EFFECTS OF X-IRRADIATION ON FOETAL DEVELOPMENT

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Summary

Pregnant mice were irradiated at doses of 250 r from 8–11 days and 300 and 350 r from 8–14 days of pregnancy. A marked effect was found on litter size and on the number of fertilized females which produced no young at term. Three groups of facial vibrissae were scored at birth. The irradiation had a marked effect between 10–12 days of pregnancy at both 300 and 350 r, causing both a doubling and a loss of these structures.

I. INTRODUCTION

Irradiation during early development can produce permanent modifications of the structure of a living organism. Although these effects are not heritable, they frequently copy the actions of known mutant genes, and have therefore been termed “phenocopies” (see Goldschmidt 1938).

Levine (1927), Murphy and de Renyi (1930), Job, Leibold, and Fitzmaurice (1935), Kaven (1938), Warkany and Schraffenberger (1947), and Wilson (1949) give details of the induction of phenocopies by X-irradiation, finding that these can be produced by doses as low as 100 r and that the type and frequency of abnormality is closely related to age at exposure. Russel (1950), in a large-scale series of experiments, verified these results, identifying the “sensitive periods” of a number of developmental sequences. The term “sensitive period” implied that each sequence of development passes through one or more stages during which it is sensitive to radiation.

The aims of the present study were to verify and extend the researches of previous workers, particularly those of Russel (1950), to include descriptions of the coat and skin since in her researches attention was concentrated on the internal structure of new-born mice.

II. MATERIAL AND METHODS

Mice were drawn mainly from an albino crossbred stock. The date of conception was determined from the occurrence of a vaginal plug. X-rays were generated at an 85 kV peak, 5 ma, with a half-value layer of 0·6 mm aluminium. Target distance was 24·5 cm and dosage rate was 43 r per minute using an 0·5-mm aluminium filter.

The following three dosages of X-rays were used —250, 300, and 350 r. Mice, scored at birth, were classed as either alive or dead and as normal or abnormal. Gross abnormalities and presence or absence of facial vibrissae were also noted and described. Further observations were made at 3 and 6 weeks of age. In addition to verifying the earlier observations, note was taken at these ages of the structure of the coat and behaviour pattern.

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Very few abnormalities of the coat were obtained, and only three mice were noted with abnormal behaviour patterns. Attention was therefore concentrated on the presence or absence of facial vibrissae which are sensitive to radiation, easy to describe, have the advantage of occurring at several fixed positions, and are remarkably constant in number (see Dun 1958).

Three groups of facial vibrissae were scored. These are the postorbitals, supra-orbitals, and postorals, termed, for simplicity, the A, B, and C positions. Two vibrissae normally occur at each of the A and C positions, one at the B position. All three positions occur on both sides of the head, making 10 vibrissae in all.

III. RESULTS AND DISCUSSION

(a) Comparison of Dosages

The effects of the three dosages of X-rays on the progeny of the pregnant mice are given in Table 1. These results, summed over all ages at irradiation, show that the X-ray induction of phenocopies has no effect at 250 r, 24 per cent. at 300 r, and 38 per cent. at 350 r. If the mice which are apparently normal, but were born dead, are considered as “invisible” or undiagnosed phenocopies then the percentage of foetuses affected by X-irradiation are approximately 5, 32, and 45 per cent. respectively; even these can be regarded as low estimates since some of the mice born alive and classified as normal are probably undiagnosed phenocopies.

(b) Effect of Time of Irradiation

The above data can be separated on the basis of the age at which the mice were irradiated. This is shown in Table 2. No abnormal mice were found at the 250 r dosage.

It is clear that the incidence of phenocopies increases to a maximum over 10–12 days of pregnancy. This is probably exaggerated by our preoccupation with the facial vibrissae which, as will be shown below, are maximally affected by
X-irradiation at 10–11–12 days. However, Russel (1950) found that far more characters had sensitive periods in the 10–12 day range than either earlier or later.

Table 2
NUMBERS OF NORMAL AND ABNORMAL MICE IN LITTERS OF PREGNANT MICE IRRADIATED AT 8–14 DAYS OF PREGNANCY

<table>
<thead>
<tr>
<th>Age at Irradiation (days)</th>
<th>300 r</th>
<th></th>
<th>350 r</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
<td>Per Cent. Abnormal</td>
<td>Normal</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>2</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>17</td>
<td>2</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>14</td>
<td>44</td>
<td>18</td>
</tr>
<tr>
<td>11</td>
<td>85</td>
<td>27</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>43</td>
<td>37</td>
<td>46</td>
<td>27</td>
</tr>
<tr>
<td>13</td>
<td>79</td>
<td>7</td>
<td>8</td>
<td>87</td>
</tr>
<tr>
<td>14</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>87</td>
</tr>
</tbody>
</table>

(c) Effect on Litter Size

The number of mice per litter, scored at birth, is shown plotted against age at irradiation for the three dosages used in Figures 1–3. A group of females from the same stock were untreated. The sizes of their litters are shown as “control” at the right of Figure 1. The mean size of each litter is shown plotted against age at irradiation for each dosage in Figure 4.

The mean number of mice per litter from untreated females was 7·6, which is regarded as larger than usual for the white albino stock. A similar, but not identical
stock, had a mean litter size of 7.1. Even allowing the inadequacy of the control data it is clear that irradiation even at the 250 r level causes a decrease in litter size. This effect is much more marked at the 300 r level, and there is a slight increase of the effect at the 350 r level.

A noticeable feature of the data is that the litter size is more affected at the earlier than the later ages at irradiation, since many mice although they are born are already dead, and resorption of dead foetuses is more likely after early irradiation. If the differences of litter size between ages at irradiation is solely due to resorption, then we would expect the frequency of mice born dead to be proportionately greater in litters of the mice irradiated at later stages of pregnancy. The percentages of mice born dead are given in Table 3.

There is no indication from these data that the percentage of dead mice is greater in litters of the mice which were irradiated at the later stages of pregnancy. It follows that the differences of litter size between different stages represent a real difference in sensitivity to radiation. The most important conclusion is that, although no abnormalities were detected at the 250 r dosage, the reduction of litter size indicates a proportion of invisible phenocopies of the order of 10–30 per cent. Further studies at lower dosages are required to examine this problem of dosage, spread over a wider range of stages of pregnancy to establish the relation of sensitivity to age at irradiation.
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These results differ from those of Russel (1950) who found that "the average litter size in each of the 16 out of 18 stage-dose groups did not differ significantly from the 6·67 ± 0·33 mean of the second litter controls". Russel's data on percentage of mice born dead show a greater lethality of her treatment, which may be a sensitivity difference peculiar to her stock. Both differences can be explained by a higher rate of resorption of dead foetuses in our stock, which would give a lower litter size and a lower mortality rate.

The above analysis of effects on size of litter is based on X-irradiation affecting the individual foetus. There is, in addition, a strong indication that irradiation affects the dam causing a termination of pregnancy. Examination of the data given in Figures 1–3 shows that the number of mice which, although fertilized, produced no progeny at term, is greater than would be expected from a simple effect on litter size. The percentages of fertilized females which did not produce any progeny are given in Table 4.

It is clear that any effect on the dam increases with earlier age at irradiation and that it may have a maximum effect at 9 days. Russel (1950) found a marked difference in the proportion of dams which failed to produce young at term, between those irradiated between ½–4½ days and those irradiated between 5½–8½ days: 54 v. 30 per cent. In dams irradiated from 9½–13½ days, 5 per cent. failed to produce young at term. It is possible that in our stock sensitivity to radiation damage of the dam is far greater, and the decrease between 9 and 8 days is only apparent. Further data are required to resolve this point.

Figs. 2 and 3.—Effect of age at irradiation on subsequent litter size for dosage levels of 300 r (Fig. 2) and 350 r (Fig. 3). – – – Mean litter size. ● As for Figure 1.
(d) Effect on Facial Vibrissae

The mean number of vibrissae at the A, B, and C positions after irradiation at the three dosage levels are given in Table 5, separated on the basis of level of dosage and age at irradiation. No effects on vibrissae followed irradiation at 250 r at any age. No effects could be detected after 13 days, i.e. number of vibrissae was normal in progeny of mice irradiated at 14 and 15 days.

Considering the totals first, it is clear that two processes are occurring. These are (i) an increase in the number of vibrissae, restricted to 9 and 10 days, and (ii) a decrease in the number of vibrissae occurring from 8–13 days.

![Mean litter size plotted against age at irradiation for the three radiation levels used.](image)

The increase in number indicates that the effect of irradiation cannot be mediated solely by destruction of critical cells, unless certain cells have the function of inhibiting the doubling of follicle primordia.

The data do not show any clear relationship between the positions and the effect of irradiation at specific ages. At all three positions the sensitive period appears at the same time, 9–10 days, and the reduction phase at 11–13 days. However, reduction occurs at 8 days and it is therefore possible that reduction of vibrissae occurs at all ages from 8–13 days, but is confounded with doubling at 9–10 days. Further studies of the effect of radiation on facial vibrissae will need to attempt resolution of this problem.

An interesting feature of these effects is that reduction of the number of vibrissae occurs following irradiation at ages when no histological differentiation is visible. Development of the vibrissae primordia cannot be detected before 12 days.
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As the A, B, and C positions repeat on either side of the head, it is possible to determine whether a specific sensitivity occurs by comparing the frequency of asymmetric effects with symmetric effects. The data are shown in Table 6, giving the

<table>
<thead>
<tr>
<th>Age at Irradiation (days)</th>
<th>250 r</th>
<th>300 r</th>
<th>350 r</th>
<th>Mean for Three Levels of Irradiation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. with</td>
<td>No. with</td>
<td>No. with</td>
<td>No. with</td>
</tr>
<tr>
<td></td>
<td>Litter</td>
<td>No Litter</td>
<td>Litter</td>
<td>No Litter</td>
</tr>
<tr>
<td>8</td>
<td>27</td>
<td>5 (16)</td>
<td>8</td>
<td>6 (43)</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>10 (50)</td>
<td>7</td>
<td>12 (63)</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>1 (11)</td>
<td>15</td>
<td>10 (40)</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>1 (20)</td>
<td>22</td>
<td>8 (27)</td>
</tr>
<tr>
<td>12</td>
<td>—</td>
<td>—</td>
<td>10</td>
<td>4 (29)</td>
</tr>
<tr>
<td>13</td>
<td>—</td>
<td>—</td>
<td>13</td>
<td>4 (24)</td>
</tr>
<tr>
<td>14</td>
<td>—</td>
<td>—</td>
<td>25</td>
<td>2 (7)</td>
</tr>
</tbody>
</table>

These data show that there is a considerable random factor in the loss of vibrissae due to radiation. This can be seen in the large number of mice which have lost only one vibrissae. This is 21 per cent. for position A, 17 per cent. for position B,
and 34 per cent. for position C*. These proportions can be used to derive expected values for the frequency of loss of two vibrissae. These are 4, 3, and 11 per cent. respectively. The actual percentages are 27, 10, and 31. It is clear that there is a significant difference between the actual frequency of mice which have lost two vibrissae and that expected, indicating that loss of vibrissae is not solely a random process. This is further demonstrated by comparing the numbers of mice which have lost two vibrissae on one side only, with those which have lost one on each side, i.e. comparison of the 2/0 with 1/1 types. There were 22 mice with the 2/0 distribution compared with 306 with the 1/1 distribution, showing that the two vibrissae at each of the A and C positions differ in the timing of their sensitivity to radiation. This is understandable since at each of the A and C positions, one of the vibrissae starts its development 12–24 hr before the other. Russel (1950), comparing the incidence

<table>
<thead>
<tr>
<th>Position</th>
<th>3/3</th>
<th>3/2</th>
<th>2/2</th>
<th>2/1</th>
<th>2/0</th>
<th>1/1</th>
<th>1/0</th>
<th>0/0</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>4</td>
<td>575</td>
<td>123</td>
<td>7</td>
<td>150</td>
<td>24</td>
<td>8</td>
</tr>
<tr>
<td>B</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>608</td>
<td>106</td>
<td>63</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>1</td>
<td>417</td>
<td>142</td>
<td>15</td>
<td>156</td>
<td>37</td>
<td>24</td>
</tr>
</tbody>
</table>

of effects on the eyes, found a significant excess of animals with both eyes affected which she states "would seem to indicate a partly common sensitive area for the two eyes, or the production of a change which predisposes the embryo as a whole to eye abnormalities." The latter seems more probable.

In general, the effects of radiation on the development of vibrissae do not differ from effects on more complex structures. The "sensitive period" is of the same order of duration and the probability of damage is also in the range found for larger, multiple structures.

(e) Effect on the Coat

Twelve newly born mice showed naked patches of skin, the affected areas showing signs of injury at birth involving formation of a scab over the area. This form of injury occurred in the progeny of mice irradiated at 350 r at 10, 11, and 12 days of pregnancy. The nakedness, unlike most genetic forms, was complete.

*Percentages calculated as follows: 2/1 as a percentage of the 2/2 type (position A), 1/0 as a percentage of the 1/1 type (position B), and 2/1 as a percentage of the 2/2 type (position C) respectively.
(f) **Behaviour Pattern**

A number of newly born mice showed extreme nervousness and a tendency to throw fits. Most of these died within a week of the first manifestation of fits, at about 30–50 days after birth, all showing changes of head shape similar to the hydrocephalous genes. Two such mice survived to adult age but they were not fully grown, did not breed, and still threw fits.

**IV. Conclusions**

The results obtained from these researches agree with those of previous workers (see Russel 1957), giving a picture of varying sensitivity to irradiation during pregnancy, this variation being shown as differences in pre-natal death, resorption, and the occurrence of abnormalities. Exposure to radiation at dosages in excess of 50–100 r is rare, but Russel (1957) has shown that a 25 r dose can produce quite marked effects on development. In so far as it is allowable to extrapolate from mice to humans, it is clear that the dangers of pelvic irradiation of human females during pregnancy are not negligible and require repeated emphasis (Murphy and de Renyi 1930).

**V. References**


