STUDIES ON X-RAY EFFECTS.

IX. THE ACTION OF SERUM FROM X-RAYED ANIMALS ON LYMPHOID CELLS IN VITRO.

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In the course of an investigation on the biological effects of x-rays it was noted that while larger doses of this agent destroy lymphoid tissue, very small exposures, after causing a slight amount of destruction, will bring about an actual stimulation of this tissue.¹ The mechanism of the stimulation phenomenon is of considerable interest owing, among other things, to the relation of the lymphoid tissue to cancer resistance. The most satisfactory stimulation has been obtained with x-rays of comparatively long wave-lengths and, therefore, of low penetrating power. In fact, the best results have followed exposure to the rays from a specially constructed tube with a window which permits the emission of a larger proportion of the soft rays than are given off by the standard tubes; and this tube is operated with a spark-gap of ½ inch.² Of the very small dose used here, approximately 57 per cent is absorbed by the first ¼ cm. of tissue, and over 92 per cent before the rays have penetrated to the depth of ½ cm., while at a depth of 1½ cm. only 0.56 per cent of the rays remains. It seems extremely doubtful, therefore, whether these rays penetrate to the deeper lymphoid organs in sufficient strength to bring about any change; yet these organs show as much evidence of stimulation

² Nakahara, W., and Murphy, Jas. B., J. Exp. Med., 1922, xxxv (in press).
or destruction as do the organs which are superficial enough to be
directly acted upon by the rays.

This observation has led to a consideration of the possibility of the
spleen and lymph gland changes being secondary to some alteration
in the circulating blood or other tissues, brought about by the action
of the x-rays. The point is one that has already been the subject of
several investigations. Linser and Helber's experiments led them to
conclude that the serum from x-rayed animals contained a leucotoxin
which on injection into other animals produces destruction of the
circulating leucocytes. The leucotoxin was destroyed by heating at
55–60°C. The toxin, according to these authors, is transmitted
from mother to fetus through the placenta. Capps and Smith reported
similar findings, with the serum from successfully treated
leucemia patients, and state that the serum from an x-ray-treated
case of leucemia when injected into an untreated case causes a definite
fall in the number of white blood cells. Curschmann and Gaupp also
state that serum from x-ray-treated cases of leucemia, in a
dilution of 1:100, causes rapid destruction of leucocytes

Later Klieneberger and Zoeppritz failed to confirm any of the
above experiments, which is true also of other observers with reference
to the so called leucotoxin in the serum of x-ray-treated
individuals.

With the evidence at hand indicating the indirect action of the
x-rays on the lymphoid tissue, it seemed of interest to reopen the
question and to determine whether or not the serum of x-rayed animals
has any effect on lymphoid cells

**The Effect of Serum from X-Rayed Animals on Lymphoid Cells in Vitro.**

A number of healthy young rats were exposed to a dose of x-rays
governed by the following factors: spark-gap 2½ inches; milliamperes
10; distance 12 inches; time 14 minutes. Immediately following

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this treatment the animals were anesthetized and exsanguinated by aspiration of the heart. The blood was placed in a test-tube, and after clotting it was centrifuged. The serum was then drawn off and again centrifuged at high speed to remove any remaining cells or fibrin. Serum was collected in the same manner from a like number of normal rats. After the blood had been drawn from the normal rats, the thymus and mesenteric lymph glands were removed under aseptic conditions. The glands were freed from adherent tissue and were then divided as nearly as possible into equal parts so that two lots were made, each having half of the thymus and half of the mass of mesenteric lymph glands from a number of different rats. One of the portions was then mixed with the serum from normal rats and the other with serum from the x-rayed rats and each was then ground thoroughly in a mortar, and the resulting suspensions were passed through filter paper under suction to remove the fibrous tissue and cell clumps. Counts were made of the two filtrates to determine the number of cells present, and then enough of the two sera was added to reduce the counts to between 10,000 and 20,000 cells per c. mm. Another count was made on the suspensions after they had been well shaken to standardize the suspension. The tubes were tightly plugged and placed in a water bath at 37°C. for 2 hours. They were then removed, well shaken, and counted, and again after 4 hours in the water bath this procedure was repeated. Films were made at the time of each count and stained with Wright's blood stain. The enumeration in each case was made by two individuals on different samples of the suspension and when there was a divergence, the mixtures were reshaken and the counting was repeated. Many counts were checked up with the high power lens of the microscope so as to make sure that fragments and debris were not included.

Table I gives the tabulated results of fourteen such experiments in which Serum A is from normal animals and Serum B from x-rayed animals.

The average of these fourteen experiments (Text-fig. 1) shows that the cells suspended in normal serum decreased by over 3,000 during the first 2 hours and by another thousand by the end of the 4 hour period. The cells in the serum from x-rayed animals increased by over 3,000 cells in the first 2 hours and showed only a slight drop be-
## TABLE 1.

<table>
<thead>
<tr>
<th>Time</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Experiment 4</th>
<th>Experiment 5</th>
<th>Experiment 6</th>
<th>Experiment 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum A</td>
<td>Serum B</td>
<td>Serum A</td>
<td>Serum B</td>
<td>Serum A</td>
<td>Serum B</td>
<td>Serum A</td>
</tr>
<tr>
<td>Before incubation</td>
<td>18,840</td>
<td>18,250</td>
<td>15,280</td>
<td>17,260</td>
<td>13,900</td>
<td>15,000</td>
<td>23,700</td>
</tr>
<tr>
<td>After 2 hrs. incubation</td>
<td>17,340</td>
<td>24,800</td>
<td>15,400</td>
<td>23,550</td>
<td>13,460</td>
<td>20,460</td>
<td>13,080</td>
</tr>
<tr>
<td>&quot; 4 &quot; &quot;</td>
<td>16,960</td>
<td>27,600</td>
<td>13,190</td>
<td>21,100</td>
<td>13,920</td>
<td>18,750</td>
<td>10,080</td>
</tr>
<tr>
<td>&quot; 24 &quot; &quot;</td>
<td>10,050</td>
<td>24,670</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Experiment 8</th>
<th>Experiment 9</th>
<th>Experiment 10</th>
<th>Experiment 11</th>
<th>Experiment 12</th>
<th>Experiment 13</th>
<th>Experiment 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum A</td>
<td>Serum B</td>
<td>Serum A</td>
<td>Serum B</td>
<td>Serum A</td>
<td>Serum B</td>
<td>Serum A</td>
</tr>
<tr>
<td>Before incubation</td>
<td>19,500</td>
<td>18,500</td>
<td>17,240</td>
<td>17,900</td>
<td>19,100</td>
<td>14,800</td>
<td>17,500</td>
</tr>
<tr>
<td>After 2 hrs. incubation</td>
<td>17,100</td>
<td>21,300</td>
<td>13,000</td>
<td>23,200</td>
<td>13,400</td>
<td>15,900</td>
<td>14,450</td>
</tr>
<tr>
<td>&quot; 4 &quot; &quot;</td>
<td>13,240</td>
<td>24,700</td>
<td>15,000</td>
<td>21,550</td>
<td>11,000</td>
<td>14,000</td>
<td>13,300</td>
</tr>
</tbody>
</table>

Serum A is from normal rats, Serum B from rats immediately after a dose of X-rays.
between the 2 and 4 hour periods. At the end of the period of observation the counts showed the suspensions still had some 3,000 cells more per c. mm. than the original suspension.

Examination of a large number of stained films made from these suspensions at the 2 hour period showed among the cells suspended in serum from x-rayed animals a fairly large number of mitotic figures (Fig. 1). The average was a little less than one mitosis to a thin film, and occasionally three or more were found in a film. In only one instance was a dividing cell found in the normal serum suspension. The amount of disintegration of the cells, judged by the number of degenerated forms found in the smears, is just as rapid in the serum from x-rayed animals as in that from normal animals. Apparently, therefore, the proliferation of the cells in contact with serum from x-rayed animals is sufficient to replace not only the disintegrated
cells but also actually to increase the total number. A large number of films prepared from the suspensions before incubation failed to show any mitotic figures, thus ruling out the question of the dividing cells being carried over in any appreciable numbers from the glands.

An unsuccessful attempt was made to extend the above observations to rabbits, but the fragility of the lymphoid cells was such that by the end of the 2 hour period no accurate counts could be made. The cells of guinea pigs showed less tendency to disintegrate and a small increase in the number of cells suspended in serum from an x-rayed animal was noted. However, the rate of disintegration was too rapid to obtain definite or consistent results. Finally, rat lymphoid cells suspended in serum from rabbits were destroyed so rapidly as to make it impossible to secure accurate counts.

The Duration of the Stimulative Quality of Serum from X-Rayed Animals.

In order to test the length of endurance of the stimulative effect of the serum from x-rayed rats, the above experiments were repeated, except that in this series the blood was taken 17 hours after the x-ray treatment was given. The results of these observations are given in Table II.

<table>
<thead>
<tr>
<th>Time</th>
<th>No. of cells in suspension.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before incubation</td>
<td>Experiment 15. 23,700 Serum A</td>
</tr>
<tr>
<td>After 2 hrs. incubation</td>
<td>14,080 Serum A</td>
</tr>
<tr>
<td>&quot; 4 &quot; &quot;</td>
<td>10,080 Serum A</td>
</tr>
</tbody>
</table>

Serum A is from normal rats, Serum B from rats 17 hours after a dose of x-rays.

It will be seen that active stimulative effect of the serum from x-rayed rats is lost by 17 hours after the treatment (Text-fig. 2), but it may be noted that the rate of disintegration is retarded somewhat in the serum from the x-rayed animals. It is not clear whether the retarding action represents an actual slowing down of the disint-
integration rate or whether there is enough stimulation substance remaining to bring about a less rapid fall in the count by cell multiplication. The finding of one mitotic figure in the comparatively small number of preparations studied suggests the latter possibility.

**The Effect of Serum X-Rayed in Vitro on Lymphoid Cells.**

A quantity of serum was prepared from normal rats in the same manner as in the preceding experiments. Half of this was exposed directly to x-rays in the same amount as that given to the animals in the previous experiments.

The cell suspensions were prepared in the same manner as described above with the normal and x-rayed serum. The results of these experiments are given in Table III.

**TABLE III.**

<table>
<thead>
<tr>
<th>Time</th>
<th>No. of cells in suspension.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum A</td>
</tr>
<tr>
<td>Before incubation</td>
<td>19,100</td>
</tr>
<tr>
<td>After 2 hrs. incubation</td>
<td>13,400</td>
</tr>
<tr>
<td>“        4 “ “</td>
<td>11,000</td>
</tr>
</tbody>
</table>

Serum A is from normal rats, Serum B is normal rat serum exposed to x-rays in vitro.

Thus the serum x-rayed in vitro proved to be devoid of stimulative effect on the suspended lymphocytes, but as in the preceding experiment there was a retardation of fall in the cell count (Text-fig. 3).

**Variations in the Response of Lymphoid Cells to Stimulative Effects.**

In all the experiments described above, the cell suspensions were prepared from the thymus and mesenteric lymph glands usually of five rats. Each mass of glands was divided into two parts so that the final suspensions contained about equal amounts of the tissue from each animal used in the experiment. It will be noted in the figures given that there was considerable variation in the amount of stimulation in the various experiments, although the dose of x-rays was the
same throughout. Hence a test was made to ascertain whether the cells of the thymus and the lymph gland shared equally in the stimulation and also whether there was striking individual variation in the degree of response between cells of different animals.

Serum from normal and x-rayed rats was prepared according to the method described in the first series of experiments. The thymus from two rats and the lymph glands from the same two animals were prepared separately so as to yield a suspension of thymus cells in serum from x-rayed rats, and a suspension of thymus cells from the same
TABLE IV.

<table>
<thead>
<tr>
<th>Time</th>
<th>Experiment 21</th>
<th>Experiment 22</th>
<th>Experiment 23</th>
<th>Experiment 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum A + lymph glands</td>
<td>11,450</td>
<td>9,000</td>
<td>14,450</td>
<td>14,250</td>
</tr>
<tr>
<td>Serum A + thymus</td>
<td>4,000</td>
<td>9,500</td>
<td>18,450</td>
<td>5,200</td>
</tr>
<tr>
<td>Serum B + lymph glands</td>
<td>9,000</td>
<td>11,400</td>
<td>14,350</td>
<td>14,250</td>
</tr>
<tr>
<td>Serum B + thymus</td>
<td>9,000</td>
<td>11,400</td>
<td>14,350</td>
<td>14,250</td>
</tr>
<tr>
<td>Before incubation</td>
<td>11,450</td>
<td>9,000</td>
<td>14,450</td>
<td>14,250</td>
</tr>
<tr>
<td>After 2 hrs. incubation</td>
<td>7,600</td>
<td>2,550</td>
<td>12,030</td>
<td>4,000</td>
</tr>
<tr>
<td>After 4 hrs. incubation</td>
<td>6,500</td>
<td>2,150</td>
<td>13,350</td>
<td>4,000</td>
</tr>
</tbody>
</table>
| Serum A is from normal rats, Serum B from rats immediately after a dose of x-rays.
animals in normal serum. Two like suspensions of the lymph glands from the same animals in the two sera were prepared for comparison. Table IV shows the result of four such experiments.

Another like experiment was carried out, to test the response of glands from different groups of rats to the same serum from x-rayed animals.

The figures for these experiments are given in Table V.

<table>
<thead>
<tr>
<th>Time</th>
<th>Serum A + lymph glands</th>
<th>Serum A + thymus</th>
<th>Serum B + lymph glands (1)</th>
<th>Serum B + lymph glands (2)</th>
<th>Serum B + lymph glands (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before incubation</td>
<td>16,970</td>
<td>14,000</td>
<td>17,800</td>
<td>2,800</td>
<td>3,750</td>
</tr>
<tr>
<td>After 2 hrs. incubation</td>
<td>11,600</td>
<td>9,100</td>
<td>11,840</td>
<td>18,800</td>
<td>4,360</td>
</tr>
<tr>
<td>&quot; 4 &quot;</td>
<td>11,070</td>
<td>8,450</td>
<td>12,500</td>
<td>16,000</td>
<td>3,950</td>
</tr>
</tbody>
</table>

Serum A is from normal rats, Serum B from rats immediately after a dose of x-rays.

It is obvious from these experiments that the thymus and lymph gland cells are affected about equally by the serum from x-rayed animals (Text-fig. 4), although the thymus cells from some animals respond more readily than the lymph gland cells from the same animals, while in others the opposite is true.

There is also considerable variability in the stimulative power of the same serum on the lymphoid cells of different individuals.

The Effect of the Serum from Animals after a Very Large Dose of X-Rays on Lymphoid Cells.

In further experiments, an attempt has been made to determine whether there is a destructive action on the lymphoid cells of serum from animals after a very large dose of x-rays. Rats were exposed for an hour to a dose of x-rays, otherwise governed by the same factors as in the preceding experiments, and the effect of the serum of these animals was tested on lymphoid cell suspension. There was no
evidence of a stimulative effect, nor was there any more rapid disintegration of the cells than was observed to take place in the normal serum.

**DISCUSSION.**

The experiments reported here fail to show any evidence of the presence of a so called lymphotoxin in the serum of x-rayed animals, even after an exposure so large as to cause almost complete destruction of the lymphoid tissue of the living animals. It is true, however, that these experiments are not an exact repetition of the earlier work along this line, but it seems probable that if any such leucotoxic substance was present in the serum of x-rayed animals, some indication would have appeared among our results. It is difficult to conceive of a lymphotoxin of such power as to be effective in dilutions of 1:100 resulting from a comparatively small dose of x-rays given to a leuemic patient. It is much more difficult to judge the reported results of the injection of serum from an x-rayed individual into animals, for there is the complicating effect of the foreign protein reaction to be taken into account, as well as the instability of the blood counts of the rabbit and guinea pig, the animals used for these tests. In regard to the latter point our experience has been that it is necessary to resort to extreme measures of precaution in order to get a fairly stable blood picture in such animals.

The source and character of the stimulus for lymphoid cells contained in the serum from x-rayed animals are questions about which there is as yet little to be said. The fact that this stimulative quality is not possessed by serum x-rayed *in vitro* suggests that the change is not a simple one in the serum itself. Furthermore, it is known that the stimulation of lymphocytes induced by x-rays *in vivo* is always preceded by a certain amount of destruction of lymphoid cells, a fact suggesting the possibility of the stimulating substance being of the nature of a disintegration product of lymphoid cells.

There is ample proof, both from the cell counts and the presence of mitotic figures, that multiplication actually takes place in cells in a fluid medium, although it has generally been supposed that a matrix of some kind is a necessity for growth. No other explanation of the results described is apparent than that cells are capable of being
stimulated to active multiplication in a fluid medium, and that such a stimulative agent is present in the serum of x-rayed animals. How great a part this agent plays in the stimulation observed to take place \textit{in vivo} after a small dose of x-rays is still to be determined.

\textbf{SUMMARY.}

Lymphoid cells, prepared from the thymus and lymph glands of rats, when suspended in the serum of x-rayed rats and incubated for 2 hours, increase in number from 15 to 30 per cent, and mitotic figures are found among these cells in fairly large numbers. A like suspension of cells in normal serum undergoes rapid disintegration and in only one instance among a large number of films examined was a mitotic figure found.

The stimulative effect of the serum from x-rayed rats endures from 1 to 2 hours after the exposure but is not detectable in the serum taken 17 hours or later after the treatment. Serum x-rayed \textit{in vitro} is devoid of stimulative action.

The lymphoid cells of rabbits and guinea pigs are so fragile as to make impossible the obtaining of counts accurate enough for experimental purposes. The serum of one species caused such rapid disintegration of the cells of another that it was impossible to determine the specificity of the reaction.

\textbf{EXPLANATION OF PLATE 15.}

Fig. 1. Mitotic figures found among the cells suspended in serum from x-rayed animals.
Fig. 1.
(Murphy, Liu, and Sturm: Studies on x-ray effects. IX.)