gad)		6	6	7.5 $\pm$ 0.6 ^d
Gad (gad/gad) $\times$ B6 ($+/+$)		8	16	3.7 $\pm$ 0.3 ^{cd}
F_1 ($gad/+$) $\times$ F_1 ($gad/+$)		3	16	7.8 $\pm$ 0.6 ^e
F_1 ($gad/+$) $\times$ Gad (gad/gad)		17	21	8.9 $\pm$ 0.3 ^f
Gad (gad/gad) $\times$ F_1 ($gad/+$)		16	26	3.7 $\pm$ 0.4 ^{ef}

Means sharing a common superscript letter are significantly different at $P < 0.001$ except those sharing b, which are different at $P < 0.05$.

Table 2. Mean litter sizes at the first and second parturitions in two types of crosses involving homozygous females of Gad/Nga(Gad), and C57BL/6 (B6) males and their F₁ hybrid males.

Types of crosses		Litter size (mean±S.E.M.)	
Strains (Genotypes)		1st parturition	2nd parturition
Females	males		
Gad (<i>gad/gad</i>)	× B6 (+/+)	3.7±0.6 (6)	3.8±0.3 (6)
Gad (<i>gad/gad</i>)	× F ₁ (<i>gad/+</i>)	2.9±0.5 (10)	3.9±0.6 (10)

Numbers in parentheses indicate no. of females examined.

Table 3. Numbers of corpora lutea, implants and live fetuses 14 days after copulation in two types of intra-Gad strain crosses¹⁾.

Types of crosses (Genotypes)		No. of corpora lutea	No. of implants	No. of live fetuses
Females	Males			
Normal (+/+ or <i>gad/+</i>)	× Normal (+/+ or <i>gad/+</i>)	9.8±0.4 (16)	8.1±0.6 (16)	6.8±0.5 (16)
Affected (<i>gad/gad</i>)	× Normal (+/+ or <i>gad/+</i>)	8.7±0.4 (13)	6.8±0.4 (13)	4.8±0.5 (13)*

Numbers in parentheses indicate no. of females examined.

1) Values are expressed as mean±S.E.M.

* Significantly different from the number in the cross of normal individuals ($P<0.02$).

Table 4. Chi-square tests for comparison of embryonic and fetal loss between two types of intra-Gad strain crosses.

Types of crosses (Genotypes)		No. of females examined	Total No. of corpora lutea	Total No. of implants	Pre- implan- tation loss	χ^2 -test for pre-implan- tation loss	Total No. of live fetuses	Post- implan- tation loss	χ^2 -test for post-implan- tation loss
Females	Males								
Normal (+/+ or <i>gad/+</i>)	× Normal (+/+ or <i>gad/+</i>)	16	156	129 (82.7)	27 (17.3)	0.97	109 (69.9)	20 (12.8)	5.30
Affected (<i>gad/gad</i>)	× Normal (+/+ or <i>gad/+</i>)	13	113	88 (77.9)	25 (22.1)	0.30<P<0.50	63 (55.8)	25 (22.1)	P<0.025

Number in parenthesis shows percentage to the number of corpora lutea in each cross.

B6 (+/+) males and homozygous females × F₁ (*gad/+*) males, suggesting that the genotypes of the males had little effect on the reduction in litter size. Segregation ratios of normal and affected offspring at F₂ and back-cross generations were in agreement with the expected ratios as observed in the previous study [12]. These results show that *gad* gene has no deleterious effect on viability *in utero* or in postnatal growth. Moreover, the homozygous males were fully fertile before onset of

the neurological symptoms, suggesting that spermatogenesis proceeds normally in the young adult. Summarizing the above results, it may be concluded that litter size is reduced to about half the normal in homozygous (*gad/gad*) females, and that this reduction is attributed to the homozygous females themselves.

Table 2 summarizes mean litter sizes at the first and second parturitions in the homozygous females. Litter sizes of the first and second parturitions were similar, although

neurological symptoms were more severe in the latter.

The numbers of corpora lutea, implants and live fetuses at Day 14 of gestation are presented in Table 3. Numbers of corpora lutea and implants in *gad/gad* females were slightly smaller than those in the normal females, although the difference was not statistically significant. The number of live fetuses, however, was significantly reduced as compared with that in the control cross ($P < 0.02$), and chi-square tests confirmed significant increase in post-implantation loss in the homozygous females (Table 4) ($P < 0.025$).

Litter size in *A^y/a* females has been shown to be significantly lower than that in non-yellow females [1]. Granholm, *et al.* suggested that depression in concentrations of gonadotrophins and/or impairment in sensitivity of ovarian cells to the hormones in *A^y/a* females may lead to lowered number of ova per ovulation, ovarian steroid deficiencies, and poor uterine environments for developing embryos [7]. In *gad/gad* females, the number of corpora lutea was slightly reduced as compared with that in normal females (Table 3), suggesting that levels of gonadotrophins and sensitivity of ovarian cells may be depressed in the homozygous females.

The present study demonstrates that reduction in litter size in *gad/gad* females was attributed mainly to embryonic and fetal losses occurring after implantation. Comparison of the number of live fetuses at 14 days of gestation with the litter size at birth indicates that additional fetal death occurs between Day 14 of gestation and parturition. Factors causing embryonic or fetal death in mice are chromosome abnormality [2], lethal genes [9], poor uterine environments [7], incompatibility between egg cytoplasm and male pronucleus [8, 10, 11], *etc.* In *gad/gad* females, reduction in litter size is not dependent on the genotypes of the males, but can be attributed to the females themselves. It seems probable that uterine function is somewhat impaired during the post-implantational period. With regard to this point, there is a possibility that impairment of uterine conditions leading to small litter size may be caused secondarily by the

progress of the neurological disorder in the homozygotes. However, this possibility can be discarded owing to the following reasons: (1) the litter size is already small at the first parturition. All first litters were observed before 3 months of age when the homozygotes looked normal or neurological symptoms were very slight if any. (2) Litter sizes at the first and second parturitions were almost the same, although the symptoms were more severe at the second parturition.

Vitamin E deficiency causes clinical and pathological features similar to those in *gad/gad* mice [12]. This vitamin was originally demonstrated to have an antisterility action, and a deficiency results in fetal death and resorption in laboratory rats [3, 5]. Ovulation, fertilization and implantation proceed normally in vitamin E deficient animals, but placenta-tion and fetal development are abnormal [4]. The impairment of post-implantational embryonic and fetal development is the common feature between vitamin E deficient rats and *gad/gad* mice. Post-implantational embryonic and fetal death in *gad/gad* females is likely to be attributed to depression in uterine function as noted before. Therefore, it would be interesting to investigate the process of placenta-tion, changes in the concentrations of various hormones during pregnancy, and the level of vitamin E in the homozygous females.

In view of reduced fertility of *gad/gad* mice, i.e. short fertile period and small litter size in the homozygous females, the following two mating systems are being performed: (1) For maintaining the *Gad/Nga* strain, one homozygous male is mated to a few normal sib females before 80 days of age. This mating produces normal progeny (*gad/+*) only, or both affected (*gad/gad*) and normal (*gad/+*) progeny. The normal progeny are mated *inter se* to give homozygotes (*gad/gad*) in the next generation. This procedure is repeated every two generations. (2) For establishing a congenic strain with the *gad* gene, a cross-intercross system would be practicable. A homozygous (*gad/gad*) male is mated to a few females of the other strain and the resulting heterozygotes are intercrossed. This cross-intercross cycle is reiterated.

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GAD (gracile axonal dystrophy) マウスの妊性について

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GAD (gad/gad) 雌マウスの妊性を検討するため系統内及び系統間交配を行なった。GAD 雌の産仔数は正常雌に比べ有意に低く、その低下は雄の遺伝子型に関係なく、GAD 雌自身に原因があることが示唆された。GAD 雌の黄体数・着床数は正常雌に比べわずかに低い程度で

あったが、妊娠 14 日目の生存胎仔数は有意に低かった ($p < 0.02$)。従って GAD 雌の産仔数の減少は、主に着床後の胚及び胎仔の死亡によるもので、その原因として子宮内環境の悪化が推定された。