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THE PRODUCTION OF CHIMERIC RATS AND ANALYSIS OF THEIR CHIMERISM

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The production of chimeric animals is an important technique for studying the development and differentiation of the ontogeny of mammals and for analyses of the aetiologies of various diseases in animal models. The techniques for production of chimeric mice were established in the 1960's. In rat chimera production, however, several problems remain to be resolved. These concern the collection of embryos, adhesion techniques and embryo-culture in vitro. In this paper, we described improved techniques for use in solving these problems and the analysis of chimeric rats.

Five-week-old female inbred rats were used for collecting embryos. Since adhesion manipulation of two rat embryos is difficult because they are flat and disk-shaped, 15% Ficoll was added to the adhesion medium. It gave adequate buoyancy to the embryos and consequently it made the movement of embryos smooth, slow and easy to control in the medium. In spite of the fact that many kinds of culture media for mouse embryos have been proposed, none have been suitable for rat embryos to develop into successful blastocysts. However, the medium proposed by Yamamura and Markert without fetal calf serum (FCS), was highly recommended for rat embryos.

Thirteen chimeric rats were obtained from the embryo aggregation of two inbred strains, ACI/Hkm(ACI) and WKAH/Hkm(WKAH), whose coat colors, major histocompatibility complex (RT1) on red blood cells and serum cholinesterase isozyme patterns were different. These properties were analyzed by computer graphic analyzer, flow cytometer with anti-RT1-A monoclonal antibody and polyacrylamide gel electrophoresis, respectively. In addition, the chimerism of germ lines was examined by a breeding test between chimeric rats and WKAH rats. Of 13 chimeras obtained, 4 of the male rats analyzed had these properties and were shown to be chimeric in every property examined. However, ACI-type of properties were superior to those of the WKAH type in every chimeric rat. In a breeding test with three chimeras, two were shown to have reproductive function, and 95% of their progeny possessed the ACI-type coat color, indicating that in chimeric rats the germ-line of the ACI-type was superior to that of the WKAH-type. Other chimera was shown to be of mixed-sex by analysis of their cholinesterase isozyme patterns and to have defective reproductive function. In these chimeric rats, the frequency of ACI-type coat color and number of red blood cells expanded with aging. Further studies were necessary to explore these aging factors in this chimeric rat.