EFFECTS ON SPERM MORPHOLOGY BY ALLELES AT THE PINK-EYED DILUTION LOCUS IN MICE[1]

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ABSTRACT

Sperm head morphology was analyzed in all genotypic combinations for alleles dark pink-eye (p^d) and p-sterile alleles, p^{6H}, p^{bs} (p-black-eyed sterile) and p^{25H}. Three of these, p^{6H}, p^{bs} and p^{25H}, were radiation induced; homozygotes and heterozygotes of these three alleles are male sterile, whereas $p^d/$—genotypes are fertile. Sperm heads were examined by light microscopy and assigned to one of five classes: A. normal and near-normal, B. triangulate and oblate, C. spatulate, D. elongate, and E. filamentous. Males of each sterile genotype had grossly abnormal sperm and each sterile genotype differed from all other sterile genotypes and from fertile genotypes in at least one class, except p^{6H}/p^{6H} compared to p^{bs}/p^{bs}.

Frequency distribution profiles (1) revealed a complex pattern of allelic interaction and do not support a deletion-complementation hypothesis, (2) do not show simple bimodality, which might suggest post-meiotic (haploid) gene expression, and (3) together with unpublished breeding data, show that p^{25H} is not a remutation of p^{6H}.

IRREGULAR sperm head morphology was described by HOLLANDER, BRYAN and GOWEN (1960a,b) in mice homozygous for a p-sterile (p^s) mutation induced by X-irradiation. HUNT and JOHNSON (1971) further described grossly altered sperm head shapes and multiple tails in mice homozygous for two radiation-induced p-sterile alleles p^{6H} and p^{25H} (they could not distinguish between the two alleles).

Sperm head morphology in mice is known to vary in a number of quantitative characteristics with head shape having a high heritability (h^2), and there are prominent differences among inbred strains (BEATTY and SHARMA 1960; BRADEN 1959; ILLISSON 1969). BRADEN (1959) tentatively reported an effect of pink-eyed dilution (p) on sperm head width (CBA-p *cf* congenic strain CBA) but WOLFE (unpublished) found no effect of p-unstable (p^{un}) on sperm head size (C57BL/6-p^{un} *cf* to congenic strain C57BL/6).

TABLE 1

Genotypes and phenotypes of mice differing at the p-locus

	Genotype	Pigmentation	Fertility
1.	p^d/p^d, p^d/p^6 p^d/p^{bs}, p^d/p^{25}	dark eyes, some coat dilution	normal fertility
2.	p^6/p^6	pink eyes, dilute coat	male sterile, female semi-sterile
3.	p^{bs}/p^{bs}	dark eyes, dilute coat	male sterile, female semi-sterile
4.	p^{25}/p^{25}	pink eyes, dilute coat	male sterile, female semi-sterile
5.	p^6/p^{bs}	dark eyes, dilute coat	male sterile, female semi-sterile
6.	p^6/p^{25}	pink eyes, dilute coat	male sterile, female semi-sterile
7.	p^{bs}/p^{25}	dark eyes, dilute coat	male sterile, female semi-sterile

Of those genotypes listed under No. 1, at birth p^d/p^6 and p^d/p^{25} have lighter eyes, and p^d/p^{bs} have darker eyes, than do p^d/p^d mice.
Nos. 2, 4 and 6 are alike in gross color phenotype.
Nos. 5 and 7 have lighter eye color at birth than No. 3.

We have made a detailed light microscopic study of sperm morphology in mice homozygous and heterozygous for a series of p-alleles in an effort (1) to better understand genetic influence on sperm differentiation and morphology, (2) to search for evidence of complementation in heterozygotes, and (3) to search for post-meiotic (haploid) gene expression. Fertile dark pink-eye allele (p^d) and three p-sterile alleles, p^{6H}, p^{bs} (p-black-eyed sterile) and p^{25H} were studied in all genotypic combinations.

MATERIALS AND METHODS

Genotypes of mice that were sampled and their more obvious phenotypes are summarized in Table 1. Alleles p^{6H}, p^{bs} (p-black-eyed sterile) and p^{25H} were radiation-induced at the MRC Radiobiology Unit, Harwell, England. Throughout this paper, alleles p^{6H} and p^{25H} are abbreviated as p^6 and p^{25} respectively. The p-sterile alleles and the allele p^d (dark pink-eye) are maintained in an outbred closed colony stock MUP.

Young mature males, age 90 to 120 days, were killed by cervical dislocation and sperm stripped from the vasa deferentia into 0.5 ml normal saline. Air dried smears were prepared after the sperm had dispersed. Replicate slides were processed in two ways. One set was examined without staining under phase contrast at 400X or 1000X magnification. These unstained sperm heads were assigned to one of five morphological classes patterned after sperm descriptions given by HOLLANDER, BRYAN and GOWEN (1960a): A. normal and near-normal, B. triangulate and oblate, C. spatulate, D. elongate, and E. filamentous. The graphic range of each class is illustrated in Figure 1. A second set was fixed for three min in methanol and stained for 20 min in an ethanolamine-methanol-Giemsa mixture as described of ILLISSON (1969). Results from stained sperm corroborated the more extensive classification of unstained sperm, and will not be presented.

All scoring of morphological types of sperm was done from coded slides without prior knowledge of a particular genotype. Statistical comparisons between classes were done on arcsin transformed percentages.

RESULTS

The percentages of sperm assigned to classes A, B, C, D, or E for each sterile genotype and for fertile genotypes ($p^d/-$) are given in Table 2 (percentages and arcsin transformed percentages) and as histograms in Figure 2(percent-

A. Normal and Near-normal   B. Triangulate and Oblate   C. Spatulate
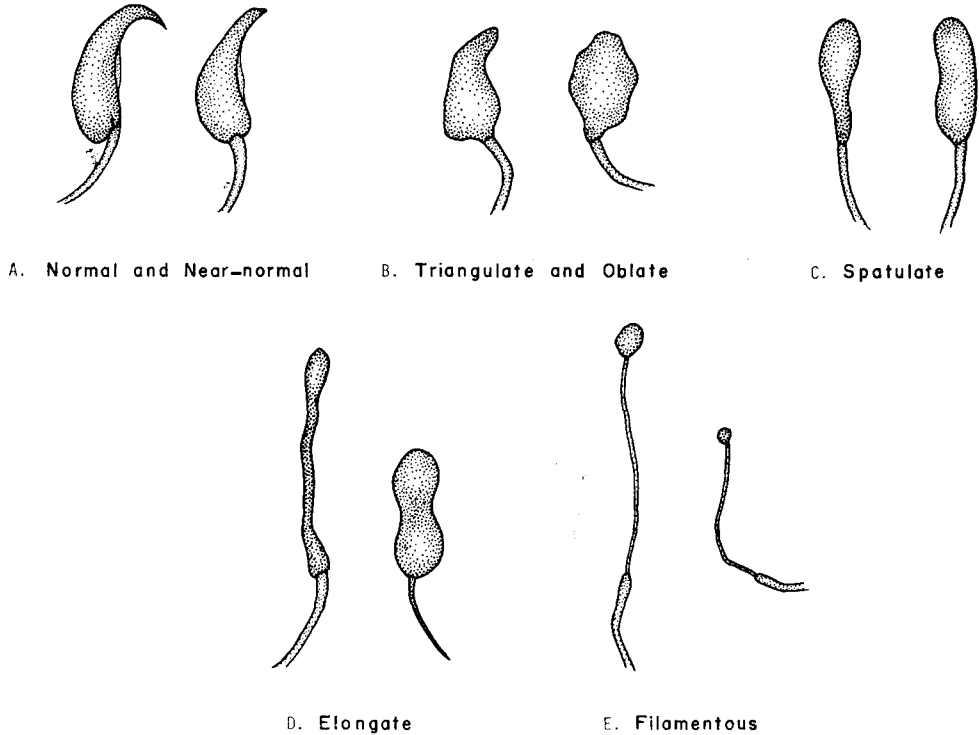
D. Elongate   E. Filamentous

FIGURE 1.—Graphic ranges for the five morphological classes of sperm: A. normal and near-normal, B. triangulate and oblate, C. spatulate, D. elongate, and E. filamentous. These establish the limits for the classification of sperm heads, data of Table 2 and Figure 2.

TABLE 2

Classification of sperm from p-sterile male mice into five morphological types

Genotype	N	A Normal and near-normal	B Triangulate and oblate	C Spatulate	D Elongate	E Filamentous
p^6p^6	4	15.6 (23.2±1.2)	3.9 (11.3±0.8)	28.5 (31.9±3.8)	15.5 (23.1±1.0)	36.5 (37.0±4.0)
p^{bs}/p^{bs}	4	16.5 (23.1±4.1)	3.1 (10.1±0.6)	29.9 (33.2±1.5)	24.0 (29.2±1.9)	26.5 (30.9±1.9)
p^{25}/p^{25}	4	1.3 (5.4±2.0)	1.2 (5.6±1.9)	42.6 (41.0±2.2)	27.0 (31.3±0.3)	27.9 (31.6±3.1)
p^6/p^{bs}	7	13.3 (21.2±1.2)	3.8 (10.3±1.8)	38.6 (38.4±1.0)	16.4 (23.7±1.2)	27.9 (31.7±1.7)
p^6/p^{25}	9	4.1 (11.4±0.8)	1.2 (5.5±0.9)	38.6 (38.4±1.2)	20.7 (27.0±0.5)	35.4 (36.5±1.4)
p^{bs}/p^{25}	5	10.1 (18.1±2.1)	3.4 (10.5±0.8)	33.4 (35.3±0.7)	26.4 (30.9±0.3)	26.7 (29.0±2.6)
$p^d/—$ (fertile)	8	83.2 (66.5±2.6)	2.0 (7.8±0.8)	10.1 (8.9±2.2)	2.4 (7.5±1.6)	2.3 (8.0±1.3)

Proportions of each type (in percent together with the mean of arcsin transformations and standard error in parenthesis) were determined from counts of 200 sperm/mouse.
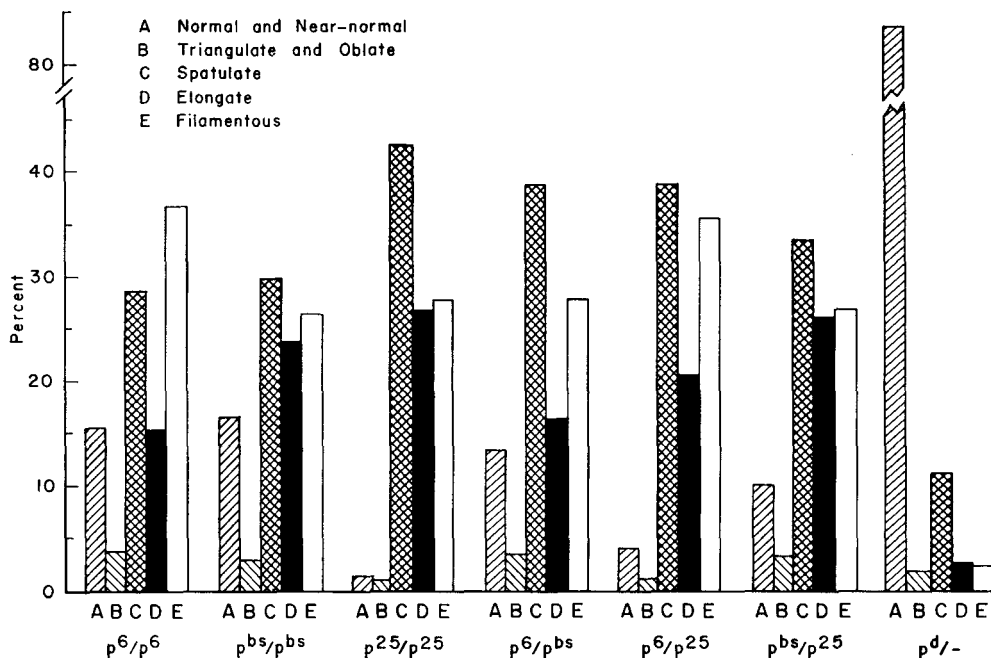
FIGURE 2.—Histograms of the frequency distributions (in percent) of sperm for each p-sterile genotype and fertile genotypes combined (p^d/—). Data of Table 2.

ages). It was possible to pool fertile genotypes p^d/p^d, p^d/p^6, p^d/p^{bs} and p^d/p^{25} since they did not differ significantly from one another for any class. It can be seen that each sterile genotype (including heterozygotes) has grossly abnormal sperm ranging from normal to long filamentous forms in the most extreme case. The percent normal and near-normal ranged from 1.3 for sterile genotype p^{25}/p^{25} to 16.5 for sterile genotype p^{bs}/p^{bs}, compared to 83.2 for fertile genotypes. Each sterile genotype differs significantly from all other sterile genotypes in at least one class, except for p^6/p^6 compared to p^{bs}/p^{bs}.

Of the homozygotes, p^{25}/p^{25} is quite obviously the most severely affected (normal and near-normal 1.3%); at the same time there are more spatulate forms (42.6%) and fewer filamentous (27.9%), just the opposite of p^6/p^6 (spatulate 28.5%; filamentous 36.5%) which has a greater number of normal and near-normal sperm (15.6%). Heterozygote p^6/p^{25} most resembles the p^{25}/p^{25} parent in its low proportion of normal and near-normal sperm (4.1% cf 1.3%). In a class by class comparison of frequencies, heterozygotes variously appear dominant or overdominant, intermediate, or recessive or hyporecessive. It was not possible to predict a priori the distribution of sperm types in heterozygotes from a knowledge of the distribution of sperm types in homozygotes. In no instance was there any suggestion of a simple bimodality of sperm types in heterozygotes, which might indicate haploid gene expression.

DISCUSSION

HUNT and JOHNSON (1971), from electron microscope studies, concluded that abnormal sperm head shape in homozygotes for the two p-sterile alleles, p^6 and p^{25}, results from abnormal proacrosome formation, and multiple tail number results from a failure in cytokinesis. The presence of spermatids with variable numbers of nuclei argued against any stage-specific effect on cytokinesis and led these investigators to hypothesize that failure of cytokinesis is secondary to other derangements of the germinal epithelium. The array of sperm head morphologies among affected genotypes observed in data reported here, differing in class frequency but not in kind, would also argue against any stage-specific effect of the p-locus on spermatogenesis.

Somewhat fewer than 50% of p^6/p^{25} males copulate with normal females and form vaginal plugs, whereas males of the other five p-sterile genotypes under consideration in this paper have never been observed to copulate or form vaginal plugs under similar conditions (WOLFE unpublished). This first suggested to us the possibility of complementation in p-sterile heterozygotes that might be revealed in a distribution of sperm types. GLUECKSOHN-WAELSCH et al. (1974) found partial complementation among certain radiation-induced lethal c-alleles; in one viable but sterile heterozygote (c^{3H}/c^{6H}) LEWIS, TURCHIN and GLUECKSOHN-WAELSCH (1975) noted the presence in the vasa deferentia of grossly abnormal sperm types similar to those described for p-sterile males. However, for a given class in our data, heterozygotes were variously dominant or overdominant, intermediate, or recessive or hyporecessive (Figure 2); the data thus provide no support for a deletion-complementation hypothesis but they do not rule out the presence of deletions.

We could find no evidence for post-meiotic (haploid) gene expression. Rather than a mixture of two primary types in heterozygotes, there was a complex quantitative variation in the frequency of different types. Lack of evidence for post-meiotic gene expression has been the general experience (ERICKSON 1974; JOHNSON and EDIDIN 1972; LYON, GLENISTER and HAWKER 1972). However, the t-alleles of mice show segregation distortion most easily explained by haploid gene expression (GLUECKSOHN-WAELSCH and ERICKSON 1970), and evidence for haploid gene expression of a t-allele controlled antigen has been reported (YANAGISAWA et al. 1974).

The data presented here, together with the unique copulatory ability of p^6/p^{25} males, clearly show that alleles p^6 and p^{25} are different from each other, and that p^{25} is not a remutation of p^6.

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