PLEIOTROPIC EFFECTS OF A MUTANT AT THE P LOCUS FROM X-IRRADIATED MICE¹

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BY means of the well-known method of irradiation followed by testing with recessive-marker stocks, many visible mutants at specific loci have been obtained in mice by several investigators. In some instances the mutants so obtained are presumably identical with the markers employed in the tester stock, but very often distinctive new alleles are involved. These latter may be of intrinsic interest in regard to knowledge of the locus, and they may also be useful additions to the available supply of markers.

Numerous mutations at the *p* locus ("pink-eyed-dilute") in linkage group I of the mouse are on record (GRÜNEBERG 1952; CARTER 1957; RUSSELL and RUSSELL 1959). Most of these resulted from irradiation of spermatogonial cells. RUSSELL and RUSSELL (1959) state that 14 of 22 such induced mutants in their studies were carried to homozygous condition, but no detailed characterization of any has been reported. In the present study such a characterization has been completed. The unusual degree of pleiotropy revealed makes the mutant significant and probably valuable for further studies, particularly of spermiogenesis and odontogenesis.

MATERIALS AND METHODS

The highly inbred albino strains "S" and "Z" were selected for irradiation. Aside from the albino gene, *c*, they possess no other mutant markers in their genotypes. Tests for induced recessive mutants were made by crossing irradiated individuals with mice of the "P" stock, which is homozygous for the marker genes *p*, *d* (dilute), *se* (short ear), *a* (non-agouti), *b* (brown) (CARTER *et al.* 1952) and also *m* (misty) (HOLLANDER, unpublished).

Since it was desired to induce mutation in mature sperms for this study, adult males were introduced into individual plastic tubes. These tubes were of one-inch inside diameter, with perforations for ventilation, and stoppers to prevent movement. To shield the anterior two thirds of the body, a lead plate was placed over the tubes. The dosage of X-rays employed was 500r from a G. E. Maxitron, 250 pkv, 30 ma, 0.25 mm Cu and 1 mm Al filters, at about 170r/minute. This dosage is sufficient to induce dominant lethal effects in nearly half the functional sperms (HOLLANDER and GOWEN 1955).

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Immediately after X-raying, each male was placed in a breeding box with four tester females. Since the male generally became sterile within about 15 days, it was discarded at about this time.

If no mutation were induced, the F_1 would be all wild type. A mutation induced at the p locus in a sperm would produce a phenotype resembling in some degree that produced by homozygous p , but presumably no other loci would be involved. Since the p and c loci are both in linkage group I, the constitution of such an F_1 individual for these loci should be $p^* c/p+$, where p^* represents the induced mutant. Chromosome aberrations (deficiency, translocation, etc.) are assumed not to be involved unless demanded by other evidence.

In order to study the phenotypic effects of the induced mutant when homozygous, one must first go through several generations of outcrossing and testing. Since the p and c loci are about 14 units apart, we assume that most of the progeny will be noncrossovers, and those receiving c will also get p^* . To detect which of the outcross progeny received c , the original F_1 showing mutation at the p locus was crossed with a homozygous chinchilla ($c^{ch} c^{ch}$) male. The c^{ch}/c progeny were light chinchilla in color, in contrast to the wild type $c^{ch}/+$. Further outcrossing of two of the presumed $p^* c/+ c^{ch}$ individuals to $C+ C+$ propagated the stock; inbreeding of the descendants produced $p^* c/p^* c$ and $p^* +/p^* c$ individuals for further investigation and breeding. The unexpected tests necessary in that connection will be detailed later in connection with treatment of the pleiotropic effects.

The mice in this laboratory are housed in an air-conditioned room; the boxes are of aluminum, with a litter of fine wood shavings, changed once a week. Food hoppers have a 1/4-inch mesh galvanized hardware-cloth side through which the mice gnaw the food biscuits (Purina Lab Chow and Wayne Lab Blox). Young mice are ordinarily weaned at the age of about four weeks. Other details of care and conditions have been typical of most mouse-breeding laboratories.

OBSERVATIONS

The F_1 mouse showing mutant pink-eyed phenotype was a vigorous female with normal fertility. She was the only such mutant observed in the F_1 population of about 3500 individuals, and there were 14 additional normal progeny from her sire, an S strain male. The litter in which she appeared was born on March 9, 1955, 23 days after the sire's irradiation.

The breeding program outlined above was followed without difficulty. Crossing over values between p^* and c were approximately 15 percent, in very good agreement with the map distance for these loci. Therefore no chromosomal disturbance of any consequence can be postulated.

In the outcross program, this female's descendants were ordinarily of large size and high prolificacy. By contrast, the $p^* p^*$ segregates proved small and sterile *inter se*. Accumulated records provide the following comparisons:

(1) *Growth and body weight*: Normals of this family ranged to over 50 grams in mature body weight, achieved within four months. Many were over 40 grams.

None of the $p^* p^*$ mice ever reached 40 grams; one exceptionally fat male weighed 38 grams, while the majority did not reach 30. Growth curves showed that the weight differential was present at birth and was consistent to maturity, and amounted to about 30 percent less than the normal litter mates.

(2) *Behavior*: The $p^* p^*$ mice were active but somewhat uncoordinated in movements. In some cases the activity was almost choreic (waltzing).

(3) *Chewing*: After weaning, some $p^* p^*$ mice starved before it was realized that they were having extreme difficulty in chewing the food biscuits through the 1/4-inch mesh of the hopper. Provision of a 1/2-inch gap obviated this difficulty.

(4) *Reproduction in females*: Although $p^* p^* \times p^* p^*$ matings produced no young, crosses of $p^* p^*$ females with normal males were more successful. At least half of the females however failed to breed, and the others produced only one to four litters. The maximum litter size was 11 young, while larger numbers were often obtained from normal sisters. The $p^* p^*$ females rarely kept the young in a nest, but usually scattered and did not nurse them. Although several litters were raised, the young were very poorly grown. It was found that fostering young of $p^* p^*$ mothers with normal nursing females was the best policy.

(5) *Reproduction in males*: As noted earlier, matings of $p^* p^* \times p^* p^*$ produced no progeny and apparently no pregnancies. Individual tests of the $p^* p^*$ males with normal females also indicated sterility. For a more thorough test, over 80 $p^* p^*$ males were mated with normal females, usually four females per male, and left at least a month. Only two males sired litters: in one case two litters, of four and one young, respectively, and in the other case a single young, dead at birth. The two males were left for a considerable time with additional mates, but appeared sterile.

Examination of the semen of $p^* p^*$ males revealed another excellent reason for sterility—high proportions of the sperms were very abnormal in head structure. Details of sperm morphology are to be reported in a separate paper. The abnormalities varied from slight shortening of the hook and posterior end of the head to complete disorganization with filamentous protrusions. The abnormalities were found even in the testis, and have been traced to faulty acrosome development in the spermatid.

In still another test, 12 $p^* p^*$ males were mated with four normal females each, and daily examinations for vaginal copulation plugs were made for two weeks. No plugs were discovered. Anatomical study of these males revealed no definite abnormality of the reproductive organs.

(6) *Teeth and aging*: As the $p^* p^*$ mice reached what in normal mice would be the "prime of life," about the age of five months, signs of senility had appeared. Many, especially females, became emaciated and died, apparently as a result of difficulty in chewing. This difficulty was not a matter of inability to get at the food, but improper wear of the incisors, sometimes with obvious malocclusion. Instead of the usual bevelling wear, the upper incisors generally developed a transverse groove into which the lower incisors pressed. In some cases an

FIGURE 1.—Comparison of sperm head morphology of sperms from a $p^* p^*$ male (left) with those from a normal male (right). Note that the normal hook and rounded posterior end of the head are altered or obliterated in the abnormal, often leaving only a loose mass of threads.

incisor broke off. Most of the mice were in such poor condition by the age of ten months that they died or were killed. Hardly any have lived over a year; one lived to 16 months. By contrast, normal mice of this stock readily live to a year and a half. A mash diet, which might have been more favorable for the $p^* p^*$ mice, has not been tried.

DISCUSSION

The mutation under discussion, which will now be symbolized p^* , must be considered detrimental though it cannot be classed as a lethal. It was noted that heterozygotes ($+/p^*$) in this stock were very vigorous and prolific, but this fact seems more readily attributable to the strain crossing involved in propagating the mutant than to overdominance at this locus. There is no evidence indicating that chromosome damage was involved, because linkage of p and c loci was undisturbed, giving the usual crossover values.

In RUSSELL and RUSSELL's report (1959), of the 14 radiation-induced mutants at the p locus which they tested homozygous, six were lethal before weaning age. These were all from irradiated spermatogonia. Other evidence, particularly the lethal mutants obtained at the d locus on chromosome II, without involving the se locus extremely close to it, led the RUSSELL's to conclude that deficiencies are rare in such material, and that point-mutant lethals are realities. RUSSELL,

FIGURE 2.—Wear patterns of incisor teeth. A.—Normal, aged nine months. B, C, D.—Examples of wear patterns of $p^s p^s$ mice at the same age as A, or younger. These abnormal wear patterns are associated with difficulty in chewing.

BANGHAM, and GOWER (1958) found a higher incidence of induced deficiencies in irradiated postspematogonial stages. It is commonly assumed that deficiencies must be lethal when homozygous, since this has been the general experience with *Drosophila*.

If we assume that p^s is not due to a deficiency, the multiplicity of its effects in the homozygote constitute an apparent case of "genuine" pleiotropy. These seem impossible to fit into the one-gene-one-enzyme hypothesis of gene action, and similar theories, namely that all the effects can be traced to a single derangement of a primordial physiological process. It is at present impossible to conceive of such a common source of the changes here observed in pigmentation, growth, behavior, tooth wear, and spermiogenesis. The ordinary p allele, so far as has been discoverable, affects only black melanin pigment formation.

The relation of pseudoallelism to the question of pleiotropy has been well-discussed by GOLDSCHMIDT (1955, pages 398–400). There is no possibility of properly testing this aspect of the question in the mouse at present.

SUMMARY

A presumably X-ray-induced mutation at the p locus, recessive to both p and wild type alleles, has been analyzed. When homozygous the mutant produces the same effect on pigmentation as $p p$, but in addition there is poor growth, slightly uncoordinated behavior, difficulty in chewing, inadequate maternal care by females, sterility in males, premature senility, and abnormal wearing of the incisor teeth. The male sterility is not quite absolute, and is partly the result of poor mating response and partly a very high proportion of sperms with abnormal head structure. Since chromosome aberration seems not to be involved in the origin of this mutant, it is considered a new allele with unusual pleiotropic effects, and is symbolized p^s .

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