Delay of the Appearance of Cytopathic Effect by Latent Viruses in Monkey Kidney Cell Cultures After Freezing and Storing at Low Temperature*

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Monkey kidney cells are often contaminated by latent viruses and when cultured in vitro, they develop progressive cytopathic changes consisting of groups of cells with extensively vacuolated cytoplasm or gradually enlarging areas of syncytial formation or the cultures may show small foci of granulated cells that progress and form holes in the cell sheet. These progressive lesions extend rapidly to the whole cellular sheet. Often cultures which initially show the vacuolated cells subsequently develop large syncytial areas. The appearance of latent contaminants in monkey kidney cell cultures is relatively late and may be detected after the cultures have served for vaccine production or for other experimental works. This makes the interpretation of results very difficult in one case and the vaccine unusable in the other.

Antisera against specific simian viruses or sera of the animal from which the kidneys were obtained may be helpful to prepare clean cultures and avoid deterioration by spontaneous contaminants (1). Wallis and Melnick (2), in 1962, reported that some simian viruses are suppressed in culture grown and maintained in aluminum chloride. These methods would facilitate experimental works, but should not be used in vaccine production. Indeed, virus for vaccines is usually grown in primary cell cultures. In the process, viruses present as spontaneous contaminants of the cell cultures are inadvertently incorporated into the virus harvests from which the vaccine is prepared.

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Therefore, it would be much easier to select cultures after an adequate test has been done to assure that endogenous viruses are not present. This might be attempted by freezing and storing cells at low temperature until a representative sample of unfrozen cells has been checked for spontaneous contaminants. If found suitable, the frozen cells would be used for experimental works or vaccine production.

**Materials and Methods**

Immature *rhesus* and *cercopithecus monkeys* were used to prepare kidney cell cultures. Monkeys were sacrificed soon after their arrival. Fresh suspensions of cells were frozen immediately after trypsinization and part of suspended cells were cultured in large prescription bottles. Freshly trypsinized cells and those dispersed from monolayers in bottles were suspended in medium for freezing. They were concentrated at $8.5 \times 10^6$ cells per ml. and dispensed in 5 ml. flasks. In some experiments, 2 ml. of concentrated cells were placed in a flask. Each flask was first closed with a rubber stopper and then sealed with a metal cap.

All cells were frozen in medium 199 enriched with 10% unheated calf serum. Glycerol or dimethyl sulfoxide were used at a final concentration of 10% to protect the cells against damages from freezing.

The freezing was done in liquid nitrogen vapor using the BF-3-2 chamber (Union Carbide, Linde). The rate of freezing varied in all experiments from 0.5 to 1.0 °C per minute. This rate was maintained from $+10^\circ$ C to $-25^\circ$ C and then maximum speed was used until $-60^\circ$ C was reached. All flasks were finally stored in a mechanical refrigerator at $-85^\circ$ C.

A 10% sample of freshly dissociated cells as well as cells dispersed from primary cultures was immediately cultured in tubes containing a final concentration of $1.5 \times 10^6$ cells per ml. These control cultures (unfrozen cells) were grown in medium 199 or Eagle's basal medium supplemented with 2% of unheated calf serum.

Frozen cells preserved after 1 to 8 months, were rapidly thawed by agitation in a 40° C water-bath. The recovered cells were dissociated by adequate pipetting and diluted in growth medium to $1.5 \times 10^6$ cells per ml. They were grown in tube cultures in the same way as the unfrozen cells.

Cultures of freshly dissociated cells and those recovered after freezing were maintained for as long as 28 days; some observations lasted as long as 6 weeks. The medium was changed every week during the first two weeks and two times a week afterwards. The growth medium was enriched with 2% of unheated calf serum. Tubes were examined two times a week for spontaneous degeneration. Cultures that showed no change in the cell monolayers were continued by subculturing the cells.

**Results and Discussion**

**Unfrozen Cells**

Eight immature *cercopithecus monkeys* were used to prepare cultures of kidney cells. A total of 200 primary cultures were initiated and maintained in tubes to look for the presence of spontaneous degeneration. All of them showed the presence of endogenous viruses. The cytopathic