Ehrlich Ascites Tumor Preservation for Fifteen Years—a Simple Method

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The Ehrlich ascites tumor was preserved for 15 years by adding to it an equal volume of 40% glycerol and placing the preparation directly into a freezer at –60° C.

The Ehrlich ascites tumor has been, and is, extensively employed for a wide variety of purposes, including microbiological pursuits. Associated with this is the need for tumor maintenance or preservation within a given laboratory, or, alternatively, repeated procurement from a source of supply. Some years ago we reported a method by which the tumor could be preserved in a frozen state for at least 1 year (1). The present communication is to report that the method has made possible the preservation of tumor cells for 15 years.

The oldest Ehrlich ascites (hyperdiploid) tumor stocks in our freezer (–60° C) were prepared on 21 March 1959, 22 February 1961, and 23 November 1961. These were thawed quickly in a water bath at 40° C. Four National Institutes of Health general-purpose mice, two females and two males all 7 weeks of age, were inoculated intraperitoneally with 0.4 ml of a given tumor stock. The number of tumor cells injected per animal amounted to approximately 2 × 10⁶. This was carried out on 27 March 1974. Within 3 weeks ascites tumors developed in all the mice.

Examination of the recovered tumors, by regular microscopy after fixation and staining or in the living state by phase microscopy, revealed cells typical of the Ehrlich tumor. They ranged in diameter from 8 to 22 μm and exhibited large, eccentric nuclei with large nucleoli. Large numbers of mitochondria were characteristically bunched on one side of the cell cytoplasm. Cells were stained by aceto-orcein, and 300 metaphase plates were counted from each of the recovered tumor stocks to determine the modal chromosome number. Before freezing, the tumor preparations showed modal chromosome counts of 46 per cell. Tumor material examined at the third serial passage of each recovered tumor stock revealed no change from the original modal count.

Viable cell counts were performed on each of the thawed tumor stocks by adding 0.1 ml of 0.25% Trypan Blue, in balanced salt solution, to 0.5 ml of tumor cell suspension and examining for stained and unstained cells. The stocks, in the respective chronological order mentioned above, showed 18, 25, and 20% viable cells.

The preservation method consists of adding 1 ml of a 1-week-old tumor (2 × 10⁶ cells) to 1 ml of 40% glycerol in a tube (13 by 100 mm), stoppering, mixing, placing at 4° C for 1 h, mixing again, and placing the tube directly into a freezer at –60° C. Lowering the temperature 1 degree per min offered no advantage, and, in fact, recovery was better after direct freezing. This obviates the need for elaborate, controlled-freezing equipment. The low-temperature freezer, within the range of –60° C, is now fairly common in laboratories and is certainly easier to procure and maintain than the equipment and supplies required for holding cells in liquid nitrogen. The preserving agent, glycerol, is readily obtainable and requires no special permit. The method is simple and highly effective at a practical level.

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LITERATURE CITED