The results confirmed the individual sensitivity of tumors transplanted into nude mice to chemotherapy and radiotherapy, and this corresponds to their sensitivity in man to treatment under clinical conditions [3]. In further investigations it will be necessary to have series of tumors of the same localization transplanted into nude mice in order to discover the most general rules.

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RESISTANCE-DEPRESSING AND METASTATIC ACTIVITY OF TRANSFORMED SYRIAN HAMSTER CELLS

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Evidence in support of the view that a tumor cell population is heterogeneous with regard to its metastasizing activity was obtained some time ago [5, 6]. This hypothesis was recently proved [4, 7]. According to the authors cited, the formation of visible metastases is the result of *in vivo* selection of a cell subpopulation which possesses certain properties essential for passage through all the stages of selection. It is evident that in the early stages of the metastatic process selection of cell variants will be based on a number of different features. It is still not clear what biological properties determine the ability of tumor cells to overcome each barrier on the road to metastasis formation. There are several such barriers in the path of spread of tumor cells from the primary focus into the blood vessels and lymphatics, until they penetrate into the tissues of different organs. The formation of metastases actually in the organs and tissues is evidently associated with the surmounting of other barriers. In our view [1-3], one of the essential properties of tumor cells for metastasis formation is their ability to depress the natural resistance (NR) of the host to the tumor. In previous experiments on Syrian hamsters the present writers showed that inactivated cells of various tumor strains (unlike normal cells) have the ability to depress the NR of the host to the tumor, and a system for determining the resistance-depressing activity of tumor cells *in vivo* was worked out [1]. It was postulated that tumor cells contain a thermostable factor capable of depressing the NR of animals to tumors and that this factor is important for the development of monoclonal tumors, for metastasization of tumors, and also for tumor growth after transplantation of single tumor cells [1-3].

Ability to depress the NR of animals to tumors was discovered during the investigation of a number of hamster tumor strains of varied origin, but it was not found when normal Syrian hamster embryonic cells were studied in the second half of embryogenesis, or in cells of a strain of embryonic hamster cells transformed spontaneously *in vitro* (strain HETR). Meanwhile the cells of this strain, if transplanted subcutaneously into hamsters in large doses, can form metastases in the lungs.

In the investigation described below the resistance-depressing activity (RDA) of the parental HETR strain and of a number of its sublines obtained from distant lung metastases, and also their metastasizing activity were studied.

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TABLE 1. Results of *in vivo* Testing of RDA of Parental HETR Strain

Group of animals	Dose of HETR cells used to test RNA ($\times 10^6$)	Results of TT with E-1 test cells: 1,4	1,4·10¹	1,4·10²	1,4·10³	1,4·10⁴	log TD_{50}	RDA
1 (intact control)	—	0/5	0/5	3/5	4/5	5/5	2,2	—
2	130	1/4	1/4	1/4	4/4	4/4	2,2	—
3	65	0/5	0/5	1/5	5/5	5/5	2,5	—
4	32	0/5	1/5	0/5	3/5	5/5	2,9	—
5	16	0/5	0/5	0/5	5/5	5/5	2,7	—
6	8	0/5	0/5	1/5	5/5	5/5	2,5	—

Legend. Here and in Table 2: TT) subcutaneous transplantation test with injection of five doses of test tumor E-1, differing by a factor of 10, 8 days after intraorbital injection of different doses of inactivated test cells of HETR strain; log TD_{50}) logarithm of 50%-take dose of test tumor cells; numerator) number of animals with tumors, denominator) number of animals into which the given dose of test tumor cells was injected.

TABLE 2. RDA of Metastatic Variant of HETR Cells (Subline HETR-MLN-3)

Group of animals	Dose of EHTR-MLN-3 cells used when testing ($\times 10^6$)	Results of TT with E-1 test cells: 1,2	1,2·10¹	1,2·10²	1,2·10³	log TD_{50}	log ER	RDA
1 (intact control)	—	0/5	1/5	4/5	5/5	1,5	—	—
2	22	5/5	4/4	5/5	5/5	<0,005	<1,45	+++
3	11	1/5	3/5	5/5	5/5	0,65	0,85	++
4	5,5	1/5	3/5	4/5	5/5	0,85	0,65	++
5	2,7	0,5	1/5	4/5	5/5	1,5	0	—

TABLE 3. Combined Results of Testing RDA and Metastatic Activity of Parental HETR Strain and its Variant

Designations of test variants of cells	Number of tests of RNA	Doses of cells used to test RNA ($\times 10^6$)	log ER	RDA	Metastatic activity*
HETR (parental variant)	12	3—130	0—0,25	—	1:10⁵
HETR-SC	3	6—110	0,4—0,8	+; ++	1:10²
HETR-SCN	2	6—55	0	—	Inactive
HETR-MLN-1	2	6—45	0	—	1:10³
HETR-MLN-3	2	3—37	0,65—1,5	++++	1:10²
HETR-MLN-4	3	3—35	0	—	1:10⁴
HETR-MLN-5	2	5—42	0,35	+	1:10⁴
HETR-MLN-6	3	5—45	0	—	1:10³
HETR-MLN-7	2	9—40	0,4	+	1:10³
HETR-MLN-8	2	6—31	0,5—1,3	++++	1:10²
HETR-MLN-9	3	8—65	0,5—1,3	++++	1:10²

*Ratio between number of metastases in lungs and number of cells of test sublines injected into blood stream.

EXPERIMENTAL METHOD

Testing the RDA of tumor cells [1] includes the following stages: 1) a single intraperitoneal injection of various doses (10^6-10^7) of the syngeneic or allogeneic test tumor cells, inactivated by heating to 56°C for 1 h and irradiated in a dose of 10,000 rads, into Syrian hamsters; 2) subcutaneous transplantation of living cells of the same or another test tumor in doses of 1 to 10^4 cells into the animals indicated above and also intact control hamsters. The transplantation test (TT) is carried out 7-45 days after intraperitoneal injection of inactivated tumor cells into the animals. It has been shown [1, 2] that tumors grow after transplantation of single tumor cells in animals which have received inactivated tumor cells (by contrast with intact animals).

Obtaining Sublines of the HETR Strain. Cells of the parental HETR strain were injected subcutaneously into Syrian hamsters. The animals were anesthetized 5-8 weeks later and HETR cells from the growing subcutaneous grafts and also their metastases in the lungs (each one separately) were retransplanted subcutaneously in normal Syrian hamsters. The cells growing from tumor nodes after transplantation in this way were returned to culture *in vitro*, under the name of sublines HETR-SC and HETR-SCN for HETR sublines obtained by subcutaneous transplantation of the parental HETR strain, and HETR-MLN (with an appropriate number) for the eight variants obtained from lung metastases of strain HETR. The parental cells of the HETR strain and its variants were propagated *in vitro* and their RDA was tested after different numbers

of passages. RDA of the test variants of cells was estimated 8 weeks after transplantation of the test cells by a 4-plus system: If the logarithm of the enhancement ratio (ER) for growth of the test tumor in the experimental animals compared with the controls following injection of maximal doses (more than 10×10^6) of inactivated test cells was under 0.3, RDA was considered to be absent and the result was expressed as –; if the value of log ER were 0.3-0.5, 0.6-1.0, and over 1.0, RDA was designated +, ++, or +++ respectively; RDA was described as ++++ if found after injection of under 10×10^6 inactivated test cells into the animals.

To determine the ability of the parental HETR strain and its sublines to give growth of colonies of tumor cells in the lungs (conventional metastases) cells of the test variants (including the parental variant) were injected into the orbital sinus of groups of Syrian hamsters in four or five doses differing by a factor of 10. The animals were killed 25-30 days later and the number of colonies visible with the aid of a magnifying glass (magnification 18-27 times) on the surface of the lung tissue of each animal was counted. To count metastases, each of the five lobes of the lung was placed between two flat glass surfaces in petri dishes. The lowest ratio for each test variant between the number of conventional metastases growing in the lungs and the number of test cells injected into the blood stream was then determined.

EXPERIMENTAL RESULTS

Repeated attempts to find RDA of cells of the parental HETR strain gave consistently negative results even when exceptionally high doses of inactivated cells of this strain were tested on the animals (one typical result is shown in Table 1). Tests of the HETR-SC and HETR-SCN cell variants in doses of between 15 and 110 million cells showed that one of them possessed RDA (from + to ++). As a result of subcutaneous transplantation of the parental HETR strain twice *in vivo*, one (of the two tested) variant possessing some degree of RDA was thus selected.

When metastatic variants of strain HETR were tested five of the eight sublines studied were found to possess RDA, three of them to the highest degree (++++). Results of the tests of RDA of the cells of one such subline and the combined results of investigation of RDA of all HETR variants are given in Tables 2 and 3. As Table 2 shows, the metastatic variant of the HETR cells (subline HETR-MLN-3) possessed marked ability to depress the animals' resistance to the tumor when only 5.5×10^6 inactivated cells were injected, whereas 130×10^6 cells of the parental strain did not have this ability. The results are evidence that during metastasis formation selection of tumor cells capable of depressing NR of the host takes place. The absence of RDA of cells of three of the eight metastatic variants of HETR tested may perhaps be attributable to the fact that metastases can be formed not only from single tumor cells endowed with RDA but also from conglomerates of tumor cells, containing both RDA^+ and RDA^- variants.

It was accordingly decided to look for the presence or absence of correlation between RDA and metastatic activity for each variant of HETR cells tested. This could be done by determining the ratio between the number of test cells injected into the blood stream and the number of visible colonies of tumor cells (conventional metastases) in the lung tissues of these animals. The results of this series of experiments are given in Table 3.

They show that variants with the strongest RDA possessed the highest metastatic activity when tested in this manner. Whereas a population of 10^5 cells of the parental HETR strain contained only one cell capable of forming metastases in the lungs, the proportion of such cells in populations of metastatic variants of this strain was between $1:10^2$ and $1:10^4$, i.e., 100-1000 times more. Correlation between the metastatic activity of the cells and the RDA must be emphasized: The higher the RDA of the test cells, the greater their metastatic activity.

The results thus demonstrate that a population of strain HETR cells spontaneously transformed *in vitro* consists mainly of variants of cells with no metastatic activity and no RDA. RDA^+ variants are selected during growth of these cells *in vivo* and, in particular, during metastasis formation. The ability of transformed cells to depress the NR of animals to tumors is evidently an essential factor in their selection for the formation of metastases in the lungs.

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