Experimental Studies of Factors Influencing Hepatic Metastases

X. Effect of Reticuloendothelial Stimulation

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SUMMARY

Effective reticuloendothelial stimulation produced by Profernin prior to the intraportal injection of Walker carcinoma cells resulted in a twofold increase in the number of animals exhibiting artificially induced metastases. In addition, the degree of hepatic involvement with tumor was approximately twice as great in these animals as in untreated controls. Although similar reticuloendothelial stimulation resulted from appropriate injections of BCG or endotoxin, no effect on the incidence or degree of hepatic involvement by these lesions was observed. All agents utilized were associated with alterations of serum glutamic oxalacetic and/or pyruvic transaminases, but only Profernin produced sinusoidal compression due to swelling of Kupffer cells. This information, together with the previously noted augmentation of such lesions observed following reticuloendothelial blockade, is considered as strong evidence against a functional role of the reticuloendothelial system in the process of metastases in the experimental model utilized. It also indicates the significance of sinusoidal compression with resultant trapping of tumor cells within the liver in accounting for the effects noted.

During the course of studies concerned with factors which might influence the development of artificially induced hepatic metastases in rats following the direct intraportal injection of known numbers of Walker 256 carcinoma cells it was observed that effective blockade of the reticuloendothelial system, as measured by blood clearance of either carbon or Evans blue, with shellac-free India ink or Thorotrast resulted in an increased incidence as well as size of such lesions (6). Since this effect was most pronounced when reticuloendothelial blockade was induced several hours before and within 24 hours after the injection of tumor cells, it was considered that this type of reticuloendothelial interference not only affected the growth but also capacity to take of the injected tumor cells. Although these findings may be attributed to the inhibition of a function of the reticuloendothelial system concerned with host resistance to the development of these neoplasms, other observations at that time tended to minimize the significance of this possibility. The marked swelling of the Kupffer cells with resultant sinusoidal compression following the injection of these blocking agents appeared analogous to our previous results of an increased incidence of such lesions in rats subjected to vena cava ligation (4). In addition, it was observed that these colloidal agents produced a significant elevation of serum glutamic oxalacetic and pyruvic transaminases which was considered indicative of hepatic parenchymal damage, a situation also demonstrated previously to be associated with an augmentation of such artificially induced hepatic metastases in the experimental model employed (3). However, in an attempt to delineate which of these factors might be principally involved in accounting for the results obtained as well as to gain further insight concerning a possible role of the reticuloendothelial system in this phenomenon, it appeared worth while to explore the effects of reticuloendothelial stimulation on the incidence of artificially induced hepatic metastases, since, indeed, if such a resistance factor were operative, one might expect to find the converse to that observed following reticuloendothelial blockade—notably a decrease in such lesions.

MATERIALS AND METHODS

Adult, female, Sprague-Dawley rats weighing approximately 150 gm. were utilized in all experi-
ments. Animals were maintained on water and Purina chow ad libitum.

Eighty-nine rats received 2 mg/100 gm of Proferrin (saccharated iron oxide) intravenously into a femoral vein daily for 4 days. Fifty of these received an intraportal injection of 5,000 Walker 256 carcinoma cells at 2 days, and 39 a similar tumor cell injection at 5 days following the last dose of Proferrin. The method of preparation and technic of injection of Walker tumor cells have been described previously (5).

One hundred sixteen rats received a single intraperitoneal injection of 0.5 mg. of BCG prepared by Dr. Hubert Bloch, Department of Microbiology, University of Pittsburgh. Forty-five of these were given injections of tumor cells as above at 2 days, and 71 at 16 days after BCG injection.

Forty-four rats received five successive daily injections of 50, 100, 150, 200, and 200 μg., and 29 a similar course of 100, 200, 300, 400, and 400 μg. of endotoxin lipopolysaccharide of E. coli (055:B5). Twenty-nine rats receiving the former were given injections of tumor cells as above at 2 days, and fifteen at 6 days after the last dose of endotoxin. Rats receiving the larger dose of endotoxin were given injections of tumor cells 2 days after the last dose.

Comparable numbers of untreated controls in each group received similar injections of tumor cells. All animals receiving tumor cells were sacrificed 14 days after injection, and the incidence of animals with hepatic tumors and the degree of hepatic involvement with such lesions were noted. The latter was arbitrarily graded 1—3+.

The clearance rate of carbon from the blood was determined according to the method of Biozzi and associates (1) on 32 normal, untreated rats receiving one intravenous injection of 32 mg/100 gm of shellac-free India ink, as well as on rats not receiving tumor cell injections at 1, 2, 3, 7, and 14 days following Proferrin; 2, 4, 8, 16, and 32 days following BCG; and 2, 4, 6, and 8 days after the last injection of the small dose of endotoxin and at 2 days following the larger dose.

Serum glutamic oxalacetic (SGOT) and pyruvic (SGPT) transaminases were determined according to the method of Reitman and Frankel (8) on additional groups of rats similarly treated with Proferrin, BCG, and endotoxin in which no tumor cell injections of carbon clearance studies were performed. The time intervals of study of these enzymes for the most part coincided with those utilized for the latter examination.

Blocks of liver, spleen, lung, kidney, adrenal, and heart were examined from all animals utilized for the evaluation of carbon clearance, SGOT, and SGPT in untreated rats as well as those after the administration of either Proferrin, BCG, or endotoxin and from selected animals that received an injection of tumor cells. All tissues were fixed in formalin and processed and embedded in the usual manner. Sections were stained with hematoxylin and eosin and, in those animals receiving Proferrin, by the Perl's method for iron.

RESULTS

As indicated in Charts 1 and 2 the clearance of carbon from the blood was augmented at all time intervals studied following the administration of

![Chart 1](image-url)

**Chart 1.**—Blood clearance of carbon following injections of Proferrin.

Proferrin and 2, 4, and 8 days after BCG. A similar effect was observed with the larger dose of endotoxin at 2 days, and apparent reticuloendothelial stimulation was observed for 6 days after the smaller dose of this agent (Chart 3). Histological examination failed to reveal discernible alteration of hepatic sinusoidal cells in animals treated with endotoxin or BCG (Fig. 1), yet carbon deposition subsequent to clearance studies in similarly treated rats appeared greater than in the livers of controls. Spleens from these animals exhibited moderate hyperplasia of reticuloendothelial cells, and carbon appeared in this organ in greater amounts than in controls. No effect of endotoxin or BCG was noted in other tissues except for the presence of small focal granulomas in the spleen and lungs of two animals receiving the latter. Except for the presence of a trace of carbon in the kidneys of a few animals this material was

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1 Merck, Sharp & Dohme, Inc.
2 Difco.
3 C 11/1481 A. Gunther Wagner, Hanover, Germany.
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found deposited exclusively in the liver and spleen of animals treated with endotoxin, BCG, and subjected to carbon clearance. On the other hand, animals treated with Proferrin exhibited swelling of the Kupffer cells which contained stainable iron within their cytoplasm with resultant sinusoidal compression (Fig. 2). Splenic reticular cells also contained demonstrable iron. Despite the accumulation of particulate iron within the liver and spleen, carbon appeared confined to these organs with rare exception (traces in endothelial cells of glomeruli and pulmonary arterioles of a rare animal) in the tissues from rats treated with Proferrin and subjected to carbon clearance studies. As with rats treated with endotoxin and the majority receiving BCG, no histologic alterations were apparent in the other tissues of Proferrin-treated rats.

Table 1 presents the results of the effect of

<table>
<thead>
<tr>
<th>Agent</th>
<th>Time inc. Tumor cells* (Days)</th>
<th>No. Rats</th>
<th>No. +</th>
<th>Per cent +</th>
<th>Degree of hepatic involvement (no.)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1+</td>
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<td>47</td>
<td>40</td>
<td>85</td>
<td>11</td>
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<td></td>
<td>50</td>
<td>22</td>
<td>44</td>
<td>21</td>
</tr>
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<td>5</td>
<td>41</td>
<td>31</td>
<td>76</td>
<td>8</td>
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<tr>
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<td></td>
<td>39</td>
<td>14</td>
<td>36</td>
<td>12</td>
</tr>
<tr>
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<td>2</td>
<td>44</td>
<td>18</td>
<td>41</td>
<td>18</td>
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<td>Controls</td>
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<td>45</td>
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<td>74</td>
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<td>71</td>
<td>27</td>
<td>38</td>
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<td>Endotoxin (100–400 μg.) controls</td>
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<td>16</td>
<td>55</td>
<td>16</td>
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<td>26</td>
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<td>54</td>
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<tr>
<td>Endotoxin (50–200 μg.) controls</td>
<td>6</td>
<td>14</td>
<td>8</td>
<td>57</td>
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* Days after last injection of agent.
reticuloendothelial stimulation upon the incidence of artificially induced hepatic metastases after the injection of known numbers of tumor cells. Treatment with Proferrin resulted in a twofold increase in the number of animals with hepatic tumors as well as degree of hepatic involvement as compared with controls, whereas BCG (2 days after last dose) and endotoxin did not appear to influence their incidence or degree of hepatic involvement by these lesions. The results of tumor cell injection 16 days after BCG are not striking and of questionable significance. Comparison of the times of injection of tumor cells with the measurements of carbon clearance as depicted in Charts 1, 2, and 3 indicates that these injections were made at periods when the reticuloendothelial system was stimulated, and in the instance of Proferrin administration this stimulation existed throughout the entire experimental period of 14 days.

As indicated in Charts 4, 5, and 6 serum glutamic oxalacetic transaminase was elevated in rats treated with Proferrin for 8 days subsequent to the last injection of this agent. SGOT was significantly elevated 16 days after BCG injection.
and SGPT at 32 days after injection. Although SGOT was elevated for 8 days after both doses of endotoxin the larger dose resulted in a significant decrease of SGPT for a similar period.

**DISCUSSION**

The results of this study indicate that effective stimulation of the reticuloendothelial system either increases the incidence as well as size and degree of hepatic involvement of artificially induced hepatic metastases in the case of Proferrin administration or, as with endotoxin or BCG, fails to influence the incidence or degree of hepatic involvement by such lesions. The effect of Proferrin in this regard is analogous to our previous observations when the reticuloendothelial system was effectively blocked by either India ink or Thorotrast (6) and is inconsistent with considerations suggesting a significant role of the reticuloendothelial system in the development of these hepatic lesions, at least in the experimental model utilized. It appears worthy of note that the reticuloendothelial system was stimulated not only at the time of tumor cell injection but also, in the instances of Proferrin and BCG administration, throughout the entire experimental period, and for at least half of this time when endotoxin was employed. It appears unlikely that the injection of the tumor cells may have altered this state of the reticuloendothelial system, since we have found no effect of such tumor growth, under identical experimental conditions, on the functional state of this system (6).

Our findings concerning the effect of reticuloendothelial stimulation on hepatic metastases are unlike those reported by Old and associates (8), who observed such stimulation to protect Swiss mice against inoculations of Sarcoma 180 and Carcinoma 175. However, the divergent findings may be due to differences in the experimental conditions of the two studies—notably species and tumor type utilized, as well as dosage of BCG and site of tumor inoculation. That certain inconsistencies concerning the role of the reticuloendothelial system and resistance do exist is evidenced by the study of Böhme, who observed that mice genetically resistant to *S. typhimurium* exhibit less reticuloendothelial activity when challenged than do susceptible mice (9). This also indicates that other factors may be concerned in producing the various effects often generally ascribed to reticuloendothelial activity. Indeed, Old and associates (7) noted that the importance of reticuloendothelial function in accounting for their results could not be accepted without reservation, since the agents utilized certainly produce widespread physiologic alterations, any one of which might have played a role.

The demonstration that stimulating injections of Proferrin result in marked sinusoidal compression due to swelling of Kupffer cells and elevation of serum transaminases which we have interpreted, in the absence of overt changes in other tissues, as indicative of hepatic damage appear to represent significant factors worthy of consideration in this regard. We have noted previously that a variety of agents utilized for the stimulation or blockage of the reticuloendothelial system appear to have a comparable effect on serum transaminases in the rat as well as rabbit. The significance of hepatic trauma in enhancing tumor growth has been emphasized previously by us (3). It has also been indicated that the sinusoidal compression following the administration of such agents as Thorotrast and India ink as well as Proferrin in this study appears analogous to studies in which vena caval ligation was attended by an increased incidence of hepatic metastases (4). The primacy of this mechanism in accounting for the results obtained in this study as well as that concerned with reticuloendothelial blockade is further supported by the recognition that no perceptible alterations by light microscopy were evidenced in the sinusoidal cells following BCG and endotoxin administration. Both of these agents although stimulating the reticuloendothelial system failed to influence the development of hepatic metastases, although, in the case of endotoxin, serum glutamic oxalacetic transaminase was elevated. The effect of BCG upon these enzymes at 16 and 32 days following injection does not appear within the time period

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**Fig. 1.**—Appearance of liver of rat sacrificed 2 days after last stimulating injection of endotoxin. The sinusoids appear patent. X 800.

**Fig. 2.**—Appearance of liver of rat sacrificed 2 days after last stimulating injection of Proferrin. The sinusoids are blocked owing to swelling of Kupffer cells containing iron (appears black). Iron stain, X 800.
of the experiment and might be considered of little consequence in this regard. We have no explanation for the dichotomy in serum glutamic oxaloacetic and pyruvic transaminase values following endotoxin administration and can find no precedence for a statistically significant low value for this enzyme. Whether this effect is due to decreased production, release, or destruction appears speculative. It does appear, however, from the results obtained that sinusoidal compression with trapping of tumor cells that usually transgress the liver, as noted by Zeidman and associates (9), represents the prime factor in accounting for the increased incidence of hepatic metastases observed after the use of some agents which stimulate or block the reticuloendothelial system.

REFERENCES

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