THE EFFECT OF VITAMIN B₁₂ ON THE LEUKOPENIA INDUCED BY RADIATION

By Robert E. Carter, M.D., Elizabeth Busch, B.A., and Verda Strang, B.A.

Vitamin B₁₂,¹ ² a highly active liver principle containing cobalt³ ⁴ and an active growth-stimulating substance for Lactobacillus lactis,⁵ has been shown to be effective in the treatment of Addisonian pernicious anemia and other macrocytic anemias.⁶ ¹⁰ Through the ability of thymidine to replace B₁₂ as a growth factor for Lactobacillus lactis, it has been postulated that B₁₂ acts as a coenzyme in the transformation of thymine to thymidine.¹¹

In view of the diverse theories of the mechanism by which radiation affects living tissue, and in view of the possibility that the formation of nucleoprotein may be interfered with,¹² a trial of the effects of vitamin B₁₂ on the leukopenia following radiation was undertaken at this laboratory.

EXPERIMENTAL PROCEDURE

Sprague-Dowley female rats weighing 100 to 150 grams were selected for this experiment. Fifty-four rats were divided into six groups of 9 animals each. Animals were housed 3 to a cage with diet and water in constant supply. The radiation was delivered by a 250 kv peak voltage x-ray machine at the rate of 50 roentgens a minute. The target-cage distance was 40 inches. No filtration was used other than that inherent in the tube, equivalent to 2 mm. of Al. The average energy of the x-rays delivered by the machine was 168 kv. Animals were contained in flat Lucite cages (1/2 inch wall thickness) during the exposure with ample room for free and random movement. The dose delivered was measured with a Victoreen r-meter, the ionization chamber being placed in the center of the filled cage during the exposure time.

Group I was exposed to 400 roentgens of x-rays. This group received a single dose of 4.0 micrograms of crystalline vitamin B₁₂ intramuscularly thirty minutes after radiation. Group II received the same amount of radiation but was given daily doses of 0.3 micrograms of vitamin B₁₂ intramuscularly starting on the day of radiation, thirty minutes after exposure, and continuing for twenty-one days. Group III received a dose of vitamin B₁₂ identical to Group I but was not radiated. Group IV was identical with Group II except that again no radiation was given. Group V was exposed to 400 roentgens of x-rays but received no vitamin B₁₂. Group VI was kept as a general control group, no radiation or vitamin B₁₂ being given. A summary of the radiation exposure and treatment of each group is given in table 1.

Blood counts were performed on animals from each group one day after radiation and at approximately three-day intervals thereafter through thirty-eight days. The exact time intervals are indicated in figures 1-4. Each point represents the average of determinations on three animals. A frequency of blood counting was selected that made it unnecessary to repeat a count on any individual animal more often than once in nine days, a rate which in itself did not alter the hemogram, as shown by the values obtained on the general control group. Blood was obtained from a small cut made at the tip of the tail, and hemostasis was carefully secured after each bleeding. The red cell count, total leukocyte count and differential leukocyte count were determined on each animal. The results from the control group were subjected to analysis and the criterion of three standard deviations from the mean was taken as the lower limit for statistical significance.

From the Los Alamos Scientific Laboratory of the University of California, Los Alamos, N. M.

This document is based on work performed under AEC Contract No. W-7405-Eng-36 with the Los Alamos Scientific Laboratory of the University of California.

The crystalline vitamin B₁₂ used in this study was supplied by Merck & Company, Rahway, New Jersey, through the courtesy of Drs. Augustus Gibson and R. C. Pogge.
limit of statistical significance. This information is included on the graphs of the red cell counts (figs. 1 and 2) and the total white blood cell counts (figs. 3 and 4).

At intervals of 9, 23, and 37 days after radiation, bone marrow biopsies were obtained from one animal in each group. The samples were obtained by anesthetizing the animal with ether, exposing a small portion of the femoral shaft, drilling through the cortex with a dental burr, and removing a portion of the marrow with a capillary pipet. The separated muscle layers were approximated with a single stitch and the skin closed with clips. Animals tolerated the procedure well, and similar biopsies on other groups of animals have shown that this procedure produces no significant alteration in the peripheral blood picture. 

Table 1.—Summary of Radiation Exposure and Vitamin B<sub>12</sub> Treatment of Various Experimental Groups

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Radiation Received</th>
<th>Vitamin B&lt;sub&gt;12&lt;/sub&gt; Administered</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>400 roentgens on day 0</td>
<td>4.0 micrograms on day 0, 30 minutes after exposure.</td>
</tr>
<tr>
<td>II</td>
<td>400 roentgens on day 0</td>
<td>0.3 micrograms daily starting on day 0, 30 minutes after exposure and continuing for 21 days.</td>
</tr>
<tr>
<td>III</td>
<td>No radiation</td>
<td>4.0 micrograms on day 0.</td>
</tr>
<tr>
<td>IV</td>
<td>No radiation</td>
<td>0.3 micrograms daily starting on day 0 and continuing for 21 days.</td>
</tr>
<tr>
<td>V</td>
<td>400 roentgens on day 0</td>
<td>No B&lt;sub&gt;12&lt;/sub&gt; given.</td>
</tr>
<tr>
<td>VI</td>
<td>No radiation</td>
<td>No B&lt;sub&gt;12&lt;/sub&gt; given.</td>
</tr>
</tbody>
</table>

* Dosage of 250 kV x-rays delivered at the rate of 50 r per minute.
† Administration via intramuscular injection.

Fig. 1.—The effect of single vs. multiple doses of vitamin B<sub>12</sub> on the red blood cell count of rats exposed to 400 roentgens of 250 kV x-rays at 50 r per minute.
R. E. CARTER, E. BUSCH AND V. STRANG

Fig. 2.—The effect of single vs. multiples doses of vitamin B₁₂ on the red blood cell count of normal rats.

Fig. 3.—The effect of single vs. multiple doses of vitamin B₁₂ on the white blood cell count of rats exposed to 400 roentgens of 250 kv x-rays at 50 r per minute.

One animal death, which was ascribed to radiation, occurred during the course of the experiment. This animal was a member of Group II. Another animal from Group I died an anesthetic death during the course of a bone marrow biopsy.
EXPERIMENTAL RESULTS AND DISCUSSION

The results of the experiment are given in figures 1-4. The dosage of radiation used was sufficient to cause a profound leukopenia (fig. 3). No statistically significant anemia resulted from 400 roentgens, but the fact that all values for the exposed groups lie below the control mean is of moderate significance in itself (fig. 1).

It is evident from the data presented in figures 1 and 3 that the blood picture of radiated animals receiving vitamin B₁₂ in single or multiple doses differed in no significant degree from the radiated controls receiving no vitamin B₁₂. Morphologic changes seen in the granulocytes and lymphocytes were similar to those occurring in radiation of several types. These changes consisted of abnormal granulation and bizarre nuclear forms in the granulocytes, and increased cytoplasmic basophilia and evidence of immaturity in the lymphocytes. No morphologic characteristics singular to the groups receiving vitamin B₁₂ were noted.

Groups receiving vitamin B₁₂ showed no difference in the degree of neutropenia or lymphopenia following radiation, as compared with the noninjected exposed groups. Similarly, vitamin B₁₂ given to nonexposed animals produced no significant change in the absolute number of neutrophils or lymphocytes.

The bone marrow biopsies showed no difference between the animals receiving vitamin B₁₂ and those not given vitamin B₁₂. The radiation administered caused a marked decrease in the cellularity of the femoral marrow nine days after exposure, following which recovery was rapid. The normoblastic and leukoblastic series were equally affected.

![Graph](image-url)
No consistently significant alteration in the hemogram of nonirradiated animals receiving vitamin B₁₂ was seen (figs. 2 and 4), which is in agreement with the blood observations by Emerson in his work on the growth-promoting activity of the vitamin in rats receiving thyroid substance.

Conclusions

Crystalline vitamin B₁₂ administered via intramuscular injection, in single and in multiple doses, produced no effect on the leukopenia induced in rats by 400 roentgens of 2.50 kv x-rays.

References

5 Shorb, M. S.: Activity of vitamin B₁, for the growth of Lactobacillus lactis. Science 117: 397, 1948.
11 Carter, R. E.: Unpublished data.

* These documents are available through the Technical Information Division, Oak Ridge Directed Operations, Oak Ridge, Tenn.
THE EFFECT OF VITAMIN B₁₂ ON THE LEUKOPENIA INDUCED BY RADIATION

ROBERT E. CARTER, ELIZABETH BUSCH and VERDA STRANG