Chromosomes of Primary Rat Hepatomas Induced by the Administration of 3'-Methyl-4-dimethyl aminoazobenzene (A Preliminary Report)[1), 2), 3)]

By

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(With 5 Text-figures)

Cytological investigations have been carried out intensively on the changes of karyotypes and morphological alteration of cancer cells, grown *in vitro* or *in vivo*. It was well demonstrated that cancer cells were known to have different chromosomal constitutions from normal tissue cells. Normal tissue cells were characterized by the species-specific and sex-specific chromosome complement, whereas cancer cells showed different karyotypic changes, such as numerical variations and morphological alterations of chromosomes from normal cells (Makino and Kano 1951, Makino 1952, Makino and Kano 1953, Makino 1957 and Hsu 1959). In his recent review, Hsu (1961) mentioned that transplantable tumors, with their long history, might have changed their genetic make-up from individual original neoplasms. It is significant therefore to investigate the chromosome constitution of primary tumors. With this view in mind, the present author has undertaken chromosome analyses of primary tumors (hepatomas) induced in rats by azo-dye in order to compare with the chromosomes derived from their transfer generations. In the present study, chromosome investigation was made with rat hepatomas treated with colchicine *in vitro*.

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Materials and Methods: Hepatomas induced by azo-dye in male inbred Wistar-King

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rats were cultured according to the following method: pieces of hepatoma tissues were removed from animals, washed twice with tissue culture media supplemented with Penicillin (200 unites per 1 ml medium) and minced with scissors to appropriate size (2–3 mm^3). The cell suspension thus prepared was transferred into culture vessels containing Mc Coy's medium with some modifications (for detail, see M. Sasaki 1961). Then colchicine was added to media containing hepatoma at the rate of 1–2γ per 10 ml medium. After 15–20 hours incubation at 37°C., the materials were collected by centrifugation at 1500 rpm. for 5 minutes. The sediment was again minced with scissors into fine cell suspension. Drops of the minced material were placed on clean slides and squashed according to the water pretreatment acetic dahlia squash method.

Pieces of the hepatoma without colchicine treatment were inoculated into peritoneal cavities of Wistar rats pretreated with cortisone acetate (6 mg per 1 kg of body weight).

Karyotype analyses were made according to a tentative system devised by the present author: normal rat chromosomes were divided into 7 groups (six autosomal groups and one sex chromosome pair) on the bases of the size and shape of the chromosomes. This system is illustrated in Figure 1 and will be described in detail elsewhere.

Observations

Studies of chromosomes derived from nine primary hepatomas and one hepatoma from the first transfer generation were made with clear metaphasic plates. Among them, eight primary hepatomas were of a solid type and two were of an ascites form. The results of chromosome counts are given in Table 1. It is shown that every hepatoma showed a wide distribution of chromosome numbers. The variations of modal numbers were subdiploid (41) in cases 4 and 9, diploid (42) in case 6, near diploid (45) in case 5 and hypotetraploid (77) in case 3. No modal number was found in 1, 2, 4, 7, and 8; their ploidy levels were hypertriploid, pentaploid and hexaploid, hypertriploid, and tetraploid, respectively. In addition, structural changes such as large metacentric chromosomes, large submetacentric chromosomes and unusually small acrocentric chromosomes were found in all 10 cases under study. However, most of these morphological alterations seemed to be simple structural changes (Figs. 2, 3, and 4). This may be well understood by the comparison between the chromosomes of Figure 1 (from normal material) and those of Figures 2, 3, and 4 (from hepatoma materials), although there occurred some numerical changes.

Peritoneal transplantation of 10 cases of hepatomas was carried out in Wistar rats, but the results were negative except case 9. The latter case was successful only in the first generation. The chromosomes of the primary hapatoma of case 9 and of its transplanted one were compared; the former showed bimodal chromosome numbers (41 and 42), while the transplanted hepatoma had one modal number (42). It is interesting that both hepatomas exhibited a similar chromosome distribution in the diploid range (Fig. 5). Detailed karyotypic analyses were made only on the primary hepatoma (case 9). A noticeable feature was that an unusually large metacentric chromosome was observed in 2 cells with 41 chromosomes among 3 cells analyzed.

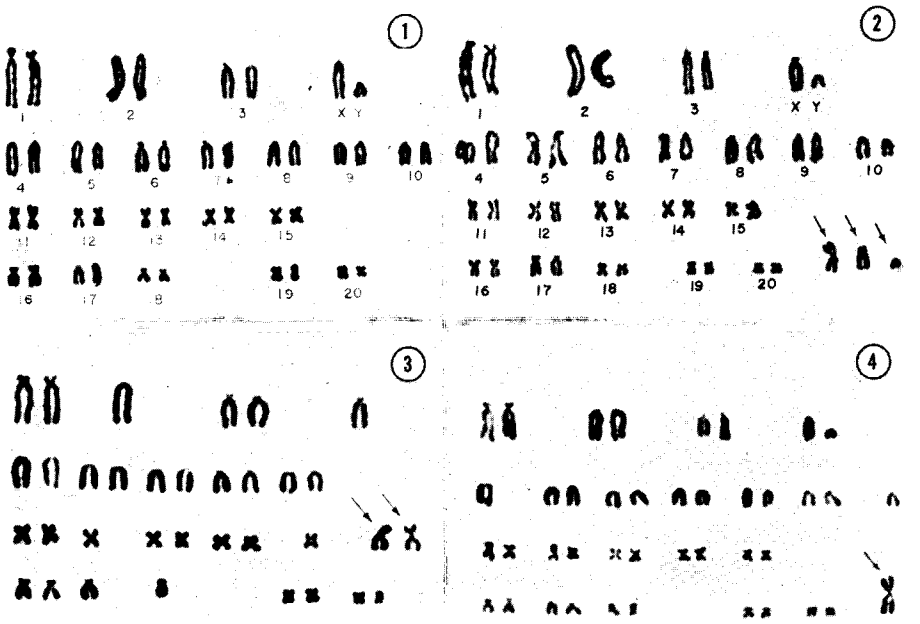

Fig. 1. *K*aryotpye of a normal male rat.
Figs. 2–4. *K*aryotypes of primary rat hepatomas. Fig. 2, 45 chromosomes (case 6). Fig. 3, 34 chromosomes (case 4). Fig. 4, 41 chromosomes (case 9). Arrows indicate abnormal chromosomes.

Discussion and conclusion

Karyological investigations of rat tumors have been usually made in transplantable ascites tumors. In those tumors, chromosome constitutions differed widely from normal tissue cells numerically and morphologically. In most cases chromosomes of those rat tumors so far examined lie in subdiploid range, though there are a few cases which showed the modal numbers around 60 or 70 (Makino 1957, Levan 1959, and Hsu 1961). Remarkable morphological alterations of chromosomes and extensive structural changes have also been observed in a number of the tumors, though their idiograms could not be directly compared those of the normal cells (Tjio and Levan 1956). The present study, however, revealed in rat primary hepatomas that there existed several stemline cells of heteroploid nature; they fell in subdiploid, diploid, near-diploid and hypotetraploid ranges. Morphological alteration of chromosomes were also observed in the primary rat hepatomas. These changes seem to involve simple structural changes, such as a mutual centric fusion of two acrocentric chromosomes. In this respect, the

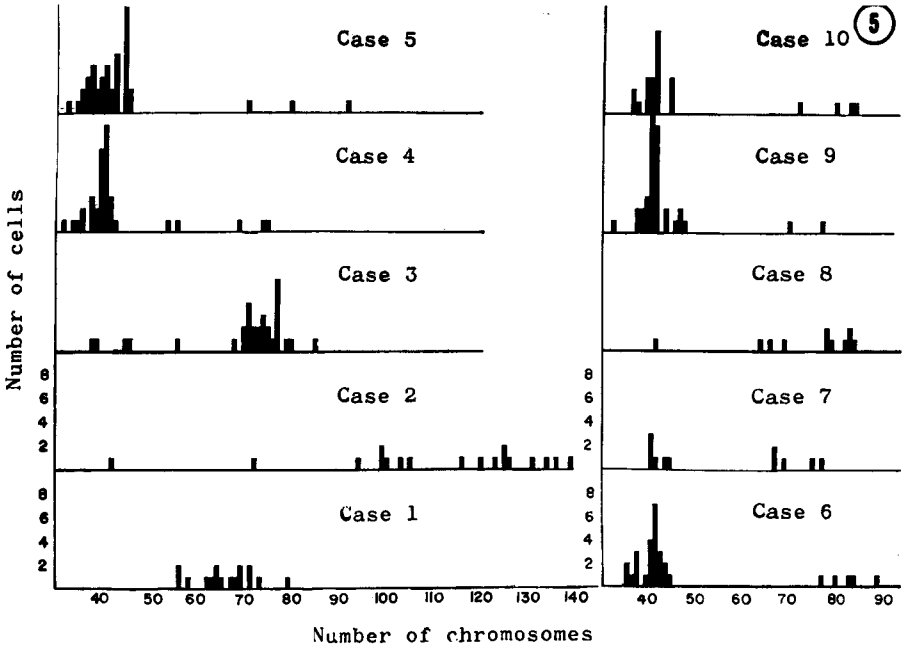

Fig. 5. Chromosome-number distributions obtained from 9 cases of primary rat hepatomas and from one case of the first transfer. Cases 1, 2, 3, 4, 6, 7, 9, and 10 are solid form, and cases 5 and 8 are ascites form. Case 10 is the first transfer derived from case 9.

morphological changes of the present hepatomas seem not to be comparable with the chromosome alterations occurring in many transplantable tumors. The present findings seem to support the view of Hsu (1961) that the chromosomal changes observed in transplantable tumors, with their long history, might have changed their genetic make-up from individual original neoplasms. However, conclusive statements should be postponed until a sufficient number of materials is obtained.

Summary

Chromosome constitutions were investigated in nine primary rat hepatomas induced by azo-dye and one hepatoma at the first transfer in rats. The results indicated that several different stemlines occurred in those primary hepatomas, and that morphological alterations of chromosomes here detected may be attributable to simple structural changes.

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