5. V. P. Rybakov, A. V. Timofeev, and Yu. A. Romanov, Byull. Éksp. Biol. Med., No. 10, 492 (1981).
6. V. P. Rybakov, Byull. Éksp. Biol. Med., No. 11, 97 (1983).
7. N. F. Semenova, Byull. Éksp. Biol. Med., No. 2, 91 (1971).
8. N. F. Semenova, Byull, Éksp. Biol. Med., No. 11, 94 (1975).

ARTIFICIAL SELECTION FOR HIGH METASTATIC POTENTIAL IN TRANSPLANTABLE RAT RHABDOMYOSARCOMA RA-2 CELL POPULATION

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KEY WORDS: clonal analysis in vivo; metastatic potential; artificial selection for high malignancy

Strains of transplantable tumors of varied histogenesis, whose cells possess affinity for a particular target organ have now been obtained with the aid of selection in vitro and in vivo [4-6]. The affinity of the cells is manifested as both spontaneous and experimental metastasization [4]. In the latter case, intravenously injected cells invade only that organ of the recipient animal for affinity to which they have been selected, and experimental metastases are formed only in the target organ. This particular feature of tumors with organ affinity enables the fraction of cells capable of forming experimental metastases to be determined with the highest possible degree of accuracy, i.e., enables the metastatic potential (MP) of the tumors to be determined.

In all studies of organotropic primary tumors an increase in MP has been observed during selection for organotropism. However, after completion of selection for organotropism (2-15 cycles), no further selection was carried out to increase MP, so that it is not possible to judge on a sufficiently sound basis the character of inheritance of the MP trait in tumor cell populations or the possibility of increasing MP of organotropic malignant tumors by means of artificial selection.

This paper gives the results of long-term (10 years), repeated (180 cycles) selection in vivo for increasing MP, recorded by the method of lung colonies, in a population of rhabdomyosarcoma cells, induced by 20-methylcholanthrene, and which yielded evidence to show that selection for high MP is effective even after the cells have acquired organotropism.

On the basis of data on the efficacy of selection for high MP, and on the degree of phenotypic and hereditary heterogeneity of the population selected on the basis of MP and its karyotypic heterogeneity, the inheritance of the MP trait was analyzed in a tumor cell population.

EXPERIMENTAL METHOD

Rhabdomyosarcoma RA2 was induced by injection of an oily solution of 20-methylcholanthrene into the thigh muscle of noninbred female albino rats (from the "Rappolovo" nursery).

Selection for increasing the ability of RA2 cells to form experimental metastases in the lungs was carried out in vivo by the lung colonies method [7]. For this purpose, suspensions of single tumor cells were prepared from subcutaneous transplants of a primary tumor, the number of viable cells was counted, and they were injected into the lateral caudal vein

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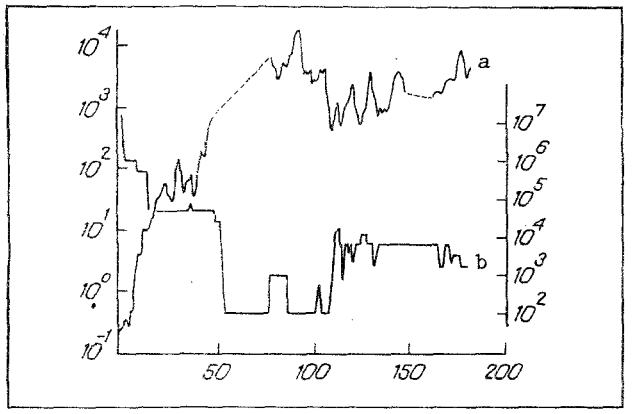

Fig. 1

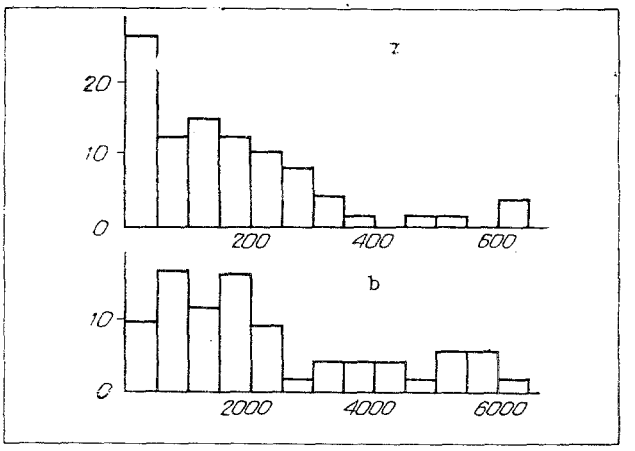

Fig. 2

Fig. 1. Dynamics of artificial selection for high MP in rat rhabdomyosarcoma RA2 cell population. Abscissa, cycles of selection; ordinate: on left – number of experimental metastases in lungs of rats, calculated per 10^5 tumor cells injected intravenously, on right – dose of cells. a) MP, b) dose of cells.

Fig. 2. Distribution of experimental RA2 metastases by mean values of MP on their cells in 87th-93rd (a) and 158th-169th-168th (b) cycles of selection. Abscissa, number of experimental metastases in lungs calculated per 10^5 tumor cells injected intravenously; ordinate, number of experimental metastases (in %).

TABLE 1. Changes in Metastatic Potential of Rat Rhabdomyosarcoma RA2 during Selection

Cycle of selection	Mean dose of cells	Number of animals	MP		
			lim (min—max)	CFU $\pm m$	S^2
112—124-	$4{,}9\cdot10^3$	49	20—2550	750±110	$0{,}55\cdot10^6$
158—169-	$5{,}0\cdot10^3$	82	40—5960	1580±150	$1{,}8\cdot10^6$
170—176-	$2{,}3\cdot10^3$	93	200—9900	2800±210	$3{,}53\cdot10^6$
177—188-	$1{,}0\cdot10^3$	42	110—25 100	6000±800	$26{,}9\cdot10^6$

of animals in order to obtain experimental metastases. A suspension was prepared from 25-30 tumor nodes formed in the lungs and it was injected intravenously in the next cycle of selection. The experimental metastases obtained by the method described above were clonal in origin [7, 9], giving clones with high MP selection advantages in each cycle.

The value of MP was calculated as the number of lung metastases per 10^5 intravenously injected, viable tumor cells (CFU). In the course of selection the value of MP and the distribution of the inoculated animals for the number of metastases formed were recorded. The kinetics of growth of the metastases in the lungs and distribution of individual RA2 metastases for values of MP during subcloning in vivo were investigated periodically in special experiments.

EXPERIMENTAL RESULTS

The data in Fig. 1 show that the first 10-15 cycles of selection were most effective. Later the tendency for MP to increase still remained, but the rates of its increase fell. The value of MP recorded correlated negatively with the dose of cells injected intravenously (Fig. 1), as other workers also observed [2]. This led to a sharp increase in MP in the 78th-108th cycles of selection, during a temporary switch to low doses of tumor cells (1×10^2). MP of experimental metastases, determined when standard doses of $(1\text{-}5) \times 10^3$ cells were injected at different stages of selection, is shown in Fig. 2. Clearly, despite the marked decrease in the efficacy of selection, there was a significant rise of MP in the course of subsequent selection (Table 1).

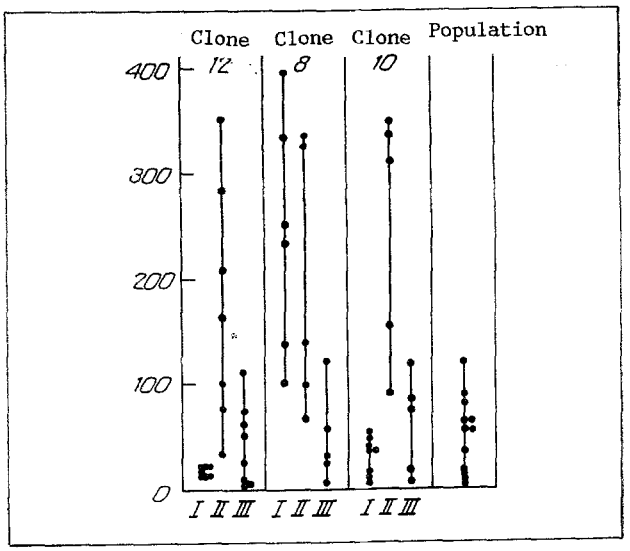

Fig. 3. Reversion of MP values of clonal lines of RA2 to mean populational level during passages. Vertical axis – number of experimental metastases in animals (filled circles) following intravenous injection of 5×10^3 cells. I-III) Passages in vivo.

Throughout the period of selection the cell population maintained not only high phenotypic (Fig. 2), but also high inherited heterogeneity: for example, the coefficient of heritability (h^2) of the MP trait in 158-169 cycles of selection was 0.55. However, the isolated clonal lines with different values of MP reverted to the mean populational level of MP by the 3rd passage (Fig. 3), evidence of the inherited instability of the "MP level" trait. This may evidently also explain the low efficacy of selection in the later stages.

The frequency of genome mutations remained high in the course of selection, namely $(12\text{-}13) \times 10^{-2}$ per cell per generation at the 28th cycle of selection and $(9.09 \pm 0.38) \times 10^{-2}$ at the 176th cycle. Accordingly, high karyotypic heterogeneity of the cells of the experimental metastases also was observed. Although a near-tetraploid level of DNA (DI 1.97-2.04) was maintained in the course of selection the cells varied in their number of chromosomes from 39 to 90, and cells of the near-tetraploid model class accounted for only 22% of the total population [3]. All karyotyped cells characteristically had multiple chromosomal aberrations, including those affecting chromosomes 4-7, for which localization of oncogenes has been demonstrated.

Successful results of artificial selection for a number of traits of tumor cells, correlating positively with manifestation of the malignant phenotype, have been described [1, 8, 10]. All these studies were conducted on tumors with a low degree of malignancy, and during a limited number of selection cycles. Our own results show that artificial selection for high MP is effective also in cell populations of highly malignant tumors, namely transplantable rat organotropic rhabdomyosarcoma RA2. By long-term selection the frequency with which MP-variants are found was increased from 0.007 in the original RA2 strain to 0.06, i.e., tenfold.

It is evident that by long-term artificial selection not only MP, but also other parameters of malignancy of strains of transplantable tumors used in experimental oncology, whether organotropic or nonorganotropic, can be enhanced.

In the course of selection clones with higher MP levels were isolated on more than one occasion (Fig. 2), but they quickly reverted to the mean populational level (Fig. 3). Long-term selection for the same trait, the MP level, did not lead to the obtaining of a karyotypically homogenous cell population; on the contrary, cells of the selected variant were distinguished by high instability of their karyotype. In the course of selection MP behaves as a quantitative trait with a high frequency of reversion to low values, and this makes selection for further raising of MP substantially more difficult.

LITERATURE CITED

1. E. L. Vendrov and G. I. Deichman, Byull, Éksp Biol. Med., No. 5, 607 (1986).
2. G. I. Deichman, Progress in Science and Technology. Series: Oncology [in Russian], Vol. 13, Moscow (1984), pp. 46-86.
3. E. V. Kaminskaya, N. M. Yartseva, and R. F. Fedortseva, Abstracts of Proceedings of an All-Union Conference on Genetics of Somatic Cells in Culture [in Russian], Moscow (1986). p. 65.
4. V. M. Senin, A. M. Buntsevich, A. V. Afanas'eva, and N. S. Kiseleva, Éksp. Onkol., No. 3, 35 (1983).
5. K. W. Brunson, G. Beattie, and G. L. Nicolsin, Nature, 272, 543 (1978).
6. I. J. Fidler, Nature New Biol., 242, 148 (1973).
7. R. P. Hill and R. S. Bush, Int. J. Radiat. Biol., 15, 435 (1969).
8. Y. Honma, T. Kasukabe, and M. Hozumi, Gann, 72, No. 6, 898 (1981).
9. F. Yu, R.-Y. Wang, and T. C. Hsu, J. Natl. Cancer Inst., 78, No. 1, 155 (1987).
10. P. A. Netland and B. R. Zetter, J. Cell Biol., 101, No. 3, 720 (1985).

ANALYSIS OF FACTORS DETERMINING SEX DIFFERENCES IN RESPONSES OF ALBINO RATS TO STRESS

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KEY WORDS: stress; sex differences; infants; castrates; neonatal androgenization

Sex differences in the sensitivity of rats to physiological stressors, discovered by the writers previously [1], are in good agreement with observed sex differences in resistance to particular types of pathology, arising in clinical practice [8] and experimentally [10]. Elucidation of the factors determining these differences depends primarily on a study of the role of sex hormones, for their level determines sex differences in a number of morphological structures [12, 15] and biochemical parameters [5, 6, 14], involved in adaptive processes.

The aim of these investigations was to study stress-induced reactions in infantile and adult intact and castrated female and male albino rats, and also in adult, neonatally androgenized females.

EXPERIMENTAL METHOD

Activity of stress-realizing mechanisms was investigated in females and males in situations of emotional and emotional-painful stress, not going beyond the limits of physiological stress factors either qualitatively or quantitatively. Emotional stress (ES) was induced by the sight and the cries of bound partners, and emotional-painful stress (EPS) by immobilization for 10 min. The intensity of the stress response was assessed on the basis of changes in the corticosterone concentration in the adrenals and blood plasma, determined fluorometrically. Responses to stress were studied in immature rats (weighing 70-100 g), in adult intact rats and rats castrated 3 weeks before the experiment, and in adult females receiving a subcutaneous injection of 1 mg of testosterone propionate at the age of 1 day. Altogether 362 rats were used.

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0007-4888/89/0011-1645$12.50