and skin fibroblasts, on the one hand, and the equal number of Ag + NOR and the equal intensity of their staining in these tissues, on the other hand, may be evidence of the relative nature of the proportionality described by some workers [9] between the intensity of silver staining of NOR and their associative capacity. It can be postulated that the active state of NOR, being inherited, is a characteristic and specific feature for each individual, essential for realization of the proliferative powers of the cell, and with respect to this feature blood lymphocytes do not differ from skin fibroblasts when cultured *in vitro*.

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LITERATURE CITED

1. A. F. Zakharov, A. Z. Davudov, V. A. Benyush, et al., in: Polymorphism of Chromosomes in Man [in Russian], Moscow (1981), p. 140.
2. N. N. Mamaev, S. E. Mamaeva, D. Bandandoiya, et al., Tsitologiya, 22, 161 (1980).
3. S. E. Bloom and C. Goodpasture, Hum. Genet., 34, 199 (1976).
4. M. Ferraro, P. Lavia, F. Pellicia, et al., Chromosomes (Berlin), 84, 345 (1981).
5. M. Hartung, C. Mirre, and A. Stahl, Hum. Genet., 52, 295 (1979).
6. H. Jotterand-Bellomo and C. van Melle, Hum. Genet., 59, 141 (1981).
7. V. D. Markovic, R. G. Worton, and J. M. Berg, Hum. Genet., 41, 181 (1978).
8. A.-V Mikelsaar and H. G. Schwarzacher, Hum. Genet., 42, 291 (1978).
9. D. A. Miller, R. Tantravahi, V. G. Dev, et al., Hum. Genet., 29, 490 (1977).
10. A. F. Zakharov, A. Z. Davudov, V. A. Benyush (V. A. Benjush), et al., Hum. Genet., 60, 24 (1982).

PRESERVATION OF PARENTAL FEATURES IN MAN—CHINESE HAMSTER CELL HYBRID

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By studying different features in hybrid and parental cells it is possible to assess the contribution of genetic and environmental factors to the phenotype of hybrid cells.

In the investigation described below the effect of long-term culture of hybrid cells in a nonselective medium on colony forming ability in selective media containing the same components as were used to select the hybrid cells during hybridization was studied.

EXPERIMENTAL METHOD

Hybrid cells of subclones MOI-8-1 and MOI-8-3 were used. The cells were obtained by fusion of normal human embryonic muscle cells (IMG 812) with transplantable chinese hamster cells (MO-1), deficient for hypoxanthinephosphoribosyl transferase and resistant to 1 mM ouabain; chinese hamster cells (O-1) resistant to 1 mM ouabain also were used.

The method of obtaining the hybrid cells was described previously [1]. The cells were cultured in Carrel flasks or on Eagle's medium with the addition of 20% bovine serum without antibiotics (henceforward this medium will be called normal medium) or on HATG medium, containing hypoxanthine (10^{-4} M), aminopterin (4×10^{-7} M), thymidine (1.6×10^{-7} M), and glycine (3×10^{-6} M). The cells were subcultured every 4-5 days. In the experiments the cells were

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TABLE 1. Survival of Cells on Normal and Selective Media (in percent)

Cells	Conditions of long-term culture	Relative plating efficiency of cells			
		normal medium	HTG	HATG	Ouabain (1 mM)
MOI-8-1	HATG, 56 passages	(17,3) 100	481,5	224,9	373,7
	Normal medium, 69 passages	(69,8) 100	123,8	74,2	95,4
MOI-8-3	HATG, 56 passages	(22,4) 100	277,3	194,2	383,8
	Normal medium, 69 passages	(79,4) 100	114,4	67,3	89,9
O-1*	HATG, 56 passages	(25,9) 100	313,7	274,4	132,2
	Normal medium, 69 passages	(90,4) 100	111,9	101,9	95,2

Legend. Absolute plating efficiency shown in parentheses. Asterisk indicates averaged data from two experiments.

seeded either on plastic Petri dishes 40 mm in diameter (Leningrad Medical Polymers Factory) or on glass Petri dishes 100 mm in diameter (Anumbra, Czechoslovakia). For each variant of the experiment either eight plastic or three glass dishes were used. To keep the pH of the medium between 7.2 and 7.4 the dishes were kept in airtight containers with an atmosphere containing 5% CO_2. To count growing colonies the cells were stained with methylene blue. The survival rate of the cells was expressed as a percentage of the absolute plating efficiency of the cells on normal medium. By absolute plating efficiency was meant the number of growing colonies expressed as a percentage of the total number of seeded cells.

EXPERIMENTAL RESULTS

To select hybrid cells HATG medium plus 10^{-3} mM ouabain was used for hybridization. It was necessary to know how long and to what degree the hybrid cells preserve parental features under nonselective conditions: a feature of human cells, namely survival on HATG medium, and a feature of chinese hamster cells, namely survival on medium containing 1 mM ouabain. At the same time the survival rate of the cells on HTG medium (HATG medium without aminopterin) was studied. The effect of long-term culture on normal medium with culture on selective HATG medium was compared. The experimental results are given in Table 1.

As the results in Table 1 show, survival of the hybrid MOI-8-1 and MOI-8-3 cells on normal medium in the experiments largely depended on the conditions of long-term culture before the experiment. Similarity in the behavior of the two hybrid subclones was noted. Their absolute plating efficiency on normal medium was quite high, if the cells were grown on normal medium (69.8 and 79.4%), but fell sharply after culture on HATG medium (17.3 and 22.4%). Hence it follows that the residual action of aminopterin had some influence on the synthesis of guanine and thymidine, i.e., the cells required time to restore synthesis of purines and pyrimidines de novo.

After culture in nonselective medium both clones preserved quite high plating efficiency on HATG medium and medium with ouabain (the values for subclone MOI-8-1 were 74.2 and 95.4%, for subclone MOI-8-3, 67.3 and 89.9% respectively). This indicates that in the populations of both subclones there was a quite high proportion of cells which preserved the genes of human hypoxanthine-phosphoribosyl transferase and hamster K^+,Na^+-ATPase.

Evidence of a toxic action of aminopterin also is given by the fact that hybrid cells incubated for a long time on HATG medium had a higher survival rate on HATG medium than on normal medium. Even higher viability of the cells was observed on HTG medium — in the absence of aminopterin. In that case the relative plating efficiency was expressed by values in excess of 100%, for the survival rate of the cells on normal medium was taken as 100%. Evidently during isolation of hybrid cells it is better not to transfer them at once from HATG medium to normal medium, but to incubate them for a short time to begin with on HTG medium so that they will multiply very rapidly, as was done in some other investigations [2].

It is not surprising that after long-term culture on HATG medium the addition of hypoxanthine, thymidine, and glycine to HATG medium increased the plating efficiency of the hybrid

cells even in the presence of aminopterin, compared with that on normal medium. However, it is a surprising fact that the addition of ouabain also increased the plating efficiency of the hybrid cells compared with normal medium (the plating efficiency of MOI-8-1 cells was 373.7%, and of MOI-8-3 cells 383.8%).

To discover whether in this case a combination of two genomes is reflected in the phenotype of the hybrid cells or whether a physiological effect of ouabain is observed irrespective of whether it acts on the genotype of the hybrid or nonhybrid cell, in the next experiments cells resistant to ouabain (O-1) were isolated from chinese hamster cell line 237. After isolation the O-1 cells were grown on HATG medium for 29 passages. Some of the O-1 cells were transferred at the 27th passage to culture with normal medium and went through a further two passages. Similar experiments were carried out with these two subpopulations of cells (Table 1).

The results showed that after long-term culture on medium HATG the O-1 cells, just like the hybrid cells, had higher plating efficiency of medium with ouabain (132.2%) than on normal medium. Possibly ouabain assists the cells physiologically to restore synthesis of purines and pyrimidines de novo more rapidly. It can be tentatively suggested that if selective HATG medium plus ouabain is used in hybridization experiments, to increase the survival rate of the hybrid cells in HTG medium ouabain should be added.

LITERATURE CITED

1. K. N. Grinberg, G. D. Iashvili, E. M. Pichugina, et al., Byull. Éksp. Biol. Med., No. 11, 85 (1982).
2. P. Draber, J. Zikan, and M. Vojtiskova, J. Immunogenet., 7, 455 (1980).