GROWTH AND METASTASIZATION OF TUMORS INDUCED IN THE SPLEEN

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Injection of 9,10-dimethyl-1,2-benzanthracene and autografting of muscle tissue into the spleen of Wistar rats lead to the formation of tumors in that organ. The tumors (rhabdomyosarcomas, polymorphic and spindle-cell sarcomas) possess all the signs of malignancy: infiltrative growth, ability to metastasize, a high proportion of successful transplanatations into homologous animals, and an increase in virulence with successive passages.

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The principles governing metastasization have mainly been studied on models of transplanted tumors, and very little work has been done on spontaneous or induced tumors [3,6,7,12,18,19]. This is because induced tumors in most cases do not metastasize or metastasize only very slightly [5,16].

There are reports in the literature showing that metastasization of transplanted and induced tumors differs in certain respects [1, 11, 13-15, 17,20]. For that reason, the production of models of metastasizing forms of induced tumors and the study of the principles governing their metastasization are of considerable interest to experimental oncology.

The object of the present investigation was to study certain aspects of growth and metastasization of tumors induced in the spleen.

EXPERIMENTAL METHOD

Experiments were performed on 195 female Wistar rats aged 4-6 months. Tumors were induced with 9,10-dimethyl-1,2-benzanthracene (DMBA) dissolved in mineral oil. The carcinogen was injected into the spleen in a dose of 1 mg. At the same time as, or ten days before, injection of the carcinogen into the spleen, the animals were inoculated with minced muscle tissue from rat's hind limb in physiological saline in a dose of 0.2 ml of a 50% suspension. Either the carcinogen or muscle tissue only was injected into the spleen of control rats. The carcinogen was also injected into the hind limb muscle of a group of rats.

The technique of the operation was as follows. The rats were anesthetized with ether, muscle tissue was excised from the right hind limb and chopped with scissors under sterile conditions and diluted with physiological saline. Simultaneously, the spleen was extracted from the abdomen of the experimental animals and placed on a sterile towel. The minced muscle tissue was then injected into the spleen by means of a syringe with a thick needle.

Material for histological investigation was fixed in 10% formalin and Carnoy's mixture. Histological preparations were stained with hematoxylin-eosin and with Heidenhain's iron-hematoxylin

EXPERIMENTAL RESULTS

Histological examination of normal muscle tissue when autografted into the spleen showed that it remained viable on the 4th, 7th, and 10th days.

On the 4th day after transplantation the picture was as follows: a lymphocyte reaction was visible at the periphery of graft and numerous muscle tubes, myosyncytia, and myoblasts were present in the center.

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TABLE 1. Metastasization of Tumors Induced in the Spleen and Hind Limb Muscle of Rats

Character of Experiment	Number	Latent period of tumor growth (in months)	Metastases in						
			Lungs	Liver	Kidneys	Omentum	Abdominal lymph glands	Mesentery	Diaphragm
Autografting of muscle tissue into spleen; injection of DMBA into graft 10 days later	30/6	45–50 days	0	2	3	2	4	4	4
Autografting of muscle tissue mixed with DMBA	30/3	35–40	0	0	0	0	0	0	0
Injection of DMBA into spleen (control I)	30/0	7	0	0	0	0	0	0	0
Injection of muscle tissue only into spleen (control II)	10/0	7	0	0	0	0	0	0	0
Injection of DMBA into hind limb muscle (control III)	50/50	3–4	28	0	0	2	8	2	9

Note. Here and in Table 2, numerator represents number of rats in experiment, denominator number of rats developing tumors.

On the 7th day after transplantation fewer muscle tubes were present, and the dominant features were myosyncytia, although a few myoblasts were still present. Mitoses in the myoblasts were occasionally visible in the field of vision. In the peripheral zone surrounding the graft, connective-tissue cells formed a capsule consisting of one or two layers of cells separating the graft from spleen tissue.

On the 10th day after grafting newly formed muscle tubes of different shapes (because of the absence of stretching) could be seen, but most of the cells were mono- or polynuclear myoblasts. Mitoses were observed in the myoblasts in the field of vision*.

It may thus be concluded from the findings described above that muscle tissue, when grafted into the spleen, goes through several stages of development, mitoses being most numerous on the 10th day. This fact, and data in the literature on the stages of development of muscle tissue after autografting [4,8], in addition to reports that mitotically dividing cells are most sensitive to the action of a carcinogen [2,9,10], led us to inject the carcinogen in our experiments on the 10th day.

The results of the first experiment are given in Table 1. They show that DMBA, when injected 10 days after autografting of muscle tissue into the spleen, caused the formation of tumors in 6 of 30 cases. In their histological structure the tumors were rhabdomyosarcomas and polymorphic and spindle-cell sarcomas. In 4 of 6 cases the developing tumors metastasized into various abdominal organs. The latent period of growth of these tumors ranged between 45 and 50 days.

When muscle tissue was injected into the spleen mixed with DMBA, tumors developed in three of 30 rats. In their histological structures the tumors were polymorphocellular sarcomas. No metastasization of these tumors were observed.

*After consultation with O. N. Rumyantseva.

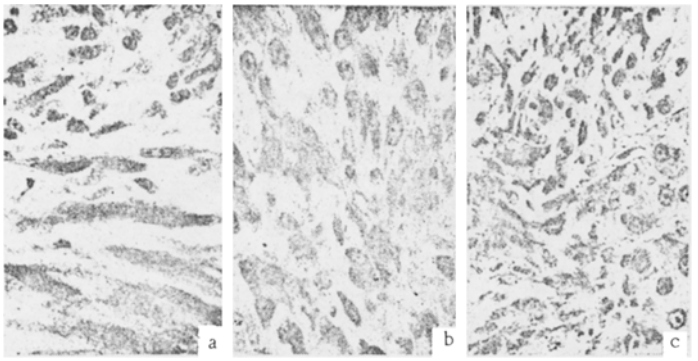

Fig. 1. Histological structure of a tumor induced in the spleen, a tumor obtained by passage, and a metastasis. a) Tumor No. 602/13 in spleen obtained by transplantation of muscle tissue followed (after 10 days) by injection of DMBA; b) 3rd passage of tumor No. 602/13 induced in spleen; c) metastasis in liver of tumor No. 602/13 induced in spleen. Hematoxylin-eosin. 200×

TABLE 2. Passage of Induced Tumor No. 602/13 in Spleen of Wistar Rats

Passage	Number of rats	Transplantability (%)	Latent period of growth (in days)	Metastases in: Lungs	Liver	Kidneys	Omentum	Abdominal lymph glands	Mesentery	Diaphragm
1	10/5	50	6—8	0	0	1	4	4	4	4
2	20/14	70	3—4	0	3	1	14	9	4	8
3	15/11	73,3	3—4	0	7	4	9	7	9	7

When carcinogen only was injected into the spleen, no tumors developed in any of the 30 cases throughout the period of observation (7 months). Histological examination of the spleen tissue at the site of injection of the carcinogen revealed connective-tissue scars and areas of necrosis.

After injection of the carcinogen into the hind limb muscle tumors developed in all 50 rats (after a latent period of growth of 3-4 months). In their histological structure these tumors were rhabdomyosarcomas, fibrosarcomas, and polymorphocellular and spindle-cell sarcomas. In 28 cases (56%) the tumors metastasized in the lungs, into 2 cases in the omentum and mesentery, in 8 cases in the abdominal lymph glands, and in 9 cases in the diaphragm.

The results show that after autografting of muscle tissue into the spleen followed (after 10 days) by injection of DMBA into the graft, in some cases induced tumors developed after comparative short time intervals and metastasized in the internal organs of the experimental animals, i.e., they showed all signs of malignancy (Fig. 1, a).

In contrast to the tumors induced in the hind limb muscle, tumors induced in the spleen arose after shorter time intervals but in a smaller percentage of cases, and they metastasized more intensively.

We also studied the ability of tumors induced in the spleen and transplanted into the spleen of other rats to form metastases. The results of transplantation of one such tumor (No. 602/13) are given in Table 2.

It is clear from Table 2 that after the second passage the malignancy of the tumor was increased. In the first passage tumors developed in 50% of cases, in the second in 70%, and in the third in 73.3% of cases (Fig. 1, b).

It is interesting to note that in the tumors of the third passage the number of mitotically dividing cells was much greater than in the tumor of the first passage.

These results show that in the course of passage of an induced tumor in the spleen, changes are observed in some of its biological properties (shortening of the latent period of growth, an increase in the proportion of successful transplantations, intensified metastasization).

The results thus show that after injection of muscle tissue and DMBA into the spleen tumors developed which show all signs of malignancy. Unlike tumors induced in the muscle of the rat's hind limb, which mainly metastasized in the lungs, tumors induced in the spleen metastasize in the liver and other abdominal organs.

We consider that tumors induced in this manner in the spleen are a convenient model for the study of metastasization of primary induced tumors.

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