CHARACTER OF METASTASIZATION OF SARCOMAS 45 AND 536 WHEN INOCULATED BY DIFFERENT METHODS

V. S. Shinkarenko

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Intratesticular injection of 1 ml of a suspension of tumor tissue into rats was followed by rapid growth of the tumor at the site of inoculation and by spread of tumor nodes into the greater omentum, the hilus of the liver, the mesentery, and the walls of the peritoneal cavity. Similar spread of nodes was observed after intraperitoneal inoculation of the sarcomas. The lifespan of the animals in these experiments was 13-15 days. One month after intratesticular inoculation of 0.2 ml of tumor suspension metastases were found in the lungs of 74% of animals with sarcoma 45 and 90% of those with sarcoma 536. Tumor cells were shown to appear in the blood stream at the time of inoculation, but metastases formed in the lungs later, after the formation of the primary node. The system described can therefore be regarded as an adequate model of the process of metastasization in the lungs of rats.

KEY WORDS: *transplantable tumors; metastasization; experimental model of tumor.*

Data in the literature on the frequency and character of metastasization of transplantable tumors of laboratory animals do not apply to all the strains used in experimental cancer research [6, 8, 9]. A change in the site of implantation of a tumor that rarely metastasizes if inoculated by the usual methods can considerably increase the frequency of metastasization [2, 5].

The objects of the present investigation were: 1) to seek a method of inoculation of rat sarcomas 45 and 536 which would be followed by a high frequency of distant metastases, and 2) to assess the adequacy of the resulting model of metastasization. The strains of tumor mentioned above are widely used in experimental chemotherapy, but they rarely metastasize after the usual subcutaneous inoculation [2, 3].

TABLE 1. Spread of Tumor Nodes of Sarcomas 45 and 536 Depending on Method of Inoculation

Strain	Group No.	Method of inoculation	Volume of suspension injected (in ml)	Time after inoculation (in days)	Mean diameter at site of injection (in mm)	Number of animals				
						total	with tumor nodes			
							in greater omentum	in mesentery	on walls of peritoneal cavity	in lungs
Sarcoma 45	1	Intraperitoneally	1,0	7	—	32	32 (100%)	12 (38%)	29 (91%)	—
	2	Intratesticularly	1,0	7	10±1,3	29	24 (83%)	19 (66%)	23 (79%)	—
	3	"	0,2	28	21,7±0,7	43	—	—	—	32 (74%)
Sarcoma 536	1	Intraperitoneally	1,0	7	—	28	28 (100%)	8 (27%)	28 (100%)	—
	2	Intratesticularly	1,0	7	13±2	31	27 (87%)	22 (71%)	28 (90%)	
	3	"	0,2	28	23,5±0,9	40	—	—	—	36 (90%)

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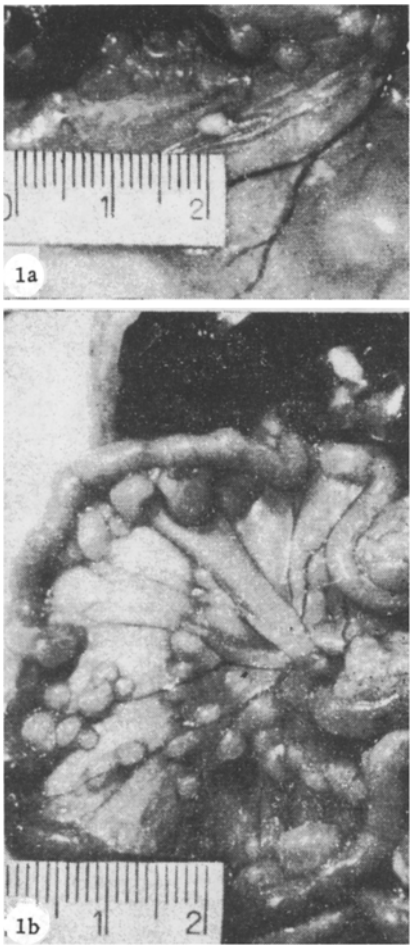

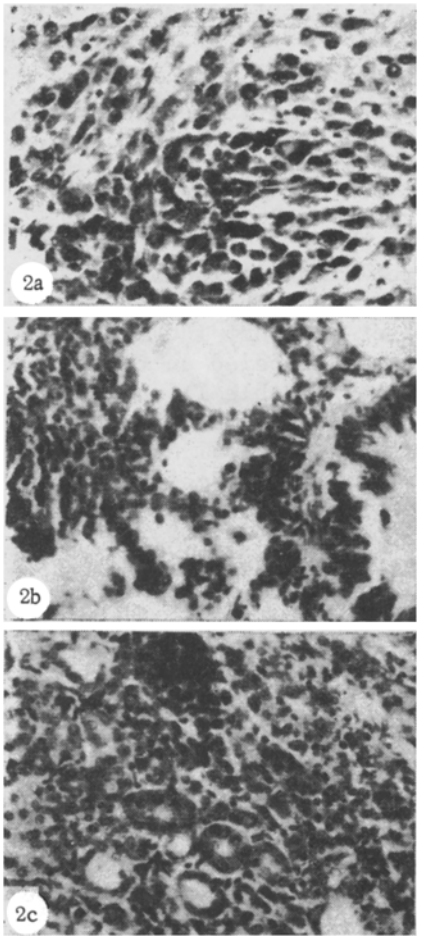

Fig. 1 Fig. 2

Fig. 1. Sarcoma 536, 7 days after inoculation with 1 ml of suspension of tumor tissue: a) intraperitoneal inoculation: sarcoma nodes on posterior wall of peritoneal cavity; b) intratesticular inoculation: nodes located along mesenteric blood vessels.

Fig. 2. Sarcoma 536, 28 days after intratesticular inoculation of rat with 0.2 ml tumor tissue suspension: a) primary node with spindle-cell structure; b) metastasis in lung; c) metastasis in kidney. Hematoxylin-eosin, objective 20×, camera ocular 10×.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 120-150 g were inoculated with sarcoma 45 or sarcoma 536*. The animals of group 1 were inoculated intraperitoneally with 1 ml of a suspension of tumor tissue in physiological saline. The animals of group 2 were inoculated intratesticularly with the same volume of suspension. The rats of group 3 were inoculated intratesticularly with a smaller volume of tumor (0.2 ml). The testis was removed from some of the animals of group 3 (10 rats with each type of sarcoma) 30 min after inoculation, under ether anesthesia. The suspension of tumor tissue was prepared from a subcutaneous node [3].

The animals were killed 7 and 28 days after inoculation and the size of the tumor nodes at the site of inoculation was measured at autopsy. The liver, kidneys, spleen, lungs, lymph glands, and mesentery were examined under the stereoscopic microscope for the presence of

*Sarcomas of strains 45 and 536 were obtained from the Laboratory of Experimental Chemotherapy of Tumors (Head, Professor V. A. Chernov), All-Union Pharmaceutical Chemical Research Institute.

tumor nodes both on the surface and in the interior of the organs. Pieces of the organs taken for histological examination were fixed in Carnoy's fluid and sections were stained with hematoxylin and eosin.

In a special series of experiments 0.2 ml of a suspension of tumor tissue stained with a luminescent dye (Acridine Orange) was injected intratesticularly into anesthetized rats and luminescence biomicroscopy of the mesenteric vessels was carried out simultaneously [7].

EXPERIMENTAL RESULTS

Intraperitoneal inoculation of the sarcomas was followed by their rapid growth and the animals died on the 13th-15th day. Conglomerates of tumor nodes up to 65 mm in diameter invading the greater omentum, intestinal loops, and other abdominal organs, were found in the animals. In rats autopsied on the 7th day, tumor nodes measuring up to 30 mm were found in the greater omentum (Table 1) and nodes 3-5 mm in diameter were present in the hilus of the liver, on the mesentery and diaphragm, and on the posterior and, less frequently, the lateral walls of the peritoneal cavity (Fig. 1a). Rapid growth of the tumor around the testis and spread of tumor nodes into the peritoneal cavity were observed after intratesticular injection of 1 ml of a suspension of tumor tissue (animals of group 2). The character of distribution of the nodes among the organs and the lifespan were almost identical with those of the animals of group 1. Differences consisted of the smaller size of the nodes and the greater uniformity of their spread over the peritoneal cavity. In this case, the connection between these nodes and blood vessels could be clearly demonstrated in the mesentery (Fig. 1b).

In the rats of group 3, into which the tumor was inoculated intratesticularly in a volume of 0.2 ml, the take rate was 96% for sarcoma 45 and 100% for sarcoma 536. Miliary metastases 0.2-0.8 mm in diameter were found in the lungs of 74% of animals with sarcoma 45 and 90% with sarcoma 536 4 weeks after inoculation. In two rats with sarcoma 536 metastases were found in the liver, and in one rat in the kidney (Fig. 2).

Animals from which the testis was removed after inoculation were killed 2 months later. No evidence of tumor growth could be found in them.

Histological examination showed that the nodes of both sarcomas at the site of inoculation were mainly spindle cell in structure. Regions with polymorphic structure were found in the nodes of sarcoma 536 (Fig. 2a). Metastatic nodes in the lungs consisted of cells similar in shape to cells of the primary nodes, but smaller in size (Fig. 2b). In the lymph nodes of the animals of group 3 tumor cells were found in 4% of cases. The sinuses of the lymph nodes were sharply dilated and filled with numerous plasma cells; a change to plasma cells also was found in the medullary cords, evidence of an immune response of the organism [4]. The results suggest a blood-borne path of metastasization of these sarcomas.

The experiments showed that intratesticular injection of large doses of tumor cells results in much of the tumor material being outside the testis, and since in rats the testis is easily replaced in the peritoneal cavity, the inoculated material can spread in this way. In the writer's opinion, this accounts for the similar results of inoculation in the rats of groups 1 and 2. The regular arrangement of the nodes in the mesentery along the blood vessels suggests that conglomerates of tumor cells, migrating over the peritoneal cavity, are implanted mainly in areas with an abundant blood supply. This system provides a convenient model for the intravital study of the development and functioning of a blood-borne network of tumor nodes, for the mesentery is accessible for investigation by biomicroscopy.

These experiments showed that after intratesticular inoculation of small volumes of tumor (rats of group 3) the tumor develops in the testicular tissue and does not spread into the peritoneal cavity. In this case, compared with the subcutaneous method of inoculation, the frequency of metastasization of sarcomas 45 and 536 in the lungs was considerably higher. The preferential localization of metastases in the lungs can be explained on the grounds that the lungs are the first "capillary filter" in the path of the tumor cells and their emboli, carried by the venous blood from the primary node through the right heart into the pulmonary circulation.

In the experiments with biomicroscopy of the mesenteric vessels of the rats during intratesticular inoculation of a suspension of tumor tissue stained with Acridine Orange, individual fluorescent cells were seen to appear in the blood stream. This indicates that

at the moment of inoculation tumor cells enter the blood stream and can be carried to any organ. Should tumor nodes develop from these cells, they cannot be regarded as metastases of the primary node. They are equally primary implanted nodes. However, absence of tumor growth in animals in the experiments in which the testis was removed after inoculation is evidence that metastases are laid down not during inoculation of the tumor, but later, after formation of the primary node. This fact confirms the view that for metastases to form it is necessary for many tumor cells, not just solitary cells, to settle and the influence of the primary node on this process is also essential [1].

The times when metastases start to form depend on the formation of blood vessels of the tumor [10], invasion of the venous system by the growing tumor, and proliferation of tumor cells in the veins. In view of these circumstances the model described above can be regarded as an adequate system for the reproduction of metastasization in rats and for use in the differential evaluation of the prophylactic and therapeutic action of antitumor preparations on metastasization in the lungs.

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