RADIATION-INDUCED PRESUMED SOMATIC MUTATIONS IN THE HOUSE MOUSE[1]

LIANE BRAUCH RUSSELL AND MARY H. MAJOR

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Received October 2, 1956

EXPERIMENTS on the induction of somatic mutations can provide information in at least two main fields of interest: (1) in the comparative study of mutation induction in different types of cells; and (2) in the study of cell lineage, where the induced somatic mutations can be used as a valuable tool.

Somatic mutations involving specific loci have been repeatedly produced by radiation in Drosophila (PATTERSON 1928, 1929a, 1929b, 1930; TIMOFÉEFF-RESSOVSKY 1929; GULBEKIAN 1934; HASKINS and ENZMANN 1937; LEFEVRE 1948, 1950) and in plants (e.g., SPARROW *et al.* 1952). In mammals, however, although several dozen spontaneous coat color mosaics have been observed (CASTLE 1922; WRIGHT and EATON 1926; BITTNER 1932; DUNN 1934, review; FELDMAN 1935; BHAT 1949; CARTER 1952; MORGAN and HOLMAN 1955), the induction of mosaics by irradiation has not been reported except in our preliminary communication (RUSSELL and MAJOR 1952). Consequently, several points of technique had to be worked out prior to the start of the experiment. The method adopted makes it possible to distinguish the genetic effects produced from developmental effects of radiation which might otherwise obscure them.

The results indicate that somatic mutations at specific loci were induced by radiation in prospective pigment cells. The mutation rate in these cells could be calculated and compared with the rate found in mouse spermatogonia for the same loci (W. L. RUSSELL, 1951). Various features of the results suggest some working hypotheses and some tentative conclusions about cell lineage in mouse development.

MATERIALS AND METHOD

In summary, the method consisted of irradiation of embryos heterozygous for four coat color genes and examination of the adult fur for mosaic patches. A parallel control series involved irradiation of embryos homozygous for the wild type alleles of the four loci studied. Spots of altered color in the latter series should represent mutations to dominants anywhere in the genome, or else non-mutational, i.e., developmental, changes (e.g., abnormal differentiation of pigment cells). When the frequency of these is subtracted from the total mosaics in the heterozygous series, one should be left only with changes due to the expression of the four coat color recessives.

Stocks and mating system

Females of the inbred C57BL strain $\left(\frac{a}{a}\ \frac{+^{p}+^{c}}{+^{p}+^{c}}\ \frac{+^{d}+^{se}}{+^{d}+^{se}}\ \frac{+^{b}}{+^{b}}\right)$ were mated either to inbred NB strain males $\left(\frac{a}{a}\ \frac{p\ c^{ch}}{p\ c^{ch}}\ \frac{d\ se}{d\ se}\ \frac{b}{b}\right)$ or to C57BL males. (a = non-agouti;

[1] Work performed under Contract W-7405-eng-26 for the U. S. Atomic Energy Commission.

p = pink-eye, diluted coat color; c^{ch} = chinchilla; d = dilution; se = short ear, no effect on coat color; b = brown). Offspring of both types of mating are black. The first litters raised served as non-irradiated controls. Following weaning of the first litters each female was returned to her original mate and the second copulation was timed by observation for vaginal plugs. Irradiation was administered during the second pregnancy. From 65 irradiated females, 60 litters were obtained. The number of unirradiated litters (i.e., first litters) observed was 57.

Stage irradiated

In a somatic mutation experiment, each individual observed represents a large number of cells in which a mutation could have occurred, instead of a single cell as in a germinal mutation experiment. It is, therefore, of advantage to irradiate embryos at a time when the number of pigment precursor cells is already large. However, if this number is *too* large, the spot resulting in the adult fur will be too small to be perceptible in every case.

To determine the embryonic stage that would best meet these criteria, a preliminary experiment was carried out. C57BL females, mated to NB males, were irradiated in various stages of pregnancy, ranging from day $6\frac{1}{2}$ to day $12\frac{1}{2}$ postfertilization, and 136 young were raised. Mosaic spots in the coat were observed in four animals, all of which had been irradiated as day-$10\frac{1}{2}$ embryos. (One spot was observed among 75 control young.) It was concluded that mosaics induced in embryos older than $10\frac{1}{2}$ days were too small to be perceptible, while the frequency of mosaics produced by irradiation of younger embryos was not high enough to have produced a case in the preliminary experiment and, thus, not high enough to be practical. It was, therefore, decided to irradiate embryos at $10\frac{1}{4}$ days, i.e., on the morning of the 10th day following observation of the vaginal plug.

Dose

The stage determined to be most suitable from the point of view of mosaic production is, unfortunately, also the most sensitive to radiation with respect to incidence of neonatal death (RUSSELL and RUSSELL 1954; RUSSELL 1954). Since 200r at $10\frac{1}{2}$ days postfertilization was already known to cause about 70 per cent neonatal death (probably no prenatal death), the present experiment was begun with 150r. With that dose, the survival of animals to an age at which they could be observed for coat color mosaics was found to be slightly lower than two thirds of the control value (table 1). With two thirds of that dose, i.e., with 100r, survival was almost equal to controls. Thus, from the practical point of view of yield per effort, the two doses should be equivalent. However, since it seemed probable that complications from factors such as abnormal differentiation would be greater with 150r, the bulk of the data was obtained with 100r.

Radiation factors were as follows: 250 kvp X-rays, delivered by a Coolidge self-rectifying tube, half-value layer of 0.4 mm copper, inherent filtration 3 mm aluminum. Using a current of 15 milliamperes, the intensity obtained at 84 cm distance was 91 ± 3r per minute. The pregnant females were exposed in individual compartments of a perforated lucite box resting on a 10.8-cm-high masonite scatter block.

TABLE 1

Survival of mice irradiated 10¼ days postconception

	C57BL ♀ × NB ♂			C57BL ♀ × C57BL ♂		
	0r	100r	150r	0r	100r	150r
Number of ♀ ♀ treated	31	38[b]	4	25	16	7[c]
Number of young born	211	255	34	153	89	35
Young born/litter	6.8	7.5	8.5	6.1	5.6	5.8
Percent survival to observation[a]	95.7	92.2	61.8	92.2	92.1	57.1

[a] Detailed observation for coat color mosaics was made between 32 and 58 days of age (see text).

[b] Includes four females that had no litters, probably because of misdiagnosis of pregnancy.

[c] Includes one female that had no litter.

Observation

Early in the experiment, the first observation for coat color mosaics was at 1–2 weeks of age, but while four of the mosaics were noted at that age, eight others, visible at a later age, were missed, probably owing to the hair, particularly in ventral regions, not yet being full length. Detailed examination of the animals was, therefore, made sometime between 32 and 58 days of age. Non-mosaic animals were discarded at that time. Mosaic animals were saved and again observed 4–10 weeks later to insure that the spots were not transient, e.g., due to moulting patterns. An additional observation was made on a few mosaic animals at about one year of age but it was found that old-age graying occasionally obscures the spots at that age.

The following records were made, at the time of the first or second observation of the mosaic: sketching of the position of the spot(s); outline of the spot(s); estimate of the proportion of spot area occupied by mutant hair; and gross description of the spot color. Hairs were plucked for histological examination. Measurement of the spot with calipers and tracing of the outline on squared paper to determine mosaic area were always done in fully mature mice, namely, between 8 and 21 weeks of age.

The area of the total coat was measured in a few skins and found to be about 45 cm^2. Since small variations in total coat area will not markedly affect the calculated proportion of coat occupied by the spot, the total coat area was not determined for each individual mosaic mouse but taken to be 45 cm^2 in all cases.

Small white belly spots, which occurred in the C57BL × C57BL matings, were recorded only if the spot was made up of at least five hairs.

The ears of all animals were examined, since offspring of the C57BL × NB cross are heterozygous for short-ear, *se*. A few of the animals were examined at birth for possible changes in the eyes. Somatic mutations involving eye tissue should be observable for three of the loci: *p* (*p*/*p* = pink eyes), *c*, and *b* (c^{ch}/c^{a} and *b*/*b* both reduce the width of the pigment ring at birth).

RESULTS

1. Frequency of mosaics

Among 701 animals whose adult coats were examined, there were 31 with altered spots of coat color. This excludes small white mid-ventral spots which will be dis-

TABLE 2

Frequency of various spots in the coats of mice that had been irradiated at 10¼ days postconception

	C57BL ♀ × NB ♂			C57BL ♀ × C57BL ♂		
	0r	100r	150r	0r	100r	150r
Number of animals observed:						
♂♂	104	110	9	70	50	9
♀♀	98	125	12	71	32	11
Total	202	235	21	141	82	20
Percent with spots of altered coat color:[a]						
♂♂	1.0	14.5	(0)	0	2.0	(11.1)
♀♀	0.0	8.0	(8.3)	1.4	0.0	(0.0)
Total	0.5	11.1	(4.8)	0.7	1.2	(5.0)
Percent with white midventral spots:						
♂♂	0.0	0.0	0.0	2.9	22.0	33.3
♀♀	0.0	0.0	0.0	9.9	34.4	63.6
Total	0.0	0.0	0.0	6.4	26.8	50.0

[a] Excluding white midventral spots listed below.

cussed below (sec. 5). The frequency of mosaic animals in the various groups is shown in table 2. The five mosaics found in the preliminary experiment (see Methods) are not included in this table.

The 150r groups are small (see Methods) and will be discussed no further from the point of view of frequencies. In offspring of the C57BL × NB cross that had been irradiated with 100r as 10¼-day embryos, the incidence of mosaic animals was about 11 percent. This incidence is significantly higher than the 0.5 percent found in the controls of the same cross ($t = 4.6$; $P \ll 0.01$) and also significantly higher than the 1.2 percent found in offspring of C57BL × C57BL matings irradiated with 100r ($t = 2.8$; $P < 0.01$). The difference between the sexes in the C57BL × NB, 100r data is not significant ($t = 1.6$; $0.1 < P < 0.2$).

If p = spontaneous frequency of animals with coat color changes due to developmental causes or to somatically arisen coat color dominants,

q = 100r-induced frequency of animals with coat color changes due to developmental causes or to somatically arisen coat color dominants (it is assumed that q is identical in C57BL × C57BL homozygotes and C57BL × NB heterozygotes),

r = spontaneous frequency of animals with somatic expression of the recessives at the *b*, *c*, *p*, and *d*-loci,

s = 100r-induced frequency of animals with somatic expression of the recessives at the *b*, *c*, *p*, and *d*-loci,

then, p = frequency of mosaic animals in the (C57BL × C57BL), 0r group... (1)

p + q = frequency of mosaic animals in the (C57BL × C57BL), 100r group.. (2)

p + r = frequency of mosaic animals in the (C57BL × NB), 0r group..... (3)

p+q+r+s = frequency of mosaic animals in the (C57BL × NB), 100r group. (4).

Therefore, the frequency of animals with somatic expression of the recessives at the *b*, *c*, *p*, and *d*-loci induced by 100r can be obtained from (4)−(3)−[(2)−(1)], i.e., 0.1106 − 0.0050 − (0.0122 − 0.0071), or 0.1006 ± 0.0253. It should, of course, be remembered that this frequency applies to animals irradiated as $10\frac{1}{4}$-day old embryos, i.e., having spots averaging a definite proportion of the coat (see below): earlier or later irradiation would, within limits, be expected to give lower and higher frequencies of mosaic animals, respectively.

To determine whether or not the mosaic animals are distributed randomly among litters, it is necessary to test several littersizes simultaneously since, for any one littersize in the C57BL × NB, 100r group, there are never more than nine litters. Two independent statistical methods were used[2], each of which simultaneously tested littersizes of 6, 7, 8, and 9 (a total of 26 litters, or 203 animals). By neither of the methods did the mosaics show a significant departure from random distribution among litters.

2. Appearance of mosaic spots

The outline of mosaic spots was, in most cases, extremely diffuse and could not be described by simple geometrical terms, such as circular, triangular, etc. Often the mutant hairs were concentrated into two or even more main areas which, however, in all but two animals were either adjacent or were close to each other with a few mutant hairs bridging the gap. In two animals there was a complete gap between main areas of mutant hair, but the areas were of the same color and the gap no larger than one cm. It may, therefore, be assumed that each mosaic animal observed in this study represents but a single event of change.

Within mutant areas, there were varying degrees of mixture of mutant and apparently nonmutant (i.e., black) hairs. This is illustrated in figure 1. Whether there is a mixture of mutant and nonmutant cells within individual hairs has not yet been determined.

The following colors of mutant spots were recorded from gross observation: dark gray (two animals); gray (seven animals); light gray or gray-white (nine animals); brown or tan (three animals); yellow-brown (nine animals). It is obviously very difficult to give accurate color descriptions for small spots which, moreover, consist of mixtures of various proportions of mutant and nonmutant hairs. Considering only known alleles at the loci studied, and their action on an *a*/*a* background, spots representing mutations at the *b*-locus should appear brown; at the *c*-locus from dark gray to quite light gray; at the *d*-locus blue-gray; and at the *p*-locus medium gray. From gross examination of spots alone, it therefore seems probable that the colors recorded represent mutations at at least two of the loci, namely, *b* and *c*. From histological examination of hairs, which is difficult (because of hair mixture) and not yet completed, it can be stated that the *p*-locus was definitely involved in at least one case. This case, grossly classified as "gray-white" was, furthermore, interesting in that the pigment in the mutant hair appeared as in $\frac{+\ p}{c^{ch}p}$, rather than as in $\frac{c^a p}{c^{ch}p}$, as it presumably would have done in case of somatic chromosome loss or deletion large

[2] We are grateful to Dr. A. W. Kimball of the ORNL Mathematics Panel for carrying out these tests.

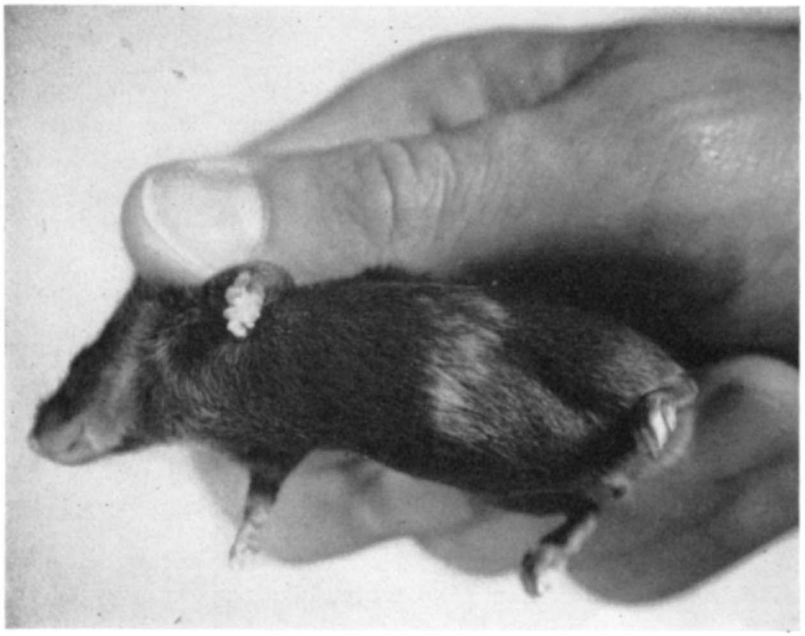

FIGURE 1.—An example of one of the larger mosaics. This animal, ♂636, was an offspring of the C57BL × NB cross and was irradiated with 100r X-rays, $10\frac{1}{4}$ days postconception. The mutant spot occupied 0.0093 of the body surface and was grossly classified as "light gray."

enough to involve both *c* and *p*-loci (crossover distance between *c* and *p*: 16 in females, 12 in males).

3. Location of mosaic spots

For the most part, spots appeared to be randomly distributed over the mouse surface, as shown in figure 2, which is a composite drawing showing the location of all nonblack areas (other than white mid-ventral spots) in offspring of the C57BL × NB cross. However, it was also clear that some spots represented abnormal differentiation, rather than genetic change. Thus of the three spots observed in offspring of C57BL × C57BL matings, two had some connection with nipples: one (in the 0r group) was an extension of the yellow-brown group of hairs which normally surrounds the female nipple; the other (in the 100r group) was the development of such a nipple spot in a male. In the C57BL × NB cross, also, there was one case of nipple-spot extension in a female. In addition, there were two other cases of yellow-brown spots close to normally yellow-brown areas: one on the snout; and one anterior to the penis. These three cases are, however, included with the rest for all quantitative purposes, since ideas as to their origin are only speculative and the C57BL × C57BL matings presumably provide an adequate control for color changes due to abnormal differentiation rather than expression of the recessives.

No mosaic eyes were observed. However, while pink-eye mosaic eyes might be detectable, it is exceedingly doubtful that small sections of b/b or c^{ch}/c^{a} tissue would

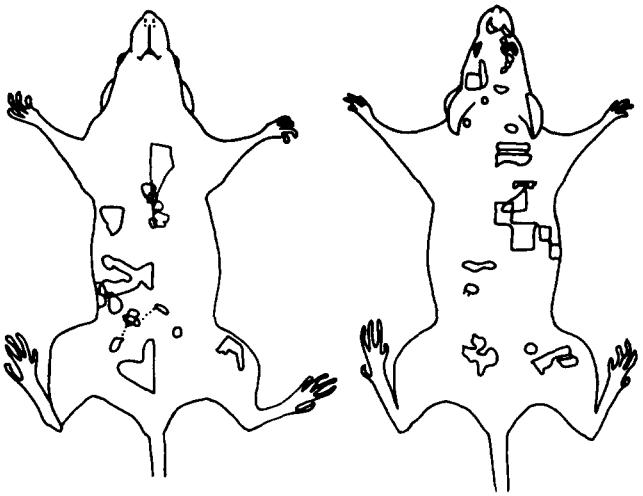

FIGURE 2.—Composite drawing showing location of spots in all mosaic offspring of the C57BL × NB cross.

show up in the adult black eye; and the number of animals observed young enough to reveal possible changes in the pigment ring was not very large.

All ears examined were normal. There was thus no evidence for somatic expression of the heterozygous *se*.

4. Proportion of coat occupied by mosaic spot. Mutation rate

A certain degree of inaccuracy entered into determination of spot size, owing to the fact that the spot perimeter was diffuse and that, moreover, the percentage of mutant hair within this indistinct perimeter could only be estimated. The proportion of adult coat occupied by mutant hair, which was computed for each animal, is therefore only approximate. The reciprocal of this proportion may be taken to give an estimate of the number of prospective pigment cells that were present at the time the mutation, or other change, occurred. Such an estimate will be valid only if the cell in which the ultimately visible change was induced does not contribute differentially less progeny on account of that change. However, such an assumption can probably be made with relative safety (see Discussion).

Table 3 shows the distribution of mosaic animals in the various groups with respect to proportion of adult coat occupied by hair of altered color. The total range for 30 mosaic animals was 0.000173 to 0.0145. If the reciprocals of these figures are taken to represent prospective pigment cells present in day-$10\frac{1}{4}$ embryos, the extremes of the range, 69–5769, are separated by approximately six cell divisions.

The probability of finding a mosaic in a given group does not depend solely on number of embryos in that group and on mutation rate, but also on number of prospective hair pigment cells per embryo. Therefore, the distribution of mosaics shown in table 3 does not directly indicate the distribution of day-$10\frac{1}{4}$ embryos with respect to number of prospective pigment cells. This latter distribution, which is

plotted in figure 3, may be derived as follows, using the results of the C57BL × NB, 100r group:

Let $p_1, p_2, \ldots$ etc. = proportion of total fur having altered color (in individual mosaic animals);

$n_1, n_2, \ldots$ etc. = number of mosaic animals characterized by p_1, $p_2, \ldots$ etc., respectively;

$\frac{1}{p_1}, \frac{1}{p_2}, \ldots$ etc. = number of prospective pigment cells present at the time the ultimately visible change occurred;

x = rate of ultimately visible change/cell/100r

$$= \frac{\text{altered cells}}{\text{total cells}}.$$

Since each mosaic animal was probably due to a single altered cell, then, for embryos of any given number, $\frac{1}{p}$, of prospective pigment cells,

$$x = \frac{n}{\frac{1}{p} \times \text{number of embryos with } \frac{1}{p} \text{ cells}}$$

(it is assumed that x is the same for all values of $\frac{1}{p}$ encountered in this experiment), and $\frac{p_1 n_1}{x}, \frac{p_2 n_2}{x}, \ldots$ etc. = number of embryos having $\frac{1}{p_1}, \frac{1}{p_2}, \ldots$ etc., prospective pigment cells. x was calculated from the results of the C57BL × NB, 100r group, in which 235 animals were observed:

$$x = \frac{1}{235}(n_1 p_1 + n_2 p_2 + \cdots)$$

$$= 3.1 \times 10^{-4}$$

In this calculation, the $p_1, p_2, \ldots$ etc. values used were for individual animals rather than for the means of the classes shown in table 3. (It may be noted that if a few animals with very small spots, i.e., p = very small, were missed in observations, this would not appreciably affect x, and thus not affect the mutation rate, calculated from x below.)

Figure 3, based on the above calculations, plots the distribution of embryos with respect to number of prospective pigment cells. The geometric means of the ranges in the left-hand column of table 3 were used for the abscissa $\left(\frac{1}{p}\right)$. The ordinates are thus $\frac{np}{x}$, where n denotes the frequencies shown in table 3. It may be noted that the curve in figure 3 indicates the modal number of prospective pigment cells in day-10¼ embryos to be between 150 and 200. The left-hand portion of the curve, however, cannot be drawn from the data since the number of embryos irradiated was not large enough to give mosaics in the groups that have such low cell numbers that the chance of producing a mosaic animal is very small. If the distribution is symmetrical then the total number of embryos with low cell numbers is not very large.

TABLE 3

Distribution of mosaic animals with respect to proportion of adult coat occupied by hair of altered color[a]

Area of altered color / total area (= 45 cm²)	C57BL ♀ × NB ♂			C57BL ♀ × C57BL ♂		
	0r	100r	150r	0r[b]	100r	150r
0.000160–0.000319	—	4	—		—	—
0.000320–0.000639	—	4	—		—	—
0.000640–0.001279	1	2	—		1	1
0.001280–0.002559	—	7	—		—	—
0.002560–0.005119	—	5	—		—	—
0.005120–0.010239	—	3	—		—	—
0.010240–0.020479	—	1	1		—	—

[a] White midventral spots (see table 2) are not included in this table.

[b] One mosaic animal occurred in this group but is omitted from the table since its spot was not accurately measured.

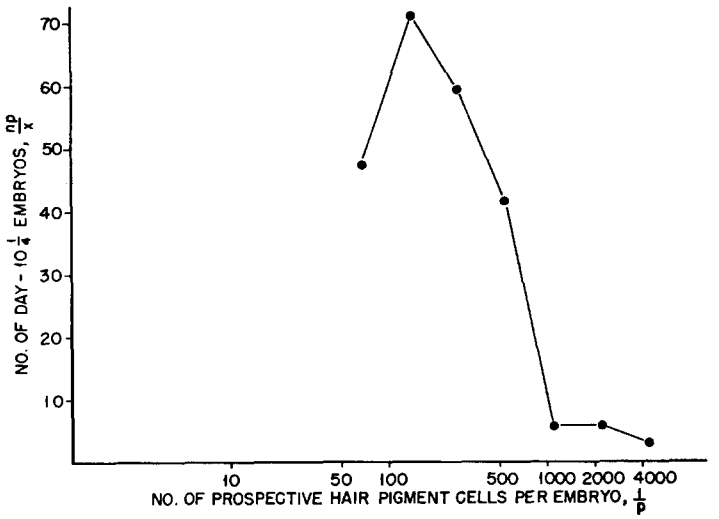

FIGURE 3.—Calculated distribution of day-10¼ embryos with respect to number of prospective hair pigment cells (see Results, sec. 4).

The mutation rate—or rate of any other change leading to expression of the four coat color recessives for which the C57BL × NB animals are heterozygous (see Discussion)—may be calculated as follows. The rate of ultimately visible change/cell/100r was shown to be 3.1×10^{-4}. It was calculated in section 1 of the Results that the frequency of mosaic animals due to radiation-induced expression of the recessives at the four loci is 0.1006, while the frequency of all animals with spots in the C57BL × NB, 100r group is 0.1106. Thus, the mutation rate/locus/r $= 3.1 \times 10^{-4} \times \frac{0.1006}{0.1106} \times \frac{1}{4} \times \frac{1}{100} = 7.0 \times 10^{-7}$. The 95 percent confidence interval, assuming no error in x, is $(4.66, 9.18) \times 10^{-7}$. Assuming 50 percent error in x (due to difficulty of spot size determination), the 95 percent confidence interval is $(2.51, 11.33) \times 10^{-7}$.[2]

5. *White midventral spots*

As shown in table 2, small white midventral spots occurred only in offspring of C57BL × C57BL matings. The increase from 6 percent in the controls to 27 percent in the group that had received 100r at $10\frac{1}{4}$ days of development is clearly significant ($t = 4.3$; $P \ll 0.01$); and the further increase from 27 percent to 50 percent between the 100 and 150r groups is significant at the $P = 0.05$ level ($t = 2.0$). It is interesting to note that in all three groups—0r, 100r, and 150r—the proportion of white midventral spots is higher in females than in males.

White midventral spots could not be produced by irradiating embryos of the C57BL × NB cross.

DISCUSSION

It seems clear from the comparison of the heterozygous (C57BL × NB) and homozygous (C57BL) groups (see Results, sec. 1) that at least a large proportion of the mosaics were due to expression of the four coat color recessives (*b*, *c*, *p*, and *d*) for which the C57BL × NB animals are heterozygous. Whether, however, this expression is due to somatic mutation of the wild-type allele or to other causes cannot be definitely stated on the basis of the present results. In the case of the spot which was diagnosed as phenotypically $p + {}^{c}/pc^{ch}$, on the basis of histological examination of hair pigment (♀ 595), some of the alternatives to somatic mutation may be ruled out. Since *p* and *c* are linked (crossover distance: 16 in females, 12 in males), this spot could not have been due to loss of a chromosome, or to somatic reduction (which would give rise to haploid cells in the spot). It could be due to somatic nondisjunction *only* if the trisomic $pc^{ch}/pc^{ch}/+^{p}+^{c}$ produces a $p+^{c}/pc^{ch}$ phenotype. It could be due to position effect of a break near the *p*-locus, but position effects have never been demonstrated in a mammal. It could be due to somatic crossing over (CARTER 1952, has suggested that spontaneous somatic crossing over can occur in mice), but only if *p* is distal to *c* and the crossover occurred between them, if *p* is proximal to *c* and there was a double crossover, or if *p* and *c* are on opposite sides of the centromere with a crossover occurring between them. Thus, while a few mechanisms by which the recessives come to express themselves can be ruled out on the basis of mosaic ♀ 595, more than one possibility remains. In the absence of further evidence, it would seem most conservative to think of the mosaics in this experiment as being due to somatic gene mutations or small deficiencies involving the loci under study.

If, in fact, the mosaics observed in the present experiment are due to somatic mutations, the somatic mutation rate (see Results, sec. 4) is 7.0×10^{-7}/r/locus [95 percent confidence limits $(2.51, 11.33) \times 10^{-7}$, assuming 50 percent error in measurement of spot size]. For comparison, the germinal rate, in spermatogonia, for the same loci (*b*, *c*, *p*, and *d*) is 2.4×10^{-7}/r/locus (W. L. RUSSELL 1951); or 2.1×10^{-7}/r/locus if one omits mutations to intermediate alleles, which are probably not included in the somatic rate (a small spot of only slightly altered color would certainly have been missed). Even though the somatic rate is open to some doubt due to the difficulty of obtaining an exact measure of the proportion of mouse surface occupied by mutant area, it is probably safe to say of the comparison that, for the

same four loci, the germinal rate induced in spermatogonia and the somatic rate, induced in $10\frac{1}{4}$-day embryos, are of the same order of magnitude. LEFEVRE (1950) found similarity between induced somatic and germinal rates at the *w*-locus in *D. melanogaster* and took this as evidence that X-ray-induced mutations differed qualitatively from spontaneous ones, since, as he says, "the spontaneous mutation rate of a given gene . . . is frequently sensitive to the influence of its cellular environment." Although the rough similarity of the X-ray-induced somatic rate to the spermatogonia rate, found in the present experiment, resembles LEFEVRE'S result, his conclusion seems open to some doubt in view of evidence from other sources that there may be variation in radiation-induced rate depending on cell type irradiated. Thus, it has recently been shown that the rate of X-ray-induced changes affecting specific loci is different at different stages in gametogenesis (ALEXANDER 1954; W. L. RUSSELL 1956 and unpublished).

In calculations of the somatic rate, the size of the cell population that was irradiated is not observed directly. One assumption that must be made is that the cell in which the scorable change was induced does not contribute differentially less progeny on account of that change; for, if it does, the determined rate will be a minimum. It seems, however, unlikely that this is the case, since it may be calculated (making reasonable assumptions with regard to number of loci) that most other somatic cells would have at least one mutation somewhere in the genome induced by 100r and would, therefore, on the average, be equally affected.

It is also necessary to consider inequality of cell contribution to the ultimately observed structure (here hair) that is due to normal cell lineage. Of all the cells of a $10\frac{1}{4}$-day embryo that will provide some descendant cells contributing to the adult hairs, not all necessarily make equal contributions. Thus, e.g., cell A may contribute its entire progeny, while cell B may contribute only a very small part, the rest of its progeny producing some other tissues. In the use of the mutant coat/total coat ratio to compute the size of the cell population at the time of irradiation, such a situation would lead to error in *individual* cases, but the errors should cancel out over several cases if the chance of causing mutation in cells A and B was equal.

The material used in this experiment was not suitable for determining whether the mosaics involved germinal as well as somatic tissue. Since the embryos were already heterozygous for the genes in question, gonadic mosaics would not have produced new types, except in the case of (a) mutations to new, phenotypically distinguishable, alleles, and (b) changes at the *d*-locus that did not involve the closely linked $+^{se}$. In all other cases, probably the great majority, the mosaic portion of the gonad would simply carry one of the genes in homozygous instead of heterozygous condition. Since it seems very unlikely, from the evidence discussed below, that the mosaic portion of the gonad could be large, the contribution from this portion would be insufficient to upset the normally expected 1:1 ratio detectably, except with very numerous progeny from each individual mosaic. Moreover, unless one knew which of the loci was involved, one would have to test simultaneously for all of them, with a resulting 16 genotypes, some of which could be established only by further testing. In another investigation, involving spontaneous somatic reverse mutations at the *pe*-locus (RUSSELL and MAJOR 1956), i.e., a situation in which each individual

descendant from mutant portions of the gonad can be positively identified, none of 19 animals with coat color spots of approximately the size induced in the present experiment was found to have gonadal tissue involved. It may, therefore, be suggested that the radiation-induced somatic mutations in the present experiment were induced too late for the cell lineage to contribute to gonadal tissue. This also seems probable on embryological grounds.

It appears from the work of RAWLES (1947) that, at the stage at which radiation was administered in the present experiment, the cells which will ultimately become melanophores have already become determined. By day $10\frac{1}{4}$, prospective pigment cells (probably neural crest cells) have begun to spread laterally from the dorsal midline and may have reached the somite region, at least in the anterior parts of the embryo. It appears from calculations shown in figure 3 that the modal number of prospective pigment cells present at the time of irradiation (day $10\frac{1}{4}$) is between 150 and 200. These cells probably have a rather rapid division rate, since the extremes of the range of spot sizes observed could be accounted for by cell numbers differing by six divisions. Since embryos of the same chronological age may differ as much as 24 hours in developmental age (ALLEN and MACDOWELL 1940), one must conclude that, at that stage, mitoses in prospective hair pigment cells are separated by only 4-hour intervals (or even less if there were embryos with cell numbers outside the observable range). In spite of this rapid division rate, there is apparently a limit to cell mixing, since, even in the case of mutations that occurred early (i.e., animals with a high proportion of mutant hairs), there was always one main mutant area, albeit a diffuse one. The present results indicate that, in general, dorsal spots tend to be somewhat more diffuse than ventral ones, but the difference is not large and more data are needed.

Wright has pointed out that in tortoise-shell guinea pigs the boundaries between red and black areas are sharp if the spotting factor, *s*, is present. In spontaneous rodent mosaics reported in the literature, the outlines of the mutant spot also appear sharper when animals have white spotting due to the genes *s* or *bt*; and DUNN (1934) has pointed out that the mosaic spots often occur in the white-spotting areas. An experiment is underway to compare radiation-induced mosaics on spotted and self backgrounds.

On the C57BL × NB background, no white spots were produced (although the occurrence of occasional white hairs in the light gray spots cannot be ruled out). It would, therefore, appear either that irradiation on day $10\frac{1}{4}$ causes no killing of prospective pigment cells; or, that if some of these cells *are* killed, their places can be filled in by a surplus of surviving cells. The latter hypothesis is supported by the results of the C57BL × C57BL matings. This genetic background (in contrast to C57BL × NB) may be thought of as normally providing only barely enough pigment cells to fill up the surface, since in about six percent of the animals the last region in which the pigment cells meet in their latero-ventrad migration is left deficient, and a small midventral white spot results. Since 100r to embryos of the C57BL × C57BL matings increases the incidence of these midventral white spots to 27 percent, some pigment cell killing must be assumed; and since the radiation causes no white spotting elsewhere on the body, it may be suggested (if RAWLES' time relations can be applied

to our case) that there is some mechanism that insures that no gaps are left during prospective pigment cell migration from the neural crest; and that, as a result of this, the deficiency is only noted at the end of migration, i.e., midventrally.

SUMMARY

1. Embryos of a C57BL × NB cross, heterozygous for four coat color genes, were irradiated with 100 or 150r of X-rays at $10\frac{1}{4}$ days postconception, and the adult fur was observed for mosaic patches. A parallel control series involved irradiation of embryos of C57BL × C57BL matings, homozygous for the wild-type alleles at the four loci. Non-irradiated offspring of both types of mating were also studied. Altogether, 701 animals were observed.

2. Two types of spots were found on the normally black coats: (a) nonwhite spots, distributed randomly over the mouse surface, which occurred, with different frequencies, in all groups studied (see below, points 3–7); (b) small white spots which were always midventral and which were found only in C57BL × C57BL matings. While the latter type of spot occurs rather frequently spontaneously, its incidence is significantly increased by irradiation. This is interpreted as indicating that radiation causes some killing of prospective pigment cells but that this becomes apparent only in a genetic background (such as our subline of C57BL) that normally provides only a barely sufficient number of such cells.

Subsequent points of the summary apply to nonwhite spots only.

3. Nonwhite spots were, for the most part, distributed randomly over the mouse surface. However, it was also clear that some spots, e.g., those adjacent to nipples, represented abnormal differentiation, rather than genetic change. The three types of controls used probably make proper allowance for such spots.

4. The frequency of mosaic animals in the C57BL × NB, 100r group was 11.1 percent. After taking account of the three types of controls, the frequency due to expression of the recessive at one or another of the four loci was calculated to be 10.1 percent for 100r.

5. The mutant areas were, in most cases, rather diffuse and were occupied by varying degrees of mixture of mutant and probably nonmutant hairs. The colors observed are consistent with the view that the various mosaics represent the expression of one or another of at least three, and probably all four, of the loci studied.

6. The proportion of the coat occupied by mutant hair ranged from 0.0145 to 0.000173 in different mosaic animals. Assuming that the reciprocals of these figures may be taken to represent prospective pigment cells present at the time of irradiation, the extremes of the range are separated by six cell divisions. It can be calculated that the modal number of prospective pigment cells in $10\frac{1}{4}$-day embryos is between 150 and 200.

7. It is clear from the results that most of the mosaics were due to expression of the coat color recessives for which the C57BL × NB animals are heterozygous. At present, one cannot be certain as to the mechanism by which these recessives come to express themselves. Several mechanisms are ruled out, but more than one possibility remains. In the absence of further evidence, it seems most conservative to consider the mosaics as due to somatic mutations or to small deficiencies involving

the loci under study. The somatic mutation rate can be calculated as 7.0×10^{-7}/r/locus, and 95 percent confidence limits, assuming 50 percent error in measurement of spot size, are $(2.51, 11.33) \times 10^{-7}$. The germinal rate induced in spermatogonia (W. L. RUSSELL 1951) for the same four loci is 2.4×10^{-7}/r/locus.

ACKNOWLEDGMENTS

We are grateful to DR. W. L. RUSSELL for discussions during the progress of this work and for suggestions in the preparation of the manuscript. We also wish to thank DR. E. F. OAKBERG who did the photography for figure 1, and DR. A. W. KIMBALL who checked the statistics. We are grateful to DRS. E. NOVITSKI and R. F. KIMBALL for critical reading of the manuscript.

LITERATURE CITED

ALEXANDER, M. L., 1954 Mutation rates at specific loci in the mature and immature germ cells of *Drosophila melanogaster*. Genetics **39:** 409–428.

ALLEN, E., and E. C. MACDOWELL, 1940 Variation in mouse embryos of eight days gestation. Anat. Record **77:** 165–173.

BHAT, N. R., 1949 A dominant mutant mosaic house mouse. Heredity **3:** 243–248.

BITTNER, J. J., 1932 A color mosaic in the mouse. A mutational mosaic (brown-dilute brown) coat pattern in a mouse. J. Heredity **23:** 421–423.

CARTER, T. C., 1952 A mosaic mouse with anomalous segregation ratio. J. Genet. **51** (1): 1–6.

CASTLE, W. E., 1922 On a non-transmissible tricolour variation in rats. Carnegie Inst. Wash. Publ. **320:** 51–54.

DUNN, L. C., 1934 Analysis of a case of mosaicism in the house mouse. J. Genet. **29:** 317–326.

FELDMAN, H. W., 1935 A mosaic (dark-eyed intense-pink-eyed dilute) coat colour of the house mouse. J. Genet. **30:** 383–388.

GULBEKIAN, CH., 1934 Mutations induced by X-raying the soma of *Drosophila melanogaster*. Biol. Zh. (Mosc.) **3:** 332–341.

HASKINS, C. P., and E. V. ENZMANN, 1937 Note of a new and apparently useful biological indication of X-ray dosage. Radiology **28:** 189–195.

LEFEVRE, G., JR., 1948 The relative effectiveness of fast neutrons and gamma rays in producing somatic mutations in Drosophila. (Abstr.) Genetics **33:** 112–113.

1950 X-ray induced genetic effects in germinal and somatic tissue of *Drosophila melanogaster*. Am. Naturalist **84:** 341–365.

MORGAN, W. C., and S. P. HOLMAN, 1955 Eight mosaic mice in thirty years. J. Heredity **46** (2): 47–48.

PATTERSON, J. T., 1928 The effects of X-rays in producing mutations in the somatic cells of *Drosophila melanogaster*. Science **68:** 41–43.

1929a The production of mutations in somatic cells of *Drosophila melanogaster* by means of X-rays. J. Exp. Zool. **53:** 327–372.

1929b X-rays and somatic mutations. J. Heredity **20:** 260–267.

1930 Proof that the entire chromosome is not eliminated in the production of somatic variations by X-rays in Drosophila. Genetics **15:** 141–149.

RAWLES, MARY E., 1947 Origin of pigment cells from the neural crest in the mouse embryo. Physiol. Zool. **20** (3): 248–266.

RUSSELL, LIANE BRAUCH, 1954 The effects of radiation on mammalian prenatal development. Chapter 13. *Radiation Biology* 1: 861–918. Edited by A. Hollaender. McGraw-Hill Co., Inc. New York.

RUSSELL, LIANE BRAUCH, and MARY H. MAJOR, 1952 A preliminary report on radiation-induced presumed somatic mutations in the house mouse. (Abstr.) Genetics **37:** 621.

1956 A high rate of somatic reversion in the mouse. (Abstr.) Genetics **41:** 658.

RUSSELL, LIANE BRAUCH, and W. L. RUSSELL, 1954 An analysis of the changing radiation response of the developing mouse embryo. J. Cellular Comp. Physiol. **43:** Suppl. 1: 103–149.

RUSSELL, W. L., 1951 X-ray-induced mutations in mice. Cold Spring Harbor Symposia Quant. Biol. **16:** 327–336.

1956 Comparison of X-ray-induced mutation rates in *Drosophila* and mice. Am. Naturalist **90:** 69–80.

SPARROW, A. H., M. DENEGRE, and W. J. HANEY, 1952 Somatic mutations in Antirrhinum produced by chronic gamma irradiation. (Abstr.) Genetics **37:** 627–628.

TIMOFÉEFF-RESSOVSKY, N. W., 1929 The effect of X-rays in producing somatic genovariations of a definite locus in different directions in *Drosophila melanogaster*. Am. Naturalist **63:** 118–124.

WRIGHT, S., and O. N. EATON, 1926 Mutational mosaic coat patterns of the guinea-pig. Genetics **11:** 333–351.