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# Stable Chromosome Changes in a Human Malignant Melanoma[1]

**T. R. Chen and Margery W. Shaw**

*Department of Biology, Graduate School of Biomedical Sciences, and M. D. Anderson Hospital and Tumor Institute, The University of Texas at Houston, Houston, Texas 77025*

## SUMMARY

A cell culture was established from a human malignant melanoma. Karyotype analyses using some of the newer staining techniques revealed a modal chromosome number of 45 with the presence of a ring, a large marker chromosome with an interstitial C-band, and at least five other structural rearrangements resulting in marker chromosomes. Most of these changes occurred *in vivo* since they were identifiable in the biopsy material 4 hr after the culture was initiated. They persisted in early and later subcultures. Although the culture grew slowly, the ring remained relatively stable while endoreduplication decreased and 2S stem cells increased with time.

## INTRODUCTION

It is well known that chromosome changes occur in cancer cells and in transformed cells growing *in vitro*. However, except for the Philadelphia chromosome in chronic myelocytic leukemia (12), specific rearrangements have not been associated with a specific class of tumors. When changes are observed it is always a question of whether they arose *de novo* during prolonged cultivation. When a normal chromosome complement is obtained, then the question of overgrowth of nontumorous cells raises doubts as to the karyotype of the tumor.

The cell culture to be reported here was established from a biopsy of a metastatic malignant melanoma of the ileum obtained from a 53-year-old white man on September 13, 1972. He had had a melanoma excised from the back several months previously and at that time was noted to have a right hilar chest mass on X-ray. At the time of admission he was taking no medication, but he had been treated for tuberculosis 7 years previously. The tissue obtained at surgery was not grossly melanotic. Initial chromosome preparations were obtained 4 hr after the biopsy specimen was received and the chromosomal complement was followed for 11 weeks.

## MATERIALS AND METHODS

The biopsy was minced and explanted and chromosome preparations were made following the method of Chen (4) with only slight modifications. In addition to conventional aceto-orcein and Giemsa stains, several of the newer methods of staining were used to reveal longitudinal differentiation along the arms of the chromosomes.

G-bands were obtained by the ASG method of Sumner *et al.* (16) modified by incubating the slides in 2 × 0.15 M sodium chloride – 0.015 M sodium citrate, pH 7.0, for 3 hr followed by dilute Giemsa stain (0.6% stock Giemsa solution). The trypsin method of Seabright (15) was used also with slight modifications including the use of dilute Giemsa stain.

Q-bands were produced with QM[2] following the method of Caspersson *et al.* (3). Some slides were double-stained for both Q-bands and constitutive heterochromatin or C-bands (1, 4) by treating QM-stained slides with the ASG method referred to above. When the Q-banding technique is followed by the G-banding method on the same chromosome preparations, C-bands, but not G-bands, will usually appear.

Anaphase bridge preparations were made by plating 2 to 4 × $10^5$ cells on coverslips (24 x 40 mm) and allowed to attach and grow for 2 to 4 days. They are then fixed in Carnoy's solution and stained with Giemsa (6). The morphology of the normal and tumor cells was photographed using a Nikon inverted microscope with camera attachment. Microscopic observations of the chromosomes were made with a Zeiss photomicroscope system, and a Zeiss monochrometer, which permits a selective emission of delimited light wavelength (set at a region around 610 nm) was used to enhance the details of the G-bands and C-bands. Pantomic X, High Contrast Copy, and Tri-X Kodak films and Agfa rapid process paper were used for the microphotography.

## RESULTS

The primary cell culture and subsequent subcultures proliferated slowly, requiring 2 to 3 weeks for confluent growth after a 2-fold split. The tumor cells were distinguishable from normal fibroblasts by their morphology (Fig. 1). Their cell bodies were round rather than spindle-shaped and there were long filamentous protrusions that tended toward a quasiparallel orientation, but webbing and meshwork patterns were observed. In a dense culture, clusters of loosely attached, rounded-up cells were often noted.

Since normal fibroblasts were mixed with the tumor cells

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[2] The abbreviation used is: QM, quinacrine mustard. The genetic abbreviations used are: S, stem cell complement; q, long arm of a chromosome; ?, questionable identification; r, ring chromosome; p, short arm of a chromosome; t, translocation; i, isochromosome; –, shorter than the designated chromosome arm, probably caused by deletion.

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in the initial outgrowth, greater purity of the tumor cells was established by special techniques in culture (5). By collecting and plating the cells floating in the medium and/or growing in the serum deficient medium in a semiconfluent culture, nearly homogeneous tumor cell populations were obtained. In these cultures, only 10 to 15% of normal diploid fibroblasts could be identified by chromosome counts. Conversely, normal fibroblastic growth was favored in the presence of serum (5).

The modal chromosome number of the tumor culture was 45 (1S) with a range of 42 to 47 (Table 1). However, 2 "stem lines" could be distinguished with chromosomes 45 and 46. The cells with 46 chromosomes had the 45 (1S) karyotype (described below) with an additional G-sized marker chromosome. On the other hand those cells with counts lower than 45 are not considered as stem lines because there is no consistent absence or presence of specific chromosomes. Most likely, therefore, these cells are accounted for by the artifactual loss of chromosomes during the slide preparation or natural loss caused by mitotic irregularities.

Polyploidy and endoreduplication were not uncommon. These comprised about 15 to 18% of the tumor cells. In the earlier passages, endoreduplication predominated, while later there was a shift to an increase in polyploid (2S) cells.

Although the 1S chromosome number was near diploid (45), the structure of the chromosomes (as determined by banding patterns) revealed many detectable rearrangements. There was only 1 identifiable member of Pairs 1, 2, 8, 9, 10, 11, and 13 present. Also, 6 marker chromosomes were identified in the 45 stem cells with a 7th marker added in cells with 46. These are illustrated in Fig. 2 and described below. These descriptions are based on most probable interpretations of the chromosomal segments as revealed by banding patterns, but it should be kept in mind that there is an element of arbitraries in the banding analysis.

**$M_1$: t (1q 9q 9q 10q ?).** This chromosome is longer than No. 1 and possesses an interstitial C-band (*arrow*) at the junction of 9q and a presumed 10q. This band most probably represents the prominent heterochromatic region of No. 9.

**$M_2$; r (2).** The ring is most probably formed as a result of breakage near the telomeres of No. 2 with subsequent healing of the proximal ends. Loss of chromosomal material appears to be negligible, since the ring circumference is comparable to the length of No. 2. Variation in ring morphology and number is illustrated in Fig. 3 and summarized in Table 2.

**$M_3$: t (9q 1p).** This chromosome is slightly shorter than No. 2. The C-band karyotype (Fig. 2b) illustrated the 9q heterochromatin.

Table 1

*Distribution of chromosome numbers in tumor cells and frequencies of stemline cells (S) and their multiples (nS) in different culture passages*

The data obtained on November 29, 1972, were based on 1 culture which was plated in regular medium and subsequently transferred to serum-free medium.

| | Chromosome counts | | | | | | | Frequency of polyploidy | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Date | 42 | 43 | 44 | 45 | % with 45 | 46 | 47 | S | nS[a] | % of nS cells |
| 9/21/72 | 4 | 10 | 18 | 100 | 62 | 27 | 2 | 400 | 71 | 15.1 |
| 10/24/72 | 1 | 1 | 4 | 62 | 79 | 9 | 1 | 256 | 59 | 18.7 |
| 11/21/72 | | | 1 | 42 | 86 | 6 | | 82 | 15 | 15.5 |
| 11/29/72 | | 2 | 3 | 52 | 88 | 2 | | 76 | 57 | 42.9 |

[a] Includes endoreduplicated cells, 2S cells, and higher orders of ploidy.

Table 2

*Variation of ring chromosomes in 2S and endoreduplication metaphases*

| | 2S cells | | | | | Endoreduplicated cells | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Date | No rings | 1 ring | 2 ring | Large ring | Total cells observed[a] | No ring | 2 rings | Large ring | Other[b] | Total cells observed |
| 9/21/72 | | | 18 (75%) | | 30 | | 23 (71.9%) | 7 (21.9%) | 2 (6.3%) | 41 |
| 10/24/72 | 2 (5.1%) | 4 (10.3%) | 27 (69.2%) | 6[c] (15.4%) | 49 | 1 | 8 | | | 10 |
| 11/21/72 | | | 9 | | 13 | | 2[d] | | | 2 |
| 11/29/72 | | | 14 (82.4%) | 3 (17.7%) | 47 | | 9 | 1 | | 10 |

[a] Some cells were not scored because the ring morphology was not clear.
[b] Rings appeared to be interlocked.
[c] One cell contained 2 large rings.
[d] One cell contains 1 large ring and 1 normal-sized ring.

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**$M_4$: i (8q).** An isochromosome composed of 2 8q's.

**$M_5$: t (13q? 11q−).** This structural rearrangement probably involves the entire long arm of 13q and the distal two-thirds of 11q. Both elements are connected by a chromatid segment of unknown origin.

**$M_6$: t (?11q−).** Chromosome 11 lacks the distal two-thirds of 11q but is replaced by a 22q-sized chromosome segment of unknown origin.

**$M_7$: G-sized Marker.** This small chromosome was seen only in the tumor cells with 46 or more chromosomes. It was absent in the modal 45-stem cell line.

The most striking marker, which remained relatively stable during continuous cultivation, was the ring chromosome (Fig. 3). There was no tendency for the ring to be lost in later passages (Table 2).

The ring chromosome and the other markers were observed in the chromosome preparations made from the biopsy only 4 hr after incubation in culture medium with Colcemid added. After 2.5 months of continuous cultivation, including several subcultures, the modal population (1S = 45) has remained unchanged. The 46-chromosome stem line constitutes 3.4% of the total tumor cells.

Anaphase bridges were observed in approximately 50% of the dividing tumor cells. There were 113 cells scored with bridges and 115 cells without bridges. In contrast, a normal diploid fibroblast culture evidenced bridges in only 2% of the cells examined. No side arm bridges resulting from subchromatid exchanges were observed (2). Most of the bridges appeared to be dicentric with centromere orientation at opposite poles, probably resulting from sister chromatid exchange within the ring.

## DISCUSSION

Interstitial C-bands are not present in the normal human karyotype (1). However, they have been seen in 3 established mouse cell lines, LMTK−, A9, and RAG (6). They have also been observed in primary cultures from the Indian muntjac, *Muntiacus muntjak*, Chinese hamster, *Cricetulus griseus*, Afghanistan wild sheep, *Ovis ammon cyclocerus* (7), and Hawaiian house mouse, *Mus musculus* (T. C. Hsu, personal communication). No chromosome with interstitial C-bands was ever found in established human heteroploid lines such as KB, D98, IMR-32, and other recently established tumor lines (T. R. Chen, unpublished data) or in normal human karyotypes (6). The interstitial heterochromatic segment must have preexisted *in vivo* and did not arise *de novo* in culture since it was observed in the 4-hr chromosome preparations and was preserved intact during continuous cultivation. It appears, therefore, that additional constitutive heterochromatin to a chromosome or to the chromosome complement may not cause the disruption of chromosomal segregation as had been reported previously (6).

Two other studies have been reported from patients with malignant melanoma. Huang *et al.* (8) studied the peripheral lymphocytes and found that most cells were diploid. This is not unexpected, since the tissue studied was not derived from the tumor. In a short report, Witkowski (18) claimed to have observed some karyotypic changes in melanoma but no descriptive details or figures were presented.

During the period of time studied, the ring chromosome proved to be quite stable. Ring chromosomes have been reported in patients with various malignant tumors (10) and can be produced by therapeutic irradiation (17). Several investigators have studied ring stability. McClintock (11) described the bridge-breakage-fusion phenomenon in ring chromosomes in corn, and Schwartz (14) concluded that ring stability is dependent on its structure rather than its size. This was reiterated by Kistenmacher and Punnett (9). Pathak and Sinha (13) have postulated that monocentric, dicentric, and polycentric rings can be perpetuated without somatic crossing over.

In the 2S cells the frequency of single rings, 2 rings, no rings, and interlocked rings varied in different passages (Table 2; Fig. 3) but cells with 2 rings predominated. This is to be expected in a stable ring culture and accounts for the persistence and perpetuation of the original ring. Only a few large rings and interlocking rings were observed.

It is quite possible that the high frequency of anaphase bridges observed explains the slow rate of proliferation of the tumor line. If many of the daughter cells receive incomplete genomes which are selected against, then the high rate of cell mortality would contribute to a lengthening of the doubling time.

Other tumors which are well adapted to resist host selection will be examined for the stable chromosomal properties observed in the cell strain reported here. Hopefully, a generalization may emerge whereby stable cultures of tumor cells *in vitro* with few rearrangements *de novo* will shed light on the prognosis of the disease. Also, attempts to reverse the positive selection for tumor cells at the expense of normal cells in mixed populations can be studied by testing with appropriate chemotherapeutic agents *in vitro*.

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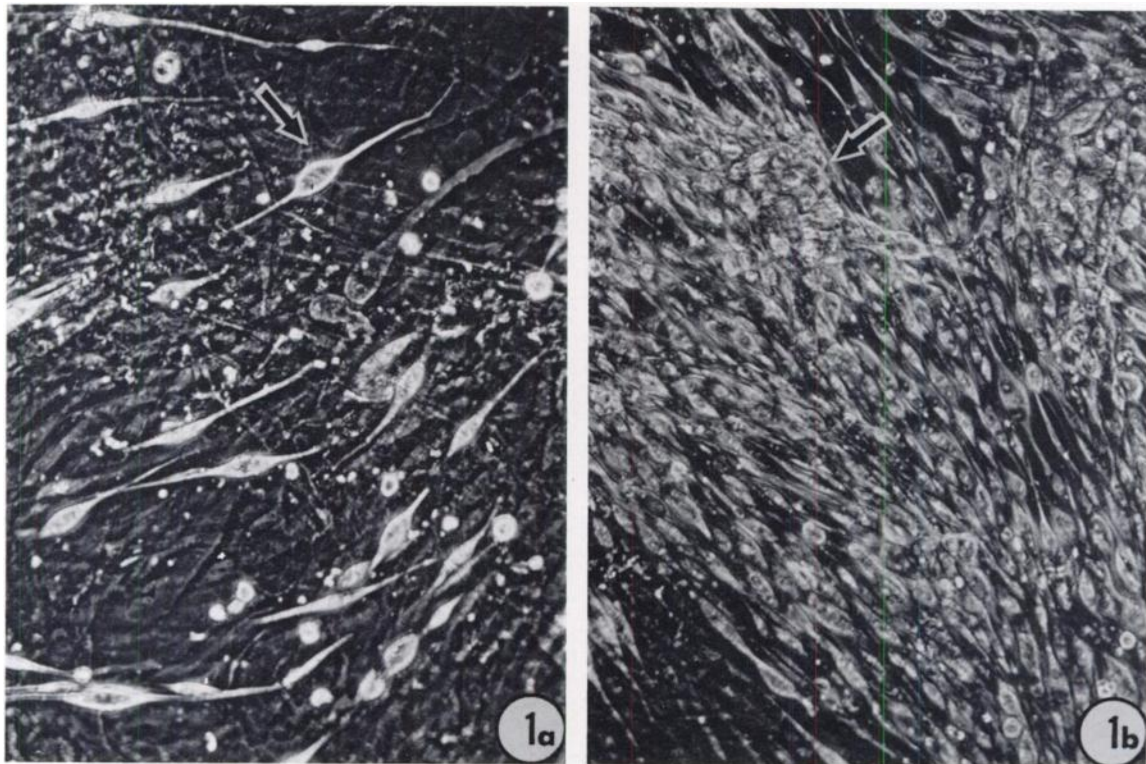
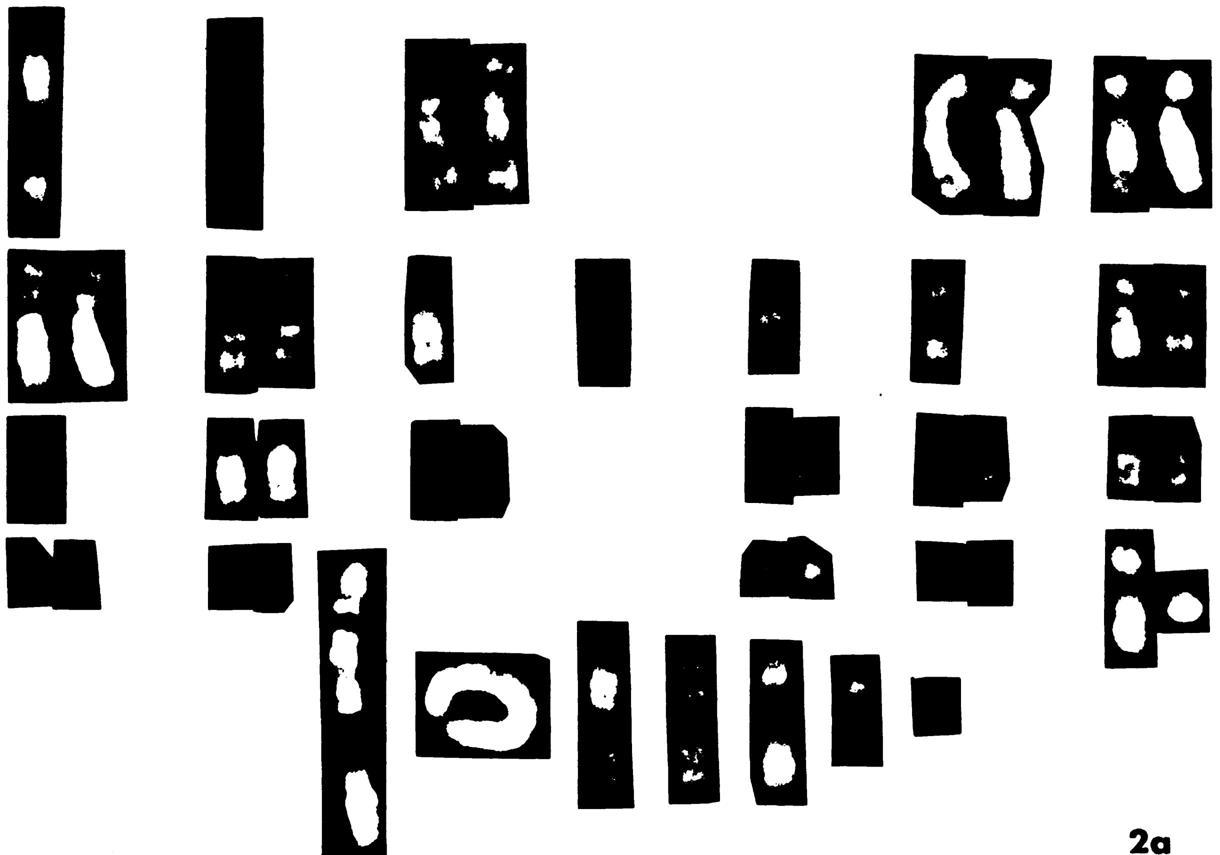
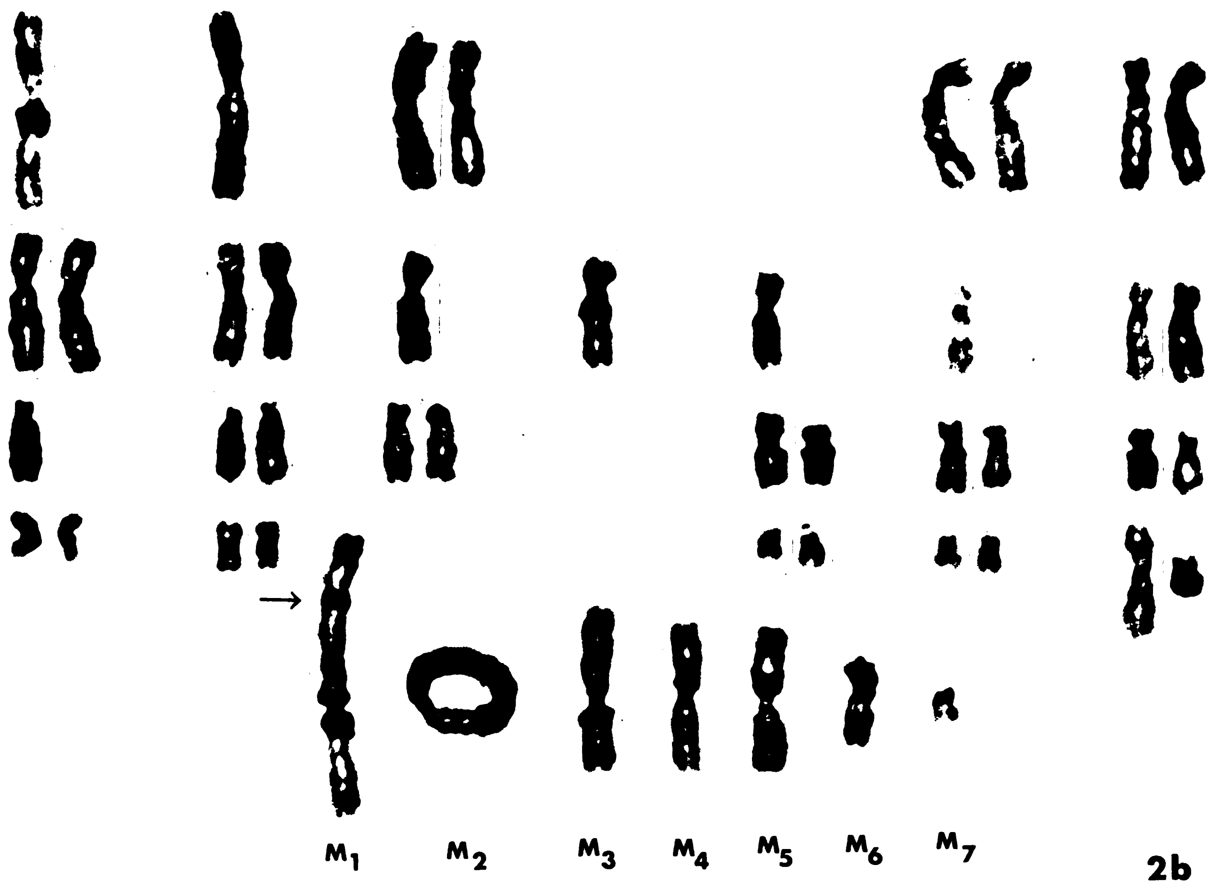



Fig. 1. Morphology of the normal and tumor cells. *a*, area in a confluent culture in which normal cells predominate. The few tumor cells (*arrow*) present are readily recognized by their characteristic round bodies and long filamentous protrusions. *b*, a different area from the same culture with predominantly tumor cells showing clusters (*arrow*) of loosely attached, rounded-up cells.

Fig. 2. Karyotypes from stem-line tumor cell with 46 chromosomes showing rearrangements and marker chromosomes. See text for description of markers. *a*, stained with QM, showing Q-bands. *b*, stained with Giemsa, showing C-bands. *Arrow*, interstitial C-band in $M_1$.

Fig. 3. Variation in the morphology of the No. 2 ring. *Arrows*, normal No. 2 homolog for comparison. *a*, normal-size ring in 1S stem cell. *b*, large ring in 1S stem cell. *c*, endoreduplicated ring in endoreduplicated cell. *d*, large uncondensed ring in 1S stem cell. *e*, two large rings which may be interlocked in 2S cell. *f*, one interlocking ring and 1 large ring in an endoreduplicated cell.

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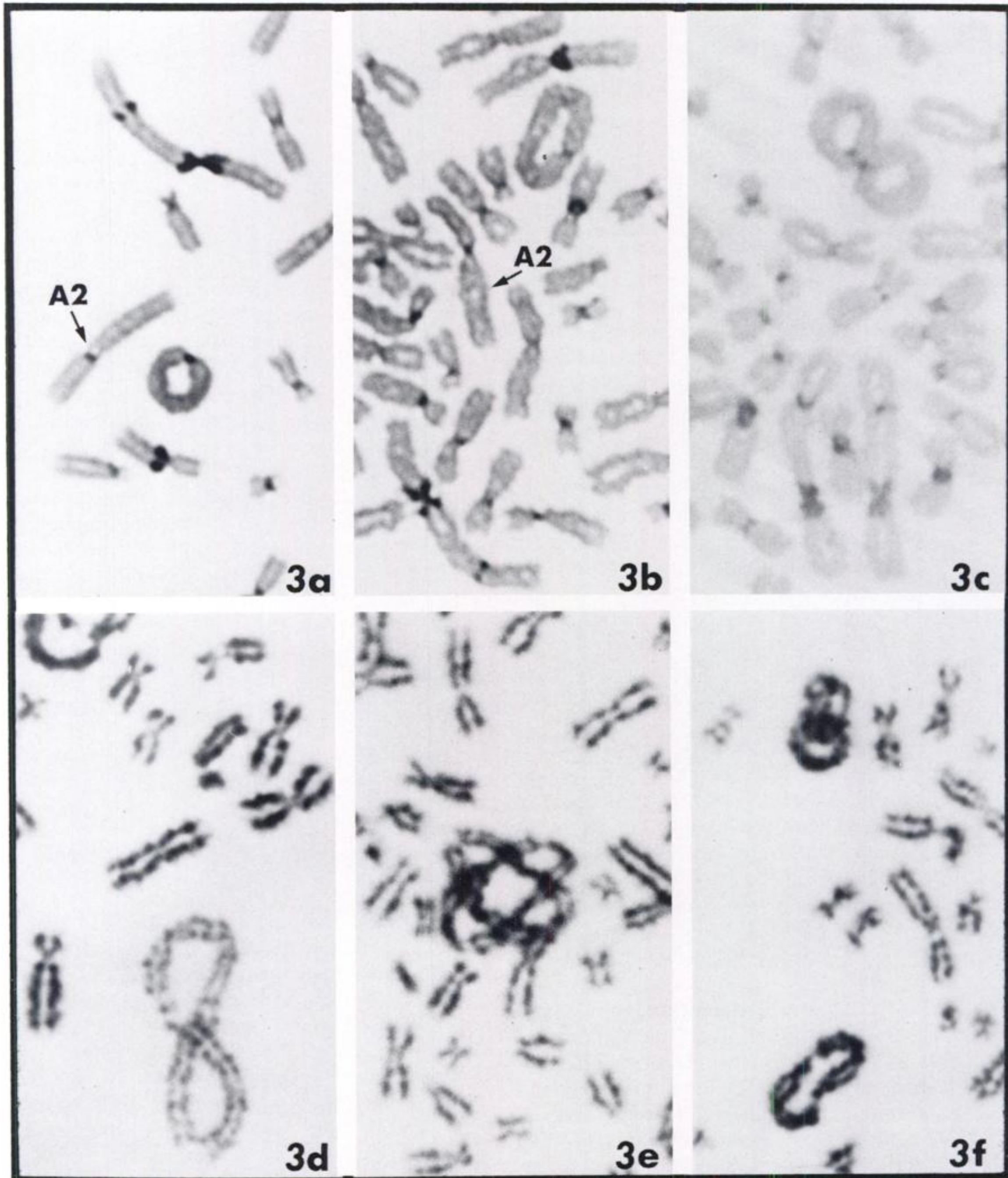


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