Mammalian Chromosomes in vitro. V. The somatic complement of the Norway rat, *Rattus norvegicus*

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Exact knowledge of somatic chromosomes in higher vertebrates, particularly in mammals, was until quite recently rather limited. Obviously this was due mainly to technical difficulty associated with unfavorable material. The tissue culture method has been used by some workers as an aid in studying chromosomes, but the advantages of *in vitro* material versus the classical sectioned and squashed preparations were fully realized only after the report of Hsu and Pomerat ('53) whose technique of prefixation treatment of cultures with a hypotonic saline spreads the dividing chromosomes so that studies on the chromosome number and morphology can be made critically, particularly for the kinetochore region.

For several years one of us (SM) has engaged in cytological investigation of several rat tumors with special attention to the chromosomal changes taking place in ordinary tissue cells in the course of tumor formation and has demonstrated in the tumor cells the existence of certain pairs of V-and J-shaped chromosomes which vary in number for each tumor characteristically (Makino, '52 a, b; Makino and Kanô, '53). Since many previously published drawings of the rat chromosomes do not indicate the exact location of the centromere, it seemed desirable to analyze the normal somatic complement in detail with the new tissue culture technique.

**Material and methods**

Roller tube cultures of embryonic lung, spleen, heart, liver and ribs from approximately two-week old fetal rats (*Rattus norvegicus*) were made according to the procedure described by Hsu and Pomerat (l. c.). On the fifth day of incubation at 37°C the cultures were treated for 20 to 30 minutes in a warm hypotonic solution made of 30 parts of regular Gey's balanced salt solution and 70 parts of Gey's solution without NaCl. They were then fixed in Helly-Zenker for 60 minutes and stained overnight with dilute

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Delafield haematoxylin (25-30 drops of stock solution in 100 ml of distilled water).

**Observations**

In the zone of outgrowth from each explant of the various tissues many cells were found in the process of division. Cells from the lung and the rib cultures at both prometaphase and metaphase exhibited chromosomes with especially well-defined, sharp outlines. Some cells in which the kinetochore region of the chromosomes was readily distinguishable were selected for a critical study of the karyotype (Figs. 1-10). The chromosomes of these cells were analyzed with special attention to size and shape to make an approximate identification of homologous pairs.

A close microscopical examination was made as accurately as possible of the individual chromosomes. They were then drawn with the aid of a camera lucida and each of the supposed homologous pairs thus identified was
numbered in order of size. The chromosomes then were copied and placed in serial alignment according to size regardless of the locus of the centromere. Several examples are shown in Figures 12 to 16.

Figs. 6-11. Photomicrographs of somatic chromosomes of white rats, from roller tube cultures of embryonic tissues. Figs. 6, 7, 8, 9, and 10 correspond to Figs. 1, 2, 3, 4 and 5, respectively. 11, aneuploid metaphase showing 46 chromosomes, from liver culture.

The diploid chromosome number here established agrees essentially with that reported by previous authors mostly from germ cells, 2n=42 (cf., Makino’s list, ’52), but tetraploid and aneuploid complexes were also observed in cells from liver cultures.
The serial alignments of the chromosomes as given in Figures 12 to 16 show 20 pairs of autosomes (pairs 1 to 20) and the sex pair which consists of unequal X and Y elements in the male but of two equal X's in the female. The first two pairs of autosomes are especially long, the first pair apparently with subterminal centromeres showing a globular segment at one end. From the third pair on the reduction of the chromosome size is gradual if the positions of the centromeres are not considered. Pair nos. 4, 8, 11, 12, 15, 16 and 19 are characterized by having non-telomitic centromeres, pairs 4 and 8 being J-shaped with short second arms, pairs 11 and 12 nearly V-shaped with submedian centromeres, and the three smaller pairs, 15, 16 and 19, each exhibiting two arms of somewhat unequal length. The remaining chromosome pairs appear rod-shaped. In certain preparations occasional chromosomes show a minute knob-like body at one end. Some authors have regarded such a knob-like body as the short arm in various orthopteran insects. However, the true nature of the knobs is not clear because they vary in shape and size and their occurrence is not constant. In conformity with the conclusion reached in the study of grasshopper chromosomes by Makino and Momma ('50) we consider that the knob-like body here is of the same nature as the spindle spherule which is protruded occasionally from the main chromosome body and described by Schrader ('39). In other words, the knob-like body is only a rather unusual feature of the kinetic body. Further consider-
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The present investigation demonstrates that in the material obtained from tissue culture preparations of the Norway rat there are at least eight pairs of autosomes having subterminal and submedian centromeres in the somatic cells. The X-element also carries a subterminal centromere. Previous descriptions of rat chromosomes derived mainly from studies on germ cells (Allen, '18; Pincip, '27; Painter, '28; Minouchi, '28; Swezy, '28; Bryden, '32, '33; Oguma, '35; Makino, '42, '43, '52) should thus be revised since they record all the rat chromosomes as having terminal centromeres. Indeed, many published drawings did not include the exact locations of the centromere, and Makino ('42, '43) was inclined to describe all of the chromosomes as having terminal centromeres regardless of the presence of certain J-shaped elements. Recently Guénin ('48), after an analysis of the chromosomes in germ cells of the newborn rat, demonstrated nine pairs of metacentric chromosomes characterized by dissimilar arms. This corresponds with the present findings. With the application of a new squash technic Sachs ('52) has shown certain J- and V-shaped elements occurring in the testicular cells of the Norway rat. Observations made by previous workers have been limited by technical disadvantages which obscured the real structure of the chromosomes.

Makino ('52 a, b) and Makino and Kanô ('53) have investigated the chromosomal features in tumor cells of several rat sarcomas and have demonstrated that in every type of the ascites tumor studied there is a definite strain (or strains) of tumor stem-cells with its own characteristic chromosome constitution contributing to the growth of the tumor through its proliferation in a regular mitotic manner. Further, the chromosome complex of the stem-cells consists of a group of rod-shaped chromosomes and certain V- and J-shaped ones. The four kinds of ascites tumors studied by them differ from each other in the number of rods, V's and J's. It was believed that the rod-shaped chromosomes found in the tumor cells were probably unchanged normal chromosomes, whereas the V- and J-shaped ones were those that had been transformed, since they were not to be found in normal tissue cells. In the light of the present findings it would seem that a modification of this view is needed, because normal tissue cells do contain a certain number of
pairs of two-armed chromosomes. Further discussion on this point will appear in detail elsewhere after precise comparative studies of the chromosomes in normal and tumor cells are completed.

**Summary**

The application of a prefixation treatment to cells in tissue culture with a hypotonic solution facilitates crucial studies on the chromosome morphology of the Norway rat (*Rattus norvegicus*), especially for the location of the centromeric positions in the prometaphase and metaphase figures. Evidence is presented that of the diploid group of the rat eight autosomal pairs and the X-element consist of two-armed chromosomes.

**Literature**

Older literature other than noticed below has been referable to Makino 1951: An atlas of the chromosome numbers in animals, Iowa State College Press, Iowa.


— 1952a. Cytological studies in cancer, III. The characteristics and individuality of chromosomes in tumor cells of the Yoshida sarcoma which contribute to the growth of the tumor. Gann 43: 17-34.


