Effects of Local Irradiation (Co\textsuperscript{60} Teletherapy) on the Peripheral Blood and Bone Marrow

By F. A. Goswitz, G. A. Andrews and R. M. Kniseley

In contrast to the extensive published studies concerning the effects of total-body irradiation upon the peripheral blood and bone marrow,\textsuperscript{1,4,6,7,11,13,14,22,30,31} few reports deal with the changes after local (port) irradiation.

Local irradiation may produce important depression of hematologic values, and radiotherapists vary greatly in their clinical impressions of the seriousness of these effects. Even when the important factors of dose, site, and size of ports are taken into consideration, no clear pattern of relative severity of hematologic effects is seen. Sometimes declining blood values are characteristic of the primary disease and are erroneously attributed to the radiation. Nevertheless, some patients given local radiation therapy do experience a hematologic depression disproportionate to the amount of marrow irradiated. Among the possible explanations is a depressing effect on the marrow outside the treatment port, mediated by chemical means from the irradiated site.\textsuperscript{12}

Several clinical studies\textsuperscript{15,17,18,23,25,27} are available. They uniformly report pronounced hypoplasia under radiation sites but are not consistent in severity of changes observed in peripheral blood and in nonirradiated marrow.

Studies done in experimental animals and in vitro contribute some information on local effects.\textsuperscript{5,8,24,26,29} Osgood\textsuperscript{18} in his studies of irradiated bone marrow cultures, and Lawrence and associates,\textsuperscript{20} in their cross-circulation experiments in cats, found no good evidence for any indirect effects, but Ellinger\textsuperscript{19} believes indirect effects are important.

The present study was undertaken to evaluate the direct effects of local irradiation upon the treated site and to attempt to detect "indirect effects" in the peripheral blood and the marrow from control (nonirradiated) sites.

Materials and Methods

Eight patients, six with carcinoma of the urinary bladder and two with carcinoma of the lung, were treated with Co\textsuperscript{60} teletherapy as described in table 1. Except for symptomatic treatment, no therapy other than the port irradiation was given. None had clinical or x-ray evidence of bone metastases during the study. Bone marrow was aspirated from the irradiated and control sites as follows: before therapy, at approximately midtherapy, at the completion of therapy, and 1, 2, 3, or 4 months postirradiation. We could not obtain samples at all intervals from every patient. Two patients died shortly after the 1-month postirradiation sample, and one patient refused further aspirations after the first postirradiation specimen. In the patients with lung cancer, the sternum was the irradiated marrow site and the posterior iliac spine was the control; in the patients with bladder tumors the sternum was the control site and the pubic bone was the treated site.

From the Medical Division, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tenn., under contract with the United States Atomic Energy Commission.

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Table 1.—Eight Patients Who Received Local Co60 Teletherapy for Treatment of Malignant Disease

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Diagnosis</th>
<th>Size of field</th>
<th>Rotation</th>
<th>Tumor dose in roentgens</th>
<th>Duration</th>
<th>No. of treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. F.</td>
<td>Ca. bladder</td>
<td>7 cm.</td>
<td>yes</td>
<td>6000</td>
<td>5 weeks</td>
<td>22</td>
</tr>
<tr>
<td>M. H.</td>
<td>Ca. bladder</td>
<td>10 x 15 cm.</td>
<td>yes</td>
<td>6000</td>
<td>5 weeks</td>
<td>25</td>
</tr>
<tr>
<td>F. N.</td>
<td>Ca. bladder</td>
<td>7 cm.</td>
<td>yes</td>
<td>6000</td>
<td>5 weeks</td>
<td>23</td>
</tr>
<tr>
<td>C. H.</td>
<td>Ca. bladder</td>
<td>3 fields</td>
<td>yes</td>
<td>6000</td>
<td>5 weeks</td>
<td>23</td>
</tr>
<tr>
<td>C. H.</td>
<td>Ca. bladder</td>
<td>8 x 7 cm.</td>
<td>yes</td>
<td>6000</td>
<td>5 weeks</td>
<td>23</td>
</tr>
<tr>
<td>L. P.</td>
<td>Ca. bladder</td>
<td>5 fields</td>
<td>yes</td>
<td>6000</td>
<td>5 weeks</td>
<td>23</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pubic bone 3-4 cm. either side midline; depth 3-4 cm.</th>
<th>“mid” course</th>
<th>total dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. H.</td>
<td>Ca. lung</td>
<td>10 x 12 cm.</td>
</tr>
<tr>
<td>J. L.</td>
<td>Ca. lung</td>
<td>10 x 12 cm.</td>
</tr>
</tbody>
</table>

*Target-to-tumor distance: 60 cm.

It was found that the pubis could be satisfactorily aspirated and yielded cellular marrow. Corresponding complete blood counts were also obtained. One patient (M. H.) also had a bone marrow aspiration after the first day of therapy, for observation of the effects after one 200-r dose.

Marrow was aspirated into syringes containing a small amount of 4 per cent diphosphatase EDTA, and, after the preparation of the plain and the cresyl blue cover-slip films, the aspirate was filtered and the particles obtained were fixed in Helly’s solution. Histologic sections were prepared, stained with hematoxylin and eosin, and with Prussian blue for iron. The control (non-irradiated) marrows were evaluated by counting 1000 nucleated cells on the cover-slip films. Because of the hypocellularity, only 400 nucleated cells were classified for most irradiated sites. Areas believed to be most representative of the marrow were selected for the differential counting.

Some of the aspirated marrow was incubated for one hour at 37 C. with tritiated thymidine* (1.9 c./mM) diluted in Osgood’s Balanced Salt Solution to a final concentration of 1 μc per 1 ml. of marrow. After incubation, smears prepared on subbed slides were air-dried, fixed in methyl alcohol for 1 hour, and dipped in Kodak NTB2 liquid emulsion. After a 3-week exposure at 4 C., the autoradiograms were developed, fixed, air-dried, and stained with an aniline stain or a Giemsa-Gurr stain.

### RESULTS

#### Peripheral Blood

Generally the red cell and platelet values demonstrated little change during the course of the study. Three of the patients (C. H., L. P., and M. H.)

*Schwarz BioResearch, Inc., Orangeburg, N. Y.
had occasional gross hematuria, but therapy with iron and blood transfusions in two of these maintained red cell values at pretreatment levels.

In seven of the eight patients the leukocyte count (fig. 1) dropped by midtherapy, and in all eight by the end of therapy. In these eight patients the mean decrease in total white counts was approximately 3600 cells/mm$^3$ from the pretreatment value, or 37 per cent. During the period after irradiation, the leukocyte counts returned to pretreatment levels only in patient C. H.

The absolute lymphocyte counts (fig. 1) also decreased at the midtherapy and posttherapy periods with a mean fall of 1000 cells/mm$^3$, or 39 per cent. The lymphocytes tended to rise after the end of therapy. The absolute granulocyte counts (fig. 1) were more variable; most showed some decline that continued beyond the end of the treatment.

**Bone Marrow**

The marrow data are grouped to relate to time of treatment. Generally, in the irradiated sites, the later granulocyte forms, the lymphocytes and the "mononuclear" cells showed wide variations in percentages, while in the control sites their numbers were more uniform. But even for these cell types demonstrating wide fluctuations, differences attributable to the irradiation were significant.

The more consistent effects in the irradiated sites included a definite decrease in red cell precursors (fig. 2), and in the more immature granulocyte precursors. These effects were present at the middle of the course of radiotherapy with little change during the rest of the period of observation. At 1 and 2 months after the end of irradiation, the more mature red cell precursors had increased, but they never approached pretreatment or control site values. There was no important change in the relative percentage of mature nucleated myeloid elements during the course of study.

The percentage of plasma cells in the irradiated sites rose strikingly to 9.5 per cent at the completion of therapy. The percentage of "mononuclear cells" (monocyte, reticulum cell, histiocyte and endothelial cell) increased impressively to 16 per cent at the end of irradiation when all eight patients had at least four times more "mononuclear cells" in the irradiated sites than in the control sites. Subsequently these cells, like the plasma cells, gradually decreased, in some patients reaching pretherapy or control values.

The percentage of lymphocytes (fig. 2) showed the widest variation of all. During the irradiation the average percentage rose sharply; the falling posttherapy values are variable and difficult to interpret.

Bone marrow sections (figs. 3–6) confirmed the general hypocellularity seen on the smears after irradiation. None of the specimens obtained at later intervals showed enough regeneration to approach pretreatment levels (fig. 6). The control marrow specimens remained normal throughout the period of study and one control marrow section at 2 months postirradiation in patient M. H. showed a moderate hypercellularity.

In the present study, an accurate method for measurement of absolute
Fig. 1.—Peripheral blood values in eight patients given port Co60 teletherapy for treatment of malignant disease. M represents the approximate midpoint of the course of therapy and E the end of the course of therapy (usually 5 weeks).
marrow cellularity would have allowed determination of the absolute changes in the different cell types. Lacking such a method we made an estimate of the marrow cellularity from a comparison of sections obtained before and after therapy. In irradiated sites, total cellularity fell to approximately 10 per cent of normal. Table 2 demonstrates the effect of irradiation on the various marrow cell types, based on the percentage distribution before and after therapy and on the estimated posttreatment change in total cellularity.

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**Fig. 2.**—Changes in bone marrow from irradiated and nonirradiated sites during and after port Co\(^{60}\) teletherapy. M represents the approximate midpoint of the therapy and E the end of the treatment course (usually 5 weeks). The extremes and the mean values obtained on differential counts are graphed. In center, right, dotted lines represent the sum of myeloblasts, progranulocytes and myelocytes; solid lines represent the sum of metamyelocytes, band and segmented forms.
Iron-stained sections showed that the amount of hemosiderin in the marrow macrophages (fig. 3) was always increased during and immediately after irradiation. At later intervals the hemosiderin was still sometimes slightly to moderately increased above normal, although in some specimens the amount was normal. In the control sections the amount of hemosiderin remained normal except at 2 months postirradiation when a wide range was encountered.

Megakaryocytes in the irradiated sites were sparse or absent in all patients from midtherapy to the end of the period of study. Only two or three at most were found on an entire cover slip. The control specimens demonstrated no change. The marrow sections confirmed these findings.

Qualitative changes in some of the cells from the irradiated marrow sites were not so striking as the quantitative changes; abnormalities occasionally noted were giant-sized metamyelocytes, pyknosis of metarubricyte and granulocyte nuclei, vacuolization of some cytoplasm and nuclei of granulocytes, and a few atypical lymphocytes with large amounts of cytoplasm. No such changes were ever found in the control sites.

The exact timing on the "midtherapy" marrow samplings varied, but this did not appear to affect the findings. Most of the alterations apparently occur during the first half of the treatment period. However, in one patient in whom an aspirate was obtained 1 day after the first dose of 200 r, there was little change from pretreatment values.
The autoradiographic study of the very hypoplastic marrows from irradiation sites was somewhat unsatisfactory because of the relatively small number of cells available for study and the large percentage of unidentifiable, nondescript mononuclear cells; also, the autoradiograms themselves were of less uniformly good quality than those made on marrow specimens from control sites. The overall percentage of cells labeled was less than in nonirradiated areas but was as high as approximately 10 per cent of all nucleated cells in the pubis of patient M. H. 2 months after completion of treatment. Autoradiograms from irradiation sites 1 and 2 months after treatment in patients C. H. (fig. 5a) and C. Hl. showed significant numbers of labeled cells in the following groups: red cell precursors, early granulocyte forms, cells with oval or elongated nuclei (believed to be stromal or endothelial forms), reticulum or other mononuclear forms, and many smudge cells.

**Discussion**

The marrow specimens from control sites showed essentially no change; thus no morphologic evidence of an indirect effect of irradiation was demonstrated, and thus the finding of Denstad* was not confirmed. However, only one of our patients had a leukopenia of the degree in which he found the indirect effect. The degree of depression of peripheral blood values presumably depends more on port size than on the total dose, since all doses used for cancer therapy are sufficient to produce a profound effect on the marrow in the fields of radiation.

The underlying mechanisms of the hematopoietic effects of local irradiation are probably exceedingly complex. The percentages of decrease in peripheral blood cell values are sometimes much greater than the percentage of the body's total hematopoietic marrow in the field of radiation. The relatively low doses of scattered radiation received by the marrow outside the port seem unlikely to be a major factor. Possibly effects on soft tissue, perhaps specifically on the tumor, cause the sequestration of leukocytes from the circulating blood and thus reduce the numbers of leukocytes in the blood. If this mechanism plays an important role, however, one would expect more evidence of compensatory changes—that is, greater immaturity of granulocytes in the blood and untreated marrow.

The observed changes in the irradiated marrow sites are undoubtedly related to the life span of the various cell types and to their radiosensitivity. Irradiation produces its effects chiefly by interfering with blood cell reproduction rather than by immediate direct killing of cells. Thus the duration and time of the individual radiation exposures in relation to the timing of mitosis may be important. Possibly significant numbers of cells leave the marrow as a result of irradiation or, conversely, circulating cells are called into the irradiated marrow sites. The latter effect might explain the relative increase in lymphocytes seen after irradiation, a finding quite out of line with the often emphasized radiosensitivity of these cells.

The increased hemosiderin in the irradiated sites is an effect not previously described, as far as we could determine. Stohlman* states that 24 hours after a sublethal dose of irradiation, erythrocytic precursors most capable of mitoses...
are at a minimum; thus the majority of the Fe59-incorporating cells remaining are the late normoblasts and reticulocytes. Similar results were obtained by Lajtha and Suit.19 Also in a study of total-body irradiation in humans, Levin et al.21 found a marked delay in disappearance of iron from the plasma during the week following irradiation. Tubiana and associates,28 in a study with Fe59
in patients given local radiotherapy to the pelvis, found a much depressed localization in the irradiated site, associated with normal or increased total iron utilization for erythropoiesis. This suggests that the increased iron deposits we found at irradiated sites were not derived from available plasma iron. It seems more likely that they came from delayed death of hemoglobin-
Fig. 5a.—C. H. Autoradiogram of pubic marrow 1 month after 6000 r. Note tritium-labeled thymidine in vitro uptake in some red cell precursors. Selected field showing viable cells in a marrow generally very hypocellular (Wright's stain X 1080).

Fig. 5b.—C. Hl. Pubis. Two months postradiotherapy, 6000 r. Note numerous hemosiderin granules and general hypocellularity. Some hematopoietic elements are found in this sample (H & E X 220).
EFFECTS OF LOCAL IRRADIATION

Fig. 6a. — F. N. Pubis. At end of radiotherapy, 6750 r (H & E X 200).
Fig. 6b. — F. N. Pubis. Three months postradiotherapy, 6750 r (H & E X 200).
Hypocellularity persists, but note evidence of hematopoiesis.

A significant feature of this study is that bone marrow given "cancerocidal" doses of radiation is not devoid of cells during or at the end of the course of

containing erythrocytic precursors, or even from local destruction of circulating red cells.

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Table 2.—Relative Sensitivity of Bone Marrow Cells to Irradiation
(pre-to-post Rx Ratio)

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Pretreatment Mean Values</th>
<th>Posttreatment Mean Values</th>
<th>Sensitivity: pre-to-post Rx Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubriblast, prorubricyte</td>
<td>2.1</td>
<td>0.2</td>
<td>105:1</td>
</tr>
<tr>
<td>Rubricyte, metarubricyte</td>
<td>19.7</td>
<td>2.3</td>
<td>86:1</td>
</tr>
<tr>
<td>Myeloblast to myelocyte</td>
<td>13.9</td>
<td>2.2</td>
<td>63:1</td>
</tr>
<tr>
<td>Metamyelocyte to mature granulocyte</td>
<td>47.8</td>
<td>38.6</td>
<td>12:1</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>12.3</td>
<td>27.3</td>
<td>5:1</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>1.4</td>
<td>9.9</td>
<td>1:1</td>
</tr>
<tr>
<td>“Mononuclear” cells</td>
<td>1.6</td>
<td>16.8</td>
<td>1:1</td>
</tr>
</tbody>
</table>

*Posttreatment the total cellularity was estimated to be one-tenth of normal. This factor and the changes in percentage distribution were used to obtain the ratios.

therapy. Although the duration is much longer, the degree of hypoplasia at these sites of approximately 5000 r seems not remarkably greater than that seen at the time of greatest depression in patients who recover from 400–500 r of total-body irradiation. The failure to develop more complete aplasia at local sites of high irradiation doses might suggest that the nonirradiated marrow somehow supports or furnishes cells to the damaged site. Within a month after completion of treatment, representative precursors of granulocytic, erythrocytic, and megakaryocytic series were present and viable. Moreover, thymidine uptake indicated that proliferative activity, although much reduced from normal, was present in a significant proportion of such cells. Yet we know that these marrow sites usually remain hypoplastic for a long time. This may mean that viable DNA-synthesizing cells have suffered genetic damage, which prevents them from serving as precursors for an adequately proliferating marrow population. Such an explanation is difficult to accept, since one might expect that at least part of the cells would retain proliferative capacity and eventually repopulate the site to pretreatment levels. One would also expect that the untreated marrow would provide circulating stem cells to repopulate the damaged marrow; in rodents it has been demonstrated that there are cells in the circulating blood capable of repopulating marrow.12 Kurnick,18 however, has presented evidence that he interprets as showing that autologous marrow precursors from nonirradiated sites do not repopulate locally irradiated areas in the human being unless injected intravenously during a period of depression of peripheral blood cell values. Another possibility, which appeals to us more, is that the inhibition of complete repopulation, whether from local primordial cells or from circulating precursors, is due primarily to some type of persistent impairment, perhaps vascular, of the marrow environment. Furthermore, there is reason to believe that heavily irradiated marrow is not completely unresponsive to the stimuli that control the size of the total marrow organ. The findings of this study, particularly the presence of DNA-synthesizing hematopoietic cells in heavily irradiated marrow, cast doubt on the potential usefulness of stored autogenous marrow infusions after large-port radiation therapy.
EFFECTS OF LOCAL IRRADIATION

SUMMARY

1. Local Co\(^{60}\) teletherapy caused a reduction in leukocytes and lymphocytes in the peripheral blood.
2. The bone marrow demonstrated no morphologic change in nonirradiated control sites.
3. Local irradiation produced a pronounced and persistent hypoplasia in the treated sites during and after irradiation, with a great reduction in the numbers of megakaryocytes and precursors of red and white cells. During the period of greatest radiation effect the persistent cells were chiefly plasma cells, “mononuclear cells,” and lymphocytes.
4. Even after “cancerocidal” radiotherapy, irradiated bone marrow shows some capacity to regenerate as evidenced by appearance of precursors of various cell series and their ability to incorporate tritium-labeled thymidine.
5. Hemosiderin increased in varying degrees in irradiated sites but showed no change in the control sites.
6. Satisfactory marrow samples can be aspirated from the pubic bone.

SUMMARIO IN INTERLINGUA

1. Local telethera\(^{60}\) causava un reduction del leucocytos e lymphocytos in le sanguine peripheric.
2. Le medulla ossee monstrava nulle alteration morphologic in non-irradiate sitos de controlo.
3. Irradiaticn local produceva un pronunciate e persistente hypoplasia in le tractate sitos durante e post le irradiation, con un grande reduction in le numero del megacaryocytos e precursors de erythrocytos e leucocytos. Durante le periodo del plus forte effecto irradiational, le persistente cellulas esseva primarimente plasmocytos, “cellulas mononuclear”, e lymphocytos.
4. Mesmo post radiotherapia de character “cancerocida”, le irradiate medulla ossee monstra un certe capacitate de regenerar se, evidentiate per le apparition de precursors de varie series cellular e per le capacitate de illos de incorporar in se thymidina marcate con tritium.
5. Hemosiderina se augmentava in varie grados in le irradiate sitos sed monstra nulle alteraticn in le sitos de controlo.
6. Satisfacente specimens de medulla pote esser aspirate ab le osso pubic.

ACKNOWLEDGMENTS

We wish to express our gratitude to Dr. Frank Comas who gave the radiation therapy to these patients and calculated the marrow irradiation dose, to Dr. Bill M. Nelson for his helpful suggestions, and to Martin Yarbrough, William Gibbs, Louise Russell, Martha Clevenger, and Joyce Hewins for their technical assistance.

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EFFECTS OF LOCAL IRRADIATION


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