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THE CHROMOSOME COMPLEMENT OF THE RHESUS MONKEY (MACACA MULATTA) DETERMINED IN KIDNEY CELLS CULTIVATED IN VITRO*

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With 3 Figures in the Text

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Introduction

As part of a cytological study on tissue culture strains designated as "altered monkey kidney" (AMK) by PARKER et al. (1957), it was desirable to study the chromosome complement of normal Rhesus monkey.

The chromosome number of this monkey has previously been reported as 42 by DARLINGTON and HAQUE (1955) and CHU and GILES (1956). The former workers have also identified the Y chromosome. The present paper describes studies on the chromosomes of monkey kidney cells cultivated in vitro, and extends the analysis of chromosome morphology to the point where most chromosomes of the complement can be individually identified.

Preparation of cell suspension and slides

Details of the preparation of cell suspensions and their in vitro propagation have been described elsewhere (SIMINOVITCH, ROTHFELS and PARKER). In general, cultures were prepared by trypsinization of kidney cortex from wild adult Rhesus monkeys of known sex, obtained from India. The trypsinized cells were dispersed into a medium consisting essentially of lactalbumin hydrolysate, yeast extract, 6% ox serum and salts (COOPER 1955). Twenty ml. aliquots of properly diluted suspension were placed into 10 cm diameter Petri dishes each containing two slides. The Petri dishes were kept at 37°C and flushed continuously with air containing 5% CO₂. Viable cells settled within 12 hours and began to proliferate on the glass. The medium was changed after 36 to 48 hours and cells were profitably examined three to six days after explantation.

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† While this paper was in press "A study of primate chromosome complements" by E. H. Y. CHU and NORMAN H. GILES appeared [Amer. Natur. 91, 273—282 (1957)] in which a photograph and some description of the chromosome complement of M. macaca is given.
Suitable slides, with a rather sparse growth of actively multiplying cells were pretreated for 30 minutes with a one quarter strength Tyrode solution (Hsr 1952). Fixation followed in acetic alcohol (1:3) for 10 minutes. Following fixation, slides were allowed to dry completely and this resulted in a flattening of the cells and separation of the chromosomes without undue scattering. Large numbers of intact metaphases in which the chromosomes were well spread were thus obtained without manual squashing. The preparations were stained in 2% natural orcein (G. T. Gurr, London) in 50% acetic acid and examined as temporary mounts or in euparal following a dry ice mounting schedule (Conger and Fairchild 1953). These cytological techniques are described more fully elsewhere (Rothfels and Siminovitch, 1958).

**Chromosome number**

In suitable preparations the bulk of the divisions were clearly normal and diploid. The rare polyploid nuclei and the more common endoreduplicated ones (Levan and Hauschka 1953) will not be considered in this paper since they will be described elsewhere (Siminovitch, Rothfels and Parker). The vast majority of the non-polyploid cells had exactly 42 chromosomes. In one of the better slides, one hundred consecutive (i.e. non-selected) metaphases were counted for chromosome number. Ninety seven were found to be 42. One, with a count of 43 proved to have a chromosome broken through the centromere, possibly during preparation of the slide. The remaining two nuclei had 41 chromosomes. Occasional counts of 41 were also made in apparently intact cells in other slides. These appear to be genuine and presumably arise through non-disjunction. In good slides no counts lower than 41 were found.

**Chromosome measurements**

In order to obtain measurements for the quantitative description of chromosome morphology, metaphases are required in which the chromosomes are well spread, with clearly defined centromeres, and free of overcontraction and obvious differential stretching. Nuclei from a male (Fig. 1) and from a female (Fig. 2) approximating this condition and actually used in analysis are shown as examples.

Once suitable nuclei were selected, tracings were obtained, either as camera lucida drawings of individually centered chromosomes using a 20 × ocular (Levan and Hauschka 1953); or, from prints or projections of 35 mm negatives taken on Dupont Microcopy. In our experience tracing of negatives projected in a micro film reader was the least laborious and most accurate of the methods tried.

In the tracings of chromosomes, the two chromatids were treated separately. The ends of the chromosomes were usually well defined but the centromeres, which formed non-staining gaps of considerable extent, required a standardized treatment.