Heterologous Transplantation, Chromosome Analyses, and DNA Measurements of the Human Carcinoma Tissue Culture Line, H.Ep. #2 [1, 2]

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SUMMARY—The human epidermoid carcinoma No. 2 (H.Ep. #2), which had been kept continuously in tissue culture since 1953, was transplanted into adult, conditioned golden hamsters. Morphologically, the transplanted tumors resembled closely the original tissue from the patient. Chromosome analyses were performed on two series of H.Ep. #2 cells, both originating from Fjelde stock cultures. The first series comprised the first stock culture from Fjelde (TCF), the corresponding first hamster tumor line (H.Ep. #2/H1), and material from the latter brought back into tissue culture (H.Ep. #2/H1-TC). The second series included the second tissue culture material (H.Ep. #2/TC2) and the ensuing second hamster tumor line (H.Ep. #2/H2). Chromosome numbers of cells of the first series varied between 69 and 80, with a large peak at 74–75 and a smaller peak at 77. Four marker chromosomes were present. In the second series, a large peak was found at 69–70 and a smaller peak at 74–75. Besides the 4 markers from the first series, the cells within these peaks possessed 2 new markers, each of them characteristic for one of the peaks. The different peaks in the frequency distribution of the chromosome numbers were interpreted as representing 2 stemlines. In the first series this assumption was based on chromosome numbers only, whereas in the second series it was corroborated by the presence of the individual marker chromosomes. DNA was measured on Feulgen-stained nuclei of H.Ep. #2 cells grown *in vivo* and *in vitro*. Both tumor cell lines showed their DNA values to be aggregated in two peaks: one around 3.5c and the other around 7c. Thus a correlation was established between the amount of DNA and the hypertriploid chromosome number of H.Ep. #2 cells.—J Nat Cancer Inst 36: 673–683, 1966.

SINCE ISOLATION from a patient in February, 1953, at the Sloan-Kettering Institute, Department of Dr. A. Moore, the human larynx carcinoma

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H.Ep. #2 (human epidermoid carcinoma No. 2) has been kept continuously in tissue culture by Fjelde. This communication reports the heterologous transplantation of this tissue culture line into adult, conditioned golden hamsters (*Mesocricetus auratus*) and presents results of chromosome analyses and DNA measurements performed on tumor cells grown both in tissue culture and heterologous animal hosts.

MATERIALS AND METHODS

The relationship of the different Zürich tissue culture sublines to the corresponding solid tumors grown in conditioned hamsters is as follows:

TCF—Fjelde stock material
H.Ep. #2/TC1—first subline obtained from Fjelde stock and maintained at the Zürich laboratory (chromosomes of this line were not analyzed)
H.Ep. #2/H1—hamster tumor line No. 1, established by material implanted from H.Ep. #2/TC1
H.Ep. #2/H1-TC—tissue culture subline, established by transplanted tumor material put back into tissue culture
H.Ep. #2/TC2—second subline obtained from Fjelde stock cultures
H.Ep. #2/H2—hamster tumor line No. 2, established by material implanted from H.Ep. #2/TC2.

The golden hamsters used for transplantation were obtained from the Centraal Proefdierenbedrijf T.N.O., Zeist, Holland, and maintained under ordinary laboratory conditions. The tissue culture lines, derived from stock cultures kept by Fjelde, were maintained as monolayer cultures in tissue culture medium 199 with 10 percent calf serum and additional penicillin and streptomycin. Prior to transplantation, the cells were centrifuged at 400 rpm (24 × *g*) for 10 minutes and resuspended in one-third of the supernatant. A total of 0.25 ml of this tissue culture cell suspension was implanted into the cheek pouches of 3-month-old golden hamsters of both sexes, according to the technique originally described by Lutz *et al.* (*1*). The animals were conditioned with Cortone acetate (Merck Sharp & Dohme, Haarlem, The Netherlands) by the procedure of Toolan (*2, 3*).

Chromosome preparation.—Tissue culture: Twenty-four hours after the medium was changed, colchicine was added to the cultures for 1 hour (final concentration 2 μg/ml), and air-dried preparations were made by the method of Ockey (*4*).

Solid tumors: Since these tumors were rather hard, a hyaluronidase system was developed by one of us (A.Z.) which proved most satisfactory for obtaining single cell suspensions. Thin slices, cut from the tumor with a razor blade, were placed on a magnetic stirrer for ½-hour in a cold (4° C) aqueous solution containing 1 percent sodium citrate, 0.1 percent colchicine, and 150 IU hyaluronidase, "Hyason" (N.V. Organon—OSS, Holland). The resulting suspension was filtered through a double layer of gauze and centrifuged at 2000 rpm (626 × *g*) for 5 minutes, and air-dried preparations were made.

Chromosome studies were performed on Fjelde stock cultures (TCF) and on the Zürich tissue culture sublines, H.Ep. #2H1/-TC and H.Ep. #2/TC2. Karyotypes of 437 well-spread metaphase plates were analyzed. Chromosome studies on transplanted tumor material were done on 88 metaphase plates of the 9th, 10th, and 11th generation of H.Ep. #2/H1 and on 81 plates of the 2d and 8th generation of H.Ep. #2/H2. The parameters used for the characterization of the different *in vivo* and *in vitro* lines were chromosome number and the presence of marker chromosomes designated m1–m6 according to their decreasing length.

DNA measurements.—DNA was measured on Feulgen-stained interphase nuclei of H.Ep. #2 cells propagated *in vitro* and in conditioned golden hamsters. The tissue culture cells were grown on coverslips; from the solid tumors, smears were made by use of the hyaluronidase system described. Smears and tissue culture coverslips were fixed in 70 percent alcohol for 30 minutes, hydrolyzed with N HCl at 60° C for 12 minutes, stained with the Graumann-Schiff reagent for 1 hour (*5*), and mounted with Cargille's oil (refractive index N_D 1.540). The relative amount of DNA in 210 interphase nuclei of H.Ep. #2 tissue culture cells and of transplanted tumor cells, respectively, was evaluated with the Deeley integrating microdensitometer (*6*) by use of the extinction of 0.75 and a wavelength of 520 mμ.

To obtain DNA values of known haploid and diploid cells, the DNA content of 30 sperm cells and of 40 thoracic duct lymphocytes of the golden

hamster was determined. We used these cells as a reference system, under the assumption that mammals form a homogeneous group with regard to the DNA content of their nuclei (*7*).

RESULTS

Histology of Transplants

Transplantation of the 2 tissue culture lines, H.Ep. #2/TC1 and H.Ep. #2/TC2, in conditioned hamsters resulted in the growth of hard nodular tumors (0.5–2.0 cm in diameter) by the end of the 3d week. The tumor cells, growing in clusters, were surrounded by dense connective tissue (fig. 1). Central necrosis was pronounced; giant tumor cells were always present. Neither of the tumor lines could be propagated in unconditioned hamsters, nor could they be grown in conditioned weanling rats. At present H.Ep. #2/H1 is in its 24th transplantation generation and H.Ep. #2/H2 in its 14th; both are growing equally well in the cheek pouch and subcutaneously.

Chromosome Analyses

The frequency distribution of the chromosome numbers of the different H.Ep. #2 lines grown *in vivo* and *in vitro* is listed in table 1. The Fjelde stock culture (TCF) (fig. 2) showed chromosome numbers between 71–80, with a large peak at 74–75 and a smaller peak at 77–78. Four marker chromosomes could be recognized: m1, a very long metacentric; m2, a very long subtelocentric; m5, a very short metacentric; and m6, a minute chromosome. Karyotypes of H.Ep. #2/H1 (fig. 5) and of H.Ep. #2/H1-TC correspond to TCF with regard to chromosome number and marker chromosomes.

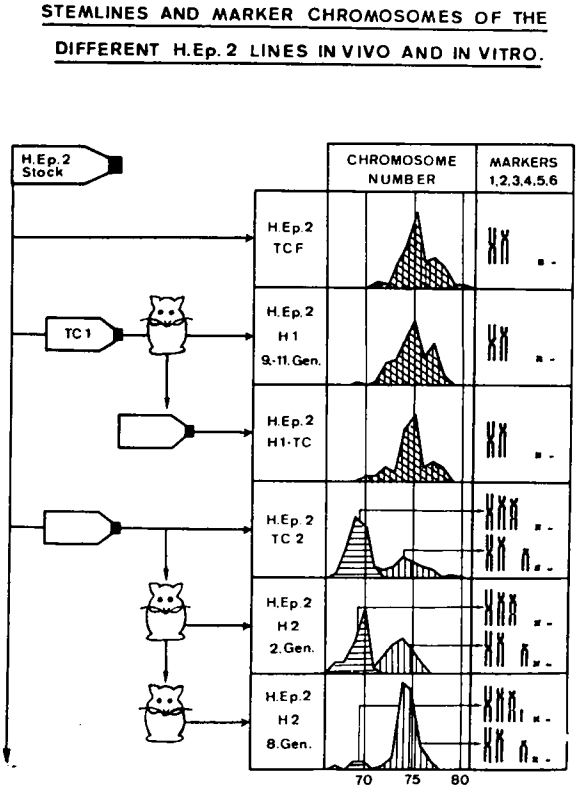
The second tissue culture subline (H.Ep. #2/TC2) (figs. 3 and 4) showed a variation of chromosome numbers between 67 and 79, with a large peak at 69–70 and a smaller peak at 74–75. Cells within these peaks, besides markers 1, 2, 5, and 6, exhibited a different new marker: Cells with chromosome numbers between 67 and 73 had marker 3, a long subtelocentric with a secondary constriction in the long arm; cells with chromosome numbers between 73 and 79 showed marker 4, a long acrocentric chromosome. Karyotypes of the second hamster tumor line (H.Ep. #2/H2) in general were similar to those of H.Ep. #2/TC2. However, during the 8th hamster generation, the peak at 74–75 was higher than that at 69–70. The marker chromosomes still corresponded to the two different cell lines. These findings are presented as a synopsis in text-figure 1.

The cells analyzed were polyploid as follows: 2.4 percent in TCF, 3.0 percent in H.Ep. #2/H1-TC, 2.2 percent in H.Ep. #2/TC2, and about 5.0 percent in H.Ep. #2/H2.

A detailed study on differences between H.Ep. #2 and the normal human karyotype, including chromosome measurements, group analyses, and autoradiography, is in preparation.

DNA Measurements

The DNA content of sperm cells and thoracic duct lymphocytes of the golden hamster and of

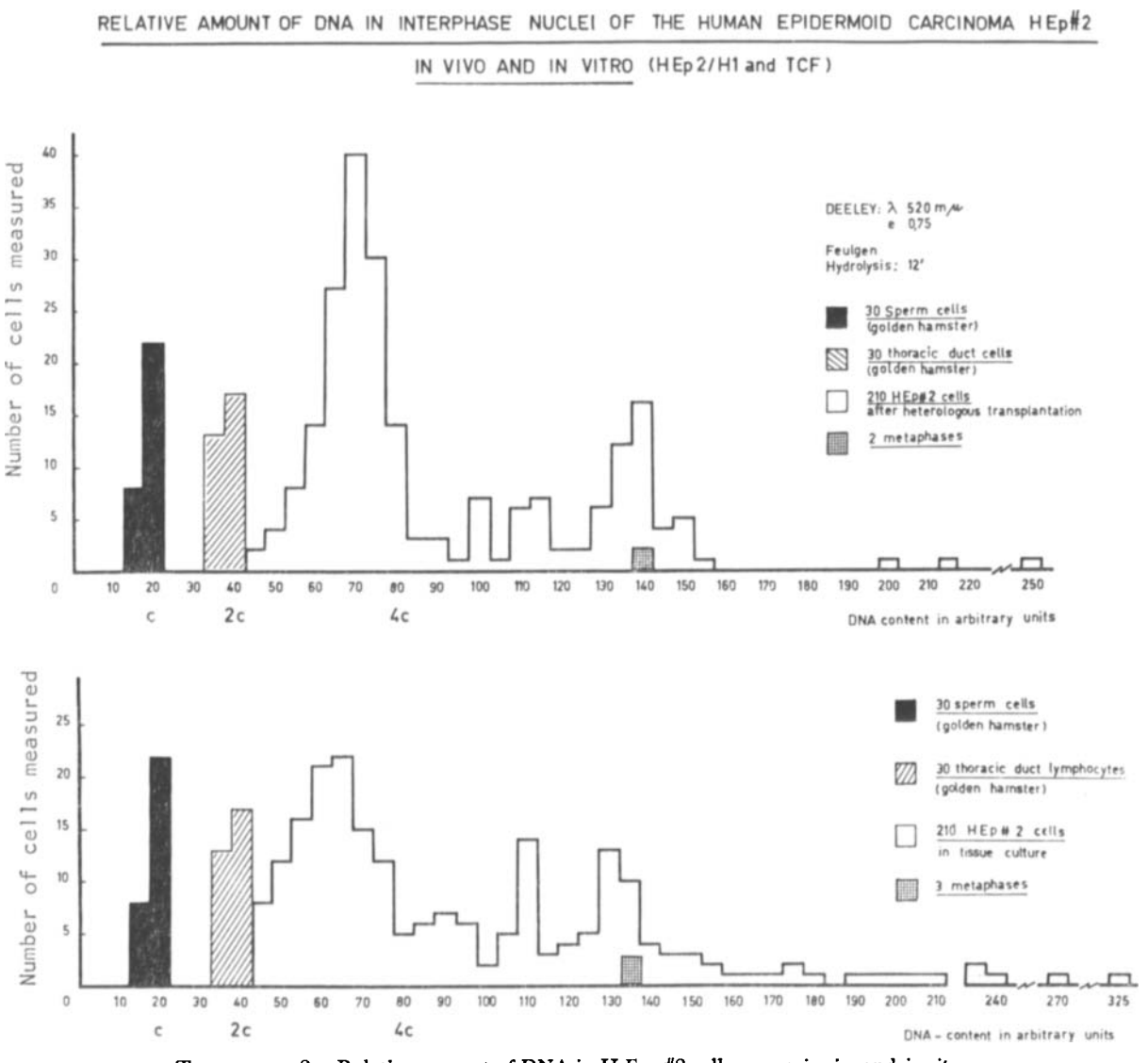


TEXT-FIGURE 1.—Synopsis of results of chromosome analyses performed on different H.Ep. #2 lines grown *in vivo* and *in vitro*.

TABLE 1.—Chromosome distribution in different lines of H.Ep. #2 propagated *in vivo* and *in vitro*

H.Ep. #2 lines	Number of chromosomes																	Total No. of cells
	>67	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	<81	
H.Ep. #2/TCF, 1964–65*						2 1		2 10	7 14	16 22	8 5	8 7	9 2	1 1	2			55 67
						3	2	12	*21*	*38*	13	*15*	*11*	2	2			122
H.Ep. #2/H1, 9th–10th generation	1			1		1	8	9	*16*	*23*	11	*15*	3				1	88
H.Ep. #2/H1-TC	6				2	2	6	4	*22*	*28*	6	*8*	6		4		6	100
H.Ep. #2/TC2*	3 1	2 1	11 14	24 30	22 23	3 6	5 2	2 7	8 10	6 7	7 3	1 6	1	2		1	5	100 115
	4	3	25	*54*	*45*	9	7	9	*18*	*13*	10	7	1	2		1	5	215
H.Ep. #2/H2, 2d generation		2	2	*5*	*11*	1	3	5	*6*	4	2							41
H.Ep. #2/H2, 8th generation		1		*2*	*2*		1	3	*22*	*20*	6	2					1	60

*Two different analyses.



TEXT-FIGURE 2.—Relative amount of DNA in H.Ep. #2 cells grown *in vivo* and *in vitro*.

H.Ep. #2 cells propagated in tissue culture and in heterologous hosts is illustrated in text-figure 2. The mean DNA value of sperm cells and lymphocytes was in the region of 20 and 40 arbitrary units, respectively, which corresponds with c and 2c. The DNA content of the tumor cells, grown both *in vivo* and *in vitro*, was higher than that of the normal cells and showed a similar pattern, except for a larger spread of values in tissue culture cells. The greatest frequency of DNA values aggregated in two peaks: one in the region of 3c (60–70 arbitrary units) and the other around 7c (135–140 arbitrary units). It was assumed that the first peak contained cells in the G1 phase and the second, tumor cells which had completed their DNA replication. The 5 metaphases measured fell within the second peak.

DISCUSSION

In February, 1953, the H.Ep. #2 tissue culture line was established by Fjelde at the Sloan-Kettering Institute (*8*). The tumor material came from a metastatic node in the neck of a male

patient with carcinoma of the larynx. Besides this line, derived directly from the patient, another H.Ep. #2 strain was initiated, originating from animal transplants (*9*). The latter material had been provided by Toolan who had started an H.Ep. #2 tumor line in conditioned weanling rats (*10*). The animal line, which produced slow-growing, hard tumor nodules, was discontinued after 12 generations because of poor growth. When H.Ep. #2 cells, after having grown vigorously in tissue culture for 17 months, were reimplanted into conditioned animals, they immediately resumed their previous slow-growing pattern (*10*). This animal tissue culture line also was later discontinued.

The work reported in this paper has been done on material that had been continuously propagated in tissue culture for the past 12 years (*11*, *12*) and that had never been implanted into animals. The present transplantation in conditioned hamsters produced tumors with growth characteristics corresponding to those reported by Toolan (*10*). The histologic pattern also closely resembled that of the original specimen taken from the patient and of the tumors grown by Toolan 12 years ago [figs. 15 and 16 in (*10*)].

The chromosomes of H.Ep. #2 cells were first studied by Biesele in 1953 (*13*) who separated tumor cells from the explanted tumor tissue by using a capillary pipette during the first days of culture. Biesele estimated the stemline number as being around diploid. This indicates that, in the patient, H.Ep. #2 had maintained a numerically normal human karyotype. The next analyses were performed by Levan in 1956 (*14*) who determined the tumor to be in the hypertriploid region with chromosome numbers between 74 and 80. De Grouchy and Cottin confirmed this in 1961 (*15*). The shift in chromosome number, therefore, must have taken place during the 3 years in tissue culture which had elapsed between Biesele's and Levan's analyses. A tendency toward polyploidy after longer or shorter periods of cultivation *in vitro* has been observed in most tumors studied so far. Heterologous transplantation, on the other hand, has not regularly produced polyploid tumor lines. Some of the older heterologously transplantable human tumors (H.S. #1, H.Emb.Rh. #1, H.Ad. #1) have retained their near-normal chromosome number in conditioned animal hosts. Structural deviations of these chromosomes, however, have been observed (*16–18*).

The most extensive chromosome study on H.Ep. #2 cells was reported by Norryd and Fjelde in 1963 (*19*). Analyses of 50 metaphase plates confirmed the consistency of the karyotype with a hypertriploid stemline of 77 chromosomes and a variation between 69 and 81. Of the cells, 3.9 percent were polyploid. These authors described 3 markers: 2 very small chromosomes (a + b) and a long subtelocentric (c). These markers correspond to our markers 5, 6, and 2.

Analyses of the different H.Ep. #2 cell lines kept in our laboratory *in vivo* and *in vitro* showed 2 peaks in the frequency distribution of the chromosome numbers. These findings suggest the presence of 2 stemlines. Whereas the distinction between these 2 cell lines in cells of TCF, H.Ep.#2/H1 and H.Ep.# 2/H1-TC was not definite, the presence of the 2 individual marker chromosomes in the second series (H.Ep. #2/TC2 and H.Ep. #2/H2) clearly differentiated 2 stemlines. Results of present cloning experiments might substantiate these findings. Chromosome analyses on cells of subsequent animal generations will show if both stemlines survive. Findings on the 8th tumor generation rather suggest that, during transplantation, one of the stemlines might be eliminated.

We can only speculate on the origin of the new marker chromosomes. However, the use of a different serum in our laboratory has to be considered, especially since, during the adaptation of H.Ep. #2 to a chemically defined medium, similar markers appeared (*20*). It is interesting to note that heterotransplantation did not visibly alter the structure of the chromosomes, whereas the small difference in the composition of the tissue culture medium could have been responsible for the induction and selection of structurally altered karyotypes.

The relative DNA values obtained for Feulgen-stained interphase nuclei of H.Ep. #2 cells, grown both *in vitro* and *in vivo*, show a correlation to the modal chromosome number: Tumor cells, aggregated in the first peak, have a DNA content placing them in the region of 3.5c which corresponds to the established hypertriploid stemline. These findings are in good agreement with those reported by Richards and Atkin (*21*) who correlated

the amount of DNA in 14 primary human tumors with the respective chromosome numbers.

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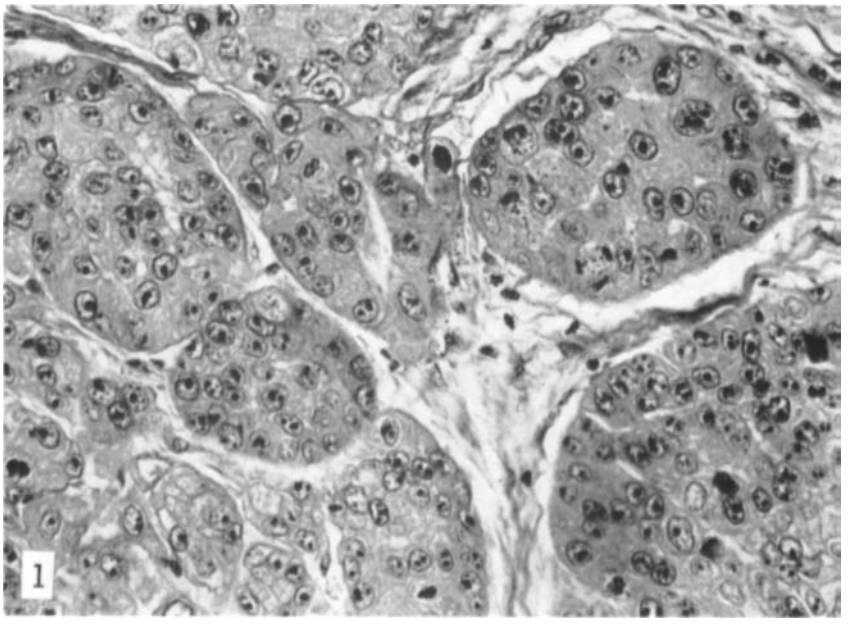

FIGURE 1.—H.Ep. #2/H1: Transplant in the cheek pouch of a conditioned golden hamster. Tenth generation. Hematoxylin and eosin. × 270

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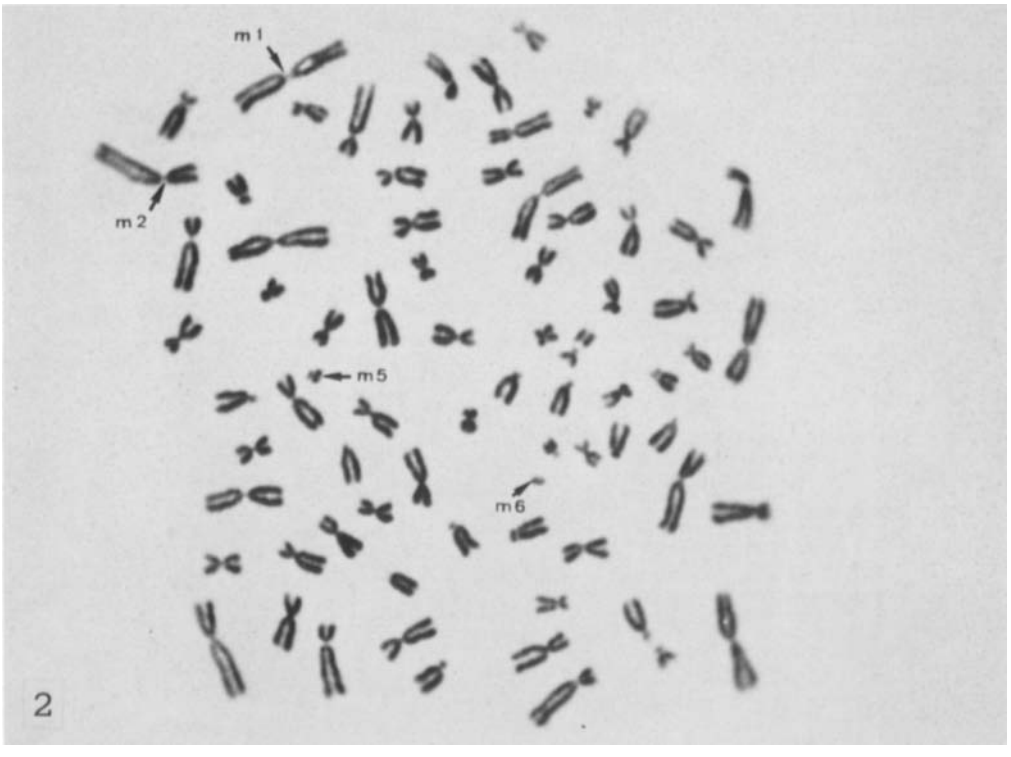

FIGURE 2.—Metaphase plate from H.Ep. #2/TCF with 74 chromosomes and 4 markers—1, 2, 5, and 6.

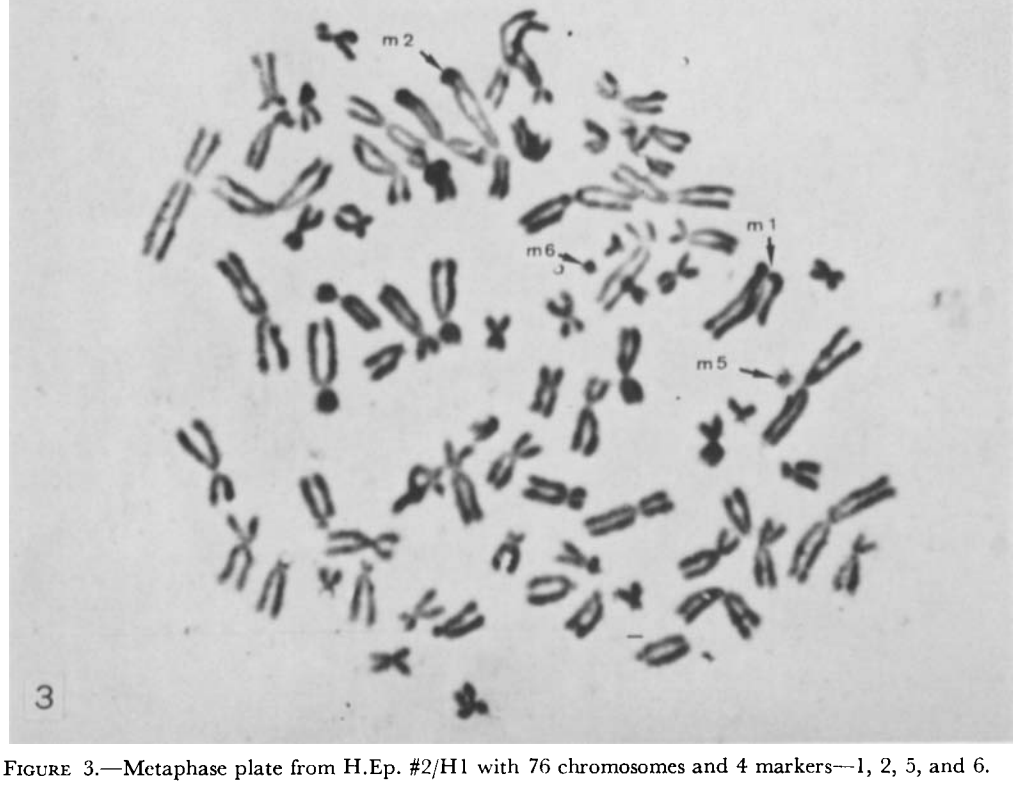

FIGURE 3.—Metaphase plate from H.Ep. #2/H1 with 76 chromosomes and 4 markers—1, 2, 5, and 6.

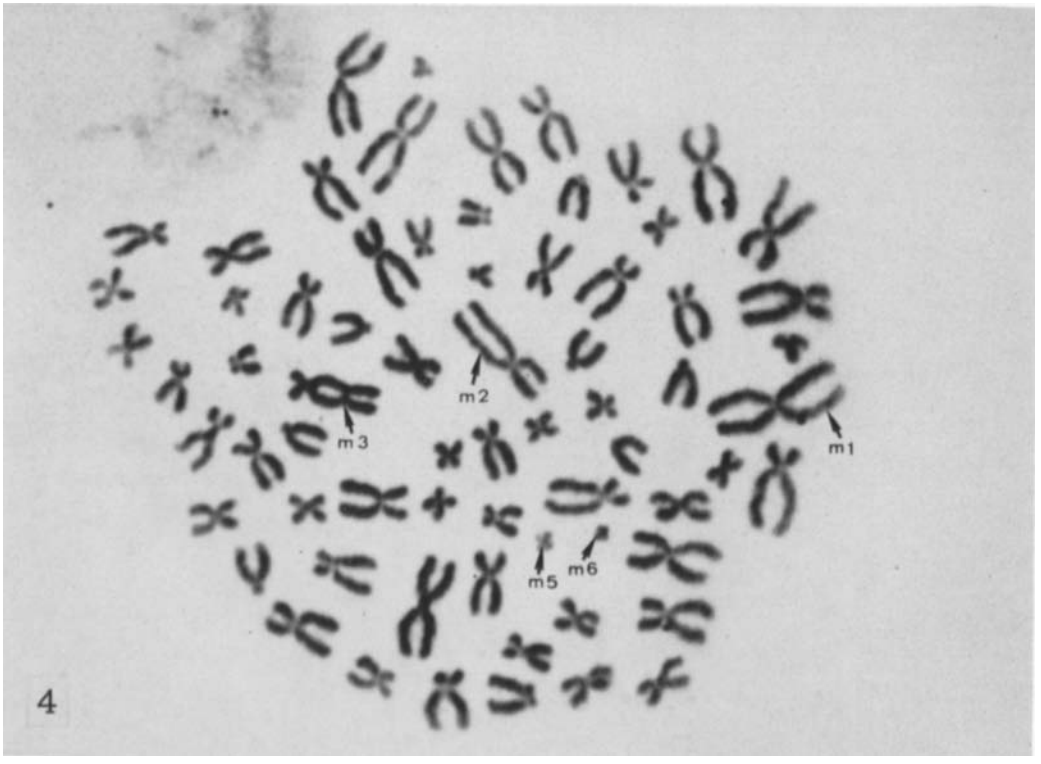

FIGURE 4.—Metaphase plate from H.Ep. #2/TC2 with 69 chromosomes and 5 markers—1, 2, 3, 5, and 6. *Note* marker 3.

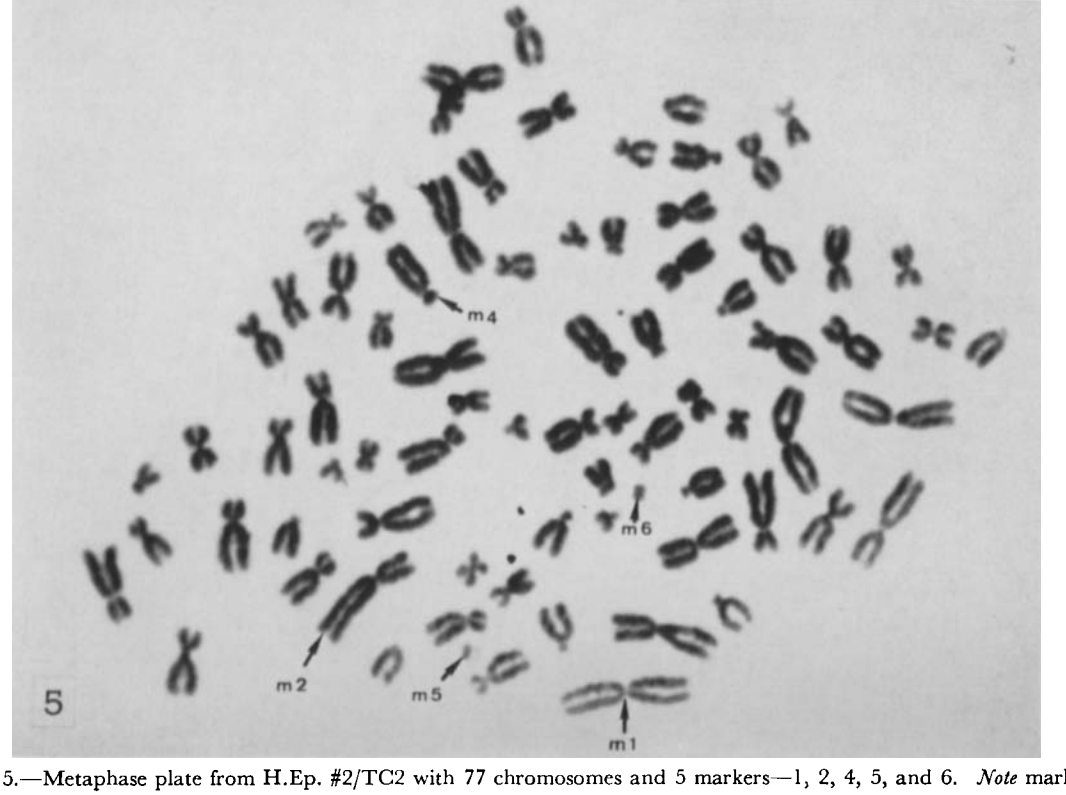

FIGURE 5.—Metaphase plate from H.Ep. #2/TC2 with 77 chromosomes and 5 markers—1, 2, 4, 5, and 6. *Note* marker 4.