Influence of Altitude on Late Effects of Radiation in RF/Un Mice: Observations on Survival Time, Blood Changes, Body Weight, and Incidence of Neoplasms

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SUMMARY

RF female mice were sham-irradiated (as controls) or given 150 or 300 R X-rays to the whole body at 10 weeks of age and kept for the duration of their lives either at sea level or high altitude (14,900 feet).

On ascent to high altitude, the mice showed a rapid, marked erythropoietic response, which was not detectably impaired by earlier irradiation. With advancing age, the erythrocyte count and hemoglobin level gradually increased in the mice at high altitude, whereas the reverse occurred in the mice kept at sea level. As the mice aged the granulocyte count also increased, but the mononuclear cell count decreased; neither change was detectably affected by irradiation or altitude.

The life-span of the mice decreased with increasing radiation dose and was shorter in all groups (including controls) at high altitude than at sea level. Body weight gain was impaired in mice at high altitude, and the overall incidence of neoplasms was lower than in the mice at sea level.

Incidence of thymic lymphomas and granulocytic leukemias increased with irradiation, whereas the reverse was true with other lymphomas and leukemias. In general, at any given dose level all such growths were less common at high altitude than at sea level.

Incidence of lung tumors decreased with irradiation in mice maintained at sea level but increased in mice maintained at high altitude, especially when the incidence was adjusted to correct for differences in survival time. Pulmonary carcinomas were more common at high altitude than at sea level.

Incidence of ovarian tumors was increased by radiation but was unaffected by altitude. Ovarian telangiectatic and angiomatoid lesions, on the other hand, were more prevalent at high altitude than at sea level.

Miscellaneous neoplasms of other types and at other sites were observed sporadically in all experimental groups, but at a frequency too low to disclose any definite effects of radiation and altitude.

Telangiectasias in various organs and mural thrombosis of the endocardium were prevalent at high altitude, although rare at sea level. Similarly, interstitial myocarditis, rare at sea level, was more common at high altitude and was occasionally associated with thrombosis of the endocardium. Occurrence of these lesions was not significantly affected by radiation. Nephrosclerosis occurred in nearly all aging mice at sea level, with or without irradiation; it occurred less commonly in mice at high altitude, in which its incidence was increased by irradiation.

INTRODUCTION

Hypoxia during irradiation decreases the radiosensitivity of microorganisms, cells, and tissues (36, 37) and protects to some degree against radiation-induced shortening of life (42). It also influences the development of spontaneous (8, 57, 58), estrogen-induced (61), and urethan-induced (15, 59) neoplasms, the growth of transplantable tumors (4), and the frequency of metastases (62, 63).

The purpose of this investigation was to study the postirradiation effects of hypoxia induced by high altitude on the development of neoplasms and other late somatic effects of radiation in mice.

MATERIALS AND METHODS

A total of 780 female RF/Un mice, 10 weeks old, were exposed to 0 (sham), 150, or 300 R whole-body X-rays at Oak Ridge National Laboratory, with the following radiation factors: 300 kV peak; 30 ma; target skin distance, 93.7 cm; filtration, 3 mm Al (Be window); half-value layer, 0.44 mm Cu; rate, 80 to 100 rad/min, with backscatter. After irradiation, the mice were retained at Oak Ridge for 2 weeks to allow recovery from the acute effects of X-ray exposure.
and were then shipped by air to Lima, Peru. After arrival in Lima, each exposure group was further subdivided into 2 subgroups (Table 1), 1 of which remained at sea level; the other was sent by automobile to the high altitude laboratory at Morococha (elevation, 14,900 feet; atmospheric pressure, 446 mm Hg; oxygen tension, 13%). The trip from Lima to Morococha required less than 4 hr. After distribution to their 2 separate locations, the mice were caged in groups of 5 and allowed free access to a standard diet similar to Purina laboratory chow, supplemented with 5% brewers’ yeast and cod liver oil. Twice weekly, greens and carrots were also added. Drinking water from a common source was available ad libitum to both altitude groups. A temperature of 72° and a humidity of 60% were maintained at both altitudes. Animals were weighed once a week.

For serial examination of the peripheral blood, groups of 20 mice were selected in each treatment group. The blood studies consisted of determining erythrocyte count, hemoglobin, hematocrit, reticulocyte count, MCV,$^3$ mean corpuscular hemoglobin concentration, total leucocyte count, and differential white blood cell count. The 1st blood examination was made on the day after the mice arrived at Lima. Thereafter, all mice were examined at monthly intervals, except for the mice at high altitude which received a 2nd examination 1 week after arrival at Morococha.

Blood samples were obtained by incising a tail vein with a razor blade after the tail had been warmed and the mouse had been immobilized in a mouse holder. Blood was collected in standard National Bureau of Standards-certified blood pipets and cells were counted by use of hemocytometers with double Neubauer-ruled counting chambers. Red blood cells were counted in 10 small squares (0.0025 cu mm) and white blood cells were counted in 10 large squares (1.0 cu mm), 5 squares of a given size being counted on each side of the same counting chamber. Erythrocytes were diluted with Hayem’s fluid. Corpuscular values were calculated by means of the mathematical formulas of Wintrobe (84). Fluid for diluting white cells was N/10 hydrochloric acid solution, the use of which allowed the colorimetric acid-hematin method to be applied for determination of hemoglobin with the Hadden-Hausser hemoglobinometer. Hematocrits were determined in heparinized capillary-hematocrit tubes (32 x 0.8 mm) and the International microhematocrit centrifuge. Reticulocytes were stained with brilliant cresyl blue (dried drop method), and the blood was smeared and stained by the Wright-Giemsa method. For each determination, the reticulocytes in a total of 2000 red blood cells were counted.

All mice were necropsied after being found dead or, in some instances, after being killed in extremis. The lymph nodes and various organs and tumors were weighed. Tissue specimens for histological examination, taken to verify or amplify gross pathological diagnosis, were fixed in Helly’s fluid and embedded in paraffin. Paraffin sections were stained routinely with hematoxylin and eosin.

RESULTS

Blood Changes

The erythrocyte count (Chart 1A) increased rapidly in mice exposed to high altitude, from a little over 10 million at sea level to about 12 million at the end of the 1st week, 13 million at the end of the 1st month, and 14 million at the end of the 2nd month after ascent to high altitude. At this point the curves tended to plateau, increasing only slightly thereafter. At sea level, the increase in the 1st month was small, from an initial count of 10 million to 11 million. Thereafter, the count decreased gradually, to a final average of 9.5 million at 14 months. No effect attributable to radiation was evident. Hematocrit (Chart 1B), as well as the hemoglobin (Chart 1C), followed patterns similar to that of the erythrocyte count.

![Chart 1. Erythrocyte count, hematocrit, and hemoglobin in relation to age, altitude, and radiation dose. •, nonirradiated; o, 150 R; o, 300 R.](image)

Reticulocyte count (Chart 2) increased at high altitude within the 1st week, from an initial average of 2% at sea level to more than 3% after ascent to high altitude. It then declined during the remainder of the 1st month to slightly below its original level. After that, it increased again and

$^3$The abbreviation used is: MCV, mean corpuscular volume.
continued to rise until the 14th month. At sea level, there was also an initial transitory rise in the reticulocyte count during the 1st month in the mice exposed to 300 R, followed by a decline to normal in the 2nd month. Thereafter, the curves for all groups of mice at sea level increased gradually, following a pattern similar to that for the animals at high altitude. The MCV in all groups of mice (Chart 3A) tended to increase, with marked oscillations, from an initial average of 41 cu μ to an average of 46 cu μ at 14 months. Curves at high altitude were less variable than those at sea level. No definite radiation effect was noted. The mean corpuscular hemoglobin (Chart 3B) and the mean corpuscular hemoglobin concentration (Chart 3C) declined during the first 3 months at high altitude and remained lower thereafter than at sea level.

The leukocyte count (Chart 4A) was depressed initially in irradiated mice, but showed no change related to radiation after the 2nd month. No change in leukocytes related to altitude was observed at any time. The percentage of mononuclear cells (mostly small lymphocytes, with a few large lymphocytes and monocytes) increased during the first 3 to 4 months at both altitudes, and then declined gradually from a maximum of about 80% to about 60% at 14 months (Chart 4B). The opposite trend was observed in the percentage of granulocytes (Chart 4C); i.e., an initial decrease during the 1st month, followed by a progressive increase.

**Body Weight**

Mice at high altitude gained less weight than those at sea level (Chart 5). Irradiation also seemed to impair weight gain.
at the highest dose level used, namely 300 R (Chart 5). In all groups, the weight increased steadily until the mice were 50 weeks old. Thereafter it remained relatively constant until late in life, declining earlier in all groups at high altitude than in any of those at sea level, in proportion to the shorter survival time of the former.

Survival Time

Mice of all experimental groups, including the nonirradiated controls, died earlier at high altitude than did the corresponding groups at sea level (Table 1, Chart 6). Survival time also decreased with increasing radiation dose, irrespective of altitude. The altitude-dependent differences in survival between corresponding irradiation groups were not so large as the difference between the 2 groups of nonirradiated controls. This suggests that the adverse effects of radiation and of high altitude were not synergistic.

In some shipments of mice sent from Oak Ridge to Lima, a few mice died in transit and several others died during the 1st days after arrival in Peru. The longevity of mice surviving such shipments was not as great as that of mice in shipments that showed no early mortality (Chart 7). Such differences in survival were largest at 300 R.

Mean survival time of mice with neoplasms exceeded that of mice without neoplasms in the irradiated groups, although not in the controls (Table 1). Hence the life-shortening action of irradiation and of high altitude cannot be accounted for on the basis of induction or acceleration of neoplasia alone.

Neoplasms

The overall incidence of neoplasms was increased by radiation and reduced by high altitude (Table 1); however,
Altitude and Radiation Carcinogenesis

the effects of radiation and altitude differed markedly from one type of neoplasm to another. In their clinicopathological features, the various types of neoplasms were no different from those reported previously in mice of the same strain (10, 11, 77).

Leukemia and Lymphoma. The incidence of leukemia and lymphoma varied, depending on the hematological type of the disease, the radiation dose, and the altitude. In general, all types of leukemias and lymphomas occurred less frequently at high altitude than at sea level; i.e., the combined incidence averaged over all radiation dose levels at high altitude was only about 35%, as opposed to 74% at sea level (Table 1).

Incidence of granulocytic leukemia was increased by irradiation in all groups, from 2 to 7% in nonirradiated controls to 22 to 24% in mice exposed to 300 R (Table 2). Associated with this increase in the incidence of the disease was a corresponding decrease in its latency, whether judged by the mean age at death of affected mice (Table 2) or by their age distribution (Chart 8). Latency was also shorter at

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**Chart 7.** Survival in relation to age as influenced by radiation dose, altitude, and mortality associated with transportation. Solid symbols, groups showing high mortality associated with transportation (20 mice/group); open symbols, groups showing low mortality associated with transportation. ■ and ○, nonirradiated control; ▲ and △, 150 R; ■ and ○, 300 R.

**Chart 8.** Cumulative age-adjusted [Sachs (68)] incidence of myeloid leukemia in relation to age, radiation dose, and altitude. Solid symbols, values for mice at high altitude; open symbols, values for mice at sea level. ■ and ○, nonirradiated controls; ▲ and △, 150 R; ■ and ○, 300 R.

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**Table 2**

Occurrence of leukemia and lymphoma of various types in relation to radiation exposure and altitude

<table>
<thead>
<tr>
<th>X-ray exposure (R)</th>
<th>Incidence of Granulocytic leukemia (%) ± S.E.</th>
<th>Mean age at death (wk)</th>
<th>Incidence of Lymphomas (%) ± S.E.</th>
<th>Mean age at death (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Sea level</td>
<td>7.3 ± 2.3</td>
<td>85.1</td>
<td>10.5 ± 2.5</td>
<td>69.7</td>
</tr>
<tr>
<td>0 High</td>
<td>2.5 ± 1.4</td>
<td>71.0</td>
<td>2.5 ± 1.4</td>
<td>68.0</td>
</tr>
<tr>
<td>150 Sea level</td>
<td>21.6 ± 3.7</td>
<td>68.4</td>
<td>19.2 ± 3.5</td>
<td>60.5</td>
</tr>
<tr>
<td>150 High</td>
<td>12.9 ± 3.1</td>
<td>64.2</td>
<td>4.8 ± 1.9</td>
<td>36.5</td>
</tr>
<tr>
<td>300 Sea level</td>
<td>21.8 ± 3.8</td>
<td>68.0</td>
<td>26.6 ± 4.0</td>
<td>48.3</td>
</tr>
<tr>
<td>300 High</td>
<td>23.7 ± 3.9</td>
<td>56.9</td>
<td>9.3 ± 2.8</td>
<td>38.9</td>
</tr>
</tbody>
</table>

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**All types combined**

<table>
<thead>
<tr>
<th>Incidence (%) ± S.E.</th>
<th>Mean age at death (wk) ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.0 ± 4.1</td>
<td>80.3</td>
</tr>
<tr>
<td>14.0 ± 3.2</td>
<td>80.4</td>
</tr>
<tr>
<td>23.1 ± 3.8</td>
<td>78.9 ± 3.1</td>
</tr>
<tr>
<td>23.2 ± 3.4</td>
<td>73.5</td>
</tr>
<tr>
<td>11.3 ± 2.8</td>
<td>71.9</td>
</tr>
<tr>
<td>38.7 ± 4.4</td>
<td>64.2 ± 3.1</td>
</tr>
<tr>
<td>16.0 ± 3.4</td>
<td>70.6</td>
</tr>
<tr>
<td>9.3 ± 2.9</td>
<td>60.6</td>
</tr>
<tr>
<td>42.4 ± 4.5</td>
<td>54.4 ± 2.4</td>
</tr>
</tbody>
</table>
high altitude than at sea level. Incidence likewise appeared lower at high altitude than at sea level in 2 of the 3 radiation dose groups, but the differences were not statistically significant.

Similarly, the incidence of thymic lymphoma increased and its latency decreased with irradiation (Table 2). Incidence and latency were also reduced at high altitude as compared with sea level (Table 2).

The incidence of nonthymic lymphomas and reticulum cell sarcomas decreased with increasing radiation dose, as did their latency (Table 2). At all radiation dose levels, the incidence and latency of these neoplasms were lower at high altitude than at sea level.

Because mice died earlier at high altitude than at sea level from causes other than leukemia, the age-adjusted incidence of leukemia was analyzed by the life-table method of Sachs (68). Results of this analysis (Chart 9) indicate that the cumulative incidence was lower at high altitude than at sea level even after correction for intercurrent mortality.

Severity of the lesions, as judged by the size and weight of affected organs and the degree of neoplastic infiltration of liver, spleen, kidneys, ovaries, and other tissues, tended to be generally greater in mice at sea level than in mice at high altitude. Apart from these differences, however, the neoplasms were similar among the different groups, resembling those described previously in mice of the same strain (12, 76, 80).

Lung Tumors. These tended to decrease in incidence with irradiation at sea level, but to increase, with or without irradiation, at high altitude (Table 3). Although the differences in the observed incidence values were not statistically significant, they were magnified when the values were adjusted to correct for discrepancies in survival (Table 3). Latency of the growths was reduced by both radiation and altitude, whether judged by the mean age at death (Table 3) or the age-specific death rate (Chart 10) of affected mice.

Only at high altitude, however, was the age-specific death rate in the irradiated populations elevated for the duration of their life-spans (Chart 10). The number of lung tumors

Table 3

Lung tumors in relation to radiation and altitude

<table>
<thead>
<tr>
<th>X-ray exposure (R)</th>
<th>Altitude</th>
<th>Observed</th>
<th>Adjusted</th>
<th>Mean age at death (wk)</th>
<th>Mean body weight (g)</th>
<th>Average No. of tumors/affected mouse</th>
<th>Average size (mm)</th>
<th>Incidence of carcinoma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Sea level</td>
<td>28 (15)</td>
<td>22.6 (±3.7)</td>
<td>99.6 (±26.6)</td>
<td>24.4 (±1.0)</td>
<td>1.08</td>
<td>5.4</td>
<td>1.6</td>
</tr>
<tr>
<td>0</td>
<td>High</td>
<td>29 (7)</td>
<td>24.0 (±5.9)</td>
<td>81.6 (±21.1)</td>
<td>24.9 (±1.0)</td>
<td>1.29</td>
<td>4.3</td>
<td>2.5</td>
</tr>
<tr>
<td>150</td>
<td>Sea level</td>
<td>25 (18)</td>
<td>20.0 (±3.6)</td>
<td>85.3 (±3.8)</td>
<td>26.1 (±1.7)</td>
<td>1.2</td>
<td>2.4</td>
<td>0.8</td>
</tr>
<tr>
<td>150</td>
<td>High</td>
<td>41 (11)</td>
<td>33.1 (±4.1)</td>
<td>79.5 (±22.5)</td>
<td>25.8 (±0.7)</td>
<td>1.2</td>
<td>3.1</td>
<td>4.9</td>
</tr>
<tr>
<td>300</td>
<td>Sea level</td>
<td>20 (15)</td>
<td>16.8 (±2.3)</td>
<td>72.3 (±4.0)</td>
<td>25.1 (±2.0)</td>
<td>1.3</td>
<td>2.2</td>
<td>0</td>
</tr>
<tr>
<td>300</td>
<td>High</td>
<td>32 (14)</td>
<td>27.1 (±5.1)</td>
<td>62.2 (±2.4)</td>
<td>24.4 (±0.7)</td>
<td>1.1</td>
<td>3.8</td>
<td>4.2</td>
</tr>
</tbody>
</table>

*a* Figures in parentheses indicate mice with concurrent leukemia or lymphoma.

*b* Incidence adjusted for intercurrent mortality by actuarial procedure of Lindop and Rotblat (43).

*c* High altitude vs. sea level, nonirradiated.

*d* High altitude irradiated vs. high altitude nonirradiated.
Chart 10. Age-specific incidence of pulmonary tumors, as influenced by age, irradiation, and altitude. Solid symbols, mice at high altitude; open symbols, mice at sea level. • and ○, nonirradiated controls; ▲ and △, 150 R; ● and ◦, 300 R.

The size of lung tumors tended to decrease with irradiation at sea level, but not at high altitude (Table 3). Tumors were classified histologically as pulmonary adenomas in all but 17 cases. Of the 17 growths classified as cancerous, only 3 occurred in mice residing at sea level (Table 3), and none were highly anaplastic or had infiltrated tissues outside the lung. The criteria for diagnosis of cancer, therefore, included pleomorphism, nuclear atypia, and increased numbers of mitotic figures (Figs. 1 to 4). No squamous cell carcinomas or small cell carcinomas were observed.

Ovarian Tumors. These were consistently increased in frequency by irradiation, and the effect was maximal at 150 R (Table 4). Although there was relatively little corresponding reduction in mean latency (Table 4), the first neoplasms occurred much earlier in irradiated mice than in controls (Chart 11). Altitude had no detectable effect on the overall occurrence of tumors, whether spontaneous or radiation induced.

Tumors comprised a variety of cell types, including most of those reported by Bali and Furth (3). The most common growths were tubular adenomas and granulosa cell tumors (Table 5). In many instances the tumors were complex, consisting of a mixture of cell types, in which case only the predominant type is tabulated. In addition to the occurrence of frank angiomas, many X-irradiated ovaries showed vascular changes of their stroma, which included telangiectasia, often with rupture of affected vessels and formation of hematomas or blood-filled cysts. Changes of the latter type, as well as the angiomas themselves (Table 5), were more common at high altitude than at sea level.

Ovarian tumors in relation to irradiation and altitude

<table>
<thead>
<tr>
<th>X-ray exposure (R)</th>
<th>Altitude</th>
<th>Incidence, all types</th>
<th>Classification (No. of mice)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>Adjusted</td>
</tr>
<tr>
<td>0</td>
<td>Sea level</td>
<td>4</td>
<td>3.2 (±1.6)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>4</td>
<td>3.3 (±1.6)</td>
</tr>
<tr>
<td>150</td>
<td>Sea level</td>
<td>57</td>
<td>45.6 (±4.5)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>46</td>
<td>37.1 (±4.3)</td>
</tr>
<tr>
<td>300</td>
<td>Sea level</td>
<td>38</td>
<td>31.9 (±4.3)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>36</td>
<td>30.5 (±4.5)</td>
</tr>
</tbody>
</table>

a Incidence adjusted for intercurrent mortality by method of Lindop and Rotblat (43).

b Figures in parentheses indicate mice with concurrent leukemia or lymphoma.
the total number being larger at sea level (15 cases) than at high altitude (5 cases). Possibly because of their greater number, the neoplasms at sea level were also more varied in type and site than those at high altitude. No consistent effects of radiation on the incidence of these growths were evident, perhaps because of the paucity of cases in any 1 group.

**Nonneoplastic Lesions**

Lesions other than tumors were observed rather frequently in the heart and blood vessels (Table 6), kidney (Table 7), lung, liver, and, to a lesser extent, other organs.

The most commonly observed lesion of the heart was mural thrombosis of the auricular and ventricular endocardium (Figs. 5 and 6). This began as a subendocardial leukocytic infiltration, with edema and progressive deposition of fibrin. Formation of thrombus on the endocardium was more or less extensive in the auricle, and, in some cases, both the auricle and ventricle. It was classified as early when the process was exclusively subendocardial, moderately advanced when there was initial formation of thrombus, and advanced when thrombus filled the chamber and showed calcification. Incidence of mural thrombosis was strikingly greater at high altitude than at sea level (Table 6). Moderate and advanced lesions, moreover, were found predominantly in controls and in mice irradiated with only 150 R, whereas early lesions predominated in mice given 300 R. No coronary thrombosis was seen in these or other animals.

The 2nd most commonly observed lesion of the heart was interstitial myocarditis (Fig. 7), the incidence of which was also greater at high altitude than at sea level (Table 6). It consisted of mild, interstitial leukocytic infiltration and edema, most prominent in the auriculoventricular junction. Leukocytic infiltration was especially marked in association with degenerative changes of muscular fibers and nuclei.

Telangiectatic lesions were observed in the lung (Fig. 8), liver (Fig. 9), spleen, kidney, ovaries (Fig. 10), and adrenal glands. These were typical telangiectasias, the rupture of which gave rise to focal or extensive hemorrhage, especially

### Table 5

**Miscellaneous solid neoplasms in relation to irradiation and altitude**

<table>
<thead>
<tr>
<th>X-ray exposure (R)</th>
<th>Sea level: Age at death (wk)</th>
<th>High altitude: Age at death (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pituitary adenoma</td>
<td>Pituitary adenoma</td>
</tr>
<tr>
<td>0</td>
<td>Undifferentiated carcinoma</td>
<td>Adrenocortical adenoma</td>
</tr>
<tr>
<td></td>
<td>of pylorus</td>
<td>Adrenal chromaffinoma</td>
</tr>
<tr>
<td>150</td>
<td>Hepatic cholangioma</td>
<td>Thymoma (lymphoepithelial)</td>
</tr>
<tr>
<td>300</td>
<td>Carcinoma of endometrium</td>
<td>Mammary carcinoma</td>
</tr>
<tr>
<td></td>
<td>Pituitary adenoma</td>
<td>Osteosarcoma</td>
</tr>
<tr>
<td></td>
<td>Adrenocortical adenoma</td>
<td>Leiomysarcoma (uterus?) and</td>
</tr>
<tr>
<td></td>
<td>Adrenal chromaffinoma</td>
<td>Endometrial carcinoma</td>
</tr>
<tr>
<td></td>
<td>Thymoma (lymphoepithelial)</td>
<td>Papillary adenoma of kidney</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>and pituitary tumor</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>Pituitary adenoma</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>67</td>
</tr>
</tbody>
</table>

### Table 6

**Nonneoplastic lesions of the heart and blood vessels**

<table>
<thead>
<tr>
<th>X-ray exposure (R)</th>
<th>Altitude</th>
<th>Mural thrombus of endocardium (%)</th>
<th>Myocarditis (%)</th>
<th>Telangiectasis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Early</td>
<td>Moderate</td>
<td>Advanced</td>
</tr>
<tr>
<td>0</td>
<td>Sea level</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>4.9</td>
<td>10.7</td>
<td>12.4</td>
</tr>
<tr>
<td>150</td>
<td>Sea level</td>
<td>5.6</td>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>9.7</td>
<td>15.3</td>
<td>6.4</td>
</tr>
<tr>
<td>300</td>
<td>Sea level</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>16.1</td>
<td>0</td>
<td>0.8</td>
</tr>
</tbody>
</table>
in the ovaries. Incidence of these lesions was greatly increased at high altitude, only 3 mice being affected at sea level (Table 6).

Nephrosclerosis, previously described in RF mice (32), appeared in all irradiated and nonirradiated groups. Its incidence and severity were greater at sea level than at high altitude (Table 7). Pyelonephritis, the other major lesion of the kidney, was seen only occasionally (Table 7).

Many irradiated and nonirradiated mice had pneumonia. Incidence was higher at high altitude than at sea level, being 23.9% versus 14.5%, respectively, in nonirradiated mice; 19.3% versus 7.2%, respectively, in the groups treated with 150 R; and 17.7% versus 15.1%, respectively, in the mice treated with 300 R. Both typical bronchopneumonia and interstitial pneumonitis were present, sometimes with edema and focal hemorrhage.

The liver occasionally disclosed multiple, pinpoint, umbilicated, necrotic foci on its surface, which microscopically were found to consist of thromboembolic septic abscesses. Occasionally, these abscesses were larger and confluent. Incidence of these lesions was not significantly affected by irradiation or altitude.

A few mice were encountered with ileocecal abscesses and, less commonly, subcutaneous abscesses. Even less frequent were cases of septicemia, with septic abscess in various organs, including the heart. Occurrence of these rare lesions bore no discernible relation to altitude or irradiation.

**DISCUSSION**

The prompt increase in reticulocyte, erythrocyte, hematocrit, and hemoglobin at high altitude represents a typical response to hypoxia (5, 38, 41, 54, 55, 72). This response was not detectably impaired by previous irradiation. Moreover, it was greater in the irradiated RF mice of this study than in the unirradiated C58 mice studied previously (60). The MCV of erythrocytes also increased at high altitude; but the macrocytosis was only slight and was presumably not, therefore, an important mechanism of compensation for hypoxia, possibly because of the large magnitude of the concomitant erythrocytosis. Polycythemia is the major mechanism of compensation for reduction in the partial pressure of oxygen in the inspired air (38).

Erythrocytosis, which is not yet well understood, was first thought to be caused by a direct action of anoxia on the bone marrow (9). Later, it was thought to be mediated through the endocrine system, via pituitary, thyroid, and gonadal hormones (13, 18, 29, 70, 82) or via the adrenal cortex (18, 19, 28, 30, 83). Other studies have suggested the existence of a humoral factor capable of directly stimulating erythropoiesis. Forster (21) first succeeded in stimulating erythropoiesis in normal animals by injecting plasma from animals previously exposed to reduced oxygen tension. His results have since been amply confirmed and extended (6, 65, 71). Merino (50) in 1956 obtained a humoral factor from the plasma of natives residing at high altitudes (3900 and 4500 m) that caused erythropoiesis in men residing at sea level. Reynafarje (66) found erythropoietin in human plasma within 24 hr after ascent from sea level to 4500 m. Existence of erythropoietin as a stimulating factor in the polycythemia of high altitude does not, of course, exclude participation of the endocrine system in homeostasis. Erythropoietin and endocrine actions are probably coordinated in adjusting the erythropoietic response.

After the initial elevation in mice placed at high altitude, erythrocyte, hematocrit, and hemoglobin values continued to rise with advancing age. At sea level, these values declined, as has also been noted for the hemoglobin of nonhypoxic, nonirradiated aging female mice of the CBA strain (22) and males of the C57BL strain (17). The corresponding age-dependent decline in mononuclear cell count and increase in granulocyte count, both of which changes were unaffected by radiation or altitude, have been noted in aging mice of other strains (14, 60).

From the foregoing it would seem that our irradiated mice retained normal capacity to adjust their hemopoietic activity as needed for adaptation to high altitude, but the persistence of residual radiation injury in the marrow, as well as other organs, was evident in impaired growth, increased incidence of leukemia and other late-occurring diseases, and life shortening from nonneoplastic as well as neoplastic diseases.

The life-shortening effects were consistent with those observed in earlier experiments with high altitude (57–59) and radiation (74). The life-shortening effects of radiation, which resemble in certain respects an acceleration of the natural aging process (74), denote a complex form of

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**Table 7**

*Nonneoplastic lesions of the kidney*

<table>
<thead>
<tr>
<th>X-ray exposure (R)</th>
<th>Altitude</th>
<th>Nephrosclerosis (%)</th>
<th>Pyelonephritis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Early</td>
<td>Moderate</td>
</tr>
<tr>
<td>0</td>
<td>Sea level</td>
<td>37.1</td>
<td>49.2</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>33.9</td>
<td>14.9</td>
</tr>
<tr>
<td>150</td>
<td>Sea level</td>
<td>41.6</td>
<td>47.2</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>50.8</td>
<td>18.5</td>
</tr>
<tr>
<td>300</td>
<td>Sea level</td>
<td>48.7</td>
<td>38.6</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>32.2</td>
<td>20.3</td>
</tr>
</tbody>
</table>
residual injury that is expressed as the sum of various kinds of damage to tissues and cells (67). In keeping with the view that the aging process results in part from accumulation of the effects of nonspecific injuries sustained throughout life (40), the life-shortening effects of high altitude, irradiation, and stress during transportation were additive. There is no evidence, however, that they were synergistic.

Although the mechanism of the life-shortening effects of high altitude remains to be determined, these effects may have involved to some extent impairment of immunological defenses such that death from infections and neoplasms occurred prematurely. These effects, which may conceivably reflect nonspecific stress, are opposite to the life-lengthening (48) and anticarcinogenic (73) effects to be expected from caloric restriction alone. Hence the impaired weight gain of our mice at high altitude, which was consistent with results of previous experiments (63) and which may have resulted in part from reduced caloric intake, was not accompanied by a corresponding increase in vigor. On the contrary, the decreased body weight was offset by, or may even itself have been a manifestation of, underlying stress or metabolic decompensation. Since the adrenals are known to play a role in adaptation to high altitude (1, 53), the possible role of the adrenal gland in the effects observed in this study warrants investigation.

Observed changes in the incidence of lymphoma, granulocytic leukemia, and reticulum cell sarcoma induced by irradiation at sea level are consistent with those reported previously in animals of the same strain (76, 78). Likewise, the inhibitory effects of high altitude on the development of lymphomas in our irradiated and nonirradiated mice confirm and extend similar observations on the spontaneous occurrence of lymphomas in C58 mice (57). Altitude affected the development of granulocytic leukemia less markedly and less consistently; hence, in the absence of further data, such effects on the occurrence of this disease will remain equivocal.

Any attempts to explain the observed effects of altitude on leukemogenesis must take into account the following factors: (a) the observed reduction of longevity at high altitude and, in turn, reduction in the time available for development of leukemia; (b) the observed reduction in body weight at high altitude, associated with which there may have been inhibitory effects on neoplasia such as result from caloric restriction (73); (c) exaggerated erythropoiesis at high altitude, owing to need for compensatory erythrocytosis; and (d) endocrine changes involved in adaptation to high altitude, which may indirectly affect susceptibility to leukemia.

As concerns the influence of survival time on the incidence of leukemia and lymphoma, the incidence was reduced even when adjusted for intercurrent mortality at high altitude. Consequently, the inhibitory effects of high altitude cannot be attributed merely to interference with leukemogenesis through loss of mice by early mortality from other diseases. The mechanism of reduction in body weight at high altitude is not known. Whatever its cause, however, the reduction was not solely a terminal phenomenon but was present throughout life after the mice ascended to 14,900 feet, despite the constant accessibility of food identical in kind and amount to that offered at sea level. Conceivably, therefore, it may have contributed to the observed reduction in leukemia incidence, since curtailment of body weight by caloric restriction inhibits the development of neoplasms of various types (73). Examination of the effects of high altitude in mice pair-fed with controls at sea level would help to clarify this matter.

We suspected at the outset that life-long hyperstimulation of erythropoiesis by high altitude might enhance the development of myeloid leukemia by heightening the proliferation of hemopoietic stem cells. On the contrary, our results indicate that stimulation of erythropoiesis not only failed to enhance leukemogenesis, but in fact may have had the opposite effect, since the incidence of myeloid leukemia, as well as of lymphoma, was generally reduced in the high-altitude groups. Nevertheless, the precise role erythropoietic hyperplasia may have played in the effects we observed warrants further exploration under conditions allowing it to be controlled specifically; e.g., the effects of high altitude on leukemogenesis deserve to be studied under conditions in which hyperstimulation of erythropoiesis is prevented by repeated transfusion of erythrocytes, or in which erythropoietic hyperplasia is elicited at low altitude by hypoxia or other stimuli.

The role that endocrine changes may have played in the observed reduction of leukemia incidence at high altitude is difficult to assess in the absence of specific data on endocrine activity. To the extent, however, that adaptation to high altitude involved hypersecretion of corticoids (52, 69), the reduced incidence of lymphomas and reticulum cell sarcomas would be in keeping with the effects of adrenal corticoids in mice of the same strain (76).

The effects of high altitude on pulmonary tumorigenesis seemed to be contrary to those of radiation, suggesting an enhancing action of neoplasia in keeping with the cocarcinogenic effects of high altitude (58, 59) and hypoxia (15) on the development of spontaneous and urethan-induced lung tumors in strain A mice (58, 59). Our results are complicated, however, by inconsistencies in the effects of radiation on pulmonary tumorigenesis observed in previous experiments with mice; i.e., although chronic whole-body irradiation increased the incidence of lung tumors in mice of the LAF1 (45—47), A (44, 45), and CBA strains (51), acute whole-body irradiation rarely increased the frequency of such tumors (77), usually reducing their incidence in mice of the RF (10, 11) and other strains (35, 43, 64, 79). Moreover, acute irradiation consistently inhibited the oncogenic action of nitrogen mustard (34) and urethan (16, 20, 31) on the mouse lung. Despite the inconsistencies noted, the suggestion that high altitude may reverse the action of radiation implies that the comparative and interactive effects of radiation and high altitude on pulmonary tumorigenesis warrant further study.

The higher frequency of pulmonary carcinomas at high altitude, as opposed to sea level, constitutes further evidence of an enhancing effect of high altitude on pulmonary tumorigenesis. Although the basis for this effect remains to
be determined, it might be related to the increased invasiveness and metastasis of experimental tumors grown at high altitude (62).

Induction of ovarian tumors by radiation in our mice conforms to earlier results in mice of the same (77) and other (24) strains. The changes leading to such neoplasia are well established, radiation injury to the ovary of the mouse being irreversible and cumulative (7, 24, 25, 27). Although, in general, no appreciable differences were observed in the occurrence, histological pattern, and distribution of ovarian tumors as a function of altitude, telangiectatic or angiomatous formations were more frequently seen in mice maintained at high altitude than in those at sea level. In other organs also (Tables 6 and 7), such vascular lesions were more prevalent at high altitude. This implies, perhaps, that such vascular changes may have been stimulated in some way by high-altitude hypoxia, possibly through hypervolemia and congestion and by the increase of the capillary bed of tissues in the process of acclimatization (56, 81). It is also possible that hormonal factors may have contributed, at least in some instances, since similar lesions have been noted in hypervolemic mice with functional ovarian tumors induced by X-rays (26) and as secondary effects of the transplantation of such tumors (23).

Mural thrombosis of the endocardium was another vascular condition that showed a strikingly greater incidence at high altitude than at sea level, possibly for similar reasons. The inflammatory appearance of the early subendocardial lesion and of the adjacent myocardium suggests that the condition may be related to interstitial myocarditis, which was also more common at high altitude than at sea level. Angevine and Furth (2) described a myocarditis that affected only male mice of their RF stock with “antemortem thrombi occasionally attached to the auricular endocardium.” This condition was associated with degenerative changes of the myocardium and with hemorrhage in the pleural cavity and in the testes. In the present experiment we dealt only with females, and both the myocarditis and the mural thrombosis were never found to be associated with pleural hemorrhage. The possibility that both types of lesions were related cannot be ruled out, however, and calls for further investigation. Mural thrombosis of the auricle, as such, occurs sporadically in male and female mice of many strains (e.g., see Upton et al., Ref. 79).

In contrast to the discussed findings, nephrosclerosis was significantly more prevalent and severe at sea level than at high altitude (Table 7). This condition, prevalent spontaneously in RF mice (32), has now been described in association with necrotizing polyarteritis in aging RF (75) and NZB/BL mice (49). The latter association supports the earlier suggestion (33) that its pathogenesis may be related to an autoimmune disturbance, as further evidenced by induction of the lesion through transplantation of spleen cells from affected donors or from aging donors (49). To the extent that an immunological mechanism may be involved, the lower incidence at high altitude may reflect immunological depression, possibly associated with stress, hypercorticism, and undernutrition, as noted. The likelihood of a disturbed or depressed immune mechanism at high altitude is also suggested by the higher incidence of pneumonitis at high altitude than at sea level, although purulent inflammation of other organs and tissues, which occurred infrequently, showed no discernible relation to altitude or irradiation. The failure of radiation to increase the frequency and severity of nephrosclerosis in our sea-level animals is presumably attributable to their high natural incidence of the disease. The increased incidence associated with radiation in our high-altitude animals, in which the spontaneous frequency was lower, is consistent with effects noted previously in mice of other strains (79).

From the foregoing, it is clear that some of the long-term effects of radiation may be modified appreciably by subsequent exposure to high altitude, but not all effects are influenced similarly. Some are enhanced, others are inhibited, and still others are not detectably affected at all. Mechanisms of the interactions of the effects of radiation and those of altitude are still to be determined. Without further information about the effects in question, derived from studies designed to control the many variables involved, generalizations about possible interactive effects must remain speculative.

ACKNOWLEDGMENTS

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Fig. 1. A nonirradiated 81-week-old mouse at high altitude showing a large lobulated tumor mass protruding from the inferior right lobe of the lung.

Fig. 2. Photomicrograph of the large lung tumor shown in Fig. 1. Although the adenomatous pattern is still preserved, the dedifferentiation was considered sufficiently pronounced to justify the diagnosis of adenocarcinoma. H & E, X 260.

Fig. 3. Carcinoma of the lung in a 28-week-old mouse at high altitude irradiated with 300 R. The tumor nodule was found in the inferior right lobe; it measured 7 x 5.5 mm in its largest diameters. The glandular pattern and the cytoplasm are barely discernible. Several mitoses are shown. H & E, X 530.

Fig. 4. The same tumor as in Fig. 3 shown at higher magnification. The atypical cells show clumping of chromatin and ill-defined cytoplasm. Several mitoses are visible. H & E, X 1250.

Fig. 5. Early mural thrombosis of the ventricular endocardium in a mouse at high altitude. The subendocardial lesion shows leukocytic infiltration and fibrin deposition. The ventricular cavity is partially filled with a recent thrombus. H & E, X 130.

Fig. 6. A more advanced thrombosis of the ventricle in a mouse at high altitude, showing extensive subendocardial granulocytic infiltration, with fibroblasts surrounding the necrotic thrombus and calciferous material filling the ventricular cavity. H & E, X 130.

Fig. 7. Heart, showing mild interstitial myocarditis in a mouse at high altitude. H & E, X 260.

Fig. 8. Section of the lung of a mouse at high altitude, showing telangiectasis. H & E, X 130.

Fig. 9. Section of the liver of a mouse at high altitude, showing telangiectasis. H & E, X 130.

Fig. 10. Section of the ovary of a nonirradiated mouse at high altitude, showing telangiectasis. H & E, X 130.
Influence of Altitude on Late Effects of Radiation in RF/Un Mice: Observations on Survival Time, Blood Changes, Body Weight, and Incidence of Neoplasms


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