in the hepatectomized mice averaged 17.6 and 26.7%, respectively, of the number of tumor cells in the control. Both indices were significantly higher than in the experiments with transplantation of hepatoma 22a, but whether this was due to the different affinity of these tumors for liver tissue is not yet clear; the factors on which this sharp inhibition of their growth depends likewise remain unexplained.

It is noteworthy that in all the experiments there were some animals which did not develop tumors, although films made from the contents of their peritoneal cavity revealed single tumor cells. This indicates that inhibition of tumor growth in these experiments was partial at the times chosen for investigation.

The results are evidence that sharp inhibition of growth of a tumor can take place in partially hepatectomized animals if it is transplanted intraperitoneally at certain periods after extensive resection of the liver.

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CHANGES IN SOME PROPERTIES OF TRANSPLANTABLE ADENOCARCINOMA OF THE COLON DURING PASSAGE

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During serial passage of an adenocarcinoma (strain AKTOL) arising as a result of spontaneous malignant change in tissues of the embryonic intestine, transplanted into syngeneic adult mice, the rate of growth of subcutaneous grafts was increased, the ability to form multiple lung tumor nodules after intravenous inoculation appeared (it was absent in the first passages), and morphological anaplasia of the tumor was increased. These changes, evidence of progression of the tumor, were not directly connected with changes in its immunogenicity.

KEY WORDS: *transplantable adenocarcinoma; pulmonary metastases; immunogenicity.*

Previous investigations have shown that during serial passage of experimental tumors arising spontaneously or induced by chemical carcinogens and viruses the character and rate of their growth and also their antigenic properties change [4-9].

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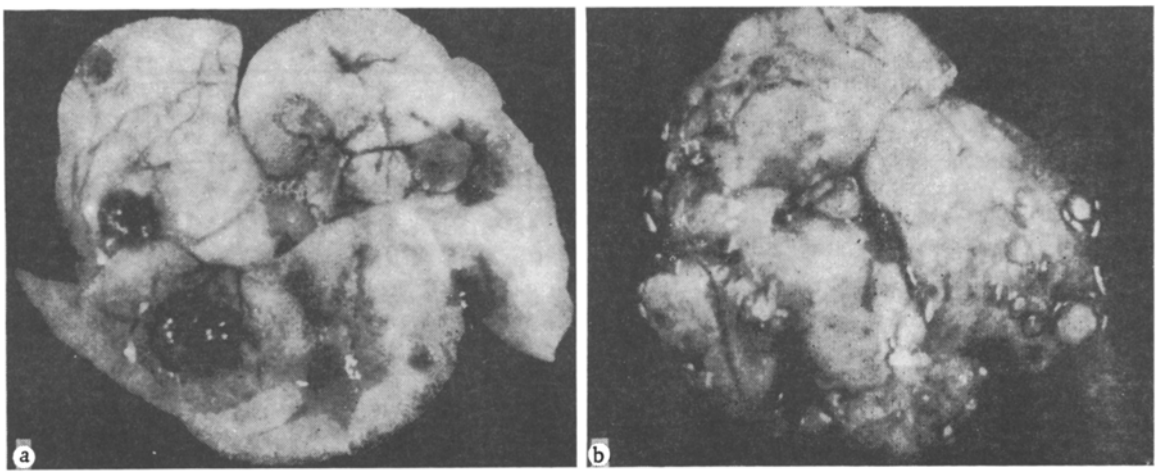

Fig. 1. Metastases in lung after intravenous inoculation with AKTOL cells of 12th (a) and 37th (b) passages.

In this investigation the properties of an adenocarcinoma (strain AKTOL) during serial passage was studied.

EXPERIMENTAL METHOD

Strain AKTOL arising as a result of malignant change in a cyst of the embryonic large intestine, transplanted subcutaneously into adult mice, was obtained from the Laboratory of Virology of the Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR [2]. Tumors were studied after 6-46 passages. Male and female BALB/c mice weighing 22-26 g were used.

Before transplantation the tumor was minced in a Plotter's homogenizer in medium No. 199 and filtered through 8-12 layers of Kapron. The number of undamaged cells was counted in a Goryaev's chamber. The tumors were measured with calipers in two mutually perpendicular directions. The product of the two measurements was taken as the approximate area.

To obtain AKTOL colonies in the lungs the tumor cells ($2 \cdot 10^5$ cells per mouse) were injected into the caudal vein.

The mice were immunized by injection of $2 \cdot 10^7$ AKTOL cells irradiated in a dose of 15,000 rad subcutaneously in the region of the inguinal and axillary lymph nodes ($5 \cdot 10^6$ cells at each of four points).

To compare the immunogenicity of the tumors after different numbers of passages, cells of the 44th passage were injected intravenously into animals immunized with cells from the 7th and 44th passages and control mice 8 days after immunization. The number of tumor colonies in the lungs was counted after 20 days and the immunogenicity of the cells of each passage judged from the difference. This method was developed in the writers' laboratory by Avdeev et al. [1].

The morphology of the subcutaneous tumors and colonies in the lungs was studied in paraffin sections stained with hematoxylin–eosin.

The significance of differences in the mean dimensions and weight of the tumors and in the number of lung colonies was determined with the aid of a Student's criterion [3].

EXPERIMENTAL RESULTS

The rate of growth of the AKTOL tumor increased parallel with the number of passages. The results of measurements of tumors of the 6th and 41st passages at different stages of development are given in Table 1. The mean area of the tumors of the 41st passage was more than 5 times greater than that of tumors of the 6th passage by the 10th day and 15 times greater by the 30th day of development (the difference in the 2nd case is highly significant). The mice were killed and the tumors weighed on the 40th day. The mean weight of the tumors of the 41st passage was 985.3 ± 136.7 mg and of the 6th passage 179.7 ± 78.5 mg ($P < 0.001$).

The character of the lung colonies of the tumor also changed significantly during serial passage. Cells of the 5th passage did not induce foci of tumor growth; after injection of

TABLE 1. Dimensions of Subcutaneous AKTOL Tumors of 6th and 41st Passages at Different Times after Transplantation (M ± m)

Index	10th day		30th day	
	6th passage	41st passage	6th passage	41st passage
Number of animals	10	10	10	8
Mean area of tumors, mm^2	1,1±0,28	5,9±2,75	9,5±3,7	146±38,4
P	>0,05		<0,01	

TABLE 2. Number of Lung Colonies after Intravenous Injection of Cells of 44th Passage of AKTOL into Mice Immunized with Cells of the 7th and 44th Passages of the Same Tumor (M ± m)

Index	Immunization		Without immunization
	44th passage	7th passage	
	1	2	3
Number of animals	7	9	7
Mean number of lung colonies per mouse	54±8,4	50,0±5,3 P_{1-2}=0,7	132±20,8 P_{2-3}<0,01 P_{1-3}<0,01

cells of the 7th-12th passages a few tumors of different sizes appeared in the lungs (Fig. 1a) During subsequent passage a tendency was observed for the number of discrete lung colonies to increase and for their size to decrease (Fig. 1b). This phenomenon was interpreted as a sign of progression of that particular strain of the tumor, for the increase in the number of lung tumor colonies was directly proportional to the acceleration of growth of the subcutaneous tumors and to the increase in the degree of their morphological anaplasia.

Histological examination showed that the tumor of the 7th passage consisted mainly of structures composed of moderately polymorphic round or polygonal cells with many mitoses. The tumor of the 45th passage was distinguished by the greater polymorphism of its cells, the appearance of elongated and fusiform cells, and the presence of very large hyperchromic nuclei and single polynuclear cells. Comparison of the structure of the subcutaneous tumors and the lung metastases of the same passage showed a higher degree of structural and cellular anaplasia of the lung foci.

The results of the experiment to detect antigenic differences in tumors of early (7th) and late (44th) passages of AKTOL are given in Table 2. They show that immunization of the mice with irradiated cells of both the 7th and 44th passage leads to a clear and statistically significant decrease in the number of lung colonies compared with their number in unimmunized animals. Meanwhile the number of lung colonies in mice immunized with tumors of different passages was virtually the same, indicating their identical immunogenicity.

The various changes in the properties of the tumor in the course of serial passage (acceleration of growth, an increase in the number of lung colonies, more marked anaplasia), undoubtedly reflecting progression of the tumor, were thus evidently not directly connected with any change in the antigenic structure of the tumor cells.

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