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The effects of mutant *p*-alleles on the reproductive system in mice*

By ROGER W. MELVOLD†
Hall Laboratory of Mammalian Genetics, University of Kansas, Lawrence, Kansas 66045

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SUMMARY

The p^{6H}, p^{25H}, and p^{bs} alleles at the pink-eyed dilution locus in mice (*Mus musculus*) cause sterility in males and semi-fertility in females when homozygous, while the p^{d}, p^{un}, p, and + alleles do not reduce fertility. Pituitaries from sterile males had significantly lower proportions of gonadotropic cells than pituitaries from fertile males. Ovaries from semi-fertile females contained large numbers of developing follicles, but no corpora lutea or corpora hemorrhagica were found. The proportion of polyovular follicles in ovaries of semifertile females was abnormally high.

Pituitary gonadotropins appear to be reduced in the sterile genotypes although the lesion cannot be localized. Possible relationships between these and other pleiotropic effects of mutant *p*-alleles are discussed.

1. INTRODUCTION

The pink-eyed dilution locus (*p*) in the mouse is best known for its effect on eye and coat pigment (Russell, 1946, 1949), but some mutant alleles at the locus have pleiotropic effects on reproduction, behaviour, viability, tooth formation, cleft palate, and other organs and systems. Castle *et al.* (1936) associated *p* with reduced body weight in certain backcross males. Braden (1959) reported a tentative effect of *p* on sperm head size, but Wolfe (1971) found no such effect under similar circumstances. Female mice carrying *p* or pink-eyed unstable (p^{un}) have elevated responses to induced ovulation (Wolfe, 1971), and bioassays indicated that the differences are due to elevated levels of pituitary and circulating gonadotropins, most likely LH (Wolfe, 1971).

Pink-eyed sterile (p^{s}) causes pigment dilution indistinguishable from *p* as well as abnormal reproduction, behaviour, and tooth formation (Hollander, Bryan & Gowen, 1960*a*, *b*). The sterility of p^{s}/p^{s} males was attributed to abnormal acrosome formation during spermiogenesis (Hollander *et al.* 1960*a*). Three radiation-induced alleles, p^{6H}, p^{25H}, and *p*-black-eyed sterile (p^{bs}), cause abnormal reproduction and behaviour, similar to p^{s}, and different degrees of pigment dilution (Phillips, 1970; Hunt & Johnson, 1971; Johnson & Hunt, 1972). Hunt & Johnson (1971) concluded that abnormal acrosome formation and a failure in cytokinesis were responsible for

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† Present address: Shields Warren Radiation Laboratory, Harvard Medical School, 50 Binney Street, Boston, Mass., U.S.A. 02115.

the giant heads and multiple tails which characterize a portion of the sperms from p^{6H}/p^{6H} and p^{25H}/p^{25H} males. Abnormal sperms are also found in p^{bs}/p^{bs} males (Phillips, 1970), but are distinguishable from the sperms of p^{6H} or p^{25H} homozygotes (Johnson & Hunt, 1972). These alleles also affect the adrenal glands and blood sugar levels (Johnson & Hunt, 1972). The p^{11H} allele, when homozygous, causes pigment dilution, cleft palate, and death (Phillips, 1973).

This investigation was undertaken to study the effects of a series *or* p-alleles on the reproductive system of the mouse and to relate those effects to other pleiotropic effects of mutant p-alleles. The histology of the pituitary and ovary was examined to learn what changes result from substitution of certain mutant p-alleles.

2. MATERIALS AND METHODS

Mice homozygous for 6 mutant alleles of pink-eyed dilution and for wild type were used. Three alleles (+, p, p^{un}) were on the C57BL/6 genetic background and were drawn from colonies maintained at the Hall Laboratory. The other 4 alleles (p^{6H}, p^{25H}, p^{bs} and dark pink-eye – p^{d}) were on the MUP genetic background and were derived from mating pairs obtained from the MRC Radiobiological Research Unit at Harwell and subsequently maintained at the Hall Laboratory. The three alleles which severely affect reproduction (p^{6H}, p^{25H}, p^{bs}) were maintained in heterozygotes with p^{d}, and homozygotes for study were obtained by mating heterozygotes. A stock of homozygous p^{d}/p^{d} mice was also kept.

Pituitaries were collected from 6 males of each homozygous genotype: three at 5 weeks of age and three at 10 weeks of age. Males were used because their pituitaries are not affected by an oestrous cycle and because the females were needed for other experiments. The pituitaries were fixed in 10% neutral formol saline, dehydrated in a graded alcohol series, cleared in toluene, and embedded in Paraplast. They were serially sectioned at 2 μ in the horizontal plane and stained for the specific identification of gonadotropic cells by the method of Wilson & Ezrin (1954) with minor modifications. Ten sections at regular intervals covering the entire anterior pituitary were chosen for counting. By means of an ocular grid, a 0·7 mm strip along the long axis and running the entire length of each of the ten sections was examined at 600× and every chromophilic cell was counted and classified. About 10000 cells were counted from each pituitary, and the mean and distribution of the proportions of gonadotropic cells in the pituitaries of each of the seven homozygous genotypes was calculated.

Both ovaries were removed from 5 mature females (10 weeks old) of each of the seven homozygous genotypes, fixed in Bouin's fluid, dehydrated in a graded alcohol series, cleared in toluene, and embedded in Paraplast. Serial sections were cut at 6 μ, stained with hematoxylin and counterstained with 1% orange-G. The ovaries were examined for follicular content and general characteristics. The proportion of polyovular follicles was determined for each ovary by counting and classifying (by the number of ova per follicle) all primary follicles in each of 10 regularly spaced sections.

3. RESULTS

With respect to the proportion of pituitary gonadotropic cells, the males fall into two groups which are significantly different at the 0·001 level of probability:

$$+/+,\ p/p,\ p^{un}/p^{un},\ p^{d}/p^{d} \gg p^{6H}/p^{6H},\ p^{25H}/p^{25H},\ p^{bs}/p^{bs}$$
(all fertile) (all sterile)

Gonadotropic cells in the pituitaries of sterile males are only $\frac{1}{3}$–$\frac{1}{4}$ as frequent, proportionately, as in the pituitaries of fertile males (Table 1) suggesting that gonadotropin levels are reduced in the sterile males.

Table 1. *Proportions of gonadotropic cells in pituitaries of male mice homozygous for various* p-*alleles*

Genotype	Reproductive capacity	Gonadotropic cells (% of total chromophilic cells) $\bar{x}$	95 % conf. lim.
$+/+$	Fertile	3·98	3·43–4·59
p/p	Fertile	4·33	3·19–5·65
p^{un}/p^{un}	Fertile	4·12	2·69–5·85
p^{d}/p^{d}	Fertile	5·03	3·01–7·55
p^{6H}/p^{6H}	Sterile	1·35	0·96–1·80
p^{25H}/p^{25H}	Sterile	1·29	1·14–1·46
p^{bs}/p^{bs}	Sterile	1·27	1·10–1·44

There are striking differences in the distribution of mono- and polyovular ovarian follicles among the seven types of homozygous females (Table 2). Polyovular follicles are normally present in the ovaries of immature mice, but disappear by sexual maturity when adult hormone balances are reached (Kent, 1960). Ovaries of 10-week-old $+/+$, p/p, and p^{un}/p^{un} females had fewer than 1 % polyovular follicles. Ten-week-old p^{d}/p^{d} females had 7 % polyovular follicles, and a strain effect may be responsible for the difference from the other three fertile genotypes. Females homozygous for p^{6H}, p^{25H}, or p^{bs} were, however, quite different, and the high proportion of polyovular follicles (26–30 %) still present at 10 weeks of age suggests abnormal hormone levels.

Developing follicles were present in all of the ovaries examined, but no corpora lutea or corpora hemorrhagica were found in the ovaries of the semi-fertile females involved in this study. Single corpora lutea were found, however, in one ovary of each of two females (one p^{6H}/p^{6H}, one p^{25H}/p^{25H}) examined for other purposes. Each ovary from a fertile female contained corpora lutea and most also contained corpora haemorrhagica.

An index based on ovarian weights and the number of primary follicles in each ovary showed that ovaries from the semi-fertile females were smaller, but contained as many, or more, follicles than ovaries from fertile p^{d}/p^{d} females (who shared the same genetic background as the semi-fertile females).

Table 2. *Ovaries of female mice homozygous for different p-alleles*

Genotype	Genetic background	Ovaries examined	Primary follicles: Ova/follicle 1	2	3	4	5	6	7	8	9	% polyovular	Corpora hemorrhagica and/or corpora lutea
+/+	C57BL/6	10	1171	4	—	—	—	—	—	—	—	0·3	+
p/p	C57BL/6	10	1461	10	—	—	—	—	—	—	—	0·7	+
p^{un}/p^{un}	C57BL/6	10	1366	8	—	—	—	—	—	—	—	0·6	+
p^{d}/p^{d}	MUP	10	2257	146	16	8	—	—	—	—	—	7·0	+
p^{6H}/p^{6H}	MUP	10	1499	400	82	25	15	3	2	1	1	26·1	−*
p^{25H}/p^{25H}	MUP	10	2497	834	167	36	17	8	1	—	—	29·9	−*
p^{bs}/p^{bs}	MUP	10	1640	444	97	29	9	3	—	2	—	26·3	−

* One female examined for other purposes found with one ovary containing a corpus luteum.

4. DISCUSSION

Previous reports on the reproductive effects of some mutant *p*-alleles in the mouse have implicated the pituitary as a likely site of primary action (Hollander *et al.* 1960*a*, *b*; Wolfe, 1971; Hunt & Johnson, 1971; Johnson & Hunt, 1972). In males, the pituitary is the source of interstitial-cell-stimulating-hormone (ICSH) – the male equivalent of LH – which is responsible, via the interstitial cells, for the maintenance of the seminiferous tubules. In females it is the source of follicle-stimulating-hormone (FSH), luteinizing-hormone (LH), and luteotropic-hormone (LtH). All three hormones are necessary, in proper sequence and amounts, for the development and maturation of ovarian follicles, ovulation, and corpora lutea formation. LtH is also involved in lactation. The secretion of these hormones is regulated, via the hypothalamus, by feedback inhibition of ovarian hormones (oestrogens and progesterones).

Semi-fertile females (homozygous for p^{6H}, p^{25H}, or p^{bs}) examined in this study apparently failed to regularly ovulate and form corpora lutea although their ovaries contained large numbers of developing follicles. Most females of these genotypes are not sterile, however, and many do breed although their oestrous cycles are variable and difficulties occur at birth along with poor maternal behaviour (Wolfe, 1973). It is possible that sufficient FSH is present to stimulate follicular development, but that the LH and/or LtH necessary for ovulation, luteinization, and some aspects of maternal care is reduced. This suggestion is compatible with reports by Wolfe (1971) that LH levels are influenced by mutant *p*-alleles and also (by extrapolation to males) by Hunt & Johnson (1971) who suggested that a pituitary defect was responsible for spermatogenic defects.

The reduced proportions of pituitary gonadotropic cells in affected males implies that pituitary gonadotropin levels are reduced in the sterile males (and possibly also in affected females), although the specific hormone(s) was not identified. Whether the gonadotropic cells are actually reduced in number, or whether only a portion of the cells present are functional (and thus histochemically detectable) is not known. In either case, the proportion of functional gonadotrophs is reduced. Preliminary radioimmunoassays agree generally with the reduced gonadotropin levels suggested here for affected males (H. G. Wolfe, personal communication).

While the data suggest that the pituitary gonadotropin levels are reduced in affected mice, the lesion is not necessarily in the pituitary, but could be in the hypothalamus, the gonads, or a general metabolic defect. In view of findings with radiation-induced alleles at other loci, parts of the syndrome may be due to deletions at neighbouring loci rather than to the *p*-locus itself.

One should begin, however, by considering whether the effects could all be due to one locus. The wide range of pleiotropic effects of some *p*-alleles complicates any attempt to define a primary site of action for the locus. It seems unlikely that all the effects can be explained by Moyer's (1963, 1966) proposal that the *p*-locus is responsible for the cross-linking unit of the melanosome matrix. Sidman & Pearlstein (1965) showed that cultured p/p and p^{un}/p^{un} (formerly called p'/p') eye tissues could become heavily pigmented in the presence of exogenous tyrosine, regardless of the

regularity of alignment of the melanosome matrix fibres. They proposed that the inability of p/p and p^{un}/p^{un} mice to form significant levels of melanin *in vivo* was not due to the absence or defect of some part of the melanin-synthesizing system, but to some shift in tyrosine metabolism which made it unavailable for melanin synthesis. The level of pigmentation associated with other p-alleles is not necessarily correlated with the melanosome structure (Melvold, 1973).

The observation of Sidman & Pearlstein (1965) raises the possible role of tyrosine in a general metabolic defect associated with the p-locus. Tyrosine is basic to the synthesis of several molecules such as dihydroxyphenylalanine (DOPA), dopamine, melanin, epinephrine, norepinephrine, and thyroxin, many of which are important in the nervous and endocrine systems. If mutations at the p-locus do affect some aspect of tyrosine metabolism, different mutations might affect the availability of tyrosine for different metabolic paths.

On the other hand, it is worth noting that the alleles which cause drastic changes in reproduction and behaviour (p^{s}, p^{6H}, p^{25H}, p^{bs}) and cleft palate (p^{11H}) are all radiation-induced. Analysis of radiation-induced alleles at the *d*, *se*, and *c* loci (Russell, 1971, 1973) indicate that the pleiotropic effects of many of those alleles are probably due to small deletions, not only of the locus in question, but of adjacent loci governing other functions. It may be that the pleiotropic effects of the radiation-induced mutant p-alleles are likewise the result of deletions involving adjacent loci concerned with reproductive or neurological functions.

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