Short-Term Storage at 4 C of Trypsinized Tissues

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Cells dispersed from tissues of certain animals remained viable for several days at 4 C although the per cent of viable cells varied among the different species.

The production of primary cell cultures is time-consuming, since it requires both the preparation of dispersed cells from the original tissue and the inoculation of the cell culture vessels. These lengthy procedures may present a problem when several tissues are to be processed simultaneously. Consequently, a method to store trypsinized concentrated cell suspensions could be useful to the investigator. Since refrigerator temperatures have been used to store whole tissues (1) and to preserve stable cells (2), a study was initiated to determine whether refrigerated temperature could maintain the viability and growth potential of concentrated primary cell suspensions.

For each suspension were determined in a hemacytometer by the trypan blue dye-exclusion method. Data are expressed as percentages of the initial cell count. To test the growth potential of stored cells, samples also were taken and diluted in Minimum Essential Medium, Eagle plus 10% calf serum and inoculated at a concentration (3 × 10^6 to 5 × 10^6/ml) of viable cells which results in a confluent monolayer in 5 to 7 days. In addition, medium from cells stored under the conditions described was tested for residual nutritional value by using it to seed freshly prepared primary cells.

Table 1 presents the results from one of a

| Tissue                      | 1-Day          | 7-Days        | 14-Days       |
|-----------------------------|----------------|---------------|---------------|
|                             | 4 C | Room temp | 4 C | Room temp | 4 C | Room temp |
| Hamster embryo              | 84 ± 3.2 | 72 ± 1.9 | 47 ± 1.4 | 21 ± 1.0 | 23 ± 1.0 | 7 ± 0.7 |
| Chick embryo                | 80 ± 1.4 | 76 ± 1.5 | 43 ± 2.6 | 19 ± 1.2 | 32 ± 3.3 | 7 ± 1.1 |
| Rhesus monkey kidney        | 85 ± 4.0 | 67 ± 1.7 | 22 ± 1.0 | 13 ± 2.5 | 9 ± 1.7 | 3 ± 0.4 |
| Rabbit kidney               | 83 ± 1.7 | 72 ± 3.0 | 10 ± 1.0 | 4 ± 0.7 | 2 ± 1.0 | <1.0 |
| Vervet monkey kidney        | 77 ± 4.1 | 63 ± 3.0 | 23 ± 2.5 | 11 ± 0.9 | 9 ± 1.7 | 2 ± 0.6 |

* Average of counts made on triplicate samples ± the standard error of the mean.

Freshly trypsinized cells from kidneys of young rabbits, adult rhesus and vervet monkeys, and from whole embryos of chickens and hamsters were maintained as cell concentrates in Hanks' lactalbumin hydrolysate plus 2 to 10% calf serum for 14 days or more. A 400-ml Kimler bottle was filled with 100 ml of a suspension of 2 × 10^6 to 6 × 10^6 cells/ml which was slowly agitated by use of a magnetic stirrer in a 4 C refrigerator. A similar suspension was kept at room temperature (ca. 23 C). Media were not replenished in containers of rabbit kidney and chick and hamster embryo cell suspensions. However, suspensions of rhesus and vervet monkey kidney were kept with and without medium changes.

The initial and subsequent counts of viable cells number of representative experiments. Cells from the various animal species tested varied as to their ability to remain viable under these conditions. Rabbit kidney cell suspensions lost their viability rapidly, whereas the viability of hamster and chick embryo cells was maintained for longer periods. As suspected, refrigerated cells remained viable longer than did cells maintained at room temperature. There were twice as many viable cells at 4 C than at room temperature at 7 days and there was a threefold difference by the 14th day.

Daily changes of the medium increased the loss of cell viability. Medium from cells stored for 14 days failed to promote the growth of freshly prepared primary cell cultures despite the pH stability of the medium during storage.
TABLE 2. Effect of temperature of initial suspending medium on cell viability

| Day | Rhesus monkey kidney | Vervet monkey kidney |
|-----|----------------------|----------------------|
|     | 23°C | 4°C | 23°C | 4°C |
| 2   | 66   | 62  | 67   | 65  |
| 4   | 45   | 43  | 46   | 50  |
| 7   | 25   | 21  | 25   | 24  |

* Ratio of number of viable cells on days indicated to number of viable cells on day 0 times 100%.

Cells refrigerated for 7 days and inoculated as described grew into monolayer cultures in 5 to 7 days at 37°C as did cells which had never been stored. However, cells kept for 14 days required 2 weeks to form a confluent cell sheet.

To examine the effect of the temperature of the initial medium used to suspend the cells before storage, an experiment was performed in which cold (4°C) and warm (23°C) media were compared (Table 2). Although, it required 1 hr and 45 min for the warm suspension to cool to 4°C, no influence on cell viability was noted.

Monolayer cultures of continuous cell lines can be stored at 4°C for 6 weeks or more without replacement of the nutrient fluids (2). Primary cell suspensions do not possess this capacity. However, certain primary cell concentrates can be kept under refrigerated conditions for 2 to 3 days without an appreciable loss of viability.

This storage procedure may provide a means of selecting temperature-sensitive mutants of viruses and protecting thermolabile viruses from inactivation during virus absorption.

LITERATURE CITED

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