Reticuloendothelial Activity during the Growth of Rat Sarcomas

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INTRODUCTION

The immune functions of the body in cancerous states have been investigated in several ways. Southam (8) has shown depression of the delayed dermal hypersensitivity response, of macrophage migration, and of homograft rejection in patients with advanced tumors, but he reports no change in the serum antibody response. Hughes and MacKay (5) confirmed depression of delayed hypersensitivity, but Lytton et al. (6) have shown a weakening of the response to tetanus toxoid.

Increase in spleen size has been noted to accompany the growth of some tumors in small animals (11); histology of these spleens resembles that seen during rejection of a homograft (11). Increased splenic activity during tumor growth is held to be a reaction to the presence of tumor-specific antigen (10, 11).

Little is known of the participation of the reticuloendothelial system in the response to tumor growth. In this paper the clearance of colloidal carbon in rats during the growth of methylcholanthrene sarcomas is reported. The tumors were chosen for study because they are easily induced and transplanted, they grow regularly at a predictable rate, and are easily measured; they may be kept growing to a large size. The use of colloid gives a quantitative measure of reticuloendothelial activity, that reflects macroscopic and microscopic changes occurring in the spleens of rats bearing methylcholanthrene-induced sarcomas.

SUMMARY

The clearance of colloidal carbon from the blood stream of rats bearing methylcholanthrene-induced sarcomas has been studied. Growth of both primary and isotransplanted tumors greatly enhances the clearance of carbon. This effect is greater with increasing tumor size. The uptake of radioactive colloidal gold has been studied following intravenous injection. The final distribution of colloid is not altered by tumor growth. The use of colloid gives a quantitative measure of reticuloendothelial activity, that reflects macroscopic and microscopic changes occurring in the spleens of rats bearing methylcholanthrene-induced sarcomas.

MATERIALS AND METHODS

Tumors were induced by the subcutaneous injection of methylcholanthrene in females of an inbred strain of Wistar rats (obtained originally from the Laboratory Animal Centre and maintained by strict brother-sister mating). Tumors were transplanted by implanting biopsies subcutaneously with a 10-gauge trocar. Tumors were examined histologically, and all were highly cellular spindle cell sarcomas; they were measured daily in two diameters at right angles.

Colloidal carbon was used as the test colloid, because this gives the most consistent results in small animals, radioactive colloids being more difficult to standardize for particle size.

The uptake of colloidal carbon in the animal under test was studied by injecting a specially prepared carbon preparation, of constant particle size (close to 250 Å), into the femoral vein of the anesthetized animal in a dose of 30 mg per 100 gm body weight. This carbon preparation was prepared by the method of Biozzi et al. (2) from Gunther Wagner C11/1431A. Blood samples of 0.1 ml were taken from each animal under anesthetic from the retroorbital plexus at one-minute intervals following injection for a period of 10 minutes; the animal was then killed. The carbon content of the samples was estimated in a Unicam SP 600 spectrophotometer, at 675 trip, after hemolysis of the sample by dilution into 4 ml of 0.1% sodium carbonate.

A graph of clearance was obtained for each animal by plotting the spectrophotometer reading for the sample against the time after injection that the sample was taken (Chart 1). From this graph the T/2 of clearance from the plasma of the preparation in the tested animal could be obtained.

The clearance of carbon varies from preparation to preparation. Each of the three experiments reported below used a single carbon preparation. The mean T/2 in the control animals is taken as 100% in each experiment; this allows comparison with other experiments using different preparations of the colloid.

To study the organ distribution of injected colloid, a radioactive colloid was used. Colloidal radiogold (198 Au) of particle size 200—350 Å (Radiochemical Centre GCS-4P) was injected to a series of tumors and a series of control animals, in the same way in which colloidal carbon had been used. Five mg of gold, 0.5 μc, were injected into each animal in 0.5 ml; 15 minutes after injection the majority of the gold had been cleared from the blood stream, and the animal was killed. Pieces of approximately 1 gm were taken from liver, spleen, tumor, and muscle. These were weighed and counted for 198 Au in an Ecko well-type scintillation counter. The weights

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2 This paper forms part of a dissertation submitted by R. W. Blamey for the degree of M. D. at Cambridge University.

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Animals & Carbon Clearance

<table>
<thead>
<tr>
<th>Animals</th>
<th>Carbon clearance (T/2 as % of control)</th>
<th>Mean difference</th>
<th>Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tumors at 4 cm diam.</td>
<td>28.15</td>
<td>71.85 ± 4.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Controls</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isotransplanted tumors at 4 cm diam.</td>
<td>28.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isotransplanted tumors at 2 cm diam.</td>
<td>57.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lastly, in Table 1 the carbon clearance in animals with tumor isotransplants of only 2 cm mean diameter is shown. There is again a considerable increase in clearance, but it is not as great as that found with larger tumors.

Table 2 shows the mean uptake of colloidal gold, $^{198}$Au, in the livers and spleens of tumor-bearing and control animals. Although clearance is quicker in rats with tumors, the final distribution is the same. A very large tumor itself takes up less than 1% of the total injection. It is therefore neither uptake into the tumor nor altered distribution of colloid that explains the quicker clearance in tumor-bearing animals.

**DISCUSSION**

Clearance of colloid is held to be a means of measuring the activity of the reticuloendothelial system. It has been shown that in infection with BCG (7), in response to the administration of sheep red cells in sensitized animals (4), and during homograft rejection (3), the clearance reflects the activity of the reticuloendothelial system as a whole.

The response of the reticuloendothelial system, measured by colloid clearance, during the growth of tumors, has been variously reported. Old et al. (7) has shown stimulation of the system in mice receiving an isotransplanted sarcoma, after an initial depression; they found an increased clearance in mice with isotransplanted methylcholanthrene sarcomas and a slight

Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mean activity in whole organ (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor bearers</td>
<td>244</td>
</tr>
<tr>
<td>Non tumorous</td>
<td>259</td>
</tr>
<tr>
<td>Liver</td>
<td>6000</td>
</tr>
<tr>
<td>Tumor (4 cm diam.)</td>
<td>8220</td>
</tr>
</tbody>
</table>

Uptake of i.v. colloidal gold, $^{198}$Au, in tumor-bearing and control animals.

**RESULTS**

Table 1 shows, first, a series of animals with primary methylcholanthrene-induced tumors that had reached 4 cm mean diameter. The animals were each paired by weight (making allowance for the weight of the tumor) with a nontumorous control. A great increase of carbon clearance is seen in the tumor-bearing animals over that in the controls.

Secondly, Table 1 shows a series of animals bearing first generation methylcholanthrene-induced tumor isotransplants of 4 cm mean diameter, similarly paired with controls. The increase in carbon clearance of the tumor-bearing animals is very close to the result obtained in animals with primary tumors of the same size.

of the whole animal, and of liver, spleen, and tumor, were also determined. The total uptake per organ was then calculated.
increase in mice with “spontaneous” mammary tumors. Stern (9) used red cells as the assay colloid and found a lowered uptake of radioactivity in liver and spleen when the effects of several tumors were assessed.

This paper demonstrates a marked increase in the rate of uptake of colloid from the blood stream of rats when methylcholanthrene-induced sarcomas are growing. This increase is greater with increasing tumor size. It is a true increase in avidity of the reticuloendothelial system for colloid, for in the experiment using colloidal gold, the overall distribution of colloid was shown to be unaltered by tumor growth.

These findings correlate with our observations on spleen size and histology in rats bearing methylcholanthrene sarcomas (paper in preparation): spleen weights are increased and spleens are markedly more cellular in tumor bearers, and spleen weight increases with increasing tumor size. Thus colloid clearance, as an index of reticuloendothelial activity, gives a quantitative measure paralleling the observed spleen changes in response to these tumors.

It is possible that the increase in clearance is a reflection of the presence of tumor-specific antigen, known to be present in the tumors used. No depression of reticuloendothelial activity was seen, even though some of the tumors studied weighed up to a fifth of the body weights of the animal.

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REFERENCES

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