It will be evident that the antigen preparation can be completely characterized only by comparing values obtained for HPAP concentration with the enzyme activity data, so that the most important parameter of the preparation can be obtained, namely its specific enzyme activity (U/mg). The most promising method for this purpose is a combination of the "sandwich" test, based on affinity-purified antibodies to HPAP, and the endogenous test using double antibodies.

The monospecific and highly sensitive immunoenzyme systems suggested for detection of HPAP can be used also for clinical biochemical investigations and also for immunochemical analysis of cell cultures in order to detect the HPAP marker, to study the role of HPAP in cell regulation, and to study the molecular bases of oncogenesis.

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DETECTION OF CELLS WITH REDUCED SENSITIVITY TO CYTOSTATICS BY THE USE OF FLUORESCENT DYES

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When mammalian cells in culture are screened for resistance to antitubulins, anthracyclines, and other cytostatic agents, cell variants resistant to all these agents (cells with multiple drug resistance) appear. Resistance is linked with reduced accumulation of cytostatics in the cells [2-4]. Evidently the system responsible for resistance to cytostatics possesses relatively low selectivity. For instance, the present writers have shown [1] that resistant cells also take up smaller quantities of fluorescent dyes (including rhodamines, acridine dyes, and so on). Reduced accumulation of dyes enables resistant variants in a cell population (in a population of tumor cells, for example) to be easily detected. This result was obtained with cells with a high level of resistance (50-1000 times as resistant). In the investigation described below cultured mouse myeloma cells, resistant to low doses of vincristine, were isolated. It was found that these cells can also be reliably distinguished from cells of the initial type by the use of the fluorescent staining method.

EXPERIMENTAL METHODS

Mouse myeloma X63 Ag 8.863 cells were cultured in medium RPMI 1640 with the addition of 10% embryonic calf serum. Before staining the cells were washed with medium without serum, after which they were incubated in a solution of Rhodamine 123 (Sigma, USA). At the end of incubation the cells were washed twice with serum-free medium and the suspension was placed under a coverslip and examined with the Optom Photomicroscope III fluorescence microscope

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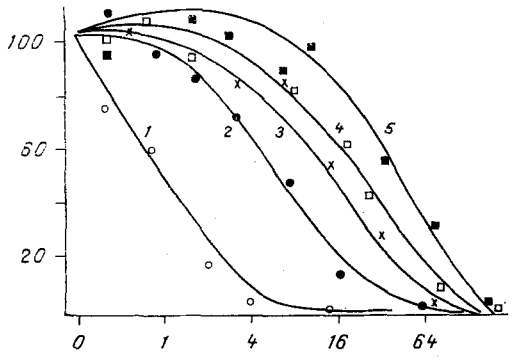

Fig. 1. Dependence of growth of VCR_0, VCR_2, VCR_{16}, and VCR_{32} cells on vincristine concentration. Abscissa, vincristine concentration (in ng/ml); ordinate, number of cells: 1) VCR_0, 2) VCR_2, 3) VCR_4, 4) VCR_{16}, 5) VCR_{32}. Number of cells growing in wells without vincristine taken as 100%.

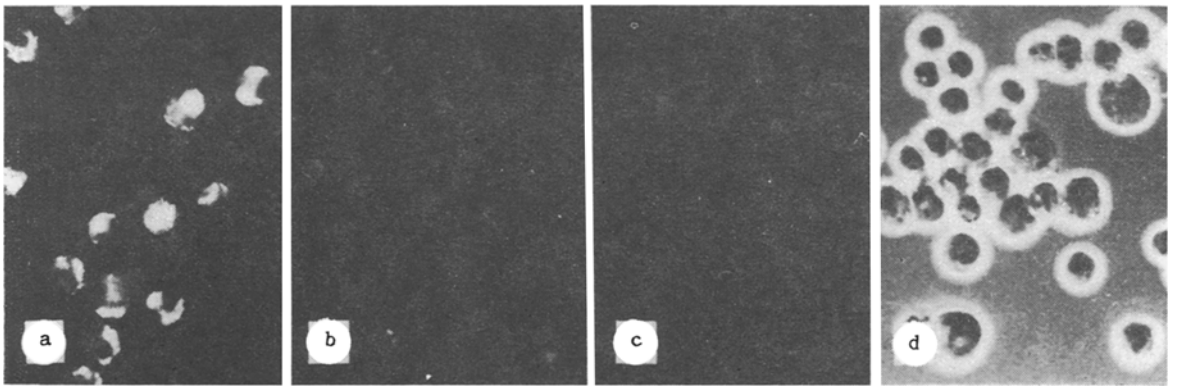

Fig. 2. Fluorescence of cells differing in their degree of resistance to vincristine, after incubation for 10 min with Rhodamine 123 (5 μg/ml) and transferred for 1 h into medium not containing the dye. a) VCR_0 cells; b) VCR_4 cells; c, d) VCR_{16} cells; c) fluorescence, d) phase contrast. Preparations photographed with identical exposure, and the same exposure also was used for printing the photographs.

with "Planapo 40×" objective. The same microscope, connected with an FMÉL-1 photometer (LOMO), was used for microfluorometry.

EXPERIMENTAL RESULTS

By gradually increasing the vincristine concentration in the medium, we were able to breed mouse myeloma cell lines growing continuously in the presence of vincristine in doses of 2, 4, 16, and 32 ng/ml respectively (lines VCR_2, VCR_4, VCR_{16}, and VCR_{32} respectively). To determine the sensitivity of these cells to the cytostatic action of vincristine they were seeded in a 96-well dish at the rate of $4 \cdot 10^3$ cells per well. Serial dilutions of vincristine (0-128 ng/ml) were added to the wells. After 1 week the number of cells in each well was counted. Dependence of cell growth on vincristine concentration is shown in Fig. 1. The vincristine concentration inhibiting growth of sensitive cells (VCR_0) by 50% was 1 ng/ml, whereas for VCR_2, VCR_4, VCR_{16}, and VCR_{32} cells it was 6.5, 26, 25, and 35 ng/ml respectively. AS a result of this screening, it was thus possible to obtain cells with a relatively low level of resistance to vincristine (6.5-35 times as resistant).

During staining of these cells with Rhodamine 123 under the conditions used previously (5 μg/ml, 15 min) [1], the mitochondria of the resistant and sensitive cells stained about equally, and only in the VCR_{16} and VCR_{32} lines were a few palely stained cells found. To

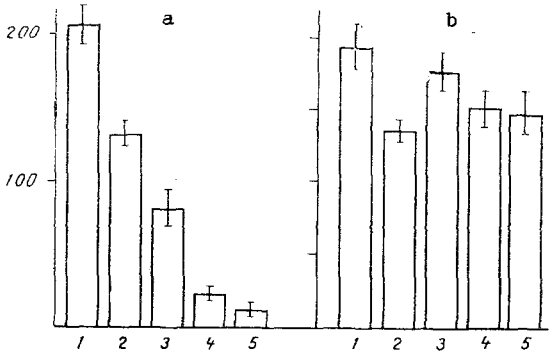

Fig. 3. Intensity of fluorescence (relative units) of cells incubated with Rhodamine 123 (5 μg/ml) and then for 1 h without dye in medium not containing reserpine (a) or containing 1 μg/ml of reserpine (b). Remainder of legend as to Fig. 1.

detect differences between the sensitive cells and cells with a low level of resistance, the conditions of staining had to be modified.

Reduced accumulation of drugs [2] and dyes [1] in resistant cells is known to be connected with the active release of these substances into the extracellular medium. We compared the rate of release of Rhodamine 123 from sensitive cells and from cells with a low level of resistance. For this purpose the cells were stained for 10-15 min in a solution with a high concentration of Rhodamine 123 (5 μg/ml), and they were then transferred into medium not containing the dye. In this case the resistant cells lost the dye quicker than the sensitive cells; the rate of elution of the dye increased with an increase in the degree of resistance. Thus sensitive and resistant cells could easily be distinguished by their level of fluorescence (Figs. 2 and 3).

Differences between sensitive and resistant cells in the rate of elution of the dye can be reduced by the use of reserpine, an inhibitor of multiple drug resistance. On the addition of reserpine in a concentration of 1 μg/ml, the process of elimination of Rhodamine 123 from resistant cells was inhibited (Fig. 3).

Thus even a low level of resistance to vincristine can be attributed to activation of the mechanism of multiple drug resistance. It can be tentatively suggested that the method of fluorescence analysis developed by the writers can identify not only cells resistant to vincristine, but also any kind of cells with a low level of multiple drug resistance.

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