EFFECT OF WHOLE-BODY $\gamma$-IRRADIATION ON THE CHARACTERISTICS AND METABOLISM OF BULL SEMEN DURING THE EARLY POST-IRRADIATION PERIOD

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SUMMARY

Whole-body $\gamma$-radiation of mature Hereford bulls with a single dose of 400 r from a cobalt$^{60}$ source did not result in any apparent change in semen characteristics (sperm concentration and initial fructose level) or in sperm activity (initial motility and fructolytic activity) during a 3-wk. post-irradiation period. Evidently, those cells which were past the radiosensitive spermatogonial stage at the time of irradiation were metabolically unaffected.

The effect of ionizing radiation on spermatogenesis has been extensively studied in laboratory animals by histological techniques (1, 2, 3, 8, 9, 13, 14, 19, 29). However, few data have been reported based on semen examinations, except for man, where some clinical reports are available (15, 17), and for the dog, where Casarett (4, 5, 6) has done extensive work on the effect of x-irradiation on semen characteristics and on actual fertility. Casarett found that whole-body x-irradiation of beagle dogs at 3.0 r per week for 40 to 60 wk. resulted in reduced sperm counts and sterility in mating tests, whereas doses of 0.3 r per week and 0.6 r per week over the same period had little or no effect. Murphree and Parish (12) reported that when mature beef bulls were exposed to a single dose of 100-400 r whole-body $\gamma$-irradiation, the first observed semen changes were an increase in per cent abnormal sperm at 6 wk. after irradiation and a decrease in sperm concentration at 8 wk. after irradiation.

These data are in accord with the concept that radiation selectively inhibits spermatogonial division. Therefore, it may be assumed that there would be no change in the characteristics of ejaculated semen until those cells which were beyond the sensitive stages of spermatogonial division at the time of irradiation have been ejaculated and those cells which were spermatogonia at the time of

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irradiation, mature and become available for ejaculation. This leaves open the question of whether radiation has a direct effect on those cells which are past the point of spermatogonial division and particularly on those cells which have completed their development and are morphologically mature (i.e., testicular or epididymal sperm) at the time of irradiation. Irradiation would not affect the concentration or morphology of these mature sperm cells, even though it might have a direct ionizing effect on their protoplasm and, therefore, on their metabolic activity. Sperm motility and sperm metabolic activity are used as standard estimates of cell activity and viability, and the possibility that radiation affects testicular or epididymal sperm may be investigated by study of these semen parameters before and after irradiation.

MATERIALS AND METHODS

The semen specimens used in this study were collected by electro-ejaculation (16) from ten Hereford bulls, maintained on native pasture during the months of June and July, 1958, at the UT-AEC Agricultural Research Laboratory, Oak Ridge, Tennessee. Two consecutive specimens of approximately 5 ml. each were collected from each bull, three times a week, for a total of six specimens per bull per week.

The original plan was to study the semen during a preirradiation period, pair the bulls on the basis of semen characteristics, and irradiate one member of each pair. However, due to the wide variation among bulls exact pairing was not feasible. The preirradiation means of the parameters of semen quality of those bulls selected for irradiation were considerably higher than the means of those bulls that were not irradiated (Table 1). Nevertheless, it was felt that these data would be of value if it was understood that direct treated-vs.-control comparisons would not be made but, rather, that Period One vs. Period Two comparisons would be made in both the irradiated and nonirradiated groups.

After a 3-wk. preirradiation period, during which semen was collected from all ten bulls, as described, five of the bulls were exposed to 400 r whole-body gamma irradiation, at the rate of 21 r per hour for 19 hr., from a cobalt-60
source designed for the irradiation of large animals (22). Semen was collected from all ten bulls for a 3-wk. post-irradiation period. The specimens were handled, diluted, incubated, and prepared for fructose assay as previously described (11). Fructose utilizations were determined after 20, 40, and 60 min. of incubation at 37 °C, after the method of Roe (18), as modified (7). Statistical analyses of the data followed standard methods (21).

RESULTS

The means of semen characteristics and of fructose utilizations for both irradiated and nontreated groups during the pre- and post-irradiation periods are presented (Table 1). No effect of irradiation on semen specimen characteristics (sperm concentration or initial fructose level) or on sperm activity (initial motility or fructose utilization) is apparent. There is some suggestion (Table 1) that initial fructose level declined in the post-irradiation period, but this is also true for the nontreated group. An examination of the fructose level data by bull means indicates that although the mean decline in the irradiated group was 1.24 mg/ml (3.27-2.03), one bull showed an increase (+0.52 mg/ml), two bulls showed decreases (−0.74 and −0.53), and two bulls showed marked decreases (−1.71 and −3.63 mg/ml). It is suggested that this may have been due to the small number of animals involved (five irradiated bulls) and to the large order of variation in individual specimen and bull mean fructose levels before and after irradiation, since the single bull with the most marked decrease (−3.63 mg/ml) in fructose level accounts for the difference, −1.24 mg/ml for the irradiated group as opposed to −0.42 mg/ml for the nontreated group, in the pre- and post-irradiation changes in fructose level.

This is supported by an analysis of the effect of irradiation on initial motility, sperm concentration, and initial fructose level (Table 2). There was no significant effect of irradiation (T_I) on any of these three parameters, nor was there any apparent effect of environment (T_E). The significant (P < 0.01) BT_I term for fructose level (Table 2) indicates the large order of bull X treatment interaction mentioned in the discussion of Table 1.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Symbol</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Sperm concentration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>motility</td>
<td>concentration</td>
</tr>
<tr>
<td>Among bulls</td>
<td>B</td>
<td>9</td>
<td>4,784*</td>
</tr>
<tr>
<td>Environmental effect*</td>
<td>T_E</td>
<td>1</td>
<td>2,509</td>
</tr>
<tr>
<td>Irradiation effect*</td>
<td>T_I</td>
<td>1</td>
<td>498</td>
</tr>
<tr>
<td>B × T_E</td>
<td>BT_E</td>
<td>4</td>
<td>364</td>
</tr>
<tr>
<td>B × T_I</td>
<td>BT_I</td>
<td>4</td>
<td>474</td>
</tr>
<tr>
<td>Ejaculates in bulls</td>
<td>E</td>
<td>336</td>
<td>338</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>355</td>
</tr>
</tbody>
</table>

* Between treatment periods, using five untreated bulls.

* Between treatment periods, using five irradiated bulls.

¢ Statistically significant (P < 0.01).

d Statistically significant (P < 0.05).
An effect of radiation on fructose utilization might be mediated through radiation effects on motility, sperm concentration, or fructose level, since it has been shown (10) that all three of these semen parameters influence sperm fructolytic activity, or else it might be a direct effect on the metabolic activity of the cell not mediated through motility, sperm concentration, or fructose level. Since there was no apparent effect of radiation on the three semen parameters during the 3-wk. post-irradiation period, we may test directly for a radiation effect on fructose utilization. An analysis of variance (Table 3) indicates that there was no effect of radiation on fructose utilization.

**TABLE 3**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Fructose utilized in 20 min.</th>
<th>Fructose utilized in 40 min.</th>
<th>Fructose utilized in 60 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among irradiated bulls B</td>
<td>4</td>
<td>2,299&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7,242&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11,884&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Irradiation effect&lt;sup&gt;T1&lt;/sup&gt;</td>
<td>1</td>
<td>731</td>
<td>3,664</td>
<td>5,444</td>
</tr>
<tr>
<td>B × T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>4</td>
<td>909&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2,561&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4,608&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ejaculates in bulls E</td>
<td>174</td>
<td>321</td>
<td>850</td>
<td>1,277</td>
</tr>
<tr>
<td>Total</td>
<td>183</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Between treatment periods, using five irradiated bulls.

<sup>b</sup> Statistically significant (P < 0.01).

<sup>c</sup> Statistically significant (P < 0.05).

**DISCUSSION**

Whole-body radiation effects on ejaculated semen parameters (sperm concentration and motility) do not become apparent for 7 to 8 wk. post-irradiation in the bull (12) and for an even longer period in the dog (4, 5, 6). Once such effects become apparent and the sperm concentration and motility are depressed, the animal may become sterile for a variable period of time (depending on radiation dose) and then may recover in quality and fertility.

This study was designed to investigate the effects of radiation on semen characteristics (sperm concentration and initial fructose level) and also on sperm activity (initial motility and fructolytic activity), during the early post-irradiation period, in an effort to determine if those cells (epididymal and testicular sperm and, possibly, spermatids) which have already developed beyond the stage of extreme radiosensitivity are affected by ionizing radiation.

No effects of gamma radiation (400 r) on initial motility, sperm concentration, initial fructose level, or 20-, 40-, and 60-min. fructose utilizations were demonstrated in this study. Sperm collected from irradiated bulls during the early post-irradiation period show the same order of motility and metabolic activity as sperm collected prior to irradiation.

**ACKNOWLEDGMENT**

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REFERENCES


