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Recessive mutation in a standard recombinant-inbred line of mice affects seminal vesicle shape

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Summary

A recessive autosomal mutation has been found in the CXBI/ByEss recombinant-inbred line but in neither of the parental strains, C57BL/6ByEss and BALB/cByEss. Its presence in the CXBI/ByJax and CXBI/ByLac sublines suggests an origin early in inbreeding. The locus, seminal vesicle shape (*svs*), appears to be linked to the albino locus on chromosome 7. The homozygote has seminal vesicles with a smooth tubular external appearance. In segregating crosses homozygotes had slightly lighter seminal vesicles but the weights of other androgen target organs were not reduced. Exogenous testosterone increased the size of the seminal vesicles but did not alter their shape. The mutation did not affect the pattern of proteins on SDS–acrylamide gel electrophoresis, which did differ between the parental strains. The locus affecting a 27000 Da protein has provisionally been assigned the symbol *svp-4*.

1. Introduction

Recombinant-inbred strains of mice are widely used for the identification of genetic variants and for their assignment to linkage groups (Bailey, 1971; Swank & Bailey, 1973; Taylor 1978). Increasingly they have been used to study complex physiological, developmental and behavioural systems. In particular they have been used to estimate the number of major loci that affect such systems that differ between the pairs of inbred progenitor strains. In such systems the identification of one or more classes of recombinant strains with phenotypes that are intermediate between those of the progenitor strains (e.g. Greenblatt, Diggs & Rosenstreich, 1984; Risser & Kaehler, 1985) has been taken as evidence for at least two loci controlling the trait. So too has the occurrence of recombinant strains with phenotypes beyond those recorded in either of the parental strains. These are phenotypes, such as naloxone sensitivity (Peets & Pomeranz, 1978), in which the progenitor strains have identical phenotypes but the demonstration of a derived recombinant strain with a different phenotype provided evidence for hidden genotypic differences between the progenitor strains. However such conclusions depend on mutation occurring rarely amongst the recombinant strains. Once homozygous, a mutation in an individual recombinant strain will give it a different phenotype to either of its progenitor strains and could thus suggest that they differ at two complementary loci.

Our studies of genetic variation affecting reproductive physiology in C57BL/6By, BALB/cBy and their CXB recombinants (Shire & Whitten, 1980; Shire, 1984; Shukri & Shire, 1986) have revealed a phenotype unique to CXBI mice. This paper shows that the unusual seminal vesicles in these mice are the result of a novel mutation and are not the consequence of recombination of alleles present in the progenitor strains. We also describe a polymorphism affecting proteins in the seminal vesicle secretions, that was uncovered whilst investigating the phenotype of the mutant mice.

2. Materials and methods

(i) *Mice*

Mice from the C57BL/6ByEss and BALB/cByEss strains and their CXB recombinant inbred derivatives (Bailey, 1971) have been studied. Reciprocal F_1 hybrids between the progenitor strains and between CXBI and both progenitors were also bred, together with backcrosses of these F_1 mice to CXBI. When crosses are defined the progenitor strains C57 and BALB are abbreviated to B and C respectively, and the CXBI recombinant strain to I. The animals were raised under the standard conditions described by

Janat & Shire (1987), except that sieved sawdust (Grade 18, Sawdust Manufacturing Co., Standon, Herts) was used as bedding. Paraffin-embedded tissue from CXBI/ByJax was kindly provided by Dr Wesley Beamer of the Jackson Laboratory. Dr Ian Lush kindly made available some CXBI/ByLac mice from his colony at University College, London.

(ii) *Tissue sampling*

Some mice were castrated at the age of 30 days under anaesthesia and given subcutaneous implants of silastic tubing containing either 5 mg of testosterone undecanoate (Restandol, kindly supplied by Dr J. L. Gibbons, Organon Laboratories Ltd) or diluent. Seminal vesicles, and other target organs, were dissected from mice killed with halothane. Seminal vesicles were quickly blotted to remove secretions before being weighed. Seminal vesicles for histology were fixed in formol saline without blotting. Paraffin sections were stained with haemotoxylin and eosin.

(iii) *Electrophoresis*

Samples of expressed secretion were immediately dissolved in 0·8 ml of a 50 g l^{-1} solution of sodium dodecyl sulphate (SDS) in phosphate buffer. These samples were then boiled for 5 min before being applied to 10% polyacrylamide gels containing 0·1% of SDS. The buffers and running conditions are described by O'Farrell & Dixon (1986). Gels were stained with Coomassie Blue and destained in 40% methanol/6·25% acetic acid. Samples of standard proteins of known molecular weight were run so that the size of the proteins in the seminal vesicle contents could be estimated.

3. Results

The seminal vesicles of CXBI mice are tubular and have a smooth appearance whilst those of the C57BL/6By mice are curved and have a convoluted surface (Fig. 1). The seminal vesicles of mice of the C57BL/10ScSn, and BALB/cBy inbred strains are similar to those of the C57BL/6By mice, as are the seminal vesicles of the other six CXB/By recombinant inbred strains. All postpubertal males of the CXBI/ByEss subline examined have had tubular seminal vesicles. The seminal vesicles of a small number of CXBI/ByLac mice, of the subline maintained at University College, London were also found to be tubular. So too were the fixed seminal vesicles from four CXBI/ByJax mice raised at the Jackson Laboratory. Normal seminal vesicles were found in all males of both reciprocal F_1 hybrids between C57BL/6By and BALB/cBy.

CXBI mice were reciprocally crossed with both B × C and C × B F_1 hybrids and with the C57 and BALB progenitor strains. None of these male progeny had tubular seminal vesicles (Table 1). Male and female progeny from these crosses were backcrossed to the CXBI strain. Male progeny with tubular seminal vesicles were found in all eight subsets of the backcrosses. Their overall incidence was 44% in the combined backcrosses. The individual backcrosses were not significantly heterogeneous on a contingency test ($\chi^2_{(7)} = 7{\cdot}7$, $P = 0{\cdot}4$). In contrast, no males with abnormal seminal vesicles were found amongst 25 progeny of I × (B × C) females crossed to B × C males or amongst 20 progeny of I × (C × B) females crossed to C × B males.

Backcrosses of F_1 hybrids with C57 to CXBI segregated at the albino locus, but only 9 of the 57 pigmented mice had tubular seminal vesicles whilst 27 albinos, out of a total of 35, had mutant seminal vesicles. The data from the 3 backcrosses (B × I) × I, (I × B) × I, I × (I × B), were not significantly heterogeneous ($\chi^2_{(6)} = 7$). A contingency $\chi^2_{(1)}$ of 31·7

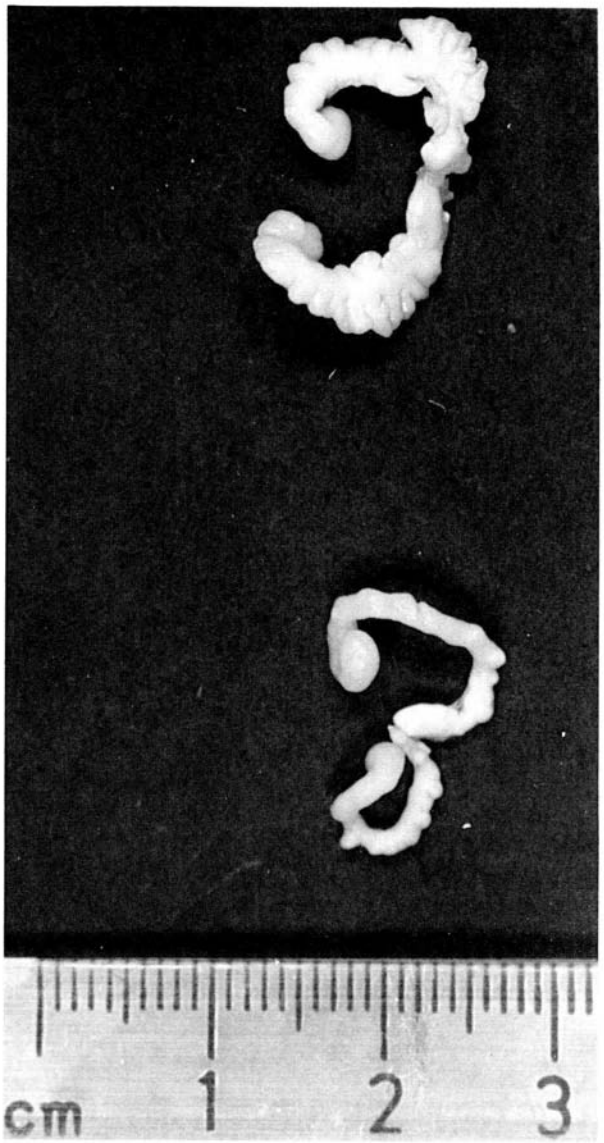

Fig. 1. Excised seminal vesicles from a C57BL mouse (above) and a CXBI mouse (below).

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Table 1. *Frequency of mice with tubular seminal vesicles in crosses*

	Incidence	%		Incidence	%
B × I	0/23	0	(B × I) × I	14/37	38
I × B	0/33	0	(I × B) × I	9/27	33
C × I	0/23	0	(C × I) × I	15/27	56
I × C	0/33	0	(I × C) × I	14/35	40
(B × C) × I	0/73	0	I × (I × B)	13/28	46
(C × B) × I	0/104	0	I × (I × C)	4/12	33
I × (B × C)	0/37	0	((B × C) × I) × I	10/27	37
I × (C × B)	0/21	0	((C × B) × I) × I	35/64	55

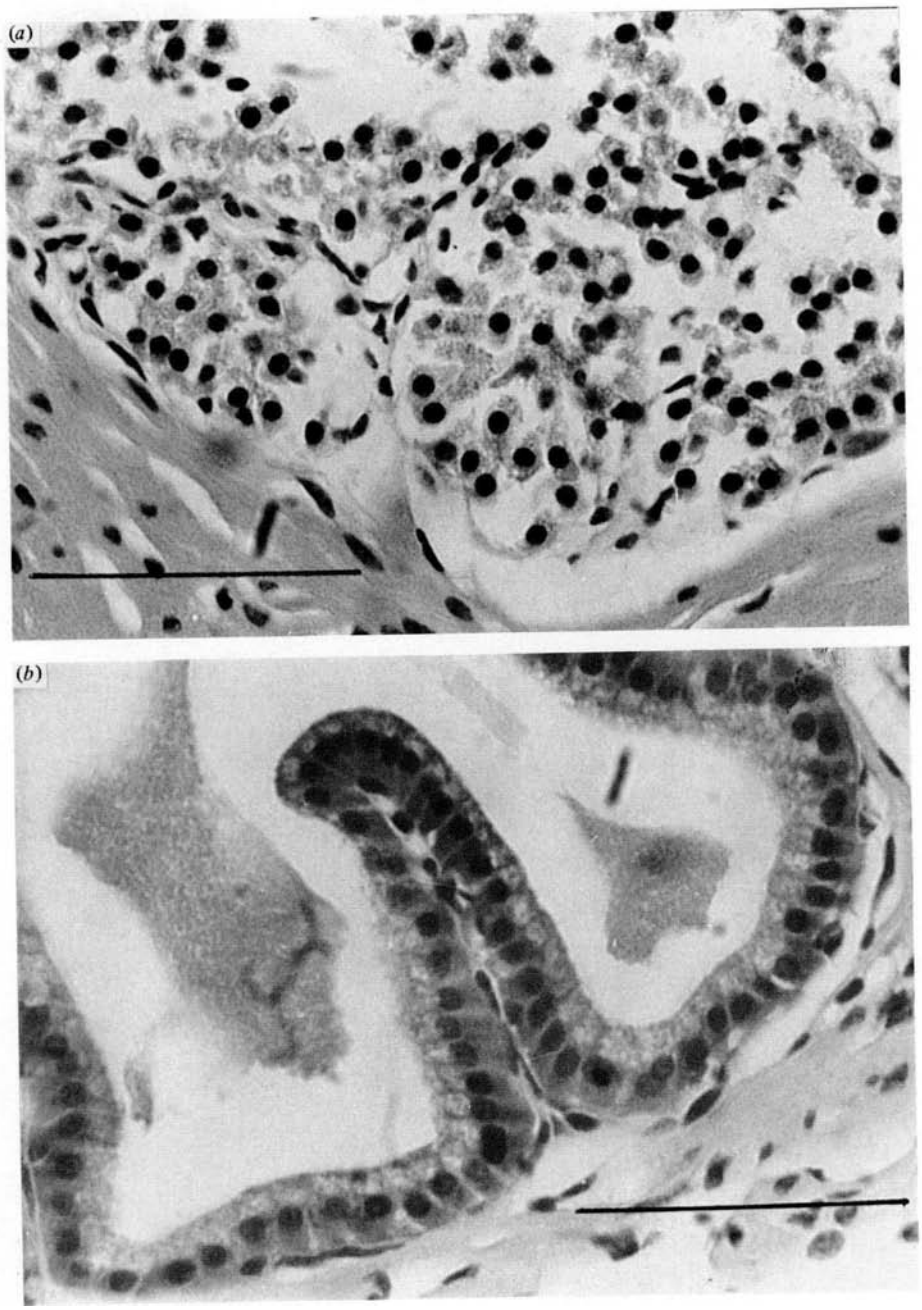

Fig. 2. Sections of seminal vesicles from C57BL (*a*) and CXBI (*b*) mice stained with haemotoxylin and oesin. Note differences in the degree of organisation of the glandular epithelium (Scale bar = 100 μm).

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Table 2. *Mean organ weights* (±S.E.) *in* ((*C* × *B*) × *I*) × *I backcross mice with normal or mutant seminal vesicles*

	Seminal vesicles (mg)	Testes (mg)	Kidneys (mg)	Submandibular glands (mg)	Bodyweight (g)
Normal ($n = 17$)	19·8 ± 0·97	166 ± 7	520 ± 17	140 ± 5	25·8 ± 0·5
Mutant ($n = 27$)	17·9 ± 0·71	169 ± 4	556 ± 16	143 ± 4	26·9 ± 0·4
$t_{(42)}$	1·61	0·4	1·5	0·5	0·4
P	0·06*	N.S.	N.S.	N.S.	N.S.

* 1-tailed.

($P < 0{\cdot}001$) for the combined data implies linkage between the mutant and albino loci.

Normal seminal vesicles, as found in BALB/cBy mice and in mice of most genotypes (Hummel, Richardson & Fekete, 1966), are elongated hollow tubes with numerous invaginations and evaginations. It is not unusual for the vesicles to be branched into two separate tubes and for the lumens to be of varying diameter and of a tortuous nature. Connective tissue extends into the lumen of the vesicle and separates the various components of the tubules. The glandular epithelium is well differentiated into distinct areas, each delineated by connective tissue and consisting of cuboidal and columnar epithelium. The lumens are of an irregular shape containing seminal vesicle fluid which in section appears as a deeply stained homogenous mass. Examination of sections showed CXBI seminal vesicles to be elongated hollow tubes with one

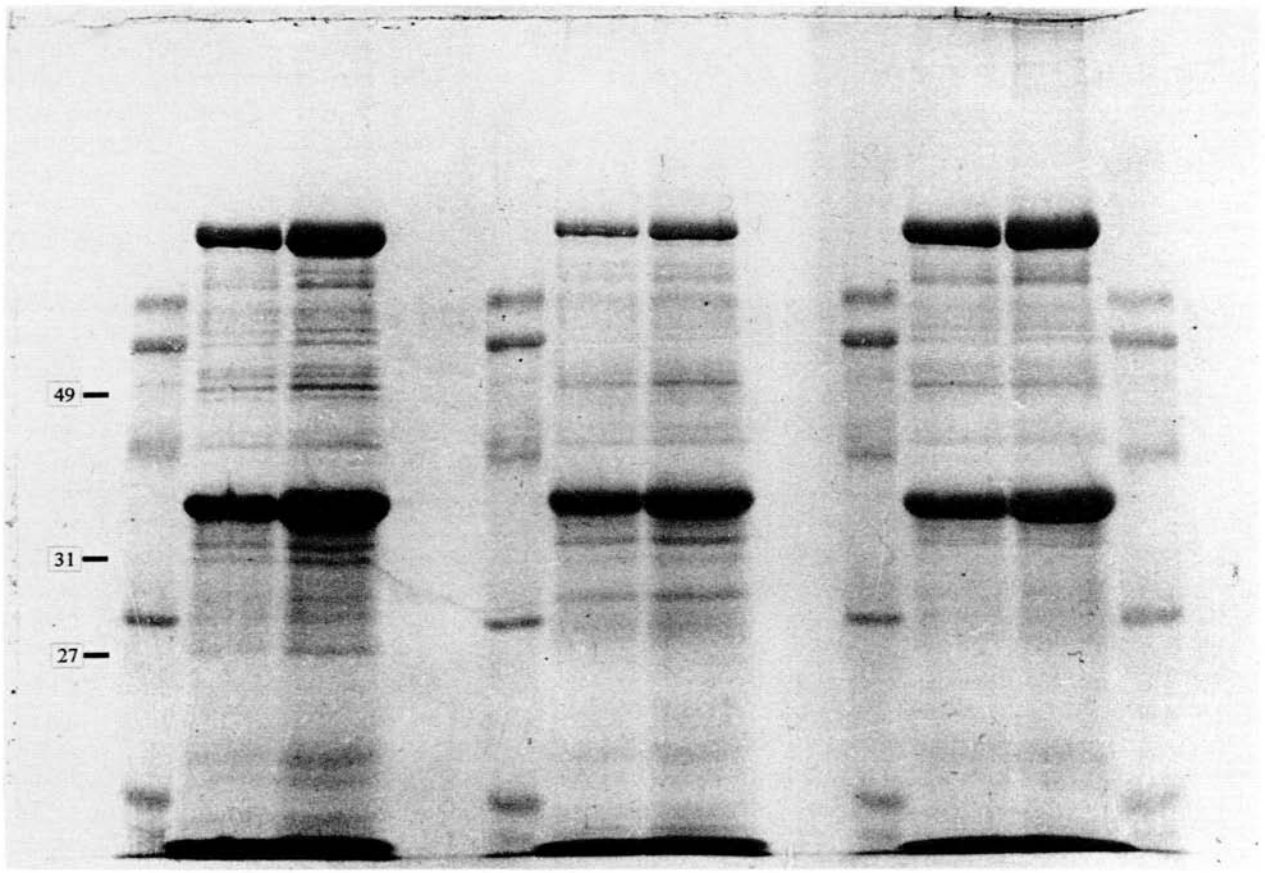

Fig. 3. Pattern of proteins in seminal vesicle contents from C57BL, CXBI and BALB mice after separation by electrophoresis on SDS–polyacrylamide (10%) gel. The two tracks for each genotype were loaded with 15 and 25 μg of protein. A minor band, of 27 kDa, that differed between genotypes is indicated, as well as two other minor bands, of 31 and 49 kDa, which showed signs of genetic heterogeneity.

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or two large lumens and few invaginations. These were lined with glandular epithelium in which there was limited folding. The glandular tissue appeared to be mainly composed of columnar cells with large nuclei and little cytoplasm. The epithelium is well organised into glandular structures when compared to sections from C57BL mice (Fig. 2). However, examination of sections from mice of the BALB progenitor strain, the reciprocal F_1 hybrids and from all six other recombinant lines showed them to have the same kind of epithelium as CXBI, but lining seminal vesicles of normal shape.

The seminal vesicles of CXBI mice aged 60 days were significantly lighter than those of either C57BL/6By or BALB/cBy male mice of the same age. Their mean weight was $14{\cdot}9 \pm 3{\cdot}4$ mg ($n = 19$) compared to $19{\cdot}3 \pm 4{\cdot}5$ ($n = 46$) and $23{\cdot}6 \pm 5{\cdot}9$ mg ($n = 33$) for the respective progenitor strains. Mean body weights were not significantly different (25·4, 24·6 and 25·1 g). Data on organ weights were collected from mice of the $((C \times B) \times I) \times I$ backcross, which had the same average genotype and were segregating for the mutation, and are shown in Table 2. Analyses of variance showed no significant reduction in body-weight, paired testis weight, paired kidney weight or paired submandibular gland weight in the mutant mice. The mutants had lighter seminal vesicles, with the difference approaching statistical significance.

CXBI males that had been castrated at 30 days of age were given implants of 5 mg testosterone undecanoate. At 60 days these treated mice had seminal vesicles with a mean weight of $20{\cdot}1 \pm 0{\cdot}8$ mg ($n = 5$), significantly larger ($t = 2{\cdot}2$, $P = 0{\cdot}05$) than those of sham-operated/intact control CXBI mice ($16{\cdot}3 \pm 1{\cdot}4$ mg; $n = 6$). All the treated CXBI mice had tubular seminal vesicles, which were as heavy or heavier than those of contemporaneous intact/sham-operated C57BL/6By controls ($t = 1{\cdot}73$, N.S.) Sections of seminal vesicles from the treated CXBI mice showed less proliferation of the glandular lining and less stimulation of the individual secretory cells than found in sections of corresponding C57BL/6By mice (Fig. 2). The basic difference in the appearance of the epithelial cells in these two genotypes was not altered by treatment with testosterone.

SDS–acrylamide electrophoresis was carried out on the seminal vesicle contents from mice of the two progenitor strains and from all seven recombinant inbred strains. Figure 3 shows a representative gel. CXBI mice resembled BALB for major and minor bands. A minor band, of approximately 27000 Da, differed between C57 and BALB. In C57 samples this band was present whilst it was absent from BALB samples. The D and J recombinant lines resembled C57BL whilst the other five recombinant lines and the reciprocal F_1 hybrids were the same as BALB. There was some evidence of genetic heterogeneity in other minor bands of apparent molecular weights of 31 and 49 kDa. There were three major bands, of approximately 80, 35 and less than 20 kDa. These did not appear to differ in apparent molecular weight between genotypes.

4. Discussion

The results of the crosses involving CXBI are compatible with a single, fully penetrant, recessive mutation being homozygous in CXBI. The normal allele is homozygous in C57BL, BALB and their F_1 hybrids. We suggest the locus be called 'seminal vesicle shape' (*svs*). The alternative possibility, that the CXBI phenotype is caused by alleles at two interacting loci that are present in repulsion in the two progenitor strains, seems unlikely. Recombinants between the two loci (with tubular seminal vesicles) would have been detected in the crosses between the CXBI mice and the F_1 hybrids; at a frequency of 25% if the two loci were unlinked. No such recombinants were found in a total of 235 such mice examined. Had even a single recombinant been found this would have placed the two loci within 1 map unit.

The absence of reciprocal differences in crosses with CXBI rules out sex linkage. The occurrence of tubular seminal vesicles in both the $I \times (I \times B)$ and $I \times (I \times C)$ progeny, in which both mother and grandmother were I females and both father and grandfather had either C57BL or BALB *Y* chromosomes, showed that a CXBI-derived *Y* was not necessary for the expression of the trait. The results of the crosses with C57BL suggest linkage of the *svs* locus with the albino locus on chromosome 7, with about 18% recombination. This would place the locus near *War* or in the region *p* to *svp-2*. It will be interesting to map the *svs* locus relative to the *svp-2* and *Gpi* loci, which are close to each other on chromosome 7 (Taylor, 1982).

The identical phenotypes of the three CXBI sublines suggest that the mutation was fixed fairly early in the history of the strain, certainly before the separation of the Jax and UK lines in 1978. This occurrence of a mutation in a recombinant-inbred line points up the need to breed hybrids when non-progenitor phenotypes have been found amongst a set of recombinant-inbred strains. Only one prior reference to a possible mutation in a recombinant strain has been found. This is to the occurrence of a single female, from a CXB strain, that unexpectedly rejected a skin graft (Bailey & Hoste, 1971).

The mutant affects shape and has a minor effect on blotted weight of the seminal vesicles, but not on other androgen target organs. The shape was not altered by increased levels of testosterone, or prolactin secreting grafts (F. Grew, unpublished data), even though the weight of the vesicles was significantly increased. Hooker & Strong (1941) found prickly surface irregularities on the seminal vesicles of all mice of the C strain, most of which had doubled seminal vesicles. Variation at two, unlinked, additive loci was suggested on the basis of observations on hybrid

generations. Males of the CHI and NH strains, which had normal seminal vesicles, developed prickly seminal vesicles when treated with testosterone propionate. Some mice developed doubled seminal vesicles. This contrasts with our finding on CXBI mice, which appear to be homozygous for a single mutation and to have seminal vesicles whose shape is unaffected by hormonal treatments.

Testosterone treatment did not alter the appearance of the secretory epithelium in mutant animals, which had the cytological appearance of being less active than in C57BL males. However this difference was not characteristic of the *svs* homozygote, but rather a trait that distinguished C57BL mice from all the other stocks studied. In this it resembles the strain distribution shown by testis weight at 60 days of age (Shukri & Shire, 1986), in which only C57BL had relatively small testes. This characteristic of C57BL may be associated with the differences in the responsiveness of C57BL seminal vesicles to exogenous testosterone (Bartke, 1974; F. Grew, unpublished data).

Although the proteins present in the seminal vesicle secretions of the CXBI mice did not differ from those in the secretions of mice of the BALB progenitor strain, differences between the progenitor strains were shown when the proteins were separated by SDS–polyacrylamide gel electrophoresis. The 27000 Da difference showed a strain distribution pattern identical to that for the *H-17, H-34* and *H-38* loci. The variant showed no association with the *Gpi* locus on chromosome 7, which is closely linked (Taylor, 1982) to the *svp-2* locus identified on cellulose acetate electrophoresis (Moutier, Toyama & Charrier, 1971). The *svp-2* locus, but not the *svp-1* locus which is linked to the agouti locus on chromosome 2 (Platz & Wolfe, 1969), differs between C57BL and BALB. These two loci code for major secretory proteins, of 18 and 15 kDa respectively (Maffei *et al.* 1984), which were too small to be resolved by the gel system used in this work. The RSV IV and RSV V genes in the rat, which are homologous to *svp-1* and *svp-2*, have been cloned (Kistler *et al.* 1981; McDonald *et al.* 1983). It is proposed to assign the symbol *svp-4* to the difference in the 27000 Da protein described here since *svp-3*, which is closely linked to *svp-1* (Moutier *et al.* 1983), defines a variant found in a single C57 subline.

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