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Reticuloendothelial Function in Normal and Tumor-bearing Rats. Measurements with a Scintillation Camera Technique*

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Abstract—*The function of the reticuloendothelial system (RES) was evaluated after the inoculation of an experimental tumor in rats. Four groups were studied according to tumor size and site. Reticuloendothelial function was evaluated by measuring the biokinetics of a standardized [$^{99}Tc^{m}$]-sulfur colloid. Estimation of the uptake rate of the labeled colloid into the liver and other parts of the RES was performed through the use of a two-compartment model. Animals with small liver or subcutaneous tumors showed an increased activity of both the hepatic and the extrahepatic RES. Animals with large retroperitoneal tumors showed a significant decrease in the RE function of the liver. In these animals the function of the extrahepatic RES was not changed compared to controls but was, however, significantly decreased compared to animals with smaller tumors. The findings may reflect a difference in the impact of tumor size on RE function extra- and intrahepatically.*

INTRODUCTION

THE RETICULOENDOTHELIAL system (RES) has many important functions in both physiological and pathological conditions. The importance of the RES in defence against tumor growth and spread has been documented in previous studies [1–5].

The Kupffer cells of the liver constitute the major part of the fixed macrophages, comprising about 80% of the fixed RES capacity. The spleen contributes another 10% to this total fixed macrophage capacity [6].

Tumor transplantation in experimental animals causes a rapid increase in reticuloendothelial activity, as measured by the blood clearance of colloid particles [2, 3]. A rapid increase in the weight of the spleen during experimental tumor growth has also been reported [2, 3]. The influence of RES activity on the growth of tumors has been described earlier [7, 8]. Suppression or stimulation of reticuloendothelial function has been shown to interfere with tumor growth [9, 10]. It is also evident that surgical trauma depresses reticuloendothelial function [6, 11]. The clinical importance of these facts is still poorly understood.

In previous studies correlating reticuloendothelial activity to tumor growth, total RES activity as measured by colloid blood clearance has mainly been used [12]. The use of radiolabeled colloids has made possible simultaneous studies of the clearance from the blood and the uptake of the colloid by different RE organs [13]. These techniques allow the performance of separate studies of the uptake by the two main stationary RE organs, the liver and the spleen.

In a previous study we investigated the efficiency of a scintillation camera technique for measurements of the reticuloendothelial function, using a [$^{99}Tc^{m}$]-sulfur colloid test as the test substance [14]. This study was undertaken to evaluate the reticuloendothelial phagocytic capacity in rats with different degrees of intra- and extrahepatic tumor load.

MATERIALS AND METHODS

Inbred Wistar rats of both sexes were used in the experiment. All rats were fed *ad libitum* on rat pellets and water.

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Twelve rats were used as controls and 21 rats were used as recipients of an *N*-methyl-*N*-nitrosoguanidine-induced adenocarcinoma. The tumor was transplanted under sterile conditions into either the liver, kidney or dorsal subcutaneous tissue. Tumor transplantation to the liver and the subcutaneous tissue was done with 1.0×10^6 cells and to the kidney with 0.5×10^6 cells. Vital cell counts were made after adding trypan blue. The tumor-bearing animals were divided into three different groups according to tumor site and size (Table 1). The time intervals between tumor inoculation and measurement of RE function are depicted in Table 1.

The test substance [$^{99}Tc^m$]-sulfur colloid ($^{99}Tc^m{}_2S_7$) was prepared according to Persson and Naversten [15]. The range of particle size was 200–500 nm, with a mean of 300 nm, and the number of particles/ml solution was approximately 10^{10} [16]. The preparation was tested in each of the experiments for the labeling efficiency with a gel chromatography column scanning technique [17], and was observed to range between 97 and 100%.

With the animal under general anesthesia with Nembutal 6 mg/kg body wt the jugular vein was cannulated and a fine catheter was introduced and fixed with the tip in the superior vena cava. About 15 MBq in a volume of 0.5 ml containing approximately 0.5×10^{10} particles was injected during 5–10 sec. Registration started immediately at the onset of injection.

After completion of the scintillation camera registration the animals were killed. The liver and spleen were dissected free and extirpated. These organs were then placed on the scintillation camera and measured for total activity content. Each tumor regardless of location was then carefully dissected from surrounding tissue. The weight of the tumor, liver and spleen were recorded.

The kinetics of the activity distribution in the animals were measured with a scintillation camera (Searle, LFOV, Searle Radiographic), equipped with a parallel 39,500 hole collimator. Simultaneous with the start of colloid injection, sequential images were recorded during a 15 min period. Three frame groups were stored with 16, 12 and 20 frames at 7.5-, 15- and 30-sec intervals respectively. Regions of interest were selected from the total image and time–activity curves were generated. The time–activity curves were stored on an RK06 disc and transferred to a computer (Digital Equipment PDP 11/45), where the uptake curves were evaluated.

An open two-compartment model was used to estimate the flow rates of the radiolabeled colloid to the liver (k_1) and other RE tissues (k_2) (Fig. 1) [14]. Using the same symbols as in Fig. 1, the rate of disappearance of the colloid in the blood (B) and the rate of uptake in the liver (L) with time can be written:

$$\frac{dB}{dt} = -B\,(k_1 + k_2) \qquad (1)$$

$$\frac{dL}{dt} = B\,k_1. \qquad (2)$$

The amount of colloid transported to the liver can then be expressed with the equation:

$$L(t) = 100\,\frac{k_1}{k_1+k_2}\{1 - e^{-(k_1+k_2)t}\}. \qquad (3)$$

The rate constants k_1 and k_2 were obtained by fitting this equation to the experimental uptake curves for each animal [14].

Analysis of the experimental data were performed for 95% confidence with Student's t test and results are given as mean values with one standard deviation.

RESULTS

Examination of 8 rats 18–21 days after tumor inoculation revealed in each case the presence of a solitary 0.8- to 2.3-g liver tumor confined to the inoculated lobe (Table 1). These animals had an average weight loss of about 5% of their weight at the time of inoculation.

The rats inoculated with tumor cells subcutaneously had a well-defined 0.3- to 2.8-g subcutaneous tumor nodule 30–36 days after

Table 1. *Material presentation, groups of examined animals, No. of tumor cells inoculated, tumor growth time and tumor weight*

Group	No.	No. of tumor cells inoculated $\times 10^6$	Time interval (days)	Tumor weight (g) Range	Mean
I. Control	12				
II. Liver tumor	8	1.0	18–21	0.8–2.3	1.6
III. Retroperitoneal tumors	5	0.5	24–28	2.9–20.7	7.0
IV. Subcutaneous tumors	8	1.0	30–36	0.3–2.8	1.3

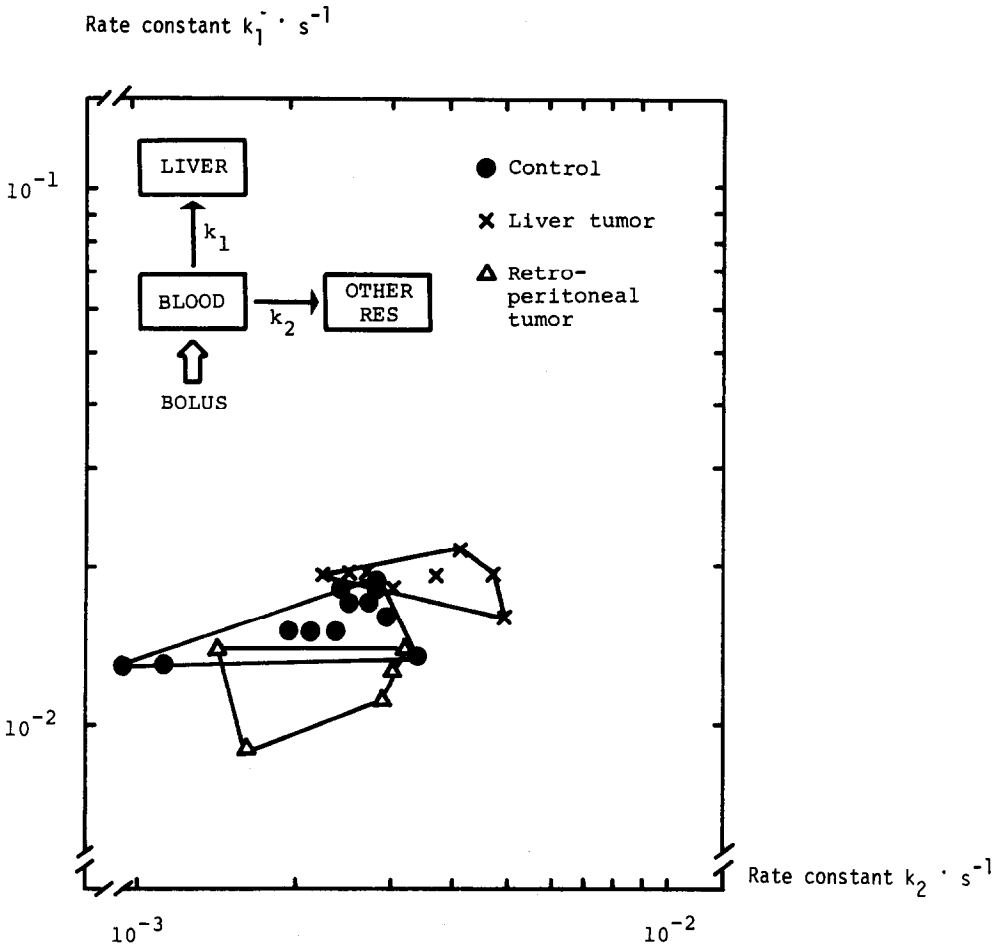

Fig. 1. Scatter diagram of the rate constants k_1 and k_2 obtained from the compartment analysis. Normal rats and rats with liver tumor and retroperitoneal tumor.

inoculation (Table 1). No signs of tumor spread were noted. These animals gained weight during the period from inoculation to examination to the same extent as the control animals.

Examination of the animals with retroperitoneal tumors revealed large tumors (2.9–20.7 g) extending beyond the kidney into the retroperitoneal space. None of the animals showed signs of tumor invasion of the liver or the spleen. These animals had a weight loss during the 24–28 days from inoculation to examination of about 10% (mean value), but no signs of cachexia were apparent (Table 1).

The relative liver weight as a percentage of total body weight in the 12 control animals was 3.6 ± 0.7%, which was not different from the relative liver weight of tumor-bearing animals (groups II–IV in Table 2). The relative weight of the spleen in the control animals was 0.26 ± 0.06%, whereas for groups II–IV the weight of the spleen was greater (Table 2).

The animals with liver tumors showed a significantly greater uptake rate in the liver (k_1) than the controls ($P < 0.005$) (Table 3). The uptake rate of the extrahepatic RES (k_2) was also significantly higher ($P < 0.005$). Animals with retroperitoneal tumors had a significantly lower k_1 ($P < 0.005$). In this group k_2 values were not

Table 2. Relative weights of liver and spleen (as % of total body weight) and P *values in comparison to controls*

Groups	Relative weight of liver (%) Mean ± S.D.	$P <$	Relative weight of spleen (%) Mean ± S.D.	$P <$
I. Controls	3.6 ± 0.7		0.26 ± 0.06	
II. Liver tumors	3.7 ± 0.3	n.s.*	1.10 ± 0.06	0.001
III. Retroperitoneal tumors	3.4 ± 0.5	n.s.	0.76 ± 0.40	0.001
IV. Subcutaneous tumors	3.6 ± 0.5	n.s.	0.60 ± 0.09	0.001

*n.s. = No significance.

Table 3. [$^{99}Tc^{m}$]-*sulfur colloid uptake rate and total organ uptake in % of given activity and* P *values compared to controls*

Groups	Colloid uptake rate (sec^{-1}) Liver $k_1 \times 10^{-2}$	$P <$	Spleen $k_2 \times 10^{-3}$	$P <$	Total organ uptake Liver (%)	$P <$	Spleen (%)	$P <$
I. Controls	1.6 ± 0.2		2.2 ± 0.7		89 ± 3		3.2 ± 1.5	
II. Liver tumors	1.9 ± 0.1	0.005	3.5 ± 0.9	0.005	84 ± 3	0.005	6.4 ± 1.5	0.001
III. Retroperitoneal tumors	1.2 ± 0.2	0.005	2.4 ± 0.8	n.s.	84 ± 3	0.01	7.6 ± 2.1	0.02
IV. Subcutaneous tumors	1.8 ± 0.3	0.05	4.5 ± 1.7	0.005	81 ± 8	0.01	5,7 ± 1.9	0.005

different from those of the control group. Rats with subcutaneous tumors showed a significantly higher k_1 ($P < 0.05$) and k_2 ($P < 0.005$). k_1 and k_2 values for normal and tumor-bearing rats are presented as scatter diagrams in Figs 1 and 2. For control rats the total activity uptake was 89 ± 3% for the liver and 3.2 ± 1.5% for the spleen. Total activity uptake by the liver was lower in all tumor animals. Total activity uptake by the spleen was higher in all tumor-bearing animals (Table 3).

DISCUSSION

Previous clearance studies of the reticuloendothelial function in relation to experimental tumor growth have not discriminated between different compartments of the stationary RE system. These studies have shown an influence of the total RE phagocytic activity related to tumor size [2, 3]. The relationship between RE function and tumor localization has not been elucidated.

In the present study tumors of similar size, located in the liver or dorsal subcutaneous tissue, independent of localization, caused an increased colloid uptake rate in the liver as well as in the spleen.

A correlation between uptake rate and total organ uptake was not invariably seen. Colloid uptake rate may reflect the functioning status of

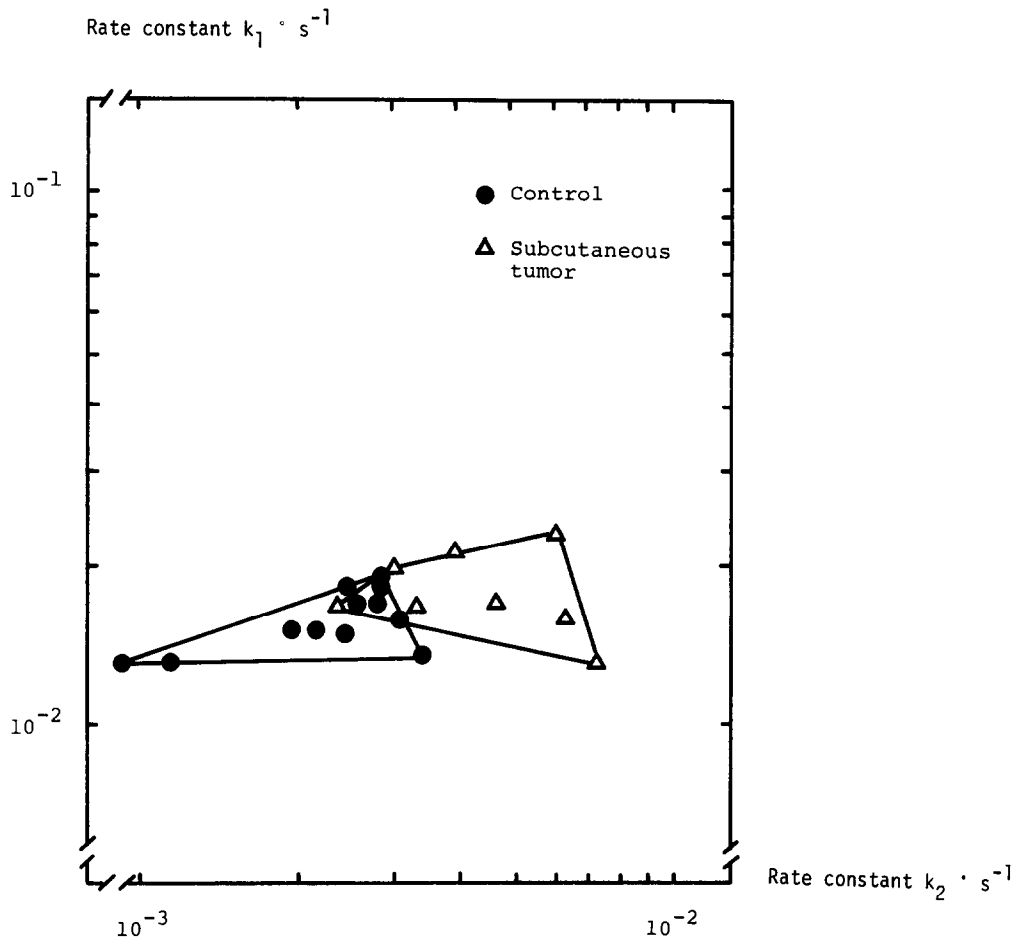

Fig. 2. Scatter diagram of the rate constants k_1 *and* k_2 *obtained from the compartment analysis. Normal rats and rats with subcutaneous tumors.*

the RE system and is influenced by factors such as opsonins. The total organ uptake reflects more the total number of functioning macrophages [6].

The total uptake of the spleen was increased to the same degree in both the group with liver tumors and the group with subcutaneous tumors. The high values of total splenic colloid uptake rate coincided with a higher relative weight of the spleen and with an increased total organ uptake of the spleen in these two groups. An increase in the size of the spleen in animals with experimental tumor growth has been described earlier, and has been attributed to an increased number of RE cells [2]. The weight of the spleen in animals with liver tumors was about double that of animals with subcutaneous tumors of similar size. An obvious explanation for this difference cannot be postulated. Since the liver tumors were all located in the periphery of the liver and did not cause any portal stasis, portal hypersplenism is not likely to be the cause of the difference.

The total organ uptake of the colloid by the liver was lower in tumor animals, regardless of tumor localization. However, no changes in the relative weights of the livers in tumor-bearing animals were seen. The lower percentage total uptake in the liver may be explained by the higher uptake of the enlarged spleen, as well as a higher uptake in the remaining parts of the RES.

In animals with large retroperitoneal tumors colloid uptake rate in the liver was significantly decreased. The total organ uptake of the colloid in the spleen and the splenic weight was also higher in this group, although the uptake rate was unchanged compared to the controls. These large tumors may cause an exhaustion of RE function, perhaps through a reduction of opsonin factors. This corresponds to earlier findings of a depression of RE function in animals with large experimental tumors [2]. The uptake rate of the extrahepatic RES was, however, significantly decreased compared to animals with smaller tumors (groups II and IV). This may reflect that the suppression of RE function with increasing tumor burden is seen earlier in the hepatic RES than in the extrahepatic RES.

The RES phagocytic capacity can be measured by the clearance of colloidal particles from the blood stream [2, 6, 12]. [$^{99}Tc^{m}$]-sulfur colloid has been used extensively in static liver scanning, as well as for studies of the reticuloendothelial phagocytic function [6, 13]. In contrast to previous methods, the technique used in the present study [14] enables separation of the hepatic and the extrahepatic RES. The spleen is by far the most important extrahepatic RE organ [6]. The present results indicate that the influence of tumor growth on the RES is mainly a function of tumor size. A varied influence of hepatic and extrahepatic RES from various tumor burdens was observed. This finding indicates the value of distinguishing between various compartments of the RES.

The present technique provides a simple and reliable method of studying RE function in the experimental animal. The good reproducibility found in earlier studies has been further confirmed in this present study.

A clinical implication of a stimulated RE system during tumor growth may be an increased uptake by the RES of monoclonal antibodies used for diagnostic purposes. Suppression of such RES uptake by pharmacological means may enhance the diagnostic efficiency of such antibodies.

Further research is necessary to determine if a lower RE function in the liver as measured by the colloid uptake rate has any clinical implication in the treatment of liver cancer.

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