Experimental Studies of Factors Influencing Hepatic Metastases

VII. Effect of Reticuloendothelial Interference*

EDWIN R. FISHER, M.D.,† AND BERNARD FISHER, M.D.

(Departments of Pathology and Surgery, University of Pittsburgh, Pittsburgh, Pa.)

SUMMARY

The administration of a saturating dose of either India ink or Thorotrast prior to or within 24 hours after the intraportal injection of Walker 256 carcinoma cells resulted in an increased incidence and size of artificially induced hepatic metastases. The enhancing effect of reticuloendothelial interference on tumor “takes” may be explained as being due to a trapping of injected tumor cells as a result of marked swelling of Kupffer cells with sinusoidal occlusion and hepatic parenchymal damage which was observed after the administration of both Thorotrast and India ink. These mechanisms abrogate the necessity of relating such an effect to an inhibitory action of these agents on a specific activity of the reticuloendothelial system associated with tumor resistance.

Established hepatic metastases of Walker 256 carcinoma fail to influence the phagocytic activity of the reticuloendothelial system as disclosed by a normal blood clearance of carbon particles. Similarly carbon clearance was normal when observed in animals 14 days after tumor cell injection but in whom hepatic metastases were not demonstrable.

The resistance of experimental animals to the transplantation and metastases of some neoplasms has been attributed by many to an immune response on the part of the host. This consideration has gained support, at least in part, from the results of studies which have revealed an enhancement of these phenomena following interference or blockade of reticuloendothelial activity by the administration of colloidal particles or other agents. Foulds (12) noted a marked increase in the incidence of artificially induced metastases in various sites following the injection of suspensions of Brown-Pearce carcinoma cells in animals treated with trypan blue. Better growth as well as the capacity to transfer some experimental tumors to otherwise resistant strains were observed by Andervont (1) after the administration of trypan blue. Dibenzanthracene-induced carcinomas have also been recognized as growing earlier in vitally stained animals than in untreated controls (14). Ghose (14) similarly noted a statistically greater incidence of metastases in C3H mice treated with trypan blue than in controls. Because trypan blue exhibited little effect on the stroma of the primary growths and on the growth rate of the tumors in vivo and in vitro, he concluded that reticuloendothelial blockade resulting from the administration of the vital dye prevented the formation of antitumor antibodies and thus allowed for more widespread dissemination. On the other hand, Kaliss and Borges (18) failed to observe any enhancement of the growth of an anaplastic carcinoma in mice following the administration of trypan blue. Bernard and associates (4) also found no effect of the administration of thorium dioxide on the incidence of metastases from the Walker tumor in the rat. The possibility that the lack of effect of reticuloendothelial blockade recorded by these investigators may be due to inadequate administration of the blocking agents has been suggested (13). Cohen and Cohen (7) have suggested that the enhancing effects on tumor growth observed following reticuloendothelial blockade may be due, in some instances, to the activation of latent infection which in itself may be carcinogenic or play a role in neoplastic development and dissemination.

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† Senior Research Fellow, U.S.P.H.S.

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Although the inhibitory effect of reticuloendothelial blockade on the formation of bacterial precipitins (17), agglutinins (24), and complement (16) has been demonstrated, comprehensive reviews (21, 22) of the role of immunologic mechanisms in neoplastic growth have offered little optimism, at least at present, to support a relationship between the latter and antibody formation. It also appears worthy of note that, in the studies cited above concerning the effect of reticuloendothelial blockade on neoplastic growth and its dissemination, measurements of the degree of blockade—or indeed its actual existence under the experimental conditions employed—were not performed.

### MATERIALS AND METHODS

Adult female Sprague-Dawley rats weighing approximately 150 gm. were utilized in all experiments. Animals were maintained on water and Purina Laboratory Chow ad libitum.

As indicated in Tables 1 and 2, rats received single intravenous injections of 32 mg/100 gm of shellac-free India ink\(^1\) or 0.5 ml. of 24 per cent Thorotrast/150 gm. at various time intervals prior to or after the direct intraportal injection of 5,000 Walker 256 carcinoma cells. A comparable number of rats received only an injection of a similar number of tumor cells, and at least ten received only India ink or Thorotrast. The method of preparation of tumor cells for intraportal injection has been described in detail previously (11). All animals receiving tumor cells were sacrificed 14 days after injection, and the incidence and size of hepatic tumors were observed. The latter was subjectively graded as 1+, 2+, or 3+. Blocks of liver, spleen, lung, kidney, and adrenal were obtained at 2, 24, and 72 hours and at 1 and 2 weeks from animals that received only India ink or Thorotrast and at 14 days from animals receiving injections of tumor cells. These were fixed in formalin and processed in the usual manner and

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**TABLE 1**

<table>
<thead>
<tr>
<th>Time of India Ink Injection*</th>
<th>Group</th>
<th>No. Rats</th>
<th>Per cent +</th>
<th>Size of Tumor</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>+1</td>
<td>+2</td>
</tr>
<tr>
<td>2 hr. prior</td>
<td>India ink</td>
<td>30</td>
<td>87</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>30</td>
<td>30</td>
<td>9</td>
</tr>
<tr>
<td>1 hr. after</td>
<td>India ink</td>
<td>30</td>
<td>100</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>20</td>
<td>37</td>
<td>11</td>
</tr>
<tr>
<td>6 hr. after</td>
<td>India ink</td>
<td>36</td>
<td>64</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>40</td>
<td>40</td>
<td>16</td>
</tr>
<tr>
<td>1 day after</td>
<td>India ink</td>
<td>29</td>
<td>59</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>30</td>
<td>37</td>
<td>9</td>
</tr>
<tr>
<td>1 week after</td>
<td>India ink</td>
<td>28</td>
<td>46</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>29</td>
<td>34</td>
<td>10</td>
</tr>
</tbody>
</table>

* In relation to tumor cell injection.

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1 C 11/1431 A, Gunther Wagner, Hanover.
embedded in paraffin. Sections were stained with hematoxylin and eosin or examined unstained.

The clearance rate of carbon from the blood was determined according to the method of Biozzi and associates (6) on ten normal, untreated animals receiving one intravenous injection of ink (32 mg/100 gm) and a similar number that received second and third doses of ink revealed carbon particles within endothelial cells of renal glomeruli, pulmonary capillaries, and adrenal sinusoids as well as Kupffer and splenic sinusoidal cells. The degree of extrahepatic deposition of carbon appeared greatest in animals receiving three doses. The administration of Thorotrast also resulted in delayed second and third injections at subsequent 2-hour intervals. The effect of injections of Thorotrast on plasma clearance of Evans blue (T-1824) was determined according to the methods described by Hyman and Paldino (15) with 0.3 ml. of a 5 per cent solution/150 gm. Clearance studies were also performed on five rats with India ink and Evans blue administered 1, 5, and 24 hours and at 1 and 2 weeks after the intraportal injection of tumor cells. Tissues from these animals were processed and stained as noted above.

Serum glutamic oxalacetic and pyruvic transaminase determinations were performed on groups of ten rats sacrificed 24 and 48 hours after receiving single doses of Thorotrast and India ink.

RESULTS

As indicated in Chart 1 clearance of carbon from the blood was delayed when a second dose of this material was injected 2 hours following its initial administration. However, a subsequent dose 2 hours later resulted in more rapid clearance of carbon than noted previously. Microscopic examination of tissues from animals receiving a single injection of ink disclosed carbon deposits in numerous Kupffer cells and occasional splenic sinusoidal cells. Those from rats receiving second

### TABLE 2

**EFFECT OF RETICULOENDOTHELIAL “INTERFERENCE” WITH THOROTRAST UPON EXPERIMENTAL HEPATIC METASTASES**

<table>
<thead>
<tr>
<th>TIME OF THOROTRAST INJECTION*</th>
<th>GROUP</th>
<th>NO. RATS</th>
<th>PER CENT</th>
<th>SIZE OF TUMOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+1</td>
</tr>
<tr>
<td>6 hr. prior</td>
<td>Thorotrast</td>
<td>39</td>
<td>87</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>48</td>
<td>42</td>
<td>13</td>
</tr>
<tr>
<td>1 hr. after</td>
<td>Thorotrast</td>
<td>40</td>
<td>83</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>40</td>
<td>38</td>
<td>14</td>
</tr>
<tr>
<td>6 hr. after</td>
<td>Thorotrast</td>
<td>39</td>
<td>84</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>39</td>
<td>41</td>
<td>12</td>
</tr>
<tr>
<td>1 day after</td>
<td>Thorotrast</td>
<td>52</td>
<td>54</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>54</td>
<td>39</td>
<td>20</td>
</tr>
<tr>
<td>1 week after</td>
<td>Thorotrast</td>
<td>29</td>
<td>31</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>30</td>
<td>26</td>
<td>6</td>
</tr>
</tbody>
</table>

* In relation to tumor cell injection.

![Chart 1](https://example.com/figure1.png)

**CHART 1.**—Blood clearances after three doses (82 mg/100 gm) of India ink repeated at 2-hour intervals (average of ten rats).
clearance of carbon and T-1824 from the blood 6 hours after injection, as depicted in Chart 2. Moderate deposition of carbon was observed in extrahepatic tissues following the administration of Thorotrast and carbon particles (Fig. 1). Only a few particles were observed within hepatic Kupffer cells. Examination of tissues from rats 2, 24, and 72 hours and 1 and 2 weeks following a single injection of ink disclosed the carbon particles in large quantity up to 72 hours. At 2 weeks only moderate numbers of hepatic Kupffer cells contained carbon particles (Fig. 2). No extrahepatic deposits were evident in these animals, although they were apparent at 2 weeks in the extrahepatic tissues of animals receiving Thorotrast followed by an injection of ink 6 hours later. Kupffer cells in all animals receiving ink or Thorotrast were notably swollen, and the hepatic sinusoids appeared compressed (Figs. 3 and 4). This was most evident in rats examined at 2, 24, and 72 hours following the injection of India ink and 6, 24, 72 hours and 1 week after administration of Thorotrast. Carbon clearance appeared normal when performed 14 days after the injection of tumor cells in rats in whom hepatic metastases were evident, as well as those few in whom such lesions were not apparent.

Tables 1 and 2 present the effect of the administration of India ink and Thorotrast on the incidence as well as size of artificially induced hepatic metastases. A marked increase in size and incidence was observed when animals were subjected to these agents either prior to or within 24 hours after the injection of tumor cells. Tumor thrombi were conspicuous in sections of liver from these animals (Fig. 2).

Chart 3 reveals that the serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) levels were significantly elevated 24 hours following the administration of either Thorotrast or India ink. SGPT was also elevated 48 hours following the injection of these agents, although SGOT values appeared within the normal range at this time.

**DISCUSSION**

The results of this study indicate that temporary saturation of the reticuloendothelial system, particularly the hepatic Kupffer cells, produces an increased incidence and size of hepatic metastases artificially induced by the intraportal injection of Walker 256 carcinoma cells. Although it is difficult to precisely assess the functional status of the reticuloendothelial system, the delayed blood clearance of carbon particles injected 2 hours after the administration of India ink or 6 hours after Thorotrast is in keeping with previous studies relating such an effect to reticuloendothelial saturation (6). Further support for such an interpretation is offered by the observations that the dose of blocking agents utilized produced deposits of carbon within endothelial cells of extrahepatic tissues—notably, renal glomeruli and splenic sinusoids. This extrahepatic distribution of carbon has been attributed to reticuloendothelial saturation by Benacerraf and associates (8). Since the most significant increase in incidence and size of hepatic metastases observed in rats subjected to blocking agents was apparent when these were administered prior to or within 24 hours after the injection of tumor cells, it appears reasonable to conclude that these modalities influence the "takability" of tu-
further injections of India ink which conceivably of others concerning its achievement (2). Blood ade during the period of these experiments is India ink disclosed an accelerated clearance rate, worthy of note and reemphasizes the contentions producing a persistent reticuloendothelial block as well as their growth. The difficulty in might produce continuous reticuloendothelial saturation resulted in a high mortality. These con-
siderations suggest that the criticism of previous studies concerned with the effect of reticuloendo-
theelial blockade on tumor growth in which isolated injections of blocking agents have been employed may be unwarranted, particularly since those studies in which continuous blockade has been purported fail to mention any measure other than histological examination to assess the functional state of this system. The necessity for such evaluation has been emphasized previously (9). Because persistent blockade of the reticuloendo-
theelial system was not accomplished in this study, we have chosen to designate the effect of these blocking agents for the experimental period employed as reticuloendothelial "interference." That both India ink and Thorotrast had some effect on the reticuloendothelial system for as long as 2 weeks following injection was apparent from their presence in Kupffer cells of animals histologically examined at this time.

Although the increase in hepatic "takes" of intraportally injected tumor cells following the administration of India ink and Thorotrast might be attributed to their effectiveness in lowering a resistance to tumor cell implantation because of inhibition of reticuloendothelial activity, there has been no definitive evidence to indicate that this system possesses such a function. Further, if indeed such a function does exist, it is well recognized that the measurement of the phagocytic capacity of the reticuloendothelial system as performed here may not necessarily reflect the status of its other activities. On the other hand, this study has provided information which may be more significant in accounting for the results observed. Histologically, following the administration of India ink and Thorotrast, Kupffer cells appear markedly swollen, and as a result hepatic sinusoids are compressed. That such an effect may alter hepatic hemodynamics, resulting in a decrease in circulation through this organ, has been indicated by the studies of Bernick and associates (5) in rats receiving Thorotrast. Since Zeidman and associates (24) have demonstrated that tumor cells may transgress the liver it becomes apparent that the blocking agents employed might effectively promote trapping of injected tumor cells. This trapping effect also appears to be reflected by the conspicuous tumor thrombi observed within histologic sections of livers from rats subjected to blocking agents and injection of tumor cells. Ligation of the vena cava has also been observed in our laboratory to result in a marked increase in artificially induced hepatic metastases (9). In addition, this study has disclosed that the administration of India ink and Thorotrast results in hepatic parenchymal damage as indicated by significant elevations of serum glutamic oxalacetic and pyruvic transaminases. A relationship be-
tween hepatic trauma and an increased incidence and growth of artificially induced metastases has been previously recorded by us (8). The general significance of reticuloendothelial blocking agents in the production of hepatic damage is now under consideration.

It is of interest that no evidence of an effect on the phagocytic action of the reticuloendothelial system was apparent in rats with or without established hepatic metastases as disclosed by normal blood carbon clearance rates at 2 weeks after the injection of tumor cells. The failure to note alteration in the phagocytic action of the reticuloendo-
theelial system in this study in rats with well developed hepatic metastases is unlike the findings of Old and associates (19). They observed in creased carbon clearance during the rapid growth phase of a variety of transplantable and some spontaneous tumors of mice and rats but normal clearance with subsequent deterioration of the host. Rats with artificially induced hepatic metastases 2 weeks following tumor cell injection in the study reported herein, however, fail to exhibit overt evidence of a decline of general health and, with the dose of tumor cells utilized, not infrequently survive 3 or 4 weeks longer. Although no apparent explanation for this difference of the effect of tumor growth on reticuloendothelial activity is apparent, it is to be noted that the experimental conditions are not anal-
ogous, since we have been concerned with the effect of hepatic involvement by neoplastic growth rather than the effect of local growths on the phagocytic activity of the reticuloendothelial system.

REFERENCES


Fig. 1.—Deposition of carbon particles in glomerular endothelial cells after injection of India ink in rat 6 hours after administration of Thorotrast. X245.

Fig. 2.—Section of liver obtained 14 days after the injection of tumor cells and India ink. Some carbon particles are still evident within Kupffer cells. Tumor thrombi are conspicuous. X75.

Figs. 3 and 4.—Appearance of livers 6 hours after Thorotrast (X370, Fig. 3) and 2 hours after India ink (X345, Fig. 4). Kupffer cells are swollen with evidence of sinusoidal compression.
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