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A technique to obtain fibroblast cells from skin biopsies of living bats (Chiroptera) for cytogenetic studies

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ABSTRACT. We developed a procedure to obtain fibroblasts from bat skin. A small fragment of the ear is removed under ether anesthesia. This material is then cut up into small pieces and cultured in standard cell culture media. Very good quality chromosome preparations for cytogenetic studies are obtained in about three weeks. Secondary cultures can be used for other biological studies. This procedure does not require sacrificing the animals.

Key words: Cytogenetics, Cell culture, Bats, Chiroptera

INTRODUCTION

In several species of small mammals, such as bats, extraction of peripheral blood provides very small volumes and most mitogenic agents are not efficient in this type of material. For this reason, chromosome preparations are frequently obtained from bone marrow cells (Ford and Hamerton, 1956) or primary cultures of different tissues (Modi et al*.,* 1987). Morielle et al*.* (1995) described a methodology to obtain bat fibroblasts from lung biopsies for cytogenetic studies. Armada et al. (1996) obtained metaphasic chromosomes from bone marrow cells grown in culture medium with 20% fetal calf serum, colchicine and ethidium bromide. Animal sacrifice is necessary for these two techniques. We developed a primary culture technique to obtain metaphase chromosomes from tissue biopsies of ear fragments of living bats.

MATERIAL AND METHODS

To obtain the ear fragments, five bats of the genus *Artibeus* were placed individually inside covered recipients containing cotton soaked with sulfuric ether. Once each bat was anesthetized, we disinfected one ear with an alcohol solution of iodine. With sterile scissors we cut out a small piece (2 mm^2) of the ear, and diced it into small pieces on a Petri dish. The fragments were transferred to a 25-cm^2 cell culture bottle, containing complete culture medium (medium MEM GLASGOW, 100 U/ml penicillin, 10^{-4}g/ml streptomycin, 10^{-4} g/ml fungizon, and 0.003 g/ml L-glutamine) and 50% bovine fetal serum, with a final volume of 1 ml. This solution forms a film that facilitates adherence of the fragments. The culture was incubated at 37ºC and the culture medium changed whenever the nutrients became depleted. Growth was observed with an inverted optical microscope. Once fibroblastic cell growth appeared, the cells were trypsinized and then suspended in a complete culture medium with 10% fetal serum, using the same routine as for cell culture. To obtain the secondary culture, where the cell monolayer is formed, the cells were trypsinized and then diluted in complete culture medium with 10% bovine fetal serum, distributed into two culture bottles and incubated at 37ºC. They were harvested for karyotypic studies when they attained logarithmic growth.

RESULTS AND DISCUSSION

The bats were not affected by the removal of a small piece of the ear. All of them survived, and their wounds healed quickly. Sufficient fibroblasts for chromosome analysis were obtained within three weeks. A high proportion of cells were in metaphase, with highly condensed chromosomes, facilitating the karyotypic analysis. The cells obtained were superior to those provided by the traditional techniques that use blood or bone marrow, and it was not necessary to sacrifice the animals.

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