Strontium-90 and intrauterine development in the rat

By B. J. HOPKINS, G. W. CASARETT, L. W. TUTTLE, & R. C. BAXTER

From the Department of Radiation Biology and Biophysics, The University of Rochester, New York

INTRODUCTION

It is well known that the developing mammalian embryo is sensitive to irradiation from external sources, causing developmental anomalies and lethal effects, and that this sensitivity can vary with stage of development (Hicks, 1953; Levy et al. 1953; Fraser, Kalter, Walker & Fainstat, 1954; Russell & Russell, 1954; Warkany, 1954; Wilson, 1954; Rugh, 1963). Irradiation of the embryo prior to implantation causes a high incidence of lethal effects with occasional developmental defects in surviving embryos. Irradiation of the embryo during the period of organogenesis produces various types of localized defects in specific organ systems including small or malformed parts or complete absence of parts. Exposure during late fetal life may produce functional or behavioral changes, but no gross anatomical defects, and little if any increase in intrauterine mortality is observed.

However, much less is known about the embryological effects of internally deposited radioactive isotopes. Sikov & Noonan (1954) observed growth retardation, developmental anomalies and lethal effects in the intrauterine rat following the administration of phosphorus-32 at various stages of gestation. It was observed that the incidence of mortality, certain anomalies, and degree of growth retardation were dependent upon dose of $^{32}$P and gestation age at treatment. The present study was done to determine the toxic effect of $^{90}$Sr on the developing rat embryo when administered at either of two different gestation ages.

1 Authors' address: Department of Radiation Biology and Biophysics, School of Medicine and Dentistry, The University of Rochester, N.Y. 14620, U.S.A.

2 Author's address: Tulane University, Delta Regional Primate Research Center, Covington, La., U.S.A.
MATERIALS AND METHODS

The experimental structure is given in Table 1. A total of 72 female rats of the Long-Evans strain were mated at 7-2 months of age. The day of conception was determined by the presence of sperm in vaginal smears on the morning following mating. The first day of appearance of a positive smear was designated as day 1 post-conception.

Solutions of $^{90}\text{Sr} + ^{90}\text{Y}$ containing 667-850 $\mu$Ci/ml of $^{90}\text{Sr}$ in 0.9% saline (pH 5.0) were used for injection. Single doses of either 382 or 191 $\mu$Ci of $^{90}\text{Sr}$ were given intravenously in 0.28-0.45 ml of solution on day 2 or day 10 post-conception. Controls were given dummy injections of equal volumes of saline that was identical in pH to the strontium solutions.

Table 1. Experimental structure

<table>
<thead>
<tr>
<th>Day post-conception</th>
<th>No. of dams</th>
<th>Dose $^{90}\text{Sr}$ ($\mu$Ci)</th>
<th>Implantation sites per dam</th>
<th>No. of embryos assayed for $^{90}\text{Sr}$ retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>16</td>
<td>0</td>
<td>11.3</td>
<td>159</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>191</td>
<td>12.3</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>382</td>
<td>11.3</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>0</td>
<td>11.4</td>
<td>159</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>191</td>
<td>12.7</td>
<td>68</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>382</td>
<td>11.8</td>
<td>96</td>
</tr>
<tr>
<td>10-11</td>
<td>12*</td>
<td>366</td>
<td>—</td>
<td>95</td>
</tr>
</tbody>
</table>

Twelve dams that had been injected with 366 $\mu$Ci of $^{90}\text{Sr}$ on day 10 post-conception were sacrificed at various intervals, beginning at 6 h post-injection, in order to determine the retention of strontium in fetal and maternal tissues. The average concentration of strontium in uterine and embryonic tissues were used to approximate radiation dose rate to these tissues at various times post-injection.

In the remaining 60 dams, 31 controls and 29 strontium-treated, a total of 725 implantation sites were counted and examined on the 20th day post-conception following injection on either day 2 or day 10 post-conception. Fetal weight, intrauterine deaths, and gross morphological defects were measured. Both dead fetuses and resorbed embryos found at the implantation sites in the excised uteri were included in the calculation of total mortality. The evaluation of defective morphological development was limited to studies of external features except in the case of skeletal anomalies. Fetuses were prepared for skeletal examination by maceration (1% KOH), staining (alizarin red-S) and clearing (glycerin) according to a standard procedure (Walker & Wirtschafter, 1957).
RESULTS

The mortality data (Table 2) show that the injection of 191 μc of $^{90}$Sr on day 2 post-conception caused a mortality incidence that was 15.4% in excess of that in the control group. The excess mortality was more than tripled (57.5%) when the dose was doubled (382 μc). When the injection was given on day 10 post-conception no apparent increase in mortality above control values was caused by a dose of 191 μc, but there was possibly a slight increase (5.5%) after a dose of 382 μc.

Since the average number of implantation sites per litter was comparable in all groups (Table 1), there is no reason to believe that the $^{90}$Sr induced increase in mortality was due to the prevention of implantation of some embryos at the doses used. Massive intrauterine hemorrhage occurred in some of the dams at about the 18th day post-conception, associated with the heavy fetal mortality in the group injected on day 2.

Table 2. Effect of $^{90}$Sr on intrauterine mortality

<table>
<thead>
<tr>
<th>Dose $^{90}$Sr (μc)</th>
<th>Post-conception day injected</th>
<th>Total implantations</th>
<th>Dead or reabsorbed</th>
<th>Mortality % day 20 post-conception</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>171</td>
<td>12</td>
<td>7.0</td>
</tr>
<tr>
<td>191</td>
<td>2</td>
<td>98</td>
<td>22</td>
<td>22.4</td>
</tr>
<tr>
<td>382</td>
<td>2</td>
<td>79</td>
<td>51</td>
<td>64.5</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>183</td>
<td>24</td>
<td>13.1</td>
</tr>
<tr>
<td>191</td>
<td>10</td>
<td>76</td>
<td>7</td>
<td>9.2</td>
</tr>
<tr>
<td>382</td>
<td>10</td>
<td>118</td>
<td>22</td>
<td>18.6</td>
</tr>
</tbody>
</table>

Examination of the 588 surviving control and treated fetuses showed that $^{90}$Sr had caused variable growth retardation among individuals with little or no effect on some. Significant weight reduction was caused at both dose levels following injection on day 2 post-conception (Table 3). The reduction at the

Table 3. Effect of $^{90}$Sr on fetal weight

<table>
<thead>
<tr>
<th>Dose $^{90}$Sr (μc)</th>
<th>Post-conception day injected</th>
<th>No. fetuses weighed</th>
<th>Mean weight per fetus (g) day 20 post-conception</th>
<th>Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>159</td>
<td>1.85 ± 0.09*</td>
<td>100.0</td>
</tr>
<tr>
<td>191</td>
<td>2</td>
<td>76</td>
<td>1.75 ± 0.12</td>
<td>94.6</td>
</tr>
<tr>
<td>382</td>
<td>2</td>
<td>30</td>
<td>1.65 ± 0.20</td>
<td>89.2</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>159</td>
<td>1.72 ± 0.12</td>
<td>100.0</td>
</tr>
<tr>
<td>191</td>
<td>10</td>
<td>68</td>
<td>1.66 ± 0.11</td>
<td>96.5</td>
</tr>
<tr>
<td>382</td>
<td>10</td>
<td>87</td>
<td>1.54 ± 0.19</td>
<td>89.5</td>
</tr>
</tbody>
</table>

* Standard deviation of litter means.
191 μc level was 5.4% and the reduction at twice this dose (382 μc) was doubled to 10.8%. According to the t test, these changes were significant at the 95 and 99% levels, respectively. Following injection on day 10 post-conception there was stunting of only an insignificant degree (3.5%) produced by the lower dose, but triple this degree of stunting (10.5%) was produced by the dose of 382 μc, the latter value being significant at the 99% level.

It was also observed (Table 4) that the incidence of morphological defects in survivors was greater after injection on the 10th post-conception day as compared with the 2nd day. Very few defective fetuses were produced by maternal injection on the 2nd post-conception day, associated with high intrauterine mortality, but a high incidence of defects developed if injection was delayed until the 10th post-conception day, associated with a low intrauterine mortality.

<table>
<thead>
<tr>
<th>Dose 50Sr (μc)</th>
<th>Day post-conception injected</th>
<th>No. fetuses examined</th>
<th>No. fetuses defective</th>
<th>Percentage defective</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>159</td>
<td>8</td>
<td>5.0*</td>
</tr>
<tr>
<td>191</td>
<td>2</td>
<td>76</td>
<td>10</td>
<td>13.2*</td>
</tr>
<tr>
<td>382</td>
<td>2</td>
<td>30</td>
<td>7</td>
<td>23.3*</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>159</td>
<td>5</td>
<td>3.1</td>
</tr>
<tr>
<td>191</td>
<td>10</td>
<td>68</td>
<td>12</td>
<td>17.6</td>
</tr>
<tr>
<td>382</td>
<td>10</td>
<td>96</td>
<td>49</td>
<td>51.0</td>
</tr>
</tbody>
</table>

* Stunted fetuses

The data also show that the incidence of morphological defects was dependent upon the dose of 50Sr as well as on the gestation age at time of injection. A dose of 191 μc of 50Sr injected on day 2 post-conception produced an incidence of defective fetuses that was 8.2% in excess of the control value, whereas after the injection of twice this dose (382 microcuries) at the same gestation age the incidence was 18.3% over the control value. There was a slight increase in the incidence of defective fetuses after a dose of 191 μc and a very large increase in incidence of defective fetuses after a dose of 382 μc administered on the 10th post-conception day, as compared with administration of the same doses on day 2. The incidence of defective fetuses was 14.5% over control values for the dose of 191 μc and 47.9% over control values for the dose of 382 μc. As in the case with x-irradiation, it is not known how many of the embryos among those that died earlier would have been classified as defective had they survived to term.

It should be noted that marked generalized stunting was included in the category of developmental defects. A fetus was considered stunted when its weight was below the limit as determined by the value of the mean control weight minus 1.96 times the observed standard deviation in the control popula-
Plate 1. Skeletons from fetuses on day 20 post-conception following $^{90}$Sr treatment on day 10 post-conception. a, Control fetus, b, $^{90}$Sr fetus. Notice general stunting. c, $^{90}$Sr fetus. Notice rib thinning, 6th rib, with unusually close position to 5th rib. d, $^{90}$Sr fetus. Notice non-ossification in vertebral centra, absence of cervical arches of vertebrae on left side, and fusion distally of ribs 1 and 2 and 4 and 5 on left.

B. J. HOPKINS ET AL.

facing p. 586
Plate 2. Skeletons from fetuses on day 20 post-conception following $^{90}$Sr treatment on day 10 post-conception. $a$, control fetus. $b$, $^{90}$Sr fetus. Notice non-ossification of thoracic and lumbar centra, fusion of adjacent thoracic and lumbar arches, dorsal function of ribs 11 and 12, and reduction in total number of ribs (11 only) on right. $c$, $^{90}$Sr fetus. Notice non-ossification of vertebral centra, bony ridge traversing spine at level of 8th rib with non-ossification in dorsal segment of the rib, and reduced ossification in the cranium. $d$, Lateral view of $c$. Notice forward angulation of spine at the level of transverse bony ridge, and reduction in size of left atlas with narrowed dorsal region.

B. J. HOPKINS ET AL.
According to this definition, 2.5% of the fetuses in the control population also were designated as stunted.

The small incidence of defects induced in fetuses by injection on day 2 consisted of general stunting only except for one tailless offspring in the group that received 191 μc. Injection on day 10 post-conception caused an increase in the total number of defective fetuses including a variety of localized anomalous changes in addition to a considerable incidence of general stunting. Similar types of defects were found at both dose levels following injection on day 10, but the incidence was greater at the higher dose level. Among the developmental anomalies observed after injection of 382 μc on day 10, in decreasing order of frequency, were taillessness (20.8%), general stunting (17.2%), microphthalmia (14.6%), skeletal defects (8.3%), and occasionally, an exaggerated depression in the region of the thorax between the scapulae. Some fetuses showed various combinations of more than one defect.

The taillessness observed was characterized by the complete or nearly complete absence of a tail. The microphthalmia ranged from a slight to moderate reduction in eye volume that was observed both bilaterally and unilaterally in individual embryos. Eyes of treated fetuses were always compared with those of controls that were similar in body weight.

With the exception of generalized stunting (Plate 1b) skeletal defects observed were confined to the ribs and vertebral column. The most frequent vertebral defects were reduction in size of thoracic centra (Plate 1d), irregular and sometimes bilateral ossification centers in the centra, and in more severe cases a complete lack of ossification in thoracic and lumbar centra (Plate 2c). The associated vertebral arches were often markedly reduced or crowded, and occasionally dorsal fusion of adjacent pairs was noted, such that the usual symmetrical arrangement of the arches with respect to bilateral pairing was disrupted (Plate 2b). Rib deformities were characterized by thinning (Plate 1c), fusion (Plates 1d, 2b), and lack of ossification dorsally in the region of articulation with the vertebral column (Plate 2b). There was one case of a bony bridge traversing the vertebral column at the level of the 8th rib and a definite forward angulation of the spine at this point (Plate 2d). Other miscellaneous skeletal defects included retarded ossification in the parietals, a marked reduction unilaterally in size of the atlas, unilateral fusion and reduced ossification in the cervical arches, and lagging ossification of the sacral vertebrae with termination of caudad ossification centers often occurring at the anterior border of the ileum (Plates 1d, 2c).

**Dosimetric considerations**

Determinations of radiation dose rate to the embryo following injection on day 10 showed that there were two periods during which the average dose rate to the intrauterine contents was at a maximum (Text-fig 1). A dose rate of 12.0 rad/day was measured in the embryo at 6 h post-injection. There was a rapid...
decrease in dose rate during the next 12 h period to an exposure rate of 0.3 rad/day at 2 days post-injection. The exposure rate remained low until late in fetal life when a rapid increase to greater than 20 rad/day occurred. This terminal increase in dose rate coincided with the appearance of significant strontium in the fetal bone as skeletal mineralization progressed.

The uterus also showed an early peak in dose rate, 16.2 rad/day, which decreased rapidly to a value of 1.2 rad/day at 2 days post-injection. The exposure to the conceptus when injection occurred on day 2 post-conception probably resulted from strontium in the uterine tissue immediately following injection,

since the conceptus at this time has no established connexion with the maternal placenta. Because neither anatomical defects nor intrauterine mortality are likely to occur from exposure late in fetal life, and because the terminal rise in dose rate was after the period of extreme susceptibility of the embryo, it is reasonable to assume that the mechanisms resulting in the lethal and teratogenic effects observed in this experiment were for the most part initiated at the time of injection while the strontium was still circulating in the maternal blood stream.

The ⁹⁰Sr body burden of the mother at 24 h following injection of 382 μc had decreased to 167 μc, or 46% of the injected dose. By day 20 post-injection (the day of sacrifice) the body burden represented 36% of the injected dose in the day-10-injected group.
DISCUSSION

The qualitative response of developing rat embryos after the injection of toxic doses of strontium-90 into the mother is similar to that reported to occur following irradiation from external sources or the internal administration of phosphorus-32. Rugh & Grupp (1960) showed that the early mouse embryo, from fertilization through third cleavage, was very sensitive to the effects of X-rays and as little as 5 to 25 r caused increases of 11–38 % in intrauterine death and resorptions. It has been shown generally that such effects increase with dose and decrease with the age of the embryo at the time of exposure (Wilson, Jordan & Brent, 1953; Russell & Russell, 1954; Sikov & Noonan, 1954; Wilson, 1954; Rugh, 1963). Despite the fact that in the present experiment those embryos treated on day 2 post-conception and examined on day 20 remained 8 days longer in a slightly radioactive environment than did those that were treated on day 10, it is strongly suggested that a difference in sensitivity of the embryo at the two different gestation ages may have been responsible for the higher mortality incidence following injection on day 2 because the radiation close to the early embryo in both groups was primarily received at the time of injection (Text-fig. 1), during a period of relatively greater sensitivity in the day-2-injected group. Although the present data show that the dose rate was very low after the first day post-injection, exposure over a longer period of time may have, in part, contributed to the higher mortality incidence in the day-2-injected group.

It has been shown, however, that those offspring which survive are often retarded in growth. Retardation of skeletal growth can be produced with X-irradiation as late as day 15 of gestation (Levy et al. 1953). Likewise, Sikov & Loftstrom (1962) demonstrated that large doses of 32P caused measurable retardation in specific bones of the fetal skeleton when injected as late as day 17 of gestation. Administration of 89Sr to pregnant mice has caused similar growth depression and prenatal death (Finkel, 1947). In the present experiment growth retardation observed in both groups (day 2 and day 10) is similar in degree and may be related to the radiation dose received near the end of gestation.

The developmental defects caused by 90Sr in these experiments also were qualitatively similar to, but did not include all types of, those gross anomalies induced by X-rays as reported by others. For example, the limb deformities described by Russell & Russell (1954) and the brain exencephalies produced in mice by Rugh & Grupp (1959) were not observed in this experiment. It is possible that differences in species of animals used may influence to some extent the type defects induced. Chang, Hunt & Harvey (1963) have reported that maternal irradiation with radioactive cobalt produced only minor abnormalities in the rabbit (cleft palate, short tail and fused digits) while similar exposure in hamsters produced severe anomalies (anopia, exencephaly, and acrania). However, the effect of 90Sr treatment at other gestation ages must be studied before a more detailed qualitative comparison with X-ray induced effects can be made.
Moreover, because of the difference in the protraction of the irradiation exposure to the embryo in the present study as compared with that in the X-ray experiments, it is extremely difficult to postulate any simple relationship between the effects of the two types of radiation.

**SUMMARY**

1. Single doses of strontium-90 (191 μc or 382 μc) were injected intravenously to pregnant rats on the 2nd or 10th day post-conception and the developing offspring were observed for deleterious effects on the 20th day post-conception.

2. Injection of either dose on the 2nd day post-conception caused a significant increase in intrauterine mortality and fetal growth retardation (stunting), but a significant increase in morphological defects other than stunting was not observed.

3. Injection on the 10th day post-conception caused little or no increase in mortality or growth retardation following a dose of 191 μc, and only slight increases after doses of 382 μc. However, gross morphological defects of various types were observed at both dose levels, but fewer varieties of defect at the lower dose. The most common defects were, in decreasing order of frequency, taillessness, retarded growth, reduced eye volume, skeletal defects, and interscapular depression.

4. The radiation dose rate to both the uterus and the embryo was high soon after injection, then declined to low levels. A second peak in dose rate to the embryo occurred during late fetal life while that to the uterus remained low.

5. The deleterious effects observed appeared to be dependent upon the total dose of ⁹⁰Sr to the dam and the gestation age at time of injection.

**RÉSUMÉ**

*Action du strontium 90 sur le développement intra-utérin du rat*

1. Des doses uniques de strontium 90 (191 ou 382 μc) ont été administrées en injections intraveineuses à des râtes gestantes le 2ème ou le 10ème jour après la conception, et on a recherché les effets nocifs sur le développement de la descendance, le 20ème jour après la conception.

2. L’injection de l’une ou l’autre des deux doses le 2ème jour après la conception provoque un accroissement significatif de la mortalité intra-utérine et un retard de la croissance foetale (rabougrissement) mais on n’a pas observé d’accroissement des anomalies morphologiques autres que le rabougrissement.

3. L’injection de 191 μc faite le 10ème jour après la conception n’a que peu ou pas accru la mortalité ou le retard de croissance, et l’injection de 382 μc n’a provoqué que de légers accroissements. Néanmoins, des anomalies morphologiques importantes de divers types ont été observées pour les deux doses (moins de variété avec la dose la plus faible). Les anomalies les plus communes
**90Sr and intrauterine development**

ont été, dans l’ordre décroissant de leur fréquence, l’anourie, le retard de croissance, la diminution de volume des yeux, les anomalies du squelette et les dépressions interscapulaires.

4. Le taux de radioactivité de l’utérus et de l’embryon était élevé peu après l’injection, puis a décroît vers les faibles niveaux. Le taux de radioactivité a présenté un deuxième pic chez l’embryon au cours de la vie foetale avancée, tandis qu’il demeurait bas dans l’utérus.

5. Les effets toxiques observés dépendent de la dose totale de 90Sr injectée à la mère, et de la période de la gestation au moment de l’injection.

The authors are indebted to Lee Schwartz and Gerald Cooper for the photographic illustrations and to Virgil Kendrick for valuable technical assistance. This report is based on work performed under contract with the U.S.A.E.C. at the University of Rochester Atomic Energy Project.

**REFERENCES**


(Manuscript received 22 November 1966)