Phagocytosis in Liver and Spleen of Rats with Lewis Lymphoma*

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SUMMARY

After Lewis rats bearing transplanted lymphomas were given injections intraperitoneally of colloidal radiogold, estimation of radioactivity in the liver gave evidence for a significant increase of uptake, whereas this was not the case for spleens of tumor-bearing rats. Because of hepatomegaly regularly present in tumor-bearing rats, the Au\textsuperscript{198} content of the whole liver was much greater than that of controls, whereas the whole spleen took up similar amounts of radiocolloid in control and tumor animals. These changes in hepatic and splenic uptake reflecting phagocytic activity were absent in rats tested 6 days after tumor inoculation, when metastatic spread had as yet not taken place. Results of this work were compared with those of similar investigations recorded in the literature which attempted to evaluate reticuloendothelial function in relation to cancer.

The possible relationship between the reticuloendothelial system and cancer has been the subject of numerous experimental and clinical investigations, the evaluation of which has met with considerable controversy (10, 13, 15). One of the important factors responsible for this state of affairs is undoubtedly the difficulty of measuring reticuloendothelial function with adequate reliability and reproducibility. So far this appears to be possible only by means of testing the ability of reticuloendothelial cells to take up certain colloid or particulate material. This communication presents results of measurements of phagocytosis of a radiocolloid in hepatic and splenic macrophages of rats bearing transplanted lymphomas.

MATERIALS AND METHODS

Inbred Lewis rats, 3–6 months old, were given inoculations subcutaneously of Lewis lymphoma, a tumor indigenous to this strain (7). Twenty-four hours before the expected date of sacrifice the animals were given an intraperitoneal injection (lower abdominal quadrant) of a colloidal solution of radiogold (Aurcoloid, Abbott), with the dose ranging from 0.2 to 0.3 \mu c/gm body weight. In most experiments, the rats were sacrificed 13–15 days after tumor inoculation, at which time the average weights of the tumors were 30 gm. in male and 15 gm. in female animals. A few experiments were terminated 20 days after tumor transplantation, by which time average weights of tumors had increased to 60 gm. in male and 35 gm. in female rats. In addition to the subcutaneous tumors, after about 10–12 days of tumor growth, metastatic involvement of regional lymph nodes, liver, and spleen was almost constantly present.

At the time of sacrifice total body weight was determined, and tumor, liver, and spleen were removed immediately and weighed. Aliquots of liver and spleen weighing from 0.25 to 1 gm. were homogenized in Ten Broeck tissue grinders and emulsified according to the method of Tabern, Lahr, and associates (6, 16) with a mixture of equal parts of formamide and water. The homogenates were transferred to volumetric flasks and brought to volumes of 25, 50, or 100 ml., respectively. This produced finely dispersed and stable suspensions, with tissue concentrations ranging from 0.5 to 2.0 per cent. Duplicate 1-ml. samples of the suspensions were plated into disposable planchets, and their radioactivity was determined.

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by means of a Geiger-Müller counter. On the basis of this radioassay the amount of radiogold present in liver and spleen was determined and calculated as the per cent of the injected radiocolloid per gm. of organ. Representative portions of liver, spleen, tumor, and tissues involved by tumor were fixed in formalin for histologic study.

The following observations concerning the experimental design and methodology are important for interpretation of the results. In pilot experiments groups of rats were sacrificed at intervals of from 3 to 72 hours after the intraperitoneal injection of radiogold, and the peritoneal cavity was washed with 30–50 ml. of saline, thus permitting estimation of residual radiocolloid absorbed from the peritoneal cavity at the stated interval. Although absorption from the peritoneal cavity was minimal 3 hours after injection, by 24 hours 80–95 per cent of the injected dose had disappeared from the cavity. Since the absorption in rats sacrificed at intervals longer than 24 hours did not consistently exceed that observed 24 hours after intraperitoneal injection, the latter time interval was selected for convenience in the main experiments.

In view of the presence of residual radiocolloid in the peritoneal cavity, the possibility had to be investigated whether surface contamination of liver and spleen might invalidate the assumption that radioassay of these organs permits the estimation of uptake of Au$^{198}$ within the organs. For this reason, in some experiments the surface of liver and spleen was thoroughly rinsed with saline prior to preparation of homogenates. No significant radioactivity was detectable in these rinses regardless of whether or not the peritoneal cavity itself had been washed with saline, as described before, prior to removal of the organs. Further proof against presence of surface contamination of the liver was obtained by preparing homogenates from the same organ by selecting two sources of tissue: (a) fragments with large areas of capsular surface and (b) fragments taken from the interior of the organ, having no or little capsular surface. Assays of radioactivity of these two homogenates agreed with one another within the range of the statistical counting error.

In the initial stages of this work information was also obtained on the possible effect on the counting rate of Au$^{198}$ of the presence and concentration of suspended tissue. By mixing varying amounts of tissue suspensions and solutions of Au$^{198}$ with known activity, it was established that tissue concentrations ranging from 0.2 to 3 per cent did not affect the counting rate. In addition, in some experiments the solution of Au$^{198}$ injected and the tissue homogenates were counted also in a well-type scintillation counter, with the results in good agreement with those obtained in parallel with the Geiger-Müller counter.

**RESULTS**

Table 1 summarizes the results of several experiments in which tumor-bearing and control animals were tested simultaneously. It is apparent that values of hepatic uptake were significantly higher in tumor-bearing than in tumor-free animals of either sex. In confirmation of observations previously reported (14), female control rats showed consistently higher hepatic uptake of radiogold than did male control rats.

When, on the other hand, in the same animals values of splenic uptake of radiogold were compared, one noted significantly lower values in tumor-bearing rats. In this connection it is important to point out that livers as well as spleens were markedly enlarged in lymphoma-bearing rats, as shown in Table 2. If the findings on uptake in liver and spleen with organ size in tumor-bearing animals are correlated, it is obvious that the increased uptake per gm. of liver associated with liver enlargement will result in an even more pronounced increase of uptake in the whole organ as compared with tumor-free controls. On the other hand, the decreased uptake per gm. of spleen in tumor-bearing rats was counteracted by the enlargement of the spleen up to 3 and 4 times its normal size. Ac-

<table>
<thead>
<tr>
<th>Sex</th>
<th>Group</th>
<th>No. rats</th>
<th>Per cent inj. Au$^{198}$/gm. of liver (Mean ± S.D.)</th>
<th>P</th>
<th>Per cent inj. Au$^{198}$/gm. of spleen (Mean ± S.D.)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Control</td>
<td>24</td>
<td>1.14 ± 0.90</td>
<td>&lt;0.001</td>
<td>1.04 ± 0.69</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Tumor</td>
<td>29</td>
<td>3.07 ± 1.04</td>
<td></td>
<td>0.42 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Control</td>
<td>34</td>
<td>2.14 ± 1.41</td>
<td>&lt;0.001</td>
<td>1.60 ± 0.72</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Tumor</td>
<td>37</td>
<td>4.79 ± 3.90</td>
<td></td>
<td>0.58 ± 0.31</td>
<td></td>
</tr>
</tbody>
</table>

In the initial stages of this work information was also obtained on the possible effect on the counting rate of Au$^{198}$ of the presence and concentration of suspended tissue. By mixing varying amounts of tissue suspensions and solutions of Au$^{198}$ with known activity, it was established that tissue concentrations ranging from 0.2 to 3 per cent did not affect the counting rate. In addition, in some experiments the solution of Au$^{198}$ injected and the tissue homogenates were counted also in a well-type scintillation counter, with the results in good agreement with those obtained in parallel with the Geiger-Müller counter.
Accordingly, calculations not presented showed that the whole spleens of control and of tumor-bearing rats contained about the same amount of radioactivity when expressed as per cent of injected radiogold.

Additional experiments were done to find out whether the changes in phagocytic activity were demonstrable already in the early stages of tumor growth or appeared only in later stages. Table 3 shows results of pertinent experiments in which rats given inoculations of tumor were divided into two groups, one of which was sacrificed after 6 days and the other after 13 days. In the 6-day group histologic study showed presence of local tumors but absence of systemic involvement, specifically in liver and spleen. Concomitantly, there was no significant difference in hepatic uptake of radiocolloid in either male or female tumor-bearing animals as compared with their controls. This is in marked contrast to the findings in rats sacrificed 13 days after tumor inoculation in which, in accord with observations recorded in Table 1, tumor-bearing rats exhibited a markedly increased hepatic uptake. Extensive metastatic spread to liver, spleen, and lymph nodes was demonstrated in these rats histologically. Uptake of radiogold in the spleen was decreased in male but not in female rats sacrificed 6 days after tumor inoculation. In tumor-bearing rats of either sex sacrificed 13 days after tumor inoculation, splenic radiocolloid was significantly lowered. As to changes in size of liver and spleen, male animals exhibited slight increase in weight of these organs 6 days after tumor inoculation, whereas the increase was remarkable 13 days after tumor inoculation. On the other hand, in female rats neither hepatic nor splenic size showed any change in the 6-day group, but they were significantly increased 13 days after tumor transplantation.

### DISCUSSION

As documented in numerous investigations, injected radiogold localizes almost exclusively in reticuloendothelial cells, with the highest concentrations of the injected dose found in the liver and spleen (4, 11, 12). Analysis of the findings of this study raises a number of questions. Specifically, three points require attention: (a) a striking increase in phagocytic activity of reticuloendothelial cells in the liver was observed in the presence of an actively growing tumor; (b) in remarkable contrast to these findings presumably referring to Kupffer cells, splenic macrophages of tumor rats showed significantly decreased phagocytic activity; (c) the changes in hepatic and splenic phagocytic activity were associated with advanced rather than early stages of tumor growth, possibly reflecting metastatic spread.

One of the obvious questions concerns the possibility of tumor cells' possessing phagocytic activity. However, this can be readily ruled out for two reasons: first, appropriate studies failed to disclose any sizable radioactivity in the lymphomas of rats injected with radiogold, and, second, the extensive

### TABLE 2

**Effect of Lewis Lymphoma on Liver and Spleen**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Group</th>
<th>No. Rats</th>
<th>Percent Body Weight Liver</th>
<th>Percent Body Weight Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Control</td>
<td>26</td>
<td>3.61</td>
<td>0.16</td>
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<tr>
<td>Tumor</td>
<td>34</td>
<td>4.97</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Control</td>
<td>41</td>
<td>3.94</td>
<td>0.16</td>
</tr>
<tr>
<td>Tumor</td>
<td>45</td>
<td>5.37</td>
<td>0.95</td>
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</tbody>
</table>

### TABLE 3

**Effect of Progressive Tumor Growth on Hepatic and Splenic Uptake of Radiogold and on Weights of Liver and Spleen**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Days after Transplantation</th>
<th>Group</th>
<th>No. Rats</th>
<th>Percent Inj. Au¹⁹⁸/ gm. of Liver (Mean ± S.D.)</th>
<th>Percent Inj. Au¹⁹⁸/ gm. of Spleen (Mean ± S.D.)</th>
<th>Percent Body Weight Liver</th>
<th>Percent Body Weight Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>6</td>
<td>Control</td>
<td>6</td>
<td>0.92 ± 0.44</td>
<td>&gt;0.5</td>
<td>1.24 ± 0.40</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tumor</td>
<td>9</td>
<td>0.91 ± 0.23</td>
<td>0.67 ± 0.23</td>
<td>2.25 ± 0.88</td>
<td>0.69 ± 0.11</td>
<td>&lt;0.01</td>
<td>5.48</td>
</tr>
<tr>
<td>13</td>
<td>Control</td>
<td>4</td>
<td>1.84 ± 0.50</td>
<td>&lt;0.01</td>
<td>2.25 ± 0.88</td>
<td>&lt;0.01</td>
<td>5.48</td>
</tr>
<tr>
<td>Tumor</td>
<td>7</td>
<td>3.07 ± 0.45</td>
<td>0.69 ± 0.11</td>
<td>2.03 ± 0.98</td>
<td>0.47 ± 0.11</td>
<td>&lt;0.01</td>
<td>6.74</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>Control</td>
<td>6</td>
<td>2.61 ± 0.51</td>
<td>&gt;0.5</td>
<td>1.75 ± 0.56</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Tumor</td>
<td>8</td>
<td>2.38 ± 0.87</td>
<td>2.38 ± 0.87</td>
<td>2.38 ± 0.50</td>
<td>0.47 ± 0.11</td>
<td>&lt;0.01</td>
<td>6.74</td>
</tr>
<tr>
<td>13</td>
<td>Control</td>
<td>7</td>
<td>2.18 ± 0.50</td>
<td>&gt;0.01</td>
<td>2.38 ± 0.87</td>
<td>&lt;0.01</td>
<td>4.03</td>
</tr>
<tr>
<td>Tumor</td>
<td>6</td>
<td>4.20 ± 1.67</td>
<td>&lt;0.05</td>
<td>2.38 ± 0.87</td>
<td>0.47 ± 0.11</td>
<td>&lt;0.01</td>
<td>4.03</td>
</tr>
</tbody>
</table>
tumor involvement of the spleen as demonstrated histologically was accompanied by decreased uptake of radiocolloid, whereas in the liver the opposite effect took place.

The experimental data presented do not permit a decision whether the increased uptake of radiocolloid in the liver of lymphoma-bearing rats resulted from increased phagocytic activity of individual macrophages, reflecting a qualitative change on the cellular level, or from a numerical increase of macrophages, or both. Review of histologic liver preparations suggested proliferation of littoral (Kupffer) cells in tumor-bearing rats, but because of the diffuse infiltration of the organ with tumor cells this can be offered only as tentative interpretation. A more clear-cut answer to this question may be expected from radioautographic studies which are presently being planned.

A crucial question concerns the significance of the observations made by us in the Lewis lymphoma-bearing rat for other host-tumor systems. A partial answer was furnished by experiments in which we estimated the uptake of radiogold in livers and spleens of Sprague-Dawley rats with Walker carcinosarcoma: while there was a trend toward higher uptake of radiocolloid in the livers of tumor-bearing rats, the findings were much less consistent and pronounced than in rats with Lewis lymphomas.

Furthermore, in previous (unpublished) studies by methods similar to those described in this paper, we were unable to observe any significant changes of phagocytic activity in liver and spleen of mice bearing either spontaneous or transplanted leukemias and carcinomas. Of particular interest are also studies of immune responses in tumor-bearing rats to be presented in detail in a separate communication:1 when sheep red cells were injected into Lewis rats with lymphomas, antibody levels in tumor-bearing rats were significantly depressed in comparison to those observed in tumor-free control rats. The stages of tumor growth in the rats studied for immunologic or phagocytic activity, respectively, were quite similar.

Argus and co-workers (1) have reported that S\textsuperscript{35}-labeled fluorene-2,7-di(sulfamido-2'-naphthalene), after intravenous injection, localized in phagocytes of liver and spleen and was present in larger amounts in organs of tumor-free CAF\textsuperscript{1}, and CSH mice than in organs of mice bearing transplanted squamous-cell or adenocarcinomas, respectively. In subsequent work these authors (2) demonstrated a similar depression in the uptake in liver, spleen, and kidney of tumor-bearing mice given injections of S\textsuperscript{35}-labeled biphenyl-4,4'-di(sulfamidobenzene-4-sulfonamide). On the other hand, two other groups of workers investigating phagocytosis in tumor-bearing animals obtained results similar to those found by us in rats with lymphomas. Halpern, Biozzi, and associates (8, 5) investigated reticuloendothelial phagocytic activity by means of their classic technic of clearance rate of intravenously injected India ink in rats with transplanted Guérin tumors and mice with Ehrlich carcinomas. In rats with Guérin epitheliomas increased phagocytic activity was already noted 8 days after tumor inoculation, and this stimulation became even more pronounced with advancing tumor growth. In mice with Ehrlich carcinomas inoculated subcutaneously, no effect on reticuloendothelial activity was demonstrable, whereas intravenous tumor inoculation produced a transient slight increase of phagocytosis, and depression of phagocytosis resulted from the presence of Ehrlich ascites carcinoma. The authors (3) suggested that this difference in effects on reticuloendothelial phagocytosis of different types and different sites of tumor growth may be related to the fact that the Guérin rat tumor has the property of metastasizing early and regularly, whereas subcutaneously growing Ehrlich carcinoma is a purely local growth. Accelerated clearance of intravenously injected carbon suspensions, resulting mainly from phagocytosis in Kupffer cells, was also reported by Old and co-workers (8, 9) for mice with a variety of transplantable tumors including Sarcoma 180 and Ehrlich carcinoma, or infected with the Friend leukemia virus. It is of particular interest, and in agreement with our findings in lymphoma-bearing Lewis rats, that in such mice, simultaneously with the increased phagocytic activity, the formation of hemolysin for sheep red cells was sharply reduced (8).

In spite of the fact that considerable additional work will be required to permit definitive statements concerning the interrelationship of neoplastic growth and reticuloendothelial function, at this time a few tentative generalizations may be offered: (a) reticuloendothelial phagocytosis in tumor-bearing animals may be increased or decreased, with the direction and extent of the change probably dependent on one, several, or all of the following variables: animal species, type and site of tumor, stage of tumor growth, type of colloid, method of assay; (b) phagocytic activity may be increased in reticuloendothelial cells of one organ, and decreased or unchanged in those of another, as was shown in the present study for hepatic and splenic uptake of radiogold in rats with Lewis lymphoma; (c) tumor-bearing animals in which increased reticuloendothelial phagocytosis is

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1 K. Stern and I. Davidsohn, manuscript in preparation.
observed may at the same time exhibit decreased antibody formation, a biologic function presumably involving reticuloendothelial tissues. From these considerations it is obvious that to speak of hyperfunction or hypofunction of the reticuloendothelial system in neoplasia—or probably any other condition—would be a misleading oversimplification. Hence, as has been elaborated in more detail in another context (13), one should at present merely refer to disturbances of certain reticuloendothelial functions the causal relationship of which to cancer is still in need of a great deal of continued experimental investigation.

REFERENCES
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