Studies on the Pathogenesis of Neoplasms by Ionizing Radiation

I. Pituitary Tumors

JACOB FURTH, NECHAMA HARAN-GHERA,† HOWARD J. CURTIS, AND RITA F. BUFFETT

(Children's Cancer Research Foundation, Children's Medical Center, Department of Pathology, Harvard Medical School, New England Deaconess Hospital, Boston, Mass.; and Brookhaven National Laboratory, Upton, N.Y.)

Pituitary tumors occurred more frequently and at an earlier age among LAF1 mice exposed to radiation from an atomic detonation ("Operation Greenhouse") than among nonirradiated controls (7). These tumors were predominantly adrenotropic and mammo-somatotropic, unlike those induced by radio-thyroidectomy, which were exclusively thyrotropic (6).

The present experiments were set up on the basis of these findings for the purpose of studying the pathogenesis and character of neoplasms induced, notably pituitary tumors, the relative contribution of neutrons and x-rays, and genetic influences of the parental strains on their induction.

The following questions were raised: (a) Is pituitary tumor development due to a direct or an indirect radiation effect? (b) What is the relative contribution of neutrons and x-rays to their induction? (c) Is fractionation of irradiation as effective as single total-body irradiation? (d) Do certain specific stresses, such as gonadectomy or adrenalectomy, enhance the rate of tumor induction and influence the character of tumor development? (e) Is susceptibility to pituitary tumor development inherited?

The design and quota were fixed, and the work was started in 1954. Animals were received in groups when 4-5 weeks of age and assigned vertically to the various groups. The quotas were filled in 1955. The experiment was terminated by natural death of the ~100 mice.

The findings will be presented in five communications with subheadings as follows: I. Pituitary tumors; II. Neoplasms of endocrine organs; III. Leukemias and non-endocrine neoplasms; IV. Genetic aspects; and V. Biometric appraisal.

In an evaluation of the incidence of a neoplasm, both mean life span of the group and histograms of disease incidence have to be considered. Since there is no accepted formula to correct for variations in mortality rates, only the observed frequency will be reported here, with sufficient data for biometricians to correct for variations in mortality rates by their own formulae. The final paper of this series by A. W. Kimball and G. Sacher will consider the problems of biometric corrections to the material reported here.

Some of the observations made are novel; others confirm earlier findings and thereby strengthen them; still others allow no conclusion, but may be of value when combined with similar experiments of others, or may give leads to future research.

MATERIALS AND METHODS

Female mice of the genetically uniform LAF1 strain, obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, were irradiated when about 7-9 weeks of age. Sick animals were killed. All others were observed until natural death. Groups of slightly over 100 mice were treated as follows:

1. Head irradiation (exposure of head and most of neck region) comprised two dose groups: 475 rad (X₄.₈) and 135 rad (X₁₂), the estimated body mass irradiation being about 17 per cent.
2. Abdomen irradiation, including the ovaries and adrenals, the chief pituitary target organs, was done in two dose groups: 380 rad (X₆.₅) and

Received for publication January 7, 1959.

* Supported by the Atomic Energy Commission and the National Institutes of Health.
† Research Fellow of the Damon Runyon Cancer Research Foundation. Present address: Weizmann Institute of Science, Rehovoth, Israel.

1 r × 0.95 = rad.
3. **Total-body x-radiation** was given to two groups: 380 rad (Xt4.5) and 452 rad (Xt4.5).

4. **Total-body neutron irradiation** was done in two matching doses: 148 rad (Nt1.5) and 176 rad (Nt1.5).

5. Specific pituitary stress was induced by bilateral adrenalectomy at about 6–8 weeks after 350 rad total-body x-radiation (Xt4.5, adrex 2), and unilateral adrenalectomy at about 6 weeks after 452 rad total-body x-radiation (Xt4.5, adrex 1). The bilaterally adrenalectomized mice were maintained on Percorten (microcrystals of deoxycorticosterone trimethylacetate) for the first 1 or 2 months following operation (1 mg/month), then on 1 per cent sodium chloride in the drinking water, with occasional further supportive treatment with Percorten as indicated.

6. Bilateral ovariectomy was followed by 452 rad total-body x-radiation (Xt4.5, g) after an interval of about 4 weeks.

7. Fractionation was done in three equal doses of 150 rad for 3 consecutive weeks (Xt4.5, f).

8. Control groups, built up parallel with irradiations, consisted of untreated normal (Xo) and bilaterally adrenalectomized mice (Xo, adrex 2).

9. Genetic contribution of the parental strains was tested at the 452-rad level; the groups set up were as follows:

- Xt4.5 L: 452 rad total-body irradiated L mice.
- XoL: normal control L mice.
- Xt4.5 A: 452 rad total-body irradiated A mice.
- XoA: normal control A mice.

**X-radiations** were done at the New England Deaconess Hospital under the supervision of Mr. Russell Cowing. The factors were as follows: 250 kv, half value layer of 0.5 mm. Cu. Target distance for total-body exposure was 100 cm. at air dose rate of 36 r/min; for head and abdomen exposures, 50 cm., and the dose rate was 137 r/min. No additional filters were used.

For total-body irradiations, animals were exposed in a circular lucite chamber divided into eight compartments, with five mice in each chamber. A standard Victoren chamber was inserted in one empty compartment at about the mid-plane of the animals. For head and abdomen irradiations, the mice were placed in individual celluloid centrifuge tubes with the Victoren chamber in air at about the mid-plane level of the animals, while head and abdomen were shielded with lead.

The fast neutron exposures were done in the thermal column of the Brookhaven reactor with a TFH "converter plate". This fast neutron spectrum is essentially a fission spectrum, having an average energy of approximately 1 mev.

An attempt was made to give a dose of neutrons which would be comparable to the single dose x-ray exposures from an acute lethality point of view. Consequently, a small group of mice was used to determine the LDs0 dose of fast neutrons.

### TABLE 1

**THIRTY-DAY MORTALITY OF THE VARIOUS GROUPS OF IAR, MICE**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. SET UP</th>
<th>30-DAY MORTALITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Per cent</td>
</tr>
<tr>
<td>Xo</td>
<td>197</td>
<td>1</td>
</tr>
<tr>
<td>Xh4.5</td>
<td>126</td>
<td>0</td>
</tr>
<tr>
<td>Xh11</td>
<td>159</td>
<td>6</td>
</tr>
<tr>
<td>Xh4.8</td>
<td>140</td>
<td>7</td>
</tr>
<tr>
<td>Xh4.4</td>
<td>148</td>
<td>14</td>
</tr>
<tr>
<td>Xh4.6</td>
<td>181</td>
<td>3</td>
</tr>
<tr>
<td>Nt4.5</td>
<td>158</td>
<td>3</td>
</tr>
<tr>
<td>Nt4.5</td>
<td>118</td>
<td>2</td>
</tr>
<tr>
<td>Nt4.5</td>
<td>135</td>
<td>3</td>
</tr>
<tr>
<td>Xr4.1</td>
<td>123</td>
<td>5</td>
</tr>
<tr>
<td>Xo adrex 2</td>
<td>145</td>
<td>7</td>
</tr>
<tr>
<td>Xr4.5 adrex 2</td>
<td>166</td>
<td>4</td>
</tr>
<tr>
<td>Xr4.5 adrex 1</td>
<td>120</td>
<td>0</td>
</tr>
<tr>
<td>Xl4.5 g</td>
<td>129</td>
<td>0</td>
</tr>
</tbody>
</table>

Adrenalectomy was done 6–8 weeks after irradiation, gonadectomy 4 weeks before irradiation.

The following abbreviations are used in designating the groups:

- X = x-radiation
- N = neutron radiation
- t = total-body irradiation
- h = head irradiation
- g = gonadectomy
- a = irradiation over the abdomen, including the ovaries and adrenals
- o = no radiation
- f = fractionated dose
- adrex 1 = unilateral adrenalectomy
- adrex 2 = bilateral adrenalectomy

Figures following the group designation indicate the dose in rad/100. Eg. 4.8 = 480 rad.

The exact value for the relative biological equivalent (RBE) for acute lethality of neutrons vs. x-rays is not known exactly for this strain of mouse, but for other strains it is about 1.7.

If the latter value is assumed, the neutron exposures were 0.66 times the x-ray exposures, when compared on an acute lethality basis; if the former RBE is assumed the match was fairly close.

It is thus possible that the original mortality vs. dose curve on which the equivalent dosage was judged was considerably in error owing to too few animals per point, and consequently the neutron doses were considerably smaller than originally intended. The few mice in the neutron groups which died within the 30-day postirradiation period almost certainly died of natural causes unrelated to the exposures.

---

1. Percorten was generously supplied by Drs. Robert Gaunt and A. A. Renzi, Ciba Pharmaceutical Products, Inc., Summit, N.J.
The gamma ray contamination in this facility has been estimated by means of a CO₂-filled graphite ionization chamber to account for about 7 per cent of the biological effect in the mice.

RESULTS

I. THIRTY-DAY MORTALITY. LONGEVITY

Thirty-day mortality.—The data are summarized in Table 1. Since the adrenalectomies were done 6-8 weeks after exposure, the respective mortality data can be pooled with the X₄₉₈ and X₄₉₅ groups, giving a mean LDₐ₀ days of 3.6 per cent for mice given 380 rad (306 mice) and 11.4 per cent for mice given 452 rad (298 mice), as compared with 0.5 per cent for unirradiated mice (342 mice). Mortality in the group given 452 rad following ovariectomy (7.3 per cent) was less than in irradiated controls (11.4 per cent). Abdomen (712 rad) and head (1235 rad) irradiation caused some 30-day deaths (both about 4 per cent).

Longevity.—Charts 1a and 1b present the mortality curves of the fourteen groups of LAF₁ mice, and Table 2 tabulates the mean life span in decreasing order.

The mean life span of the controls was the longest (102.5 weeks). Consequently, correction of the frequency of neoplasms for difference in life span by any formula will magnify the recorded increase in the variously irradiated groups.

It has been assumed (1) that life is shortened in definite relation to the quantity of irradiation received. A survey of the above charts and tables points to a complex situation and to a high degree of "biologic variation."

It is noteworthy that the controls had the longest life span (102.5 weeks). Next in order was the X₄₅₅ group, which received the smallest total tissue dose, the mean life span in this group being 3.4 per cent less than that of the controls. The only "out of line" finding is the mean life span of the X₄₅₅ group, which was longer than that of the X₄₅₅ group (81 vs. 76 weeks). This was probably owing to some unexplained "biologic variable." The greater mean life span of the N₄₅₅ group as compared with the N₄₅₅ group (86 vs. 84 weeks) is "in the right direction."

A reduction of the mean life span in the fractionated (X₄₅₅) vs. nonfractionated (X₄₅₅) group was marked (73.5 vs. 81 weeks), but the question remains to what extent shortening of the life span...
span by fractionation was owing to increase in incidence of some neoplasms such as leukemia and related hematopoietic disorders or to other factors such as "stress" (adrenal-mediated hormonal changes). The greater longevity of gonadectomized (Xt g) vs. normal mice (Xt 4.5) (84 vs. 81 weeks) may be due to prevention of development of ovarian tumors in the former group.

The marked difference in mean life span between the two abdomen-irradiated groups, Xa 3.8 and Xa 7.1 (83 vs. 66 weeks), is due mainly to nephrosclerosis in the Xa 7.1 group. This disease is due to direct kidney irradiation and has a threshold of about 475 rad.

Unilateral adrenalectomy after irradiation did not alter the mean life span. The marked shortening of the life span by bilateral adrenalectomy was due to inadequate compensation with corticoids, as indicated by autopsy findings.

Irradiation of the head with doses of about 1000 rad caused marked disturbance in odontogenesis, resulting in deaths from malnutrition. (Preliminary experiments covered the 950–1425-rad range to determine the greatest dose which could be tolerated; the large group set up received 1235 rad).

In the present series, the neutron-irradiated animals lived slightly longer than the x-rayed groups. In view of the smaller doses of the neutron groups, this would indicate that the RBE for life shortening is probably not far different from its value for acute lethality. This is in accord with other recent studies (3).

II. PITUITARY TUMOR INDUCTION

The histograms of pituitary tumor incidence in the various groups studied are shown in Chart 2.

Neutron vs. x-rays.—The neutron-treated groups had a much higher incidence of pituitary tumors than did the x-ray groups, even though the neutron doses were much smaller (Table 3). The group with the largest x-ray dose had about the same tumor incidence as the group with the smallest neutron dose. This would indicate an RBE of about 3, but the data would also indicate that the incidence is a nonlinear function of dose, so the RBE would be expected to vary with the dose.

Fractionation.—Pituitary tumor incidence was reduced by fractionation (Table 3) but by what ratio remains to be calculated. The observed tumor incidence (1 per cent) was close to that of normal controls (1.1 per cent), but the mean life expectancy of animals exposed to the fractionated dose was reduced from 103.5 to 73.5 weeks.

Total- vs. partial-body irradiation.—Table 4 shows that pituitary tumors were induced by direct irradiation and that exposure of two main target organs, the gonads and adrenals alone, did not induce them. The Xa 3.8 mice lived longer than Xa 13.5 mice, yet none developed tumors.

It is doubtful, however, whether pituitary irradiation alone will produce more tumors than total-body irradiation with the corresponding dose. In this series, most or all of the thyroid gland was also irradiated with the head, and thyrotropic

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (rad)</th>
<th>Tumor (per cent)</th>
<th>Mean survival (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutron</td>
<td>176</td>
<td>9.2</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>145</td>
<td>4.1</td>
<td>86</td>
</tr>
<tr>
<td>X-rays</td>
<td>452</td>
<td>4.9</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>580</td>
<td>2.4</td>
<td>76</td>
</tr>
<tr>
<td>X-t</td>
<td>450</td>
<td>1.0</td>
<td>73.5</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>1.1</td>
<td>102.5</td>
</tr>
</tbody>
</table>

* Fractionated, 3 × 150 rad.
pituitary tumors occurred with greater frequency in this group. Thyrotropic pituitary tumors may have resulted from combined pituitary-thyroid irradiation (Gorbman and Edelmann, cf. 6); if they are discounted from the total, tumor frequency would be about the same in the Xh4.s and Xt4.5 groups (Xh4.b, 4.9 per cent; Xt4.5, 4.8 per cent).

The life span of animals exposed to 1~35 rad to the head was much reduced, and this may explain in part the reduction in incidence of pituitary tumors over that of the Xh4.s group.

In most animals of this group, the pituitary gland and its target organs were smaller than in normal animals. Atrophy of the uterus, adrenals, and thyroid suggested reduced pituitary hormonal activity. It is possible that, when an organ exposed to very large doses of radiation undergoes atrophy associated with impaired proliferative capacity, such organs respond with a smaller number of tumors to a given carcinogen. This problem could be investigated by isolated irradiation of the pituitary with very large doses.

In our series the entire head (including the pituitary) was exposed to radiation. However, the data of Tobias, who produced pituitary tumors in rats with well-focused deuteron beams, indicate that pituitary irradiation without exposure of the hypothalamus will induce such tumors.

Influence of adrenalectomy and ovarioectomy.—Table 5 shows that pre-irradiation gonadectomy almost prevented pituitary tumor induction by x-rays. Gonadectomy markedly reduces estrogen levels (as indicated by atrophy of uteri at autopsy) and thereby minimizes the development of mammatropic pituitary tumors. However, it remains unexplained why adrenotropic tumors should not develop following this procedure.

In animals adrenalectomized following irradiation there was some reduction in tumor incidence. The mean life span of this group was the same as that of the Xt4.5 group. More tumors occurred following bilateral adrenalectomy in spite of the greatly reduced life span of this group. Complete adrenalectomy stimulates adrenotropes. It remains to be determined whether the tumors in adrenalectomized mice are predominantly adrenotropic and whether the frequency of mammatropic tumors is reduced by adrenalectomy, as might be expected.

Characterization of pituitary tumors.—Three procedures were followed to characterize (type) the pituitary tumors: (a) study of secondary changes in tumor-bearing hosts, (b) transplantation assays in normal and variously treated histocompatible hosts, and (c) bioassays. Typing proved to be a time-consuming and expensive procedure and will be more fully reported later. Identification by transplantation assays is the simplest means of typing at present.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumors (per cent)</th>
<th>Mean survival (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xo</td>
<td>1.1</td>
<td>102.5</td>
</tr>
<tr>
<td>Xh4.b</td>
<td>4.9</td>
<td>70</td>
</tr>
<tr>
<td>Xh4.s</td>
<td>4.3</td>
<td>81</td>
</tr>
<tr>
<td>Xh4.5</td>
<td>7.4</td>
<td>99</td>
</tr>
<tr>
<td>Xh12</td>
<td>1.8</td>
<td>74</td>
</tr>
<tr>
<td>Xh4.9</td>
<td>0</td>
<td>89</td>
</tr>
<tr>
<td>Xh7.2</td>
<td>0</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 6 is a tentative survey of the findings thus far. Three well defined types were encountered: mammatropic, adrenotropic, and thyrotropic, as indicated in earlier studies (6).

GENERAL COMMENTS

Pituitary tumor induction in mice by ionizing radiation was clearly indicated in "Operation Greenhouse." The control and base-line figures from this experiment (Xo, Xh4.b, Xh4.s, Nt1.5, Nt1.9) are in essential agreement and thus strengthen the significance of the findings in the two experiments.

Pituitary tumor in normal animals is a disease of senescence. Its onset is markedly hastened by irradiation, and its incidence is increased. Most cases occur after the mean survival time. Hastening of senescence and pituitary tumor development

---

3 A. C. Tobias, personal communication, to be published.

4 Mortality could probably be much reduced by autologous grafting of adrenals in the spleens following adrenalectomy.
roughly parallel each other. While the absolute increase in pituitary tumor incidence by irradiation is not impressive, that of hastening its occurrence is strikingly evident when one considers tumor incidence at 2 years of age: it rose ten- to 40-fold at this time in the irradiated groups as compared with controls (7).

The experiments here recorded suggest that induction of adrenotropic and mammotropic pituitary tumors is primarily a direct (scopal) effect. Partial-body shielding affords little or no protection. The leads here given require elaboration by more pointed experiments aiming at both increase and decrease of tumor development following pituitary irradiation.

**TABLE 6**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. in group</th>
<th>No. with tumors</th>
<th>Thyrotropic</th>
<th>Mammatropic</th>
<th>Adrenotropic</th>
<th>Untyped</th>
</tr>
</thead>
<tbody>
<tr>
<td>X₀</td>
<td>190</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X₁₄₂</td>
<td>124</td>
<td>5</td>
<td>1?</td>
<td>1?</td>
<td>1?</td>
<td>1</td>
</tr>
<tr>
<td>X₁₄₄</td>
<td>125</td>
<td>6</td>
<td>1?</td>
<td>1?</td>
<td>1?</td>
<td>2</td>
</tr>
<tr>
<td>N₁₀8</td>
<td>121</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>N₁₀₃</td>
<td>109</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>X₁₄₃ f</td>
<td>117</td>
<td>2</td>
<td>2, 1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>X₁₄₄ s</td>
<td>121</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>X₁₄₅ s</td>
<td>115</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>X₁₄₅ adrex 2</td>
<td>140</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>X₁₄₆ adrex 1</td>
<td>120</td>
<td>3</td>
<td>1, 1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>X₀ adrex 2</td>
<td>125</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>X₁₄₃ g</td>
<td>120</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>X₁₄₄ s</td>
<td>127</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>X₁₄₅ g</td>
<td>118</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

The italicized numbers signify characterization by bioassay; the question marks express uncertainty as to the correctness of typing.

The most striking influence noted is the prevention of pituitary tumor development by gonadectomy before irradiation. This is in harmony with the findings at “Operation Greenhouse” indicating that irradiated female mice develop over 5 times as many tumors as do males (7). The experiments suggest that fission neutrons have a somewhat greater efficiency in inducing pituitary tumors than do x-rays.

Earlier experiments have shown that tumors induced by thyroid destruction are thyrotropic (6) and are due to thyroid hormonal deficiency. Whether ionizing radiation of the pituitary plays a supporting role in their induction has been debated (6). The question also remains to be answered whether combined pituitary-thyroid irradiation may promote thyrotropic tumor development.

Characterization of pituitary tumors cannot always be done from secondary changes in tumor-bearing hosts. About 70 per cent of the pituitary tumors could be typed with fair probability on the basis of changes in the different target organs. In about 30 per cent of the cases there were no “diagnostic” hormonal effects in the pituitary target organs. The main difficulty encountered was that of identifying induced adrenotropic pituitary tumors. Grafted adrenotropic tumors are unmistakable.

Some adrenotropes appeared to stimulate the mammary glands. Adrenal corticoids are known to promote mammary gland development (cf. 2).

The character of mammotropic pituitary tumors is often suggested by gross morphological changes in the hosts, but this is not adequate for firm diagnosis, since some old female animals have hyperplastic mammary glands without pituitary tumors.

Thyrotropic pituitary tumors (other than those caused by thyroid destruction) usually cause a marked enlargement of the thyroid of the host. In a few cases, the host’s thyroid was of normal size, yet the pituitary tumors proved to be thyrotropic upon bioassay. However, the functional capacity of these tumors was low.

**SUMMARY**

Pituitary tumor induction by ionizing radiation appears to be a scopal (direct) effect.

Fission neutrons appeared to have a greater relative biological equivalent (about 2 or 3) in inducing them than x-rays.

Gonadectomy prior to irradiation prevented or inhibited pituitary tumor development.

The radiation-induced pituitary tumors ob-
served were predominantly adrenotropic and mammotropic, with the exception of the head-neck irradiation groups, in which thyrotropic tumors were frequent.

The spontaneous pituitary tumors were predominantly mammotropic.

ACKNOWLEDGMENTS

We gratefully acknowledge the aid of Mr. Russell Cowing in x-radiations and x-ray dosimetry and the technical assistance of Miss Evelyn Gadsden, Miss Esther-Mary Farrington, and Mrs. Eugenia Hirsch.

REFERENCES


Downloaded from http://aacrjournals.org/cancerres/article-pdf/19/5/550/2374551/crs0190050550.pdf by Capital Medical University user on 19 January 2024