# ISOLATION OF MUTANT HeLa AND Am-1 CELLS RESISTANT TO NITROGENOUS BASE ANALOGS

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The sensitivity of cultivated cells of strains HeLa and Am-1 to a number of analogs of purine and pyrimidine bases was studied. For this purpose the number of viable cells was counted and cell division was studied in cultures grown in medium with addition of the analogs. HeLa and Am-1 cells was found to be sensitive only to 8-azaguanine and 2,6-diaminopurine sulfate. Cultures of spontaneous mutants of these cells resistant to 8-azaguanine were isolated.

One of the causes restricting the scope of genetic analysis of somatic cells of mammals and man is the difficulty associated with the discovery of genetic markers.

A matter of the utmost importance in this respect is the isolation of mutants, resistant to analogs of purine or pyrimidine bases, and also to metabolic inhibitors, from cultures of different cells.

This problem was first studied by Szybalski and Smith [5], who isolated mutants resistant to individual analogs of purine bases and their ribosides from cultures of human bone marrow cells. Subsequently reports were published by other workers who had isolated resistant mutants from cell cultures [1-4].

This paper describes the results of a study of the sensitivity of cultures of HeLa and Am-1 cells to analogs of purine and pyrimidine bases and also the isolation of mutants resistant to 8-azaguanine from cultures of these cells.

## EXPERIMENTAL METHOD

HeLa and Am-1 cells cultivated in Eagle's medium with 10% bovine serum were used in the experiments. The sensitivity of the cells to analogs of the nitrogenous bases was determined by counting the number of viable (resistant) cells and studying cell division in cultures grown with the addition of one of the analogs (8-azaxanthine, hypoxanthine, adenine, 2,6-diaminopurine sulfate, 5-bromouracil, guanosine, Ba salt of guanosine-2,3-phosphate, guanosine-2,3-phosphatic acid, inosine, 8-aza-adenine, or 8-azaguanine) in concentrations of between 1 and 500 $\mu$g/ml.

The survival rate of the cells in the presence of analogs contained in the medium in different concentrations was judged from the number of cells collected from Carrel's flasks on the 7th-8th day after seeding in a dose of 50,000 cells/ml medium. The number of resistant cells was determined from the number of colonies (fixation with Carnoy's mixture, staining with Mayer's hematoxylin) growing after four changes of identical medium.

Cells for cytological investigations were grown on cover slips in Wasserman tubes, fixed with Bouin's solution, and stained with Mayer's hematoxylin. Cells dividing by mitosis were counted in promille per 4000 cells in each specimen. The phases of mitosis were determined as percentages of the total number of mi-

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TABLE 1. Effect of 8-Azaguanine on Cell Division in HeLa Culture

| Index (in %) | Control | Concentration of 8-azaguanine (in μg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 0.5 | 1.0 | 2.0 | 3.0 | 4.0 |
| Mitotic index | 2.8±0.55 | 2.2±0.04 | 1.7±0.25 | 1.6±0.03 | 1.6±0.25 | 1.5±0.12 |
| Prophases | 22.4±0.4 | 23.1±1.8 | 19.5±1.8 | 20.1±1.6 | 22.0±1.6 | 26.5±1.9 |
| Metaphases | 44.1±1.1 | 39.6±3.4 | 37.2±2.7 | 35.9±3.0 | 25.6±1.0 | 19.2±3.5 |
| Anaphases | 14.2±0.5 | 8.3±0.6 | 5.4±1.2 | 4.2±1.3 | 4.2±1.0 | 4.6±1.0 |
| Telophases | 21.2±0.4 | 29.0±3.0 | 37.9±5.0 | 39.8±7.0 | 48.2±4.4 | 49.6±3.5 |
| Binuclear cells | 0.2 | 2.0±0.2 | 2.1±0.18 | 2.2±0.2 | 2.1±0.3 | 4.6±0.3 |
| Trinculear cells | — | 0.06 | 0.2 | 0.2 | 0.26 | 0.24 |
| Polynuclear cells | — | 0.15 | 0.10 | — | — | — |
| Giant cells | 0.17 | 0.21 | 0.07 | 0.11 | — | — |
| Cells with single buds | 0.17 | 1.7±0.22 | 1.2±0.01 | 1.7±0.20 | 2.0±0.30 | 2.5±0.4 |
| Cells with multiple buds | — | 2.0±0.1 | 3.0±0.2 | 6.2±0.4 | 5.6±0.7 | 9.3±0.7 |
| Amitotically dividing cells | — | 1.3±0.08 | 1.4±0.08 | 1.4±0.1 | 1.8±0.3 | 1.3±0.3 |

TABLE 2. Effect of 8-Azaguanine on Cell Division in Am-1 Culture

| Index in % | Control | Concentration of 8-azaguanine (in μg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 0,5 | 1,0 | 2,0 | 3,0 | 4,0 |
| Mitotic index | 4,1±0,3 | 2,1±0,06 | 1,7±0,04 | 1,2±0,05 | 1,1±0,08 | 0,96±0,07 |
| Prophases | 17,2±1,4 | 16,0±0,2 | 20,4±1,1 | 21,4±1,0 | 34,4±5,3 | 30,5±0,5 |
| Metaphases | 49,2±2,6 | 48,1±1,5 | 49,3±1,4 | 51,0±1,0 | 40,6±5,0 | 43,6±3,6 |
| Anaphases | 12,3±0,6 | 10,4±0,3 | 5,7±0,3 | 4,1±1,0 | 2,4±1,0 | 2,0±0,8 |
| Telophases | 21,2±2,6 | 25,3±1,3 | 24,6±1,1 | 23,5±1,1 | 22,5±5,8 | 19,0±1,7 |
| Binuclear cells | 0,2±0,004 | 0,4±0,04 | 0,7±0,006 | 1,4±0,01 | 1,5±0,08 | 2,2±0,01 |
| Trinuclear » | 0,07 | 0,04 | 0,08 | 0,17 | 0,04 | 0,1 |
| Polynuclear » | 0,07 | 0,008 | 0,05 | 0,03 | 0,05 | 0,04 |
| Giant cells » | 0,07 | 0,09 | 0,07 | 0,08 | 0,08 | 0,12 |
| Cells with single buds | 1,2±0,08 | 1,0±0,3 | 0,9±0,20 | 1,3±0,3 | 1,0±0,09 | 1,2±0,20 |
| Cells with multiple buds | 0,1±0,01 | 0,3±0,1 | 0,4±0,05 | 0,5±0,10 | 0,4±0,04 | 1,5±0,05 |
| Amitotically dividing cells | 0,3±0,01 | 0,4±0,1 | 0,6±0,07 | 0,7±0,4 | 0,7±0,06 | 0,6±0,1 |

totically dividing cells. In control experiments the viability and division of cells grown in Eagle's medium with 10% serum, but without addition of the analogs, were studied.

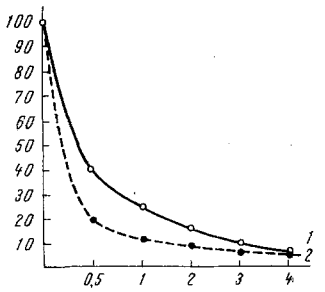


Fig. 1. Curves showing survival of HeLa (1) and Am-1 (2) cells in Eagle's medium containing 8-azaguanine in different concentrations. Abscissa, concentration of 8-azaguanine (in $\mu$g/ml); ordinate, percentage of surviving cells.

## EXPERIMENTAL RESULTS

In the first experiments the concentration of analogs in which death of most HeLa and Am-1 cells took place and only resistant forms survived was determined. It was found that the number of viable cells in the cultures was changed only in the presence of 8-azaguanine and 2,6-diaminopurine sulfate. Curves of survival of HeLa and Am-1 cells (in percentages of the control, i.e., of the number of cells grown in medium without analog) as functions of the 8-azaguanine concentration in the medium are shown in Fig. 1. These curves demonstrate that this analog affected the survival of HeLa and Am-1 cells equally.

The results of the cytological investigations are given in Tables 1 and 2.

Table 1 shows that 8-azaguanine had a marked effect on mitotic division of HeLa cells. The mitotic index fell from 2.8 to 1.5%, i.e., by 44%. The phases of mitosis were distributed as follows: the percentage of prophases was slightly increased, that of metaphases somewhat more, while the percentage of telophases rose sharply and that of anaphases fell sharply. In analog in a concentration of 4 $\mu$g/ml the percentage of metaphases fell from 44.1 to 19.2%, i.e., by more than 50%; the percentage of anaphases was reduced by almost three times, from 14.2 to 4.6; the percentage of telophases was increased by 2.5 times (from 21.2 to 49.6). The percentage of binuclear cells rose from 2.2 to 46, while the percentages of trinuclear and giant cells were almost unchanged.

Determination of the number of amitotically dividing HeLa cells showed an increased in the number of budding nuclei, both single and multiple, and also in the number of amitotically dividing cells.

The results in Table 2 show that the mitotic index of Am-1 cells also was lowered by 8-azaguanine, but less sharply. In the control culture of amniotic cells it was 4.1%, while by the action of the largest dose of the analog used (0.5 $\mu$g/ml) it was reduced by about 50%. Under the influence of 8-azaguanine in a concentration of 1 $\mu$g/ml, the number of mitotically dividing cells fell even more – to 1.7%. With large doses of the compound (2, 3, and 4 $\mu$g/ml) the number of dividing cells was only 0.96%.

A characteristic feature of the ratio between the phases of mitosis of amniotic cells was that, besides the general decrease in number of dividing cells, the percentage of prophases rose from 17.2 in the control cultures to 34.4 in the presence of large doses of analog (an increase of 100%). There was a small decrease in the percentage of metaphases (from 49.2 in the control to 40.6 in the experimental cultures) and a marked decrease in the percentage of anaphase figures (from 12.3 to 2). Variations in the number of telophases in the control and experiment were not significant.

Analysis of the data in Table 2 also shows that, with 8-azaguanine present in the medium in low concentrations, the number of trinuclear and polynuclear cells was hardly increased. The number of giant cells rose very slightly, but the number of binuclear cells vary sharply (from 0.2% in the control to 2.2% in samples containing 8-azaguanine in a concentration of 4 $\mu$g/ml). So far as the amitotically dividing cells are concerned, their number increased more slowly than that of the binuclear cells, but an increase in the number of all forms of amitotically dividing cells was observed.

Further observations on the HeLa and Am-1 cells in Eagle's medium with 8-azaguanine showed that not all colonies which initially were resistant retained their resistance to this analog during subsequent subcultures. As a result of prolonged cultivation of a series of clone cultures, one culture each of HeLa and Am-1 cells was isolated which possessed stable resistance to 8-azaguanine in a concentration of 4 $\mu$g/ml. It can thus be concluded from these experiments that HeLa and Am-1 cells are not sensitive to all analogs of the nitrogenous bases. It can be postulated that the isolated clone cultures of HeLa and Am-1 cells which possess resistance to 8-azaguanine are spontaneous resistant mutants of these cells.

## LITERATURE CITED


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