Changes in the Phagocytic Activity of Polymorphonuclear Leukocytes following Total Body X-Irradiation in the Rat

By M. Wilkinson

While X-irradiation in sufficient dosage will depress the number of circulating leukocytes, little is known about its effect on the functions of those few leukocytes which continue to be formed and delivered to the blood stream. During histologic studies on the lymph nodes of rabbits exposed to 600 r or 800 r x-irradiation and sacrificed at intervals up to twenty-four hours after irradiation, Bloom noticed that while some areas of cellular debris were infiltrated with polymorphonuclear leukocytes, these cells showed no evidence of phagocytic activity. Glenn studied the phagocytic indices (i.e., the average number of bacteria ingested per leukocyte) of rabbits following x-irradiation of varying dosage and quality. Following an optimal dose of 350 r (measured in air) delivered at 140 kv. to a small area of skin, the phagocytic index for Staphylococcus aureus showed a definite rise, maximal after forty-eight hours and subsiding to normal seven days after the irradiation. Esplin, et al., using an in vivo technic, studied the phagocytic ability of mouse leukocytes for Micrococcus pyogenes at two and six days following 350 r total body x-irradiation. At both times, the leukocytes from the irradiated animals contained more bacteria than did those from normal animals, but this may have been partly or wholly due to the leukopenia in the former group. Using mixtures of leukocytes and bacteria, it has been shown that if the concentration of bacteria is kept constant, a fall in the numbers of leukocytes will cause an increase in the percentage of active phagocytes, and in the average number of bacteria per leukocyte, though the total amount of phagocytosis in the system will be decreased. This is probably due to the greater number of bacteria available to each phagocyte in the case of the leukopenic mixture.

In the present investigation, using a modification of the technique recommended by Boerner and Mudd, the in vitro phagocytic activity of leukocytes from irradiated rats was studied over a period of two weeks. A preliminary study on mice showed a definite decrease in phagocytosis from the sixth day to the termination of the experiment on the twelfth day following 350 r x-irradiation. Owing to severe leukopenia, it was impossible to make adequate counts before the sixth day. The following experiment was then planned to confirm this finding, and by doing parallel phagocytic studies on suspensions of normal

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leukocytes in plasma from irradiated animals and on leukocytes from irradiated animals suspended in normal plasma, it was hoped to determine whether the decreased phagocytosis was due to a defect in the leukocytes or in the plasma.

**Materials and Methods**

Two groups of 9 week old female Wistar rats were used. Twenty-eight rats received irradiation, and twenty-one served as a control group.

**Radiation Data**

Radiation was delivered from an x-ray machine operating at 250 kv. and 15 Ma. with a H.V.L. of 1.5 mm. Cu. The target to mid-animal distance was 96.5 cm. and the tissue dose-rate was 42 r per minute as determined in a wood and rice dummy set-up, using a Victoreen thimble chamber. Each animal received a total body dose of 550 r.

**Anticoagulant**

One hundred mg. of purified heparin was dissolved in 20 ml. of normal saline solution and sterilized by autoclaving.

**Bacterial Suspension**

Two loopfuls of a 48 hour culture on blood-agar of avirulent *Pasteurella pestis* (strain A-1122) were suspended in normal saline solution. A bacterial count was then done, using a Petroff-Hauser chamber, and the suspension adjusted to a concentration of 400 million organisms per ml.

**Phagocytosis Technic**

Three ml. of blood was obtained from the hearts of two stunned control rats, using a syringe previously rinsed with heparin solution and containing 0.1 ml. of this solution. This blood was transferred to three 10 cm. silicone-coated Kahn tubes in the proportions of 0.5 ml. to each of the first two tubes and 2 ml. to the third tube. Three blood samples were similarly obtained from irradiated rats, and in each instance the first sample was used immediately for a phagocytic estimation. The second and third samples were centrifuged at 3000 rpm for 15 minutes. The supernatant plasma was then removed from each of the second samples by careful suction. In each case, the original volume was restored by adding plasma from the upper layers of one of the third samples, so as to achieve a suspension of normal blood cells in plasma from irradiated animals and a suspension of cells from irradiated animals in normal plasma. Thus, four samples of blood were tested for phagocytic activity, usually at daily intervals, during the first thirteen days after the irradiation. These samples were: (1) blood from normal rats; (2) blood from irradiated rats; (3) blood cells from normal rats suspended in plasma from irradiated rats; (4) blood cells from irradiated rats suspended in plasma from normal rats.

Before the actual phagocytic test, total and differential white cell counts were performed on each sample. Then, to the 0.5 ml. of blood was added 0.1 ml. of bacterial suspension. The tube was placed immediately in a water bath at 37.5 C. and subjected to a constant gentle mechanical agitation. Using a fine pipet, blood samples were withdrawn 2, 5, 8, and 10 minutes later and used to make smears. The smears were fixed in methyl alcohol and stained with Giemsa stain. An attempt was made to examine 100 neutrophile polymorphonuclear leukocytes to ascertain the percentage of cells showing phagocytosis and also the number of plague bacilli phagocytosed by 100 neutrophile leukocytes. Because of marked leukopenia in the irradiated animals, it was not always possible to find 100 neutrophile polymorphonuclear leukocytes. In such instances as many as possible were examined, and for comparison the results were expressed on the basis of a count of 100 cells. Only counts exceeding 30 cells have been included in the results.
Effects of Total X-Irradiation on Polymorphonuclear Leukocytes

Table 1.—Effect of Incubation Time on the Phagocytosis of Plague Bacilli by Neutrophile Leukocytes from Normal Rats

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>2 Min.</th>
<th>5 Min.</th>
<th>8 Min.</th>
<th>10 Min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of neutrophils showing phagocytosis</td>
<td>12 ± 1.8</td>
<td>43 ± 4.0</td>
<td>69 ± 4.6</td>
<td>81 ± 2.7</td>
</tr>
<tr>
<td>Bacilli per 100 neutrophils</td>
<td>14 ± 2.7</td>
<td>80 ± 12.6</td>
<td>299 ± 37.1</td>
<td>384* ± 49</td>
</tr>
</tbody>
</table>

* Statistical analysis by “t” test shows that the mean counts for each incubation time are significantly different (p < .05) when each is compared with the preceding count, with the single exception indicated where p < .1 and > .05.

† Standard error.

Fig. 1.—Phagocytosis of P. pestis by polymorphonuclear leukocytes from normal and irradiated rats at intervals following the irradiation.

Results

Table 1 shows the results of phagocytic tests performed on blood from control rats over a period of thirteen days. As would be expected, both the percentage of neutrophils showing phagocytosis and the number of ingested bacilli increased progressively as the period of incubation lengthened. Since the blood from the irradiated rats behaved in exactly the same manner, only the results of phagocyte counts after 10 minutes’ incubation will be presented for comparison (fig. 1 and table 2).

On the first day after irradiation, the blood from the irradiated rats showed a slightly greater number of phagocytic leukocytes and a greater number of ingested plague bacilli than did normal blood. Because of leukopenia no further counts were possible until the fifth and sixth days following irradiation, when the blood from the irradiated animals again showed a slightly higher proportion of neutrophile leukocytes containing bacteria and a higher count of ingested bacteria. During the same period the mixtures of normal cells in irradiated plasma and irradiated cells in normal plasma gave results similar to normal whole blood.
Table 2.—Phagocytic Activity of Polymorphonuclear Leukocytes at Intervals after Irradiation, Correlated with the Leukocyte Counts on the Various Samples of Blood Tested

<table>
<thead>
<tr>
<th>Days after irradiation</th>
<th>Control blood</th>
<th>Irradiated blood</th>
<th>Normal cells in irradiated plasma</th>
<th>Irradiated cells in normal plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total leukocyte count</td>
<td>neutrophil count</td>
<td>phagocyte count*</td>
<td>mean bacilli per active phagocyte</td>
</tr>
<tr>
<td>1</td>
<td>5,440</td>
<td>435±72% (284)</td>
<td>3.9</td>
<td>2,640 1,575 ±80% (486) 6.1</td>
</tr>
<tr>
<td>3</td>
<td>8,120</td>
<td>650±78% (371)</td>
<td>4.8</td>
<td>475 19</td>
</tr>
<tr>
<td>4</td>
<td>7,400</td>
<td>481±88% (545)</td>
<td>6.2</td>
<td>375 15</td>
</tr>
<tr>
<td>5</td>
<td>5,840</td>
<td>750±85% (464)</td>
<td>5.5</td>
<td>675 61±94% (550) 5.9</td>
</tr>
<tr>
<td>6</td>
<td>6,680</td>
<td>1,202±82% (269)</td>
<td>3.3</td>
<td>975 229±90% (377) 4.2</td>
</tr>
</tbody>
</table>

Means for days 1-6  
81% (387) 4.7 88% (471) 5.4 85% (429) 4.8 81% (443) 5.0

| 7                      | 5,700          | 821±91% (543)   | 6.0                | 1,450 80±47% (71) 1.5      | 4,600 481±81% (255) 3.1 | 1,225 24 69% (147) 2.1 |
| 8                      | 7,320          | 1,244±81% (394) | 4.9                | 1,375 27±50% (70) 1.4      | 7,720 1,119±83% (219) 3.6 | 1,350 7 62% (107) 1.7 |
| 9                      | 6,400          | 1,576±83% (263) | 3.4                | 1,375 55±41% (47) 1.1      | 3,120 343±82% (266) 3.2 | 925 23 59% (172) 1.3† |
| 11                     | 14,160         | 1,699±87% (684) | 7.9                | 1,000 35±42% (67) 1.6      | 12,840 706±85% (298) 3.5 | 1,075 64 49% (90) 1.8 |
| 12                     | 6,200          | 558±78% (248)   | 3.2                | 1,700 51±30% (57) 1.5      | 4,680 351±76% (239) 3.1 | 1,275 13 49% (80) 1.6 |
| 13                     | 7,040          | 1,197±60% (130) | 2.2                | 700 105±24% (38) 1.6      | 10,840 1,084±60% (104) 1.7 | 625 100 45% (67) 1.5 |

Means for days 7-13  
80% (382) 4.6 41% (58) 1.45 78% (230) 2.9 56% (94) 1.7

* First figure indicates percentage of neutrophils containing bacteria. Figure in parentheses indicates number of bactera ingested by 100 neutrophils.
† Mean number of bacilli per leukocyte showing phagocytosis.
‡ Satisfactory phagocyte counts were not made on this sample. The figures given were calculated as described by Snedecor for the purposes of the statistical analysis.
EFFECTS OF TOTAL X-IRRADIATION ON POLYMORPHONUCLEAR LEUKOCYTES

TABLE 3.—Analysis of Variance of Percentage Active Phagocyte Counts on the Four Groups of Blood Tested During the Second Week Following Irradiation

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean squares</th>
<th>Variance ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between days</td>
<td>1564.7</td>
<td>5</td>
<td>312.9</td>
<td>15.8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Between treatment groups</td>
<td>6490.8</td>
<td>3</td>
<td>2163.6</td>
<td>107.3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Error</td>
<td>302.5</td>
<td>15</td>
<td>20.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8358.0</td>
<td>23</td>
<td>363.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

An analysis of variance test was applied to the results of phagocytic counts on the first, fifth, and sixth days after irradiation, and each treatment group was compared by “t” tests using the standard error as calculated from the error variance. This showed that there was actually no significant difference between any of the groups of blood samples.

On the seventh day there was an abrupt change, and the blood from the irradiated animals showed markedly fewer phagocytic leukocytes containing extremely few bacteria. This depression of phagocytosis in the blood of the irradiated animals persisted to a similar degree throughout the remainder of the period of observation, and was still very definite on the thirteenth day following irradiation. The results of phagocytic tests performed on suspensions of normal blood cells in plasma from irradiated animals were not significantly different from the results in the control series. On the other hand the results of tests performed on blood cells from irradiated animals suspended in normal plasma showed a marked depression of phagocytosis throughout the second week after irradiation similar to, though less pronounced, than that seen in the series using whole blood from irradiated animals.

Toward the end of the second week there was a tendency in all groups for the phagocyte counts to decline. This may have been due to a change in the plague bacillus which made it less easily phagocytosed.

The percentage active phagocyte counts for the second week after irradiation were also subjected to analysis of variance (table 3), which confirmed that there was a significant day-to-day drift of the results (p < .001). The standard error of the difference between any two means was 2.59, and using “t” tests it was possible to confirm that the active phagocyte counts on irradiated whole blood and on irradiated cells in normal plasma differed significantly (p < .001) from the counts on normal blood. Also, the slight differences between the counts on irradiated whole blood and on irradiated cells in normal plasma were highly significant (p < .001).

DISCUSSION

In these studies, no attempt was made to standardize the white cell concentration before estimating phagocytic activity; however, the total number of white cells and the absolute number of neutrophile leukocytes were estimated in each sample. The results are included in table 2, so that changes in phagocytic activity can be considered in relation to changes in the leukocyte concentration. From the work of Hank, one might expect an increased percentage of phago-
cytosis in the blood samples from irradiated rats because of the profound leukopenia. Instead, there was an increase on the fifth and sixth days following irradiation. While this increase was not statistically significant, it is quite possible that if more counts had been done during the first six days after irradiation a significant increase might have been demonstrable.

From the seventh to the thirteenth day following irradiation there was a marked depression of phagocytic activity in the blood from the irradiated animals, yet during this period the neutrophil count rose very little. In view of this neutropenia, the phagocytic activity of these leukocytes must have been more severely depressed than the figures suggest. Also, under the conditions of the test, not only did fewer neutrophils show phagocytosis, but those which did phagocytose ingested very few bacilli. This can be seen by comparing the mean bacilli per active phagocyte in the control and irradiated series during the second week after irradiation (table 2).

This depression of phagocytic activity could be due to some change in the leukocytes themselves or to a change in the plasma, such as deficiency of opsonic factors or the presence of substances which inhibit phagocytosis. If the correct explanation were a simple deficiency of opsonic substances in the plasma of irradiated animals, one would expect leukocytes from irradiated animals suspended in normal plasma to phagocytose plague bacilli normally, but in fact this combination proved only slightly more phagocytic than whole blood from irradiated animals. Lack of opsonic factors, therefore, plays a definite though minor role in this defect.

The presence of substances which inhibit phagocytosis in the plasma of irradiated animals seems unlikely, for a combination of normal blood cells suspended in plasma from irradiated animals proved as phagocytic as normal whole blood.

The main defect, then, seems to be in the leukocytes themselves, which have a reduced phagocytic ability. Presumably, those primitive leukopoietic cells in the bone marrow which escape destruction by the irradiation are not undamaged and give rise to a generation of functionally deficient leukocytes which appear in the peripheral blood during the second week after irradiation. It is interesting that Taplin, et al., during studies on the blood retention of colloidal prodigiosin injected intravenously into rabbits, found increased dye levels following irradiation, with a peak between the seventh and fourteenth days. This they considered to reflect a decrease in the phagocytic activity of macrophages of the reticuloendothelial system. With neither macrophages nor the few remaining polymorphonuclear leukocytes functioning normally during the second week after irradiation, the liability to infection at this time is not surprising.

SUMMARY

The phagocytic activity of polymorphonuclear leukocytes from irradiated rats was studied over a period of thirteen days following 550 r total body x-irradiation.

Leukocytes from irradiated rats showed slightly increased phagocytosis of plague bacilli during the first six days after irradiation, but on statistical analysis this increase proved to be insignificant.
From the seventh to the thirteenth day after irradiation, these cells showed a markedly reduced capacity to phagocytose plague bacilli.

Phagocytic studies on suspensions of normal cells in plasma from irradiated animals and on cells from irradiated animals in normal plasma showed that the deficient phagocytosis during the second week following irradiation was due mainly to a defect in the polymorphonuclear leukocytes themselves, though the fact that the addition of normal plasma to cells from irradiated animals did increase their phagocytic capacity by a small but definite degree suggests that there is also a defect in the plasma of irradiated animals.

**SUMMARY IN INTERLINGUA**

Le activitate phagocytic de leucocytos polymorphonuclear ex rattos esseva studiate durante un periodo de dece-tres dies post röntgenorriadiation total a 550 r. Leucocytos ex rattos irradiate monstrava levemente augmentate phagocytosis de Pasteurella pestis durante le prime sex dies post irradiation, sed in le analyse statistic le augmento se provava insignificante. Ab le septe al dece-tertie die post irradiation, le capacitate de iste cellulas a phagocytar P. pestis esseva multo reducite.

Studios phagocytic de suspensiones de cellulas normal in plasma ex animales irradiate e de cellulas ex animales irradiate in plasma normal demonstrava que le phagocytosis deficiente durante le secunde septimana post irradiation esseva debite principalmente a un defecto in le leucocytos polymorphonuclear. Non-obstante, le facto que le addition de plasma normal a cellulas ex animales irradiate resultava in un parve sed definite augmento in lor capacitate phagocytic permette le conclusion que le plasma del animales irradiate es etiam defective.

**REFERENCES**

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M. WILKINSON