Gene Complex Controlling Growth and Fertility Linked to the Major Histocompatibility Complex in the Rat

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The Bl strain of rats carries a unique mutation which causes defects in growth and reproduction: the males and females are small, the testes are hypoplastic and aspermatic, and the females have a reduced reproductive capacity. The loci controlling these defects are linked to the major histocompatibility complex (MHC) as determined by segregation studies in backcross and F2 hybrid populations. The levels of pituitary hormones and somatomedin C in the Bl strain are elevated or normal, and the testosterone level is elevated relative to the size of the testes. These findings suggest that hormone deficiencies are not the cause of these defects. The genes governing these defects have been designated the growth and reproduction complex (Grc). The recessive gene regulating small body size has been designated dw-3 (dwarf-3), and the recessive gene influencing reproductive capacity has been designated f. The Grc and MHC are separable by recombination, and the dw-3 and f genes are also separable by recombination. Studies in the (Bl × DA)F2 hybrid indicate that the map distance between the Grc and the MHC is 0.6 cM. Segregation distortion due to a deficiency of $RT1^l$ homozygotes is seen in some F2 hybrid populations derived from the Bl strain. Litter size data suggest that the loss of the $RT1^l$ homozygotes is due to intrauterine death. There is no apparent sex influence on the inheritance of the Grc, at least as it is presently understood, since it can be transmitted by either females or males. The growth and reproduction complex in the rat may be the analog of the T/t complex in the mouse, and the importance of the region of the chromosome adjacent to the major histocompatibility complex in the control of developmental processes may be a general phenomenon in mammals. (Am J Pathol 96:185–206, 1979)

THE GENES in the major histocompatibility complex (MHC) control a set of cell surface antigens that play an important role in determining tissue compatibility, regulating many immunologic responses, and influencing the susceptibility to a variety of diseases (reviewed in Reference 1). The importance of this region of the chromosome for controlling cell surface antigens affecting tissue interactions was dramatically emphasized with the discovery that the T/t complex in the mouse, which controls a variety of developmental defects, is linked to the MHC (reviewed in Reference 2). The proximity of these two gene complexes prompted studies in other species to determine whether this pat-

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tern of chromosomal organization is a general phenomenon. Despite assiduous efforts to find an analog of the T/t complex in other species, no such region has yet been described.

In the course of examining the immunologic and genetic properties of the B-stock rats, in which the $RT1^l$ and $RT1^n$ phenotypes* of the MHC are still segregating, we found that the $RT1^l$ homozygotes were always small and had fertility abnormalities. In contrast, the growth and reproductive capacity of the $RT1^n$ homozygotes and $RT1^{l/n}$ heterozygotes were normal. Because of this consistent association, we explored the possibility that the gene(s) controlling the defects in growth and reproduction might be linked to the MHC and might be the analog in the rat of the T/t complex.

Materials and Methods

The B1 and B3 strains were derived from the B-stock population of rats which was bred for many years for homozygosity of the coat color genes.[3] For the past 6 years the stock was maintained by parallel matings of five families for five generations followed by switching of families systematically and selection for the next breeding cycle of parallel matings. The matings within families were brother × sister. The $RT1^n$ homozygotes, which were designated the B3 strain, were maintained by brother × sister mating for nine generations when used in this study. The B1 strain was maintained by intercrossing the $RT1^{l/n}$ heterozygotes (the B13 animals) and selecting animals homozygous for $RT1^l$ (B1 strain), since the B1 males are sterile. The inbred DA, F344, YO, and LEW strains, which were used in various crosses with the B1 strain, are maintained in our colony by brother × sister mating. All of the animals were kept under conventional conditions and fed rat chow and water *ad libitum*.

In studies investigating the genetic control of the growth and reproductive defects in the B1 strain, it was of interest to determine whether these defects could be transmitted from both the B1 female, which is fertile but has a reduced reproductive capacity, and the B1 male. To obtain an F2 hybrid population in which the abnormal genes were transmitted through the male, a female DA animal was bred with a heterozygous B13 male. The (DA × B1)F1 hybrid that resulted was then intercrossed to obtain the F2 hybrid generation for genetic studies.

The newborn animals were counted at birth and were sexed and counted again at weaning (3 weeks of age). They were weighed two to three times per week thereafter using a Mettler P 1200 or Torbal PL-1 balance; the parental strains were weighed beginning 1 week after birth. The testes or ovaries were removed at 14–18 weeks of age, trimmed, fixed in 10% formalin for 10 days, dried, and weighed on a Mettler H20 balance. The animal's weight at the time of autopsy was used to calculate the gonad/body weight ratio.

The animals were serotyped using the Ficoll hemagglutination method.[3] The follicle-stimulating hormone (FSH),[4] luteinizing hormone (LH),[5] growth hormone (GH),[6] and testosterone[7] were measured by radioimmunoassays using antibodies developed against these hormones, and somatomedin C was measured by a radioimmunoassay using an antibody against the human hormone.[8]

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* The recently adopted nomenclature for the major histocompatibility complex in the rat, formerly called Ag-B or H-1, is RT1 (Workshop on the Major Histocompatibility Complex of the Rat, Transplantation Proceedings 11:1174–1178, 1979): $RT1^l$, Ag-B1 ($H\text{-}1^l$); $RT1^y$, Ag-B2 ($H\text{-}1^w$); $RT1^n$, Ag-B3 ($H\text{-}1^n$), and $RT1^a$, Ag-B4 ($H\text{-}1^a$).

Table 1—Segregation of Phenotypes in the F2 Generation Derived by Intercrossing the Heterozygous B13 Animals

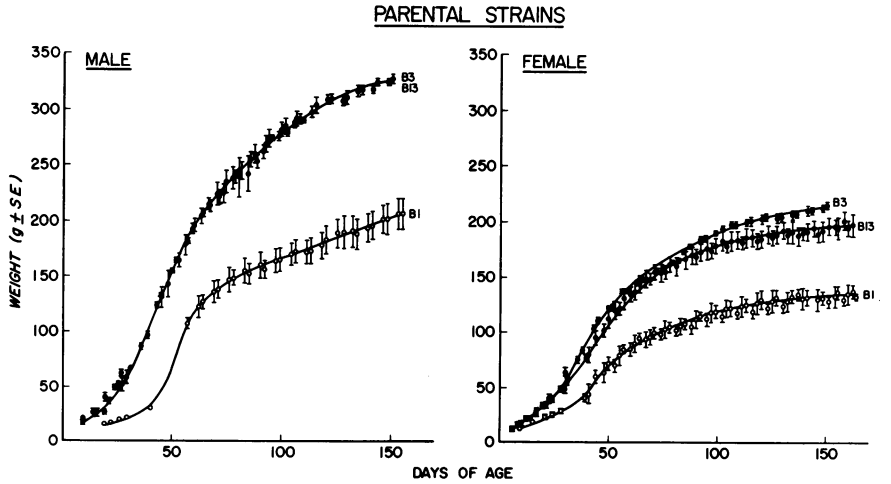
Sex	RT1 phenotype (A region)				Comparison to the expected F2 ratio (1:2:1)	
	(n/n)	(l/n)	(l/l)*	Total	χ^2	P
Female	81	153	27	261	30.103	<0.001
Male	82	141	28	251	27.064	<0.001
Total	163	294	55	512	56.844	<0.001

* All $RT1^l$ homozygotes were small.

Results

The (B13 × B13)F2 hybrids produced from 75 mating pairs were examined for the segregation ratio of the RT1 phenotypes: it did not fit the expected 1:2:1 ratio for either females or males due to a marked decrease in $RT1^l$ homozygotes (Table 1). Only one third of the expected number were present, and all of them were small. On the other hand, the ratio of heterozygotes to $RT1^n$ homozygotes did not deviate significantly from that expected (2:1). This difference was not due to postnatal death, since less than 3% of the animals died before weaning, or to a distortion of the male:female ratio (0.97).

The B1 strain ($RT1^l$) is small compared with the B3 strain ($RT1^n$) and



TEXT-FIGURE 1—The growth curves of the B3, B13, and B1 animals. The difference in size between the B3 and B13 rats and the B1 rats persists throughout the lifespan of the animals. There is also a slight difference in the sizes of the homozygous B3 ($RT1^l$) and heterozygous B13 ($RT1^{l/n}$) females. The number of animals weighed is shown in Table 2.

Table 2—Body Weight, Gonad Weight, and Litter Size of the B3, B13, and B1 Rats

Strain*	RT1 Phenotype (A region)	Body weight (g)						Testes					Ovaries				
		Male			Female			Weight (g)			Percentage of body weight($\times 10^2$)		Weight (mg)			Percentage of body weight ($\times 10^2$)	
		Avg	SD	No.	Avg	SD	No.	Avg	SD	No.	Avg	SD	Avg	SD	No.	Avg	SD
B3	(n/n)	324	7	5	213	7	5	1.291	0.221	94	39.8	6.9	24.50	6.46	108	1.15‡	0.31
B13	(l/n)	320	4	5	198	16	6	1.232	0.291	52	38.5	9.1	23.94	4.71	42	1.21	0.26
B1	(l,l)	204†	24	5	134†	10	5	0.154	0.020	72	7.5†	1.3	15.00	3.83	11	1.12	0.30

* 120–130 days old

† Significantly different from the B3 and B13 animals at $P < 0.001$

‡ $1.10 \pm 0.17 \times 10^{-2}$ percent of body weight in the DA strain

the B13 heterozygotes ($RT1^{l/n}$). The decrease in size is uniform, and it is accurately reflected in the lower body weight and in the proportional decrease in the size of the skeleton and of the internal organs. The small body size is apparent within 4 days of birth (Figure 1), is quite striking in the adult (Figure 2), and persists uniformly throughout the lifespan of the animals (Text-figure 1). The fact that the growth defect in the Bl strain was evident so early in life suggests that postnatal endocrinologic factors do not play a major role in its etiology. Of the three small Bl animals shown in Figure 1, two have no hood and one is hooded. This finding indicates that the genes controlling the decrease in body size are not linked to the genes controlling hood pattern. This is an important observation in relation to the abnormality in the rat causing the testicular feminization (Tfm) syndrome and the restriction of hood pattern (H^{re}),[9,10] which cause some abnormalities similar to those seen in the Bl strain and will be discussed below.

When normalized to body weight, the testes were small but the ovaries were not (Table 2). The Bl males were sterile throughout their lives due to testicular hypoplasia, with an arrest in spermatogenesis at the spermatocyte stage (Figure 3). The Bl females were fertile, but they had a reduced reproductive capacity as measured by litter size when mated with various other strains (Table 3). These defects are not due to decreased hormone production, since the levels of FSH, LH, GH, testosterone, and somatomedin C were normal or elevated (Table 4). The decrease in the amount of testosterone produced is apparent rather than real: it is half the normal level, but the testis weight is one fifth of normal.

Since small body size and testicular hypoplasia were always associated

Table 3—Reproductive Capacity of B1 Females Compared With That of Females From Several Other Strains

Cross	No.	Litter size				Sex ratio at weaning (male: female)
		Birth		Weaning		
		Avg	SD	Avg	SD	
B1 × F344	8	5.25	2.31	2.62	1.92	1.99
B1 × DA	5	5.40	1.52	5.20	1.79	1.36
B1 × B13	19	3.21	1.32	3.20	1.58	0.83
DA × B13	3	ND*		7.33	0.58	
YO × B13	3	8.67	0.58	8.67	0.58	
LEW × DA	12	11.08	1.56	11.08	1.56	0.94

* Not done

Table 4—Hormonal Levels in Male B1 and B3 Strain Rats

Hormone	B1 strain			B3 strain			B1 compared with B3*	
	Avg	SD	No.	Avg	SD	No.	Change	*P*-value
FSH (ng FSH.RP-1/ml)	792	120	4	477	68	5	+	<0.005
LH (ng LH.RP-1/ml)	47.9	22.4	10	14.1	7.3	10	+	<0.001
Testosterone (pg/ml)	1678	934	10	2792	1084	10	−†	<0.025
GH (ng/ml)	9.8	8.9	4	5.2	2.7	4	0‡	
Somatomedin C (units/ml)	0.48	0.09	5	0.72	0.26	4	0	

* +, increased; −, decreased; 0, no change ($P > 0.05$)
† Increased relative to the size of the testes
‡ No difference in females also: 17.9 ± 12.1 (B1) and 13.6 ± 8.8 (B3)

Table 5—Sex, Body Weight, and Testis Weight of Progeny of B1 × (B1 × DA)F1 Backcross

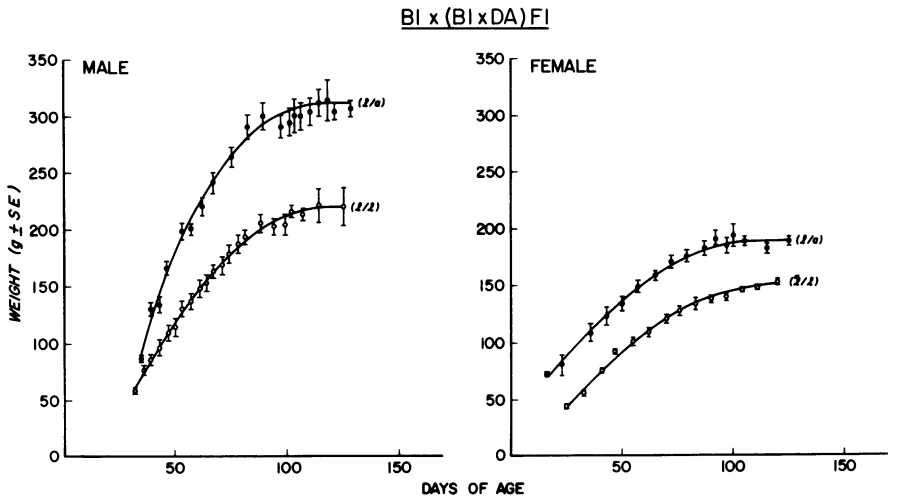
Sex*	RT1 phenotype (A region)	No. of animals†	Body weight (g)		Testes			
					Weight (g)		Percentage of body weight (×10²)	
			Avg	SD	Avg	SD	Avg	SD
Male	(l/a)	27	309	27	1.527	0.086	49.4	5.1
	(l/l)	17	218	22	0.170	0.022	7.8	1.3
Female	(l/a)	14	188	14				
	(l/l)	12	147	6				

* The animals were 120–130 days old.
† The (l/a):(l/l) ratio in the 70 animals examined was 1.41, which differs at $P < 0.05$ from the expected 1:1 ratio.

with the $RT1^l$ phenotype, the possibility that the gene(s) controlling these defects might be linked to the major histocompatibility complex was tested in the appropriate segregating populations by examining whether the defects segregated with the $RT1^l$ phenotype.† In the B1 × (B1 × DA)F1 backcross, the decrease in both body weight and testicular weight segregated with the $RT1^l$ phenotype (Table 5 and Text-figure 2). The heterozygous $RT^{l/a}$ animals had normal body and testis weights, whereas the homozygous $RT1^l$ animals were small and had hypoplastic, aspermatic testes. In this cross, there was no selection against the $RT1^l$ phenotype.

The segregation of body weight and testicular weight with the major histocompatibility complex was also tested in three F2 hybrids. The DA strain was used as the test strain in two of these hybrids because it is a

† In the designation of F1 and F2 hybrids, the maternal strain is written first.



TEXT-FIGURE 2—The weight of the animals from the Bl × (Bl × DA)F1 backcross as a function of age. The heterozygous $RT1^{l/a}$ animals are significantly heavier than the homozygous $RT1^{l}$ animals, and this difference is evident at all ages. The relative differences between the weights of the males of both phenotypes increases with age, whereas the relative differences between the females is constant. The number of animals weighed is shown in Table 4.

good breeder with a high reproductive capacity: the original maternal parent was the Bl strain in the (Bl × DA)F2 hybrid and the DA strain was the original maternal strain in the (DA × Bl)F2 hybrid. In both cases there was a reduction in the body weight and the testicular weight in the $RT1^{l}$ homozygotes (Table 6 and Text-figure 3). The body weights and testicular weights of the $RT1^{a}$ homozygotes and the $RT1^{l/a}$ heterozygotes were the same and were normal. Thus, this defect is observed regardless of whether the offspring inherit it from the maternal or paternal parent.

To test whether there was any unique interactive effect of the $RT1^{l}$ phenotype with the genes causing the growth and reproductive defects, the Bl strain was mated with the F344 strain, and this F1 hybrid was used to generate an F2 hybrid population. Since both parents have the same RT1 phenotype, segregation of the genetic defect with the MHC could not be tested in this cross. The F2 population did contain both normal size and small animals (Table 6 and Text-figure 4). Thus, the presence and expression of the growth and reproductive defects did not appear to be influenced specifically by the $RT1^{l}$ phenotype.

The phenotypes found in the three F2 crosses are shown in Table 7. The phenotypic ratios in the (Bl × DA)F2 cross did not differ significantly from expected 1:2:1 ratio. However, the phenotypic ratios in the other two F2 hybrids did differ significantly from the expected. Of the four F2

Table 6—Body Weights and Testicular Weights of F2 Hybrids of B1 Strain and DA or F344 Strains

Strain or cross	Age (days)	RT1 phenotype (A region)	Body weight (g)			Testes				
						Weight (g)			Percentage of body weight ($\times 10^2$)	
			Avg	SD	No.	Avg	SD	No.	Avg	SD
DA	120-130	(a/a)	269	13	5	1.298	0.147	18	48.3	5.9
F344	120-130	(l/l)	280	23	13	1.401	0.120	22	50.0	5.9
(B1 × DA)F2	100-115	(a/a)	300	29	15	1.414	0.108	86	47.1	5.8
		(a/l)	300	29	27	1.428	0.140	204	47.6	6.6
		(l/l)	208*	24	14	0.161	0.018	64	7.7*	0.9
(DA × B1)F2	65-100	(a/a)	297	20	3	1.205	0.225	12	40.6	8.1
		(a/l)	281	27	4	1.188	0.196	44	42.3	8.0
		(l/l)	186*	9	3	0.163	0.032	4	8.8*	0.4
(B1 × F344)F2	100-115	(l/l)(N)†	313	23	24	1.519	0.128	106	48.5	5.4
		(l/l)(S)‡	213*	18	7	0.213	0.126	20	10.0*	0.8

* Significantly different at $P < 0.01$ from the $RT1^a$ or $RT1^l$(N) homozygotes
† Normal size animals
‡ Small animals

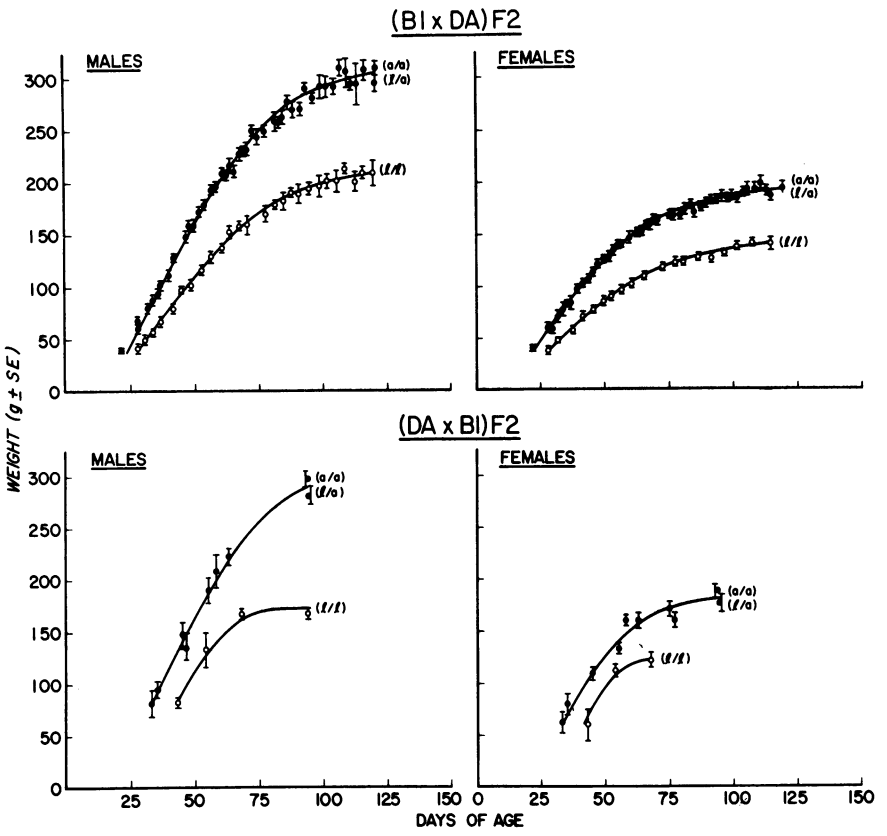
hybrids examined, three (Tables 1 and 7) showed selection against animals carrying the $RT1^l$ phenotype, which is linked to the genes controlling the defects in body size and testicular size. Thus, these genes may also affect fetal viability.

The litter sizes and sex ratios of the parental strains and of the various crosses are shown in Table 8. When the progeny of a cross received three

Table 7—Segregation of Haplotypes in Various F2 Hybrid Crosses Involving B1 Strain

Cross	RT1 phenotype (A region)	Females	Males	Total	Phenotypic ratio		
					Expected	Comparison with observed χ^2	P
(B1 × DA)F2	(a/a)	48	52	100	1:2:1	1.465	>0.3
	(a/l)	86	104	190			
	(l/l)	38	46	84			
(DA × B1)F2	(a/a)	8	14	22	1:2:1	9.764	<0.01
	(a/l)	34	37	71			
	(l/l)	10	7	17			
(B1 × F344)F2	(l/l)(N)*	44	65	109	3:1	4.705	<0.05
	(l/l)(S)†	10	12	22			

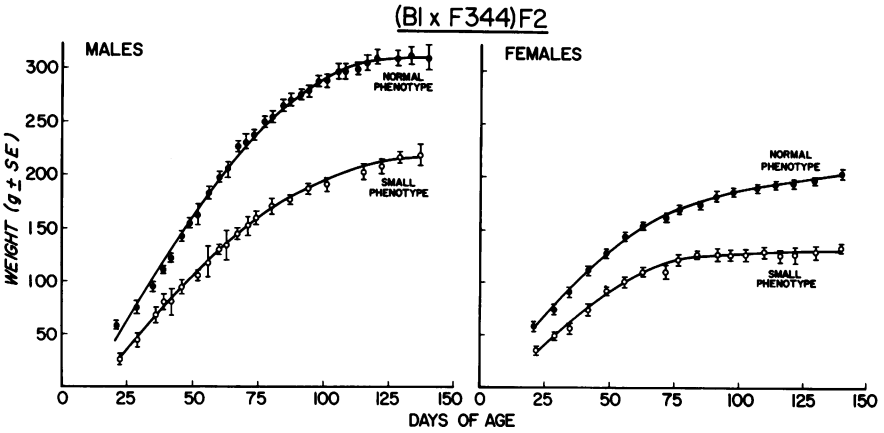
* Normal size animals
† Small animals



TEXT-FIGURE 3—The weight of the animals from the (B1 × DA)F2 and (DA × B1)F2 hybrids as a function of age. The $RT1^a$ homozygotes and the $RT1^{l/a}$ heterozygotes are significantly heavier than the $RT1^l$ homozygotes, and this difference is apparent at all ages. The F1 male animals carrying the B1 genome with which the (DA × B1)F2 hybrid was made were derived from the (DA × B13) cross (see text). Twenty-five animals were weighed for each phenotype in the (B1 × DA)F2 hybrid, and the numbers weighed for the (DA × B1)F2 hybrid are given in Table 5.

quarters of their genome from the B1 strain (the [B1 × B13] and B1 × [B1 × DA] crosses), the litter sizes were significantly reduced compared with those of inbred strains. When only half the genome came from the B1 strain, there was no significant reduction in litter size. In the case of the (B1 × DA)F2 hybrid, there is a significant heterosis effect, and the litter sizes of this cross are larger than those of inbred strains.

In the course of studying the F2 hybrids listed in Table 7, each animal was checked for the possibility of recombination among the genes controlling the growth and reproductive defects, which will be designated the growth and reproduction complex (Grc), and those controlling the serologic phenotype of the major histocompatibility complex. In 131 (B1 ×



TEXT-FIGURE 4—The weights of the animals from the (B1 × F344)F2 hybrid as a function of age. Since the RT1 phenotype was the same for all animals in this cross, the animals carrying the Grc were selected on the basis of their size at approximately 1 week of age. They were assigned to either the normal or small phenotype group and weighed with the appropriate group throughout the experiment. Examination of the weight development of each animal showed that all of the animals were assigned to the proper group when that judgment was made within the first week of life. The number of animals weighed is shown in Table 5.

F344)F2 hybrids, two recombinants were found (Table 9). In both cases there were recombinations between body weight and testicular weight. Thus, the gene(s) controlling the defect in body size and the gene(s) affecting testicular development are separable. The recessive gene con-

Table 8—Litter Sizes and Sex Ratios of Various Crosses Involving B1 Strain and of Appropriate Parental Strains

Strain or cross	Litter size						Sex ratio at weaning (male: female)
	Birth			Weaning			
	Avg	SD	No.	Avg	SD	No.	
B3	6.72	2.27	29	5.97	2.29	29	1.27
DA	6.73	2.43	40	6.32	1.23	261	0.94
F344	8.14	1.46	10	7.61	1.36	163	0.94
B1 × B13	3.21	1.32	19	3.21*	1.58	39	0.83†
B1 × (B1 × DA)F1	4.50	1.31	12	4.07*	1.22	15	1.54
(B13 × B13)F2	ND‡			5.64	2.57	42	1.14
(B1 × DA)F2	10.38	2.75	39	9.77§	2.71	39	1.16
(DA × B1)F2	9.86	2.67	7	8.56	3.13	9	1.08
(B1 × F344)F2	9.20	3.65	15	7.73	4.64	15	1.37

* Significantly decreased ($P < 0.01$) compared with B3

† There is an excess of heterozygotes in this cross, since the proportion of total B13 phenotypes is 2.14.

‡ Not done

§ Significantly increased ($P < 0.01$) compared with B3

Table 9—Recombinant Animals Found in 131 (B1 × F344)F2 Hybrids and in 374 (B1 × DA)F2 Hybrids*

			Recombinant animals†			
Cross	Animal number	Age (days)	RT1 phenotype (A region)	Body weight (g)	Testicular weight (g)	Recombination
(B1 × F344)F2	4998	120	(l/l)	168(S)‡	1.360(N)§	dw-3/F
	5516	92	(l/l)	310(N)	0.542(S)	DW-3/f
(B1 × DA)F2	5155	105	(l/l)	318(N)	1.330(N)	$RT1^l$/DW-3,F

* No recombinants were found in 110 (DA × B1)F2 hybrids.
† All recombinant animals found in these studies were males.
‡ Small
§ Normal

trolling small body size will be designated dw-3. In conformity with standard genetic practice, the wild-type gene at this locus will be DW-3. The recessive gene causing testicular hypoplasia will be designated by the symbol f; the wild type at this locus will be F.

The first recombinant found in the (B1 × F344)F2 hybrid (4998) was small but had normal testicles (Table 9, Figure 4). The size of the tubules was the same as in the normal animals, but there was evidence of a variable and somewhat reduced level of sperm production (Table 10). The other recombinant from this cross (5516) had normal body size but small testes (Table 9, Figure 4). The testicular weight was significantly smaller than that of the normal strain but larger than that found in the B1 strain or in the $RT1^l$ homozygotes in the backcross and F2 hybrid populations. The testes were also histologically different from those in both the normal

Table 10—Size and Sperm Production of Testicular Tubules from Various Strains and Recombinants

	Size of testicular tubules (μm)*		Sperm production	
Animal	Major axis (Average ± SD)	Minor axis (Average ± SD)	Amount†	Tubules with sperm
B3 strain	316 ± 41	254 ± 46	+++ to ++++	20/20
B13 heterozygote	305 ± 41	239 ± 30	+++ to ++++	20/20
Recombinants				
4998	316 ± 41	234 ± 30	+ to ++++	19/20
5155	295 ± 41	244 ± 25	+++ to ++++	20/20
5516	234 ± 41	183 ± 30	+	13/20
B1 strain	158 ± 25	142 ± 15	0	0/20

* Average of 20 tubules. The shape of the tubule was best approximated as an ellipse.
† Arbitrary scale of 0 to ++++ for those tubules with sperm. The estimates were made on Feulgen-stained sections at ×200.

animals and the small animals. The tubules had a better organized and more cellular epithelium than those of the small animals (Figure 3), and they were intermediate in size between the tubules of normal and small animals (Table 10). Sperm production was almost absent, but there were a few sperm produced in about two thirds of the tubules (Table 10). It appears as if the major arrest in spermatogenesis occurred at the spermatid stage. This finding suggests that the development of the testicular epithelium and the process of spermatogenesis are controlled by several closely linked genes and that in this recombinant all of these genes did not recombine. The slightly altered level of spermatogenesis in recombinant 4998 may also be the result of an incomplete recombination between the polygenic system controlling spermatogenesis and the gene(s) controlling body size. Since different types of recombination appear to have occurred in these 2 animals, accurate map distances between the genes regulating body size and testicular development cannot be calculated with the data currently available.

In the (B1 × DA)F2 hybrid population, there was one recombinant (5155), and it had a recombination between the MHC and Grc: it had the $RT1^l$ phenotype, but the body and testicular sizes were normal. From the data in Table 7 the recombination frequency can be estimated as approximately 0.6 cM. This animal should have one chromosome carrying the recombination ($RT1^l$/DW-3,F) and one chromosome from the B1 strain ($RT1^l$/dw-3,f). To test this hypothesis, the 5155 male was mated with a B13 female. Of the 33 progeny, 22 were $RT1^{l/n}$ and 10 were $RT1^{l/l}$. Of the $RT1^{l/l}$ animals, six were normal size and four were small; this ratio does not differ significantly from the expected 1 : 1 ratio. The four small animals should be homozygous for the recessive trait dw-3, and this hypothesis was tested by mating them with B13 animals. All of the $RT1^{l/l}$ homozygous progeny of these matings were small, as expected. Thus, progeny testing of animal 5155 through two generations clearly showed that the recombination was a true one.

Discussion

The B1 strain has a unique defect: a gene complex controlling both growth and reproduction (Grc) linked to the major histocompatibility complex. The recessive genes controlling body size (dw-3) and testicular size and fertility (f) are closely linked. In three of the five genetic crosses studied (Tables 1, 5, and 7), there was significant selection against $RT1^l$ homozygotes, which carry the Grc. In the (DA × B1)F2 hybrid, there appears to be strong selection for heterozygotes with a concomitant reduction in both homozygotes. Thus, in addition to controlling defects in

growth and fertility, the genes in the Grc are lethal in a certain portion of $RT1^l$ homozygotes.

Comparison of the litter sizes at birth with those at weaning (Table 8) shows no statistically significant difference that would explain the deficit of $RT1^l$ homozygotes. Thus, the presumption is that these embryos died *in utero*. There is also a suggestion from litter size data (Table 8) that there are other genes in the B1 animals which influence viability in addition to those of the Grc: the litter sizes of the (B1 × B13) and B1 × (B1 × DA) crosses are significantly smaller than those of the B3 strain. In the other B1 crosses, there is no such reduction in litter size. In the two crosses showing a decrease in litter size, the offspring inherited, on the average, three fourths of their genome from the B1 strain, whereas in those crosses without any decrease in litter size, half the genome came from the B1 strain. Consistent with this hypothesis is the observation that the strongest selection against the $RT1^l$ homozygotes was found in the F2 hybrids of the heterozygous B13 animals (Table 1).

There is no clear evidence for a sex influence on the transmission of the Grc, since it can be transmitted via the female or the male (Table 6). The question of whether there may be some modifying influences depending on whether the transmission was by the female or the male is still open. There is, however, the curious finding that all of the recombinants found in the various crosses studied (3/685) were male, even though approximately the same number of males and females were examined. This observation may suggest some form of sex influence on the transmission of the Grc, at least as it affects recombination.

The two recombinants found in the 131 (B1 × F344)F2 hybrids demonstrate that the loci controlling small body size (dw-3) and male sterility and testicular hypoplasia (f) are separable by recombination. There is some suggestion that each of these two loci may be complex, since in 1 case (Animal 5516, Table 9), the testes were larger than in other $RT1^l$ homozygotes but still considerably smaller than the normal and had a more developed tubular structure. This finding suggests that control of the fertility defect, at least, may involve several genes. Since these recombinants were found only in the cross with an $RT1^l$ strain (F344), there might be some interaction between the genes of the Grc and the MHC. Calculations of map distances between dw-3 and f must await further studies with larger numbers of animals and more detailed definition of the two defects.

In the (B1 × DA)F2 hybrid there was one recombination between the MHC and the Grc in the 374 animals studied, and this datum gives an estimate of the map distance between these two complexes of 0.6 cM. This

Table 11—Size and Fertility Defects in Rats

Mutant		Body size	Reproductive capacity	Other characteristics	Reference
Symbol	Name				
dw-1	Dwarf-1*	Small	Sterile and male has small testes	Insensitive to pituitary hormones; visible at 5 days of age	11
dw-2	Dwarf-2*	Small		Visible at 2 months of age	12
Grc: dw-3,f	Growth and reproduction complex*: dwarf-3, decreased fertility	Small (dw-3)	Male: sterile with small testes (f) Female: decreased (f)	Linked to MHC; segregation distortion	This paper
H^{re}	Restricted†	Normal	Male: decreased or sterile with small testes Female: normal	Variation in hood pattern linked to coat color genes; Tfm syndrome; lethal in homozygote	9, 10, 13–15
hd	Hypodactyly*	Normal	Male: sterile with small testes Female: normal		16, 17
	Hunt-Hoppert	Normal	Decreased		18, 19
	Runt	Small		Arose from a radiated male parent; lethal	20
	None	Normal	Male sterile with small testes	Sporadic defect in Long-Evans strain	21

* Recessive gene(s)
† Dominant gene(s)

distance is of the same order of magnitude as the distance between the T/t complex and the H-2 complex in the mouse, which is 1–2 cM. This analogy will be discussed in greater detail below.

The defect in the Bl strain is unique in that a gene complex controlling both growth and fertility is linked to the MHC. No similar defect has been described in the rat (Table 11) or in the human.[22-24] It is also unlike any of the size and reproduction defects reported in the mouse with the exception of those associated with the t genes (Table 12). The most extensively studied defect in the rat, and one which has some superficial

Table 12—Genetically Defined Size and Fertility Defects in Mice*

Mutant					
Symbol	Name	Body size	Reproductive capacity	Other characteristics	Reference
df	Ames dwarf	Small	Sterile	Sensitive to growth hormone	25
dm	Diminutive	Small	Decreased		25
dw	Dwarf	Small	Sterile	Sensitive to pituitary hormones	25
lit	Little	Small	Decreased	Ateliotic dwarf which responds to growth hormone	26
mn	Miniature	Small	Sterile	Lethal within 2 months	25
nu	Nude	Small	Decreased	Disturbed maturation of spermatids and deficient corpora lutea	27, 28
p^s	Pink-eyed sterile	Small	Males: almost sterile Females: fertile		25
pg	Pigmy	Small	Variable fertility depending on genetic background	No pituitary defect	25
Sl	Steel†	Small	Sterile	Homozygotes die *in utero;* severe anemia	25
Sl^d	Steel-Dickie†	Small	Sterile	Homozygotes viable; severe anemia	25
sla	Sex-linked anemia	Small			25
t		Small	Male: decreased or sterile Female: normal	Linked to MHC; specific embryonic defects; segregation distortion	2, 29, 30
W	Dominant spotting†	Small	Decreased	Severe macrocytic anemia; shortage of primary germ cells	25

* Those without gross anatomic defects. All mutations are recessive unless noted otherwise.

† Semidominant

similarity to those controlled by the Grc, is that described in mutants derived from a King-Holtzman colony of rats: a dominant gene (H^{re}) controlling restriction of hood pattern, male sterility due to seminiferous tubular dysgenesis, lethality in the homozygous state,[9,10] and the testicular feminization (Tfm) syndrome.[14,15] The male sterility in these animals and in the animals possessing the Grc is insensitive to pituitary hormones. The H^{re} gene(s) appears to be allelic with or linked to the genes of the coat color pattern series, which are not linked to the major histocompatibility complex. In contrast, the genes of the Grc are recessive, are linked to the major histocompatibility complex, do not segregate with hood pattern (Figure 1), and affect growth (Text-figure 1).

The rats carrying the Grc have defects analogous in many ways to those controlled by the t genes of the T/t complex in the mouse. First, both the Grc and the t genes are linked to the major histocompatibility complex. No other developmental defect in either the mouse or the rat has, to the best of our knowledge, been described as being linked to the MHC. Second, both the Grc and the t genes can affect reproduction by causing an alteration in segregation ratios of the offspring and a decreased litter size due to intrauterine death. Third, both defects are approximately the same order of magnitude distant from the major histocompatibility complex. The Grc is 0.6 cM from the MHC, and the t genes are 1–2 cM from the MHC.[2,29,30] The t genes apparently cause a "contraction" of the linkage map, since the distance of the dominant T genes from H-2 is approximately 15 cM. In the rat, we have not yet found a dominant mutation in the Grc complex analogous to the T genes in the mouse. In addition, the t genes suppress recombination in the region of the MHC, and the Grc genes are associated with a low rate of recombination (Table 9). Finally, the finding of recombination between dw-3 and f in the (B1 × F344)F2 hybrid only suggests specific associations between the genes of the Grc and those of the MHC similar to the specific associations between certain t genes and H-2 haplotypes.[28]

The mechanisms controlling the defects regulated by the Grc probably do not involve defects in hormone production, since the pituitary and testicular hormones and somatomedin C are produced in normal or increased amounts (Table 4). Since these genes influence such complex processes as growth and reproductive capacity, they must affect a very fundamental developmental mechanism. Our working hypothesis is that they affect the structures of cell surface molecules among which are receptors for hormones. The high hormone levels in the plasma suggest that the binding constants of the cell surface receptors are low and that high concentrations of hormones are needed to force enough hormone to bind to the cell surface to promote a level of development compatible with survival.

The presence of a gene complex linked to the MHC in the mouse, which governs developmental defects (the T/t complex), and in the rat,

which governs defects in growth and reproduction (the Grc complex), suggests that the existence of a chromosomal region influencing development and linked to the MHC may be a general phenomenon in mammals and that, indeed, the whole region might function as a "supergene."[29,31] The detection of such a region in the mouse and in the rat may have been the fortuitous result of mutations which were phenotypically detectable. In searching for a similar region in other species, developmental defects other than those classically associated with the T/t complex in the mouse should be sought. The identification of an analogous region in the human would be of particular interest, since its investigation might provide considerable insight into a variety of pathologic processes affecting reproduction and postnatal development.

Addendum

At the Second International Workshop on Alloantigenic Systems in the Rat (Freiburg, Germany, May 14–16, 1979) nomenclature changes were adopted that will affect the future designation of the strains described in this paper. The inbred B3 strain was assigned the $RT1^i$ haplotype, and it will be designated the BI strain. The B13 strain, which is inbred by brother × sister mating to maintain forced heterozygosity at RT1, will be designated the BIL strain. The Bl homozygotes, which are produced by intercrossing the BIL strain, will be assigned the designation BIL/1. Recombinants among the MHC, dw-3, and f loci will receive the sequential designations BIL/2, BIL/3, etc.

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Figure 1—A litter of (B1 × YO)F2 hybrids at 4 days of age, illustrating that the small size of the animals carrying the $RT1^{l}$ haplotype of the B1 strain is clearly visible shortly after birth and that size does not correlate with hood pattern. The YO ($RT1\gamma$) strain was chosen to mate with the B1 ($RT1^{l}$) strain in this experiment because the YO animals are quite large and their hybrids should show any size differences early in life. The 3 $RT1^{l}$ animals are indicated (*arrows*). The 12 other, normal sized animals typed as $RT1\gamma$ or $RT1^{l/}\gamma$

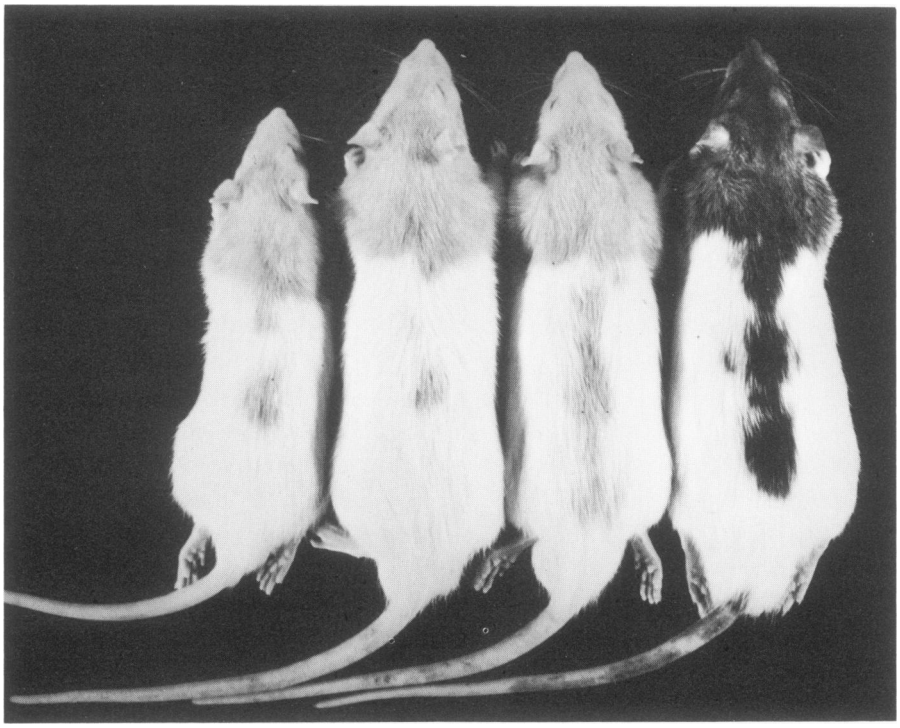

Figure 2—Adult male animals of the B1, B13, B3, and PVG strains (*left to right*). The B3 and B13 rats are approximately the same size and quite similar in size to the inbred PVG strain, which was used for comparison. The B1 strain is considerably smaller and has much smaller testicles than the other strains.

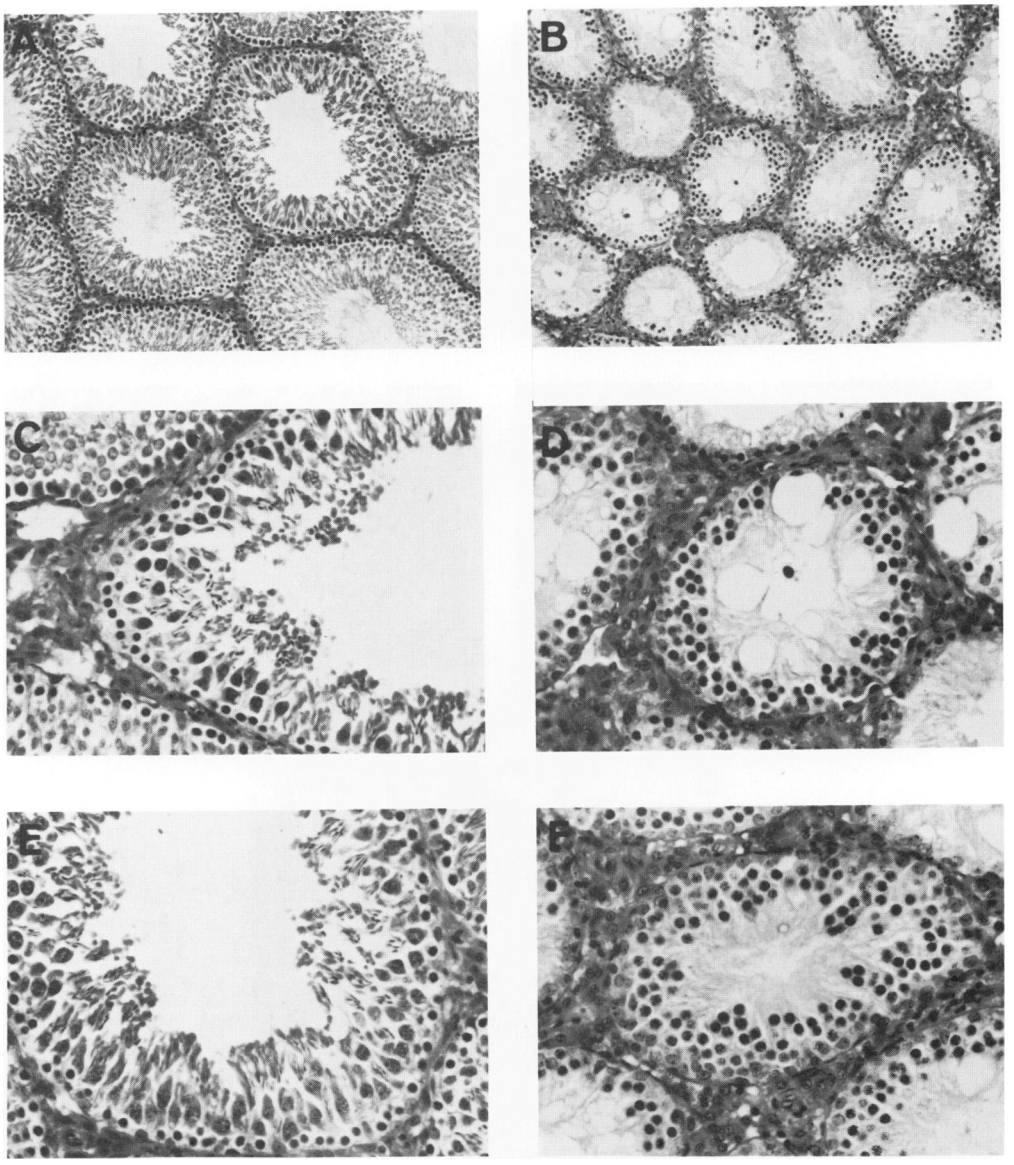

Figure 3—The testes of the normal B3 (**A, C,** and **E**) and aspermatic B1 (**B, D,** and **F**) animals. The B3 strain has a normal tubular structure (**A**), whereas the B1 strain shows considerable tubular atrophy with an apparent increase in interstitial tissue (**B**). The development of sperm (**C**) is arrested at the spermatocyte stage in the B1 strain (**D**). The normal tubular epithelial structure (**E**) is disorganized in the B1 strain (**F**), and it sometimes has vacuolated epithelium (**D**). (Hematoxylin and eosin. **A** and **B,** × 125; **C** and **D,** × 300; **E** and **F,** × 300) (With a photographic reduction of 4%)

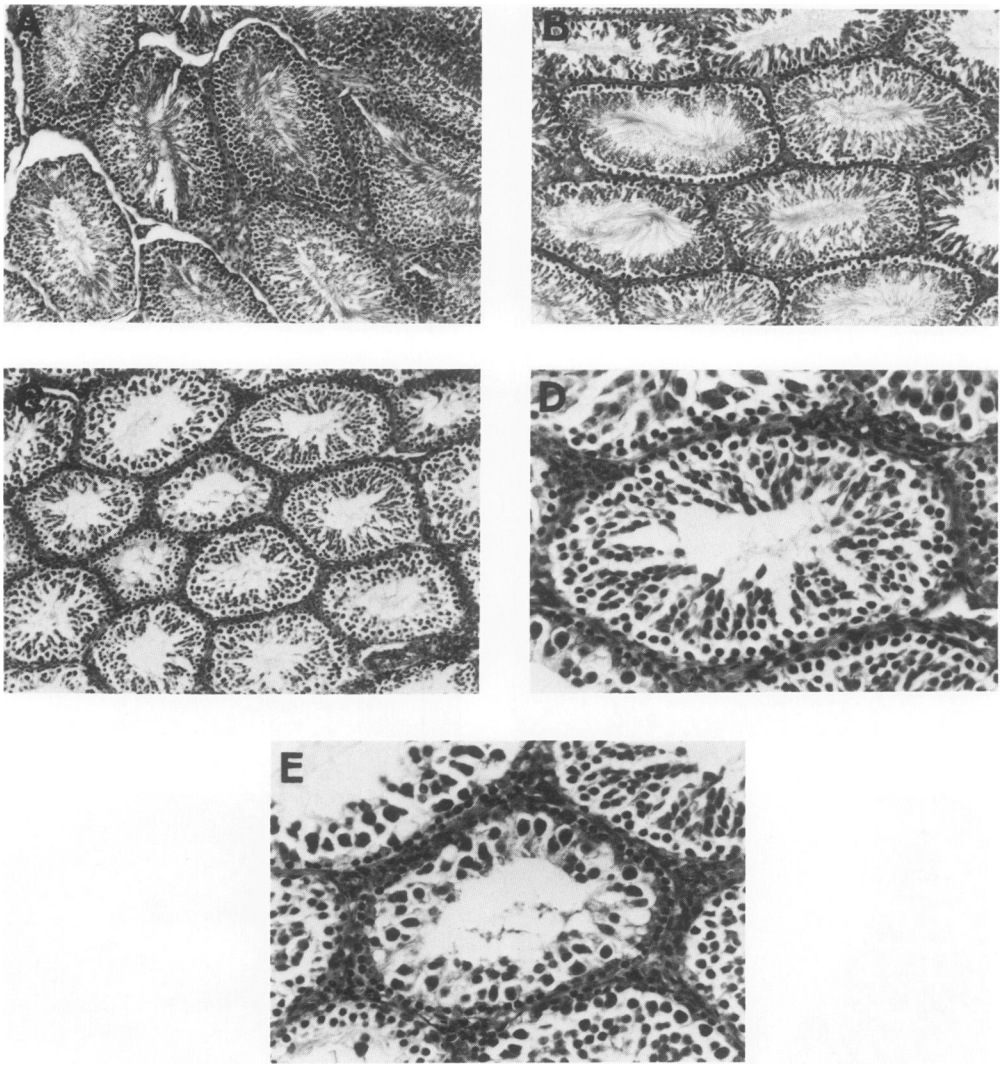

Figure 4—The testes of the three recombinant animals: Animal 5155 from the (B1 × DA)F2 hybrid (**A**), Animal 4998 from the (B1 × F344)F2 hybrid (**B**), and Animal 5516 from the (B1 × F344)F2 hybrid (**C–E**). The germinal epithelia of 5155 and 4998 are normal, although there is some evidence for slightly decreased sperm production in 4998 (Table 10). There is an extensive arrest of spermatogenesis at the spermatid stage in the tubules of 5516, so that only a few sperm are produced in approximately 65% of the tubules (Table 10). (**A–C**, H&E, ×125; **D** and **E**, H&E, ×300) (With a photographic reduction of 7%)