ONCOLOGY

METASTASIS OF SARCOMA M-1 IN RATS AND THE BIOLOGICAL PROPERTIES OF THE METASTASES

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We are not the first to study the character of growth of transplanted tumors in relation to the site of inoculation. This problem appeared earlier in work by Keysser [1911], Endler [1916] and other investigators who showed that tumors grow in different ways depending on the site of inoculation.

Recently Soviet workers in this direction have established a number of interesting features of tumor growth. A. A. Solov'ev, for example, studied the principles of spread of metastases of the Brown-Pearce tumor of rabbits and their relation to the site of development of the primary tumor. This worker injected a suspension of tumor cells into the testis, ovary, kidney, adrenals, liver, spleen, retrobulbar tissues and the anterior chamber of the eye. In this way he observed that, depending on the site of injection of the tumor, the pattern of metastasis formation was varied. O. I. Epifanova [1] in her research showed that the Ehrlich adenocarcinoma of mice grows best in the region of the forelimbs and worst in the hindlimbs. After subcutaneous inoculation this tumor as a rule does not form metastases but after intraperitoneal inoculation an ascitic variant of the malignant cells develops, in a certain percentage of cases with metastases in the liver, kidneys, spleen and other organs. The M-1 rat sarcoma, also inoculated subcutaneously, as a rule does not give metastases. However V. N. Popov [3] observed the development of metastases from this tumor in the internal organs after its inoculation in the muscle tissue of the hind limb or into the testis.

Similar results were obtained by Hlavayova and Kossey [8] with inoculation of the rat tumor BSH-1 into the testicle of experimental animals. As another example confirming the ability of tumors to grow in different ways depending on the site of inoculation may be mentioned the ascitic variants of malignant cells which are formed by intraperitoneal passage through experimental animals of subcutaneous or solid forms of tumors [7, 9, 11, 12, 13].

In view of these findings we decided to study the possibility of metastasis formation by the rat sarcoma M-1 after inoculating the tumor into rats subcutaneously and into the testicle, and also the biological properties of the metastatic tumor tissue after subcutaneous inoculation of experimental animals with it.

The rat sarcoma M-1 was first obtained by L. M. Shabad [5] by injecting rats with a solution of 3 : 4 benzpyrene in castor oil. According to the findings of this worker this tumor, from a histological point of view, is a spindle-cell and partly polymorphocellular sarcoma with low malignancy and a low percentage of successful inoculation (50%).

In our experiments the experimental animals used were sexually mature males aged from 6-10 months. In all the experiments we carried out the inoculation with tumor tissue with strict sterile precautions and with the same dosage: 0.5 ml of a 10% suspension of tumor particles in physiological saline.

EXPERIMENTAL RESULTS

Inoculation of the Sarcoma M-1 Subcutaneously

For subcutaneous inoculation 112 rats were used. Of these, 74 rats died from the developing tumors (66%),

in 15 rats the transplants at first showed some degree of growth and then regressed, while in 23 rats the transplantation was unsuccessful.

During pathological anatomical investigations of the experimental animals dying from tumors we never once found in them any metastases in the internal organs or peritoneal cavity. The tumor nodules grew in the form of continuous conglomerating masses, sometimes reaching large dimensions and a weight of 140 g or more. Usually in the depth of these tumor nodules could be found a cavity containing a blood-stained fluid. The rats died from tumors in the interval between the 30th and 39th days after inoculation.

Inoculation of Sarcoma M-1 into the Testicle

For inoculation of the sarcoma M-1 into the testicle 105 rats were used (Table 1).

In 28 animals the tumors in the testicles grew to some extent, increased perceptibly in size and then became necrotic, ulcerated and underwent complete regression. Usually in these rats the site of inoculation was fully cicatrized on the 30th-35th day from the start of the experiment.

TABLE 1

Results of Inoculation of the Rat Sarcoma M-1 into the Testicle

Series No. of experiments	No. of rats in each separate series of experiments	Tumors			Metastases	Day of death of rats from developing tumors
		inoculated	not inoculated	regressed		
		number of rats				
1	12	8	2	2	8	25—30 th
2	8	5	2	1	5	25—31 st
3	8	4	2	2	4	27—35 th
4	8	3	2	3	3	30—32 nd
5	9	4	—	5	4	29—36 th
6	16	2	8	6	2	31—35 th
7	8	5	1	2	5	25—31 st
8	10	7	1	2	7	26—39 th
9	10	8	—	2	8	21—30 th
10	8	7	—	1	7	22—28 th
11	8	6	—	2	6	25—33 rd
Total	105	59	18	28	59	21—39- th

In 18 rats the tumors did not grow in the testicles.

In 59 rats the tumors were successfully inoculated and on the 12th-17th day they could already be felt in the abdominal cavity of the animals in the form of dense, nodular formations. On pathological examination of these rats after slaughter or natural death tumor nodules of various sizes were found in the omentum, on the mesentery of the small and large intestine, on the peritoneum, the diaphragm and the surface of the liver and kidneys and in the thickness of muscle tissue situated far from the site of the original inoculation.

Not once did we observe the development of metastases directly in the lymph glands, the liver, kidneys, lungs, spleen or other internal organs. Thus, metastasis of the sarcoma M-1 differs somewhat from the development of metastases from, for example, the Brown-Pearce rabbit carcinoma, which metastasizes intensively in the internal organs of experimental animals.

Rats with developing tumors died during the period from the 21st to the 39th day from the start of the experiment. In Fig. 1 is shown an experimental rat (No. 334) in whose peritoneal cavity multiple metastases developed. It died on the 33rd day.

At necropsy the findings were as follows. There was a large tumor in the right testicle. In the omentum and mesentery of the small and large intestines was a continuous conglomeration of tumor tissue, the size of a

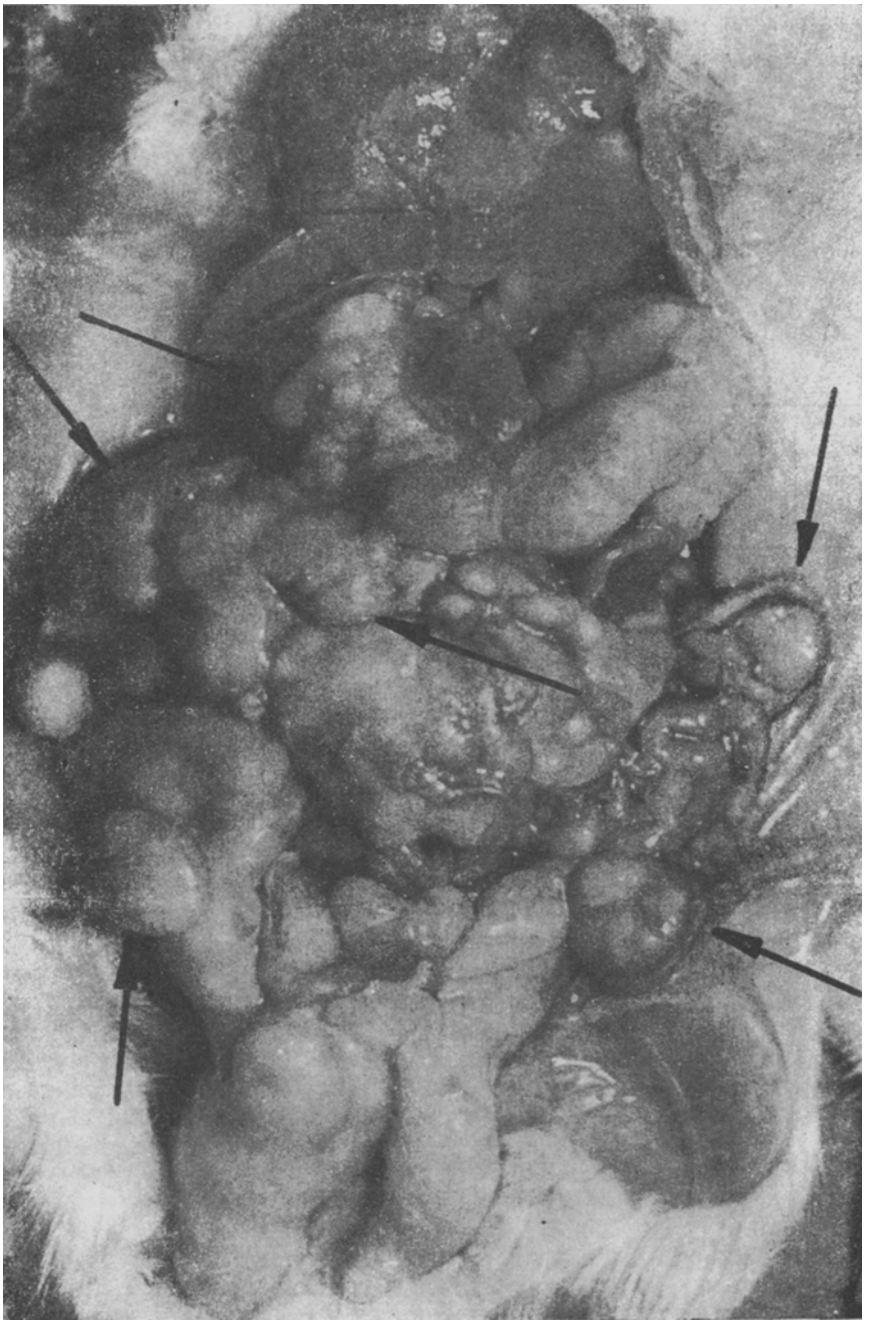

Fig. 1. Metastases of a sarcoma M-1 in a rat in the peritoneal cavity as a result of inoculation of the tumor into the testicle. The rat died on the 25th day from the moment of inoculation. The arrows indicate the situation of the metastases.

large hen's egg, completely isolated from the tumor developing in the site of inoculation. On the abdominal wall there were solitary tumor nodules of various sizes. In the region of the right kidney there was a large tumor nodule infiltrating muscle tissue. The kidney was slightly deformed and displaced. On the capsule of the left kidney were two metastases the size of peas. In the liver, kidneys, spleen, lymphatic glands and lungs no metastases were found.

TABLE 2

Distribution of Metastases from Sarcoma M-1 in Rats after Inoculation of the Tumor into the Testicle

Series No. of experiments	No. of rats giving metastases in each separate series of experiments	Metastases				
		in the omentum	on the peritoneum	on the diaphragm	in muscle tissue	on the surface of the other organs
		number of rats				
1	8	8	4	3	4	+2
2	5	5	1	4	3	—
3	4	4	1	1	1	—
4	3	3	—	—	—	—
5	4	4	—	2	1	1
6	2	2	—	1	—	—
7	5	5	2	2	3	—
8	7	7	5	3	1	1
9	8	8	1	2	5	—
10	7	7	4	3	3	—
11	6	6	1	3	2	—
Total	59	59	19	24	23	4

In Table 2 is shown the distribution of metastases from sarcoma M-1 in rats after implantation of the tumor in the testicle: in the omentum — 100% of the animals in which growth of the tumor took place, on the diaphragm — 40.6% of these animals, in muscle tissue — in 38.7%, on the peritoneum — in 32.2%, on the surface of organs (liver, kidneys) — in 6.7% of animals.

In order to make a comparative study of the biological properties of the "primary" (growing at the site of implantation under the skin) M-1 tumor and of its metastases, we carried out 5 series of experiments in which one group of animals was inoculated subcutaneously with tumor tissue from a metastasis in the diaphragm and another group of rats from the "primary" subcutaneous tumor nodule. According to the findings of E. E. Pogosiants [2], the latent period of growth of sarcoma M-1 when implanted under the skin varies from 5 to 7 days. The number of positive inoculations is 98-100%. According to L. M. Shabad, the number of positive inoculations under the skin is on the average 50%.

In our experiments, as may be seen in Table 3, the number of positive subcutaneous implantations in rats of the "primary" tumor was about 75% and the latent period of growth varied between 4 and 7 days.

The results of subcutaneous implantation in rats of tissue from a metastasis from sarcoma M-1 in the diaphragm were slightly different from those of the controls. The number of positive implantations in this case was about 32.1% and the latent period of growth varied between 8 and 12 days. Thus, during growth of the tumor in the diaphragm a weakening took place in the malignancy of the tumor tissue, which was reflected in the lower percentage of positive results of inoculation and in the lengthening of the latent period of growth.

There is no single opinion either on the morphological properties of the sarcoma M-1 growing in the subcutaneous cellular tissue. Thus, according to L. M. Shabad, sarcoma M-1 is mainly composed of spindle-shaped cells and is partly polymorphocellular. According to E. E. Pogosiants this tumor, histologically speaking, is a polymorphocellular sarcoma.

In our investigation the subcutaneous transplant of sarcoma M-1, as seen in Fig. 2, has a mixed structure: alongside polymorphous cells may be seen elongated cells and spindle-cell forms. In some places the cells are

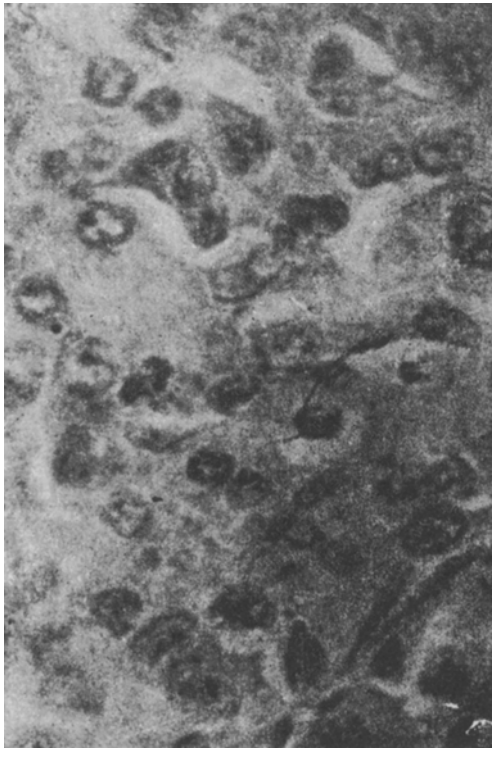

Fig. 2. Histological section of a metastasis of sarcoma M-1 in the diaphragm. Stained with hematoxylin-eosin. Magnification 536 ×.

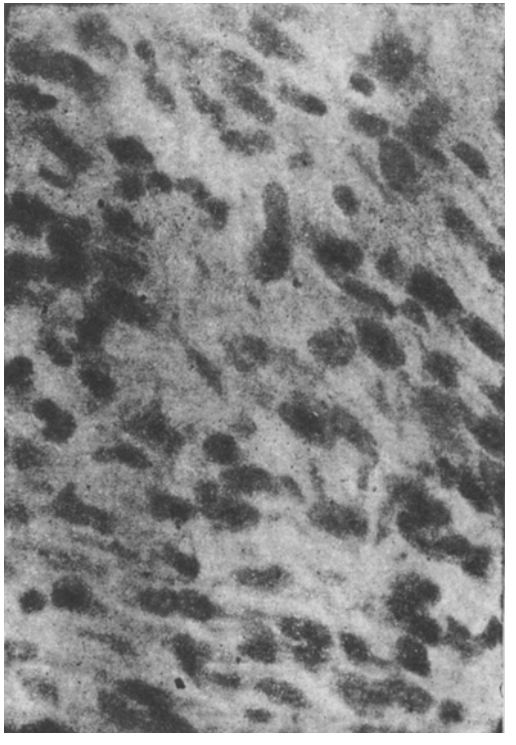

Fig. 3. Histological section of a subcutaneous transplant of sarcoma M-1. Stained with hematoxylin-eosin. Magnification 536 ×.

disposed in the same direction and form characteristic vortical structures. In the field of vision may be seen cells whose nuclei have a round or slightly elongated oval shape.

TABLE 3

Results of Subcutaneous Inoculation of Rats with Ordinary Tumor and with Tissue from a Metastasis of Sarcoma M-1 in the Diaphragm

Series No. of experiments	No. of rats in each experiment	Latent period of growth of the tumor (in days)	Results of implantation of tumor		
			positive	negative	temp. growth of tumor and subsequent regression
Experiment (inoculation of metastatic tissue)					
1	15	10—12	9	3	3
2	17	8—9	3	14	—
3	10	—	—	10	—
4	9	9—11	4	5	—
5	5	8—10	2	3	—
Total	56	8—12	18 (32.1%)	35 (62.5%)	3 (5.3%)
Control (inoculation of "primary" subcutaneous tumor)					
1	15	5—6	10	2	3
2	17	4—7	13	1	3
3	10	4—6	7	1	2
4	10	5—7	8	1	1
5	8	4—5	7	—	1
Total	60	4—7	45 (75%)	5 (8.3%)	10 (16.6%)

The histological structure of the tissue of the metastasis from sarcoma M-1 in the diaphragm is shown in Fig. 3. Here the cells are mainly polymorphous, with large nuclei. If spindle-cells are encountered, they are very few. The tissue of the metastasis is rich in stroma. Neither are the vortical structures in evidence, as in the subcutaneous transplant. The cells are arranged haphazardly.

The results described thus show that in consequence of changes in the conditions of existence, both the malignancy and the morphological structure of the M-1 sarcoma may undergo modification.

Our experiments confirm the findings of V. N. Popov that the M-1 sarcoma produces metastases when implanted in the testicle. However, in contrast to this worker, we did not once meet the development of metastases in the internal organs of the experimental animals, but observed them exclusively on the serous membranes of the organs.

SUMMARY

Sarcoma M-1, as a rule, produces no metastases when inoculated subcutaneously in rats. Metastases develop in the abdominal cavity of experimental animals when this tumor is inoculated into the testicle. Decrease of malignancy is noted in metastatic tumor on the surface of the diaphragm and is manifested in reduced percentage of positive inoculations and in prolonged latent period in subcutaneous inoculation of the tissue from the metastatic tumors in rats.

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