

PREPARATION OF PRIMARY MONKEY KIDNEYS CELL CULTURES

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Primary green monkey kidney cell cultures are used for large-scale manufacture of live oral poliomyelitis vaccine in the U.S.S.R. The kidneys are disaggregated by step-wise trypsinization (1), however, procedures such as mincing of the tissue, long-term treatment with the enzyme and centrifugation of the cell suspension markedly reduce the capacity of cells to multiply on the glass surface interfering in maximum utilization of expensive monkey kidneys. The perfusion method of kidney trypsinization is devoid of the above shortcomings (2, 3).

We have developed and satisfactorily tested under production conditions a new modification of the perfusion method for disaggregation of isolated monkey kidneys. The experiments were carried out in 20 monkeys. From the exsanguinated animals the kidneys were extirpated together with the renal part of the aorta, and one kidney was disaggregated by stepwise trypsinization (control) while the other by the perfusion method (experiment). The perfusion was done in following way: through a needle inserted into the opening of renal artery, dispersing agents were pumped by means of a peristaltic pump via a system of silicone tubes. A 0.02% versene solution (5–7 min) was followed by a 0.25% trypsin solution (8–12 min). After perfusion, the renal capsule was removed, the kidneys were hanged on a hook, placed in a bottle with nutrient medium without serum (75–100 ml), and shaken vigorously by hand several times. The detached cells were removed, a new portion of the medium added, and the procedure was repeated until the complete exhaustion of kidney parenchyma. The yield of cells per 1 g of tissue was 304.3 ± 19.4 million in the control as compared to 570.0 ± 26.2 million in the perfusion disaggregation (the percentage of viable cells being 78.9 ± 2.6 and 94.7 ± 1.7 , respectively). The latter values are the highest ever reported in literature.

The cells produced by the perfusion method were explanted into 1.5 litre flasks ($S = 340 \text{ cm}^2$) containing 250–300 ml medium at a ratio of $4-5 \times 10^4$ cells per 1 ml, while cells produced by step-wise trypsinization were seeded at a ratio of $12-14 \times 10^4/\text{ml}$. The nutrient medium consisted of a mixture of 0.5% lactalbumin hydrolysate in Hanks' solution and Eagle's medium (1:1) with 5% bovine serum. The average number of flasks supplied from 1 kidney were 250 in the test and 40 in the control, respectively. In the former, a monolayer formed within 6 days, in the latter within 8 days. The cell yield per one flat flask in both tests and controls was 35–40 million. The cultures obtained by both methods did not differ in their sensitivity to poliomyelitis virus (Sabin strains). Virus titres were 7.7 log PFU/ml for type 1, 7.6 log PFU/ml for type 11 and 7.8 log PFU/ml for type III, respectively.

Summing up, the perfusion method of monkey kidney disaggregation provides the most sparing regimen and yields 5–6 times more primary cell cultures per each pair of kidneys than the method of step-wise trypsinization. This increases the vaccine harvests per kidney to 160–170 l as compared to previous 28–35 l. Larger batch sizes reduce the number of necessary controls and, accordingly, also the price of entire vaccine production.

References

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