Attempts have been made recently to treat human leukemia by total-body irradiation with subsequent bone marrow transplantation (2, 47, 62). This suggested the desirability of studies of the levels of radiation necessary to sterilize various transplanted mouse leukemias in order to ascertain whether such sterilization could be accomplished at levels of radiation that could be protected against by bone marrow transplantation.

It has been shown that total-body irradiation in a dose range of 700—1100 r kills mice within 30 days (3, 5, 13, 14, 17, 19, 27, 34, 36, 40, 52, 54, 66). Sensitivity is higher in young animals (6). Since the irradiated animals show bone marrow aplasia, they have been given injections of isologous (1, 16, 17, 26, 54, 49, 59) bone marrow intravenously, and protection against the lethal effect of irradiation, in the dose range mentioned above, has been demonstrated. Continued growth of the transplanted marrow cells resulted in normal cellularity of the recipients’ bone marrow after 15—20 days. When heterologous or homologous marrow was used, the marrow was repopulated by the donor cells (28, 29, 39, 41, 48, 49, 59). The mice protected with homologous or heterologous bone marrow, however, died at a time later than would have been expected from irradiation damage and without showing marrow depression. This “homologous” or “heterologous” disease seems most likely to be due to the production of antibodies against the host tissues by the transplanted cells (1, 12, 15, 22, 26, 36, 54, 66, 67). Attempts have been made to overcome this complication, and amethopterin was able to attenuate, and in some cases even to prevent, homologous disease in normal mice (68). Homologous embryonal myeloid tissues has been shown to protect against lethal irradiation without producing homologous disease (4, 25, 69). Unfortunately, there was no more antileukemic activity than with isologous marrow (45).

X-ray therapy in the dose range mentioned above, administered to mice with transplanted lymphosarcomas and leukemias, usually is not able to cure the disease, as has been shown in experiments using isologous marrow to protect the...
animals against radiation damage (23, 38). There seem to be exceptions, however, since it was reported that in CBA mice with transplanted leukemia 151/1, given 1500 rads over a 25-hour period 7 days after the transplantation of the leukemia and protected against the lethal effects of this radiation by isologous marrow, there were 25 survivors out of 35 mice after 8 months (9). Immunological differences between this leukemia and the host mice were suggested as a possible explanation for these surprising results (32). Homologous marrow has been able not only to protect against lethal irradiation, but also, in some cases, to overcome the transplanted leukemia 151/1 (1), and a transplanted lymphosarcoma (23) in irradiated mice. There were only scattered cures, however, in mice with spontaneous AK-leukemia after lethal irradiation and subsequent transplantation of homologous bone marrow, and, even in these, "homologous disease" finally caused death (44).

Thus, since the "cure" of transplanted mouse leukemia by irradiation and transplantation of homologous bone marrow is followed immediately by another fatal disease, we have been interested to know what dose of x-rays alone would be able to render the leukemic cells nontransplantable. We have not been concerned, at this time, with the problems of whether and how the mice could be protected against such a dose of irradiation. There is evidence that isologous bone marrow alone would not be able to give this protection over 1500 rads, and that probably intestinal lesions would be fatal for these animals (17, 50, 56, 69). At doses of 2000–3000 rads, the mice would die from central nervous system damage (18) or from other metabolic disturbances which have been investigated in several systems (10, 30, 51, 55, 58, 64, 65). Especially interesting are experiments showing that lethal irradiation (1000 r) is followed in mice by a marked hypogammaglobulinemia, paralleling the atrophy of the lymphatic tissue (46), but that the ability of the reticuloendothelial system to phagocytise colloidal carbon is not significantly affected (60).

In the past, attempts to sterilize neoplastic cells have been made by means of chemical agents and irradiation. Karnofsky et al. (38) have shown an inactivating effect with x-ray and methylbis(β-chloroethyl)amine hydrochloride (HN2) on Sarcoma 180 when grown on the chorioallantoic membrane of the developing chick embryo. We have studied the in vivo sterilizing effect in mice of various alkylating agents and folic acid antagonists on transplanted leukemias AK 1594, AK 4, and AK 9417, and found that these leukemias would not grow on transplantation into suitable recipients after a single dose of from 4 to 10 times the single LD50 dose of various nitrogen mustard derivatives (7). With the folic acid antagonists, however, there was no inhibition of growth up to the levels of more than 100 times the acute LD50 dose (7). This lack of sterilizing effect occurred even in the leukemias that were very sensitive to inhibition by the folic acid antagonists administered on a daily or 3 times weekly schedule. This seemed to demonstrate marked difference in the mechanism of action between the alkylating agents and the folic acid antagonists (7). In irradiation experiments, it has been shown that x-ray doses up to 4000 r are not able to damage suspensions of human leukemic cells in slide chambers sufficiently to kill all the cells, insofar as phase microscopy and time-lapse cinemicrography allow such judgment (58). In melanoma S91 slices in vitro, 3000–3500 r were necessary to destroy the transplantability into new hosts, but this dosage still allowed cell growth in tissue cultures (11). In vivo irradiation of human tumors explanted into hamster pouches up to 1200 r gave inconsistent changes of the ability of these tumors to grow in new hosts (70). It was possible to sterilize Sarcoma 180 in vivo by 1500–2000 r (61, 62). Most recently it was reported that 2400 r Co60 gamma rays were not able to sterilize a lymphocytic type of leukemia in CBA mice (39). In a preliminary report we have been able to demonstrate that the level of total-body radiation necessary to sterilize transplanted mouse leukemia is different for the leukemias B82 and P388 (8). We have continued these studies, the results of which are herewith reported.

**MATERIALS AND METHODS**

Mice of either the DBA strain or BDF1 hybrids for leukemias L1210 (35), P1081, P388, P3292, P815 (24), or F1 hybrids of the BALB X C58 cross (for leukemia B82 and B82T) (9) were given injections intraperitoneally (subcutaneously for B82T) of one million cells derived either from ascitic fluid, spleen, or leukemic tumor, depending on the leukemia. After 7–10 days, when there was definite evidence of leukemia such as enlarged spleen, ascites, or subcutaneous tumor development, four mice were put each in a separate compartment of a Lucite box, 6 × 3.5 × 3.5 cm., and irradiated. These factors were as follows: 180 kvP, 25 ma. HVL., 0.8 mm. copper filter at 84–89.6 r/min, 40 cm. TSD. from each of two opposing tubes. At either 2 or 24 hours after irradiation, mice were sacrificed, and suspensions of 1 million

* M. Potter, personal communication, 1958.
leukemic cells from ascitic fluid, spleen, or solid tumor, depending on the type of leukemia, from each mouse were inoculated into five suitable recipient mice of the same respective strain. These mice were watched for the development of leukemia and were autopsied at death. Since the survival time of the untreated mice with all these leukemias, except P1081, varied between 10 and 20 days, and with leukemia P1081 was rarely over 30 days, survival of recipient mice for 100 days with no signs of leukemia was considered as evidence of sterilization of the leukemic cell suspension.

RESULTS

As can be seen from Tables 1 and 2, there was considerable variation in the susceptibility of the various strains of leukemia to sterilization by large doses of total-body irradiation. Leukemia B82, as TABLE 1

<table>
<thead>
<tr>
<th>X-Ray Dose (r)</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>B82T</td>
<td>B82</td>
</tr>
<tr>
<td>1,000</td>
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</tr>
<tr>
<td>10,000</td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

of slightly greater sterilizing effect for a given dose of irradiation if the leukemic cell suspension was transferred at 24 hours instead of at 2 hours after irradiation. The difference was so small, however, that it did not appear significant and did not hold with L1210 or P1081 at all. Leukemia P815 showed survival of the animals transplanted with leukemic cells from donors receiving 6000 r, but less than 50 per cent survival at 5000 r, whereas with leukemia P388, although in one experiment more than 50 per cent of the mice survived at doses of 6000 r, in another, even 6000 and 8000 r had no sterilizing effects.

DISCUSSION

Previous studies have shown that leukemia L1210 can be transmitted by inoculation of 100 cells, resulting in a 100 per cent take (57). For leukemia P1081, a minimum inoculation of 1000 cells is required to achieve a 50 per cent take. Therefore, sterilization in our experiments does not necessarily mean destruction of all viable cells, but may only indicate survival of a number of cells less than that needed for successful transfer. We are at a loss to explain the greater sensitivity of the subcutaneous tumor (B82T) as compared with the same strain as a generalized leukemia (B82). Transfers were made from a tumor brei in the former and a spleen brei in the latter. If the oxygen tension in the leukemic cells in mouse spleen at the time of irradiation, however, is lower than that in the subcutaneous leukemic tumor, owing to differences in supply or to differences in the type of leukemia.

<table>
<thead>
<tr>
<th>X-Ray Dose (r)</th>
<th>B82T</th>
<th>L1210</th>
<th>P1081</th>
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</tr>
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</table>

ences in metabolism, the studies of Hall et al. (31) might explain this apparent discrepancy.

Sugiura (61, 62), using Sarcoma 180, found that at a local tumor dose of 2890 r there were essentially no takes from sarcoma, whether the tumor was transplanted 15 min. to 7 days after irradiation. Similarly, at 500 r, he noted 100 per cent takes, whether the tumor was transplanted 1 hour or 14 days after irradiation. At 1500 r, however, at 2 hours there were 28 out of 40 takes, at 8 days ten out of twenty, and at 10–14 days six out of 60. At that particular dosage (1500 r) there seemed to be a difference in the percentage of takes, depending on the length of time the tumor was allowed to remain in the donor after the irradiation. No data are given, however, on the effects of radiation slightly above or slightly below this level, so that it is impossible to say whether the effects of the time between irradiation and transplantation would have extended to slightly higher (2000 r) or lower (1000 r) dosage levels. These data are consistent with those shown in Tables 1 and 2, in which, with leukemia B82T, the percentage of takes at 2000 r was lower when transfers were made at 24 hours as compared with 2 hours after irradiation. A similar situation is seen with leukemia P1081 at 3500 r, although the fact that the reverse occurred with P1081 at 4000 r and with L1210 at 3500 r makes the significance of these findings questionable.

The data of these experiments demonstrate that, although the exact dose of irradiation necessary to prevent transplantation varies somewhat with different lines of transplanted mouse leukemia, with all lines these doses are well above those which normal mice can survive even if protected with isologous marrow. Although extrapolation to man is obviously difficult and dangerous, this would suggest that doses which can be tolerated in man, even by means of successful isologous marrow transplant, would be unlikely to sterilize the leukemic cells in the advanced stages of the disease, unless human leukemic cells have a quite unique order of sensitivity to ionizing radiation. Considering the studies which have been done in vitro (58), this does not seem to be very likely. Admittedly, however, the experiment of irradiating a leukemic animal with far advanced transplanted leukemia with total-body irradiation, removing the leukemic cells or tumors, and transplanting them into an unirradiated host is far different from irradiating a spontaneous leukemia in the animal or human and protecting against the toxic effect of this irradiation by bone marrow transplant.

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