Karyotype and Tumorigenicity of 1-Methylguanine-Transformed Chinese Hamster Cells [1,2]

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ABSTRACT—Chinese hamster embryo cells transformed with the tRNA catabolite 1-methylguanine were characterized by Giemsa-banded karyotyping and by their tumorigenic potency in athymic nude mice. All seven 1-methylguanine-transformed cell lines were hyperdiploid with a modal chromosome number of 23. Three of these lines had an additional marker chromosome derived from the long (q) arm of chromosome #4, and they had alterations of chromosome #5 as well. Two of these three cell lines were tumorigenic. Nonrandom chromosome changes were observed in the other four 1-methylguanine-transformed cell lines, which included the addition of all or a portion of chromosome #6. One of these cell lines was also tumorigenic in nude mice. Specific cytogenetic changes were observed in most 1-methylguanine-transformed populations in contrast to the karyotypic heterogeneity of a benzo[a]pyrene-transformed cell line.—J Natl Cancer Inst 62: 633–638, 1979.

Previous investigations in our laboratories revealed that a minor base of tRNA, 1-methylguanine, can alter the growth properties of normal Chinese hamster embryo cells in vitro (*1, 2*). This tRNA catabolite induces in the cells a diverse range of heritable changes (*1*) that are characteristic of in vitro transformation (*3*). The changes observed include morphologic, proliferative, cytogenetic, biochemical, and neoplastic properties (*1, 2*). One 1-methylguanine-transformed cell line ($ChH\text{-}1G_2$) was shown to have an extra chromosome fragment and to be tumorigenic in athymic nude mice (*2*). The chromosome fragment was tentatively assigned to be the long (q) arm of chromosome #4 of the Hsu and Zenzes karyotype (*4*), but chromosome banding had not been done to verify its identity.

The association of changes in chromosome #4 with chemical transformation of Chinese hamster cells was reported by Kirkland and Venitt (*5*) and was later expanded by Bloch-Shtacher and Sachs (*6*) to include viral transformation. Our preliminary cytogenetic analysis of a single 1-methylguanine-transformed cell line suggested a similar correlation; therefore, Giemsa chromosome banding was undertaken with seven 1-methylguanine-transformed cell lines. We have now karyotyped these lines and analyzed their tumorigenicity in nude mice.

MATERIALS AND METHODS

Six independently derived 1-methylguanine-transformed Chinese hamster cell lines were obtained by continuous treatment of duplicates of three primary embryo cultures, which were derived from 4–6 pooled embryos, with 10 μM 1-methylguanine (*1*). The transformed cell lines obtained were designated $ChH\text{-}1G_1$ to $ChH\text{-}1G_6$. The cells were subcultured (1:4) weekly in Eagle's minimum essential medium (Pacific Biological, Richmond, Calif.) supplemented with 10% fetal calf serum (Pacific Biological) and with 10 μM 1-methylguanine to simulate a chronic exposure. $ChH\text{-}1G_1$ and $ChH\text{-}1G_2$ cells were derived from primary culture II, $ChH\text{-}1G_3$ and $ChH\text{-}1G_4$ from primary culture III, and $ChH\text{-}1G_5$ and $ChH\text{-}1G_6$ from primary culture IV. The $ChH\text{-}1G_2$ cell line was split at p 50, with one culture being maintained as described above and the other being grown in the absence of exogenous 1-methylguanine. The cultures were then passaged in parallel with the latter being designated $ChH\text{-}1G_2m$. The parental line, $ChH\text{-}1G_2$, was shown to be tumorigenic at p 47 (*2*).

A continuous cell line that arose spontaneously ($ChH\text{-}Sp_1$) from one of the 12 untreated cultures and a benzo[*a*]pyrene-transformed ($ChH\text{-}BP_1$) continuous line (*1, 2*) were also studied. The $ChH\text{-}Sp_1$ line was from primary culture II and the $ChH\text{-}BP_1$ from primary culture IV. The $ChH\text{-}BP_1$ line was obtained after treatment of the cells with 1 μM benzo[*a*]pyrene in 0.5% dimethyl sulfoxide for 2 weeks (*1*).

Metaphase chromosome spreads were obtained from cells treated with 0.05 μg Colcemid (Calbiochem, San Diego, Calif.)/ml of medium for 3 hours. The cells were harvested, microscope slides were prepared as previously described (*7*), and the Giemsa chromosome banding technique described by Zuna and Lehman (*8*) was utilized. The chromosome number distribution was established by counting 50 metaphase cells. Karyotypic analyses were performed on eight metaphase spreads per cell line after the spreads were photographed through a Leitz microscope. The Giemsa banding pattern of Chinese hamster chromosomes reported by Kato and Yoshida (*9*) was followed, and sample karyotypes can be seen in figures 1 and 2.

Tumorigenicity was tested by the injection of 10^6 cells sc into each flank of athymic nude mice. When tumors reached greater than 1 cm in size, which occurred in 2–6 weeks, the mice were killed and the

ABBREVIATION USED: p = passage.

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tumor cells were placed back into culture. Cytogenetic analyses were done to verify that these cells were of Chinese hamster origin. A portion of the tumor and tissues from autopsy were fixed in Tellyesniczky's fluid, sectioned, and stained with hematoxylin and eosin.

RESULTS

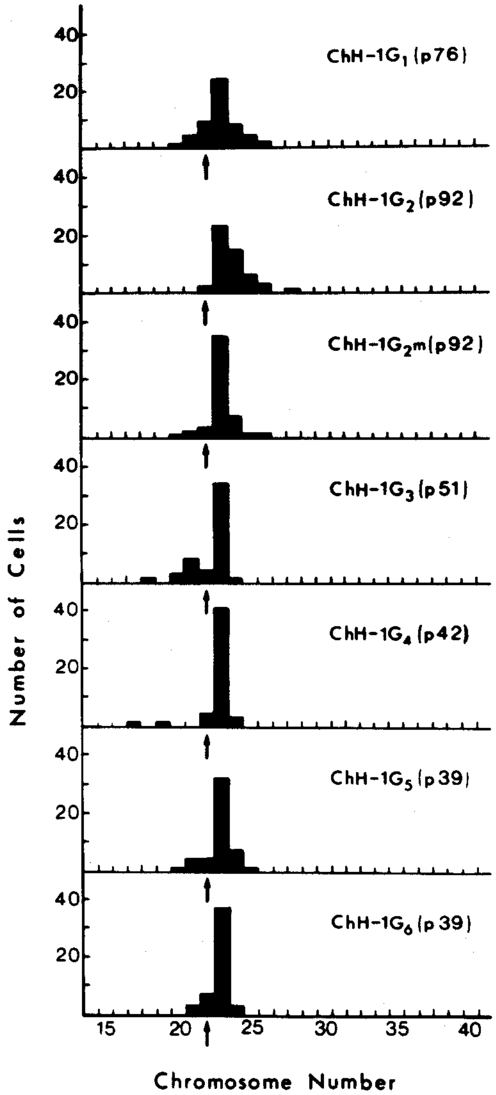
Chromosome number distributions for the 1-methylguanine-transformed cell lines are shown in text-figure 1. A modal chromosome number of 23 was observed for all of these lines.

The karyotypes for the ChH-1G_1, ChH-1G_2, and ChH-1G_2m lines, which were derived from the same primary culture, are presented in table 1. An additional chromosome fragment from the q-arm of chromosome #4 was observed in approximately 90% of the cells in this group, and a similar proportion had abnormalities associated with the #5 chromosome (fig. 1). A single sex chromosome was present in 40% of the cells examined, and the ChH-1G_1 line had an additional #9 chromosome in 90% of the cells. Random changes in the 9–11 group were observed in 50% of the ChH-1G_2 and ChH-1G_2m cells.

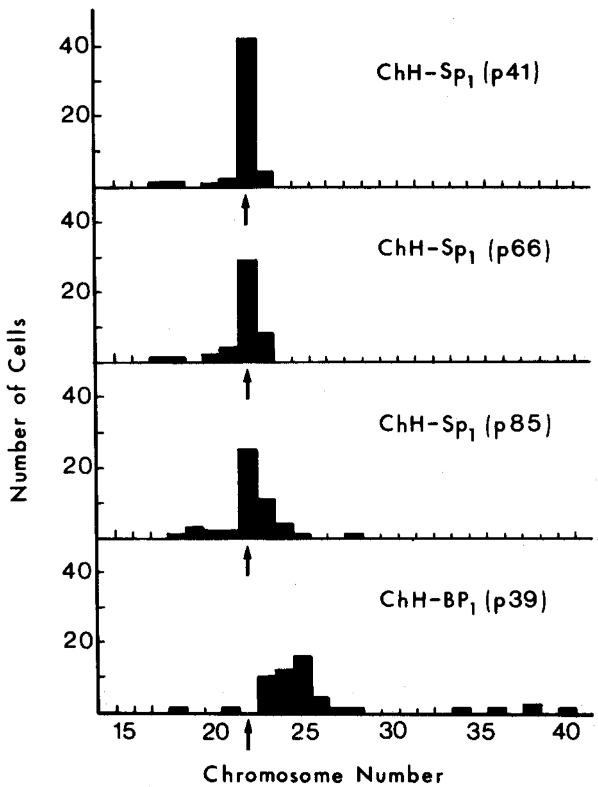
The karyotypic analyses of the ChH-1G_3 and ChH-1G_4 lines are presented in table 2. The ChH-1G_3 line had an extra chromosome that was a fragment of chromosome #6 (fig. 2), whereas the ChH-1G_4 line had an extra #6 chromosome. The sex chromosome specificity of the ChH-1G_3 and ChH-1G_4 karyotypes indicated in table 2 was verified by microscopic examination of more than 20 additional metaphase spreads for each. In no instance was an XX pair observed with ChH-1G_3 cells or an XY pair with ChH-1G_4 cells, which may be explained as transformation of a single cell (XX or XY) or possibly a selection of a clone.

The individual karyotypes for ChH-1G_5 and ChH-1G_6 cells (table 3) exhibited an additional #6 chromosome. Approximately 50% of the ChH-1G_5 cells had an abnormal X-chromosome, and the same numbers were missing one of the #7 chromosomes. No other consistent changes were observed with ChH-1G_5 or ChH-1G_6 cells.

ChH-Sp_1 cells appeared to be diploid at p 41 (*2*), but as shown in text-figure 2, increasing proportions of hyperdiploid cells were present at p 66 and p85. The chromosome number distribution for ChH-BP_1 cells is also presented in text-figure 2, and a heterogeneous, hyperdiploid population was apparent with a large



TEXT-FIGURE 1.—Chromosome number distribution of 1-methylguanine-transformed Chinese hamster cells. Fifty mitotic figures were counted per cell line. *Arrows* indicate position of diploid (2n=22) chromosome number.



TEXT-FIGURE 2.—Chromosome number distribution of ChH-Sp_1 cells at different passages and ChH-BP_1 cells. Fifty mitotic figures were counted per cell line at each passage. *Arrows* indicate position of diploid (2n=22) chromosome number.

TABLE 1.—*Individual cell karyotypes for ChH-1G1, ChH-1G2, and ChH-1G2m*

Cell line	No. of chromosomes in each group:[a]																																		
	1	a	b	c	d	e	2	a	b	c	d	X	a	b	Y	4	a	b	c	d	e	5	a	b	6	a	7	8	a	9	10	11	F	m	Total
ChH-1G1 (p 76)	2						2					2				2						1		1	2		2	2		2	2	2			22
	1	1					2					2				2	1					1	1		2		1	2		3	2	2			23
	2						2					2				2	1					1	1		2		2	2		3	2	2			24
	2						2					2				2	1					1		1	2		2	2		3	2	2			24
	2						2					1				2	1					1		1	2		2	2		3	2	3			24
	2						2					1				2	1					1	1		2		2	2		3	2	3			24
	2						2					1				2		1				1		1	2		2	2		3	2	3			24
	2						2					2				2	1					1		1	2		2	2		3	2	3			25
ChH-1G2 (p 92)	2						2					1				2	1					1		1	2		2	3		2	2	2			23
	2						1					2				2	1					2			2		2	1		3	3	2			23
	2						2					2				2	1					1		1	2		2	2		2	2	2		2	23
	2						2					2				2	1					2			2		2	2		2	2	2			23
	2						2					1	1			2	1					1		1	2		2	2		2	2	2			23
	2		1				2					1				1	1					1		1	2		3	2		2	3	2			24
	2						2					1				2	1					1		1	2		3	2		2	3	3			25
	2						2					2				1	1					1		1	2		3	2		3	3	2	1		26
ChH-1G2m (p 92)	2						2					2				1						1		1	2		2	3		2	2	2		1	22
	2						2					2				2	1					1		1	2		2	1		2	2	2			22
	2						2	1				1				2	1					1		1	2		2	2		2	2	2			23
	2						2		1			1				2						1		1	2		2	2		2	3	2			23
	2						2	1				2				2	1					1		1	2		2	1		2	2	2			23
	2						2					2				2	1					1			2		2	2		2	3	2			23
	2						2					1				2	1					1		1	2		2	2		3	2	2			23
	2						2					2				2	1					1		1	2		2	2		3	2	2		1	24

[a] 1a=loss of one-third of lower (q) arm; 1b=loss of half of upper (p) arm and half of q-arm; 1c=loss of entire q-arm; 1d=loss of half of p-arm and three-fourths of q-arm; 1e=loss of one-fourth of p-arm; 2a=loss of two-thirds of p-arm and two-thirds of q-arm; 2b=loss of one-fourth of p-arm and one-third of q-arm; 2c=loss of one-fourth of q-arm; 2d=loss of half of p-arm and one-third of q-arm; Xa=loss of one-fourth of q-arm; Xb=loss of half of p-arm; 4a=loss of entire p-arm; 4b=loss of half of p-arm; 4c=loss of one-fourth of p-arm; 4d=loss of entire q-arm; 4e=loss of three-fourths of q-arm; 5a=loss of half of p-arm; 5b=loss of one-fourth of q-arm; 6a=loss of one-fourth of q-arm; 8a=loss of half of q-arm; F=unidentified chromosome fragments; m="minute" chromosome material smaller than #11 chromosome; total=No. of chromosomes in each, excluding minutes.

TABLE 2.—*Individual cell karyotypes for ChH-1G3 and ChH-1G4*

Cell line	No. of chromosomes in each group:[a]																																		
	1	a	b	c	d	e	2	a	b	c	d	X	a	b	Y	4	a	b	c	d	e	5	a	b	6	a	7	8	a	9	10	11	F	m	Total
ChH-1G3 (p 51)	2						2					1			1	2						1			2	1	2	2		2	2	1			21
	2						2					1			1	2						2			2	1	2	2		2	2	2			23
	2						2					1			1	2						2			2	1	2	2		2	2	2			23
	2						2					1			1	2						2			2	1	2	2		2	2	2			23
	2						2					1			1	2						2			2	1	2	2		2	2	2			23
	2						2					1			1	2						2			2	1	2	2		2	2	2			23
	2						2					1			1	2						2			2	1	2	2		2	2	2			23
	2						2					1			1	2						2			2	1	2	2		2	2	2			23
ChH-1G4 (p 42)	2						2					2				2						2			3		2	2		2	2	1			22
	2						2					2				2						2			3		2	2		2	2	2			23
	2						2					2				2						2			3		2	2		2	2	2			23
	2						2					2				2						2			3		2	2		2	2	2			23
	2						2					1				2						2			3		2	2		2	3	2			23
	2						2					2				2						2			3		2	2		2	2	2			23
	2						2					2				2						1		1	3		2	2		2	2	2			23
	2						2					2				2						2			3		2	2		2	3	3			25

[a] *See* footnote to table 1.

percentage having 23, 24, and 25 chromosomes.

Detailed karyotypes for the ChH-Sp1 (p 85) and ChH-BP1 (p 39) cultures are shown in table 4. The continuous cell line that arose spontaneously (ChH-Sp1) had some random chromosome changes at p 85, as well as a loss of one #11 chromosome in 50% of the cells. However, the benzo[*a*]pyrene-transformed cell line (ChH-BP1) was even more heterogeneous than the chromosome number distribution suggested; i.e., different karyotypes were observed for the same chromosome numbers. Changes were found with chromosome #4 in all ChH-BP1 cells examined, with three-fourths of the cells having a chromosome fragment missing a portion of the p-arm and the same number having fragments missing all or most of the q-arm. Other nonrandom changes for ChH-BP1 cells included the loss of a sex chromosome and the loss of all or part of one of the #8 chromosomes.

TABLE 3.—*Individual cell karyotypes for ChH-1G₅ and ChH-1G₆*

Cell line	No. of chromosomes in each group:[a]																																		
	1	a	b	c	d	e	2	a	b	c	d	X	a	b	Y	4	a	b	c	d	e	5	a	b	6	a	7	8	a	9	10	11	F	m	Total
ChH-1G_5 (p 39)	2						2					2				2						2			2		1	2		2	2	2			21
	2						2					2				2						2			3		2	2		2	1	1			21
	2						2					1	1			2						1	1		3		1	2		2	2	2			22
	2						2					1	1			2						2			3		1	2		2	2	2			22
	2						2					1	1			2						2			3		2	2		2	2	2			23
	2						2					2				2						2			3		1	2		3	2	2			23
	2						2					1		1		2						2			3		2	2		2	2	2			23
	2						2			1		2				2						2			3		2	1		2	2	2			23
ChH-1G_6 (p 39)	2						2					1				2						2			3		2	2		2	2	2			22
	2						2					2				2						2			3		2	2		2	2	2			23
	2						2					2				2						2			3		2	2		2	2	2			23
	1	1					2					2				2						2			3		2	2		2	2	2			23
	2						1			1		2				2						2			3		2	2		2	2	2			23
	2						2					2				2						2			3		2	2		2	2	2			23
	2						2					2				2						2			3		2	2		2	2	2			23
	2						1			1		2				2						2			3		2	2		2	2	2			23

[a] *See* footnote to table 1.

TABLE 4.—*Individual cell karyotypes for ChH-Sp₁ and ChH-BP₁*

Cell line	No. of chromosomes in each group:[a]																																		
	1	a	b	c	d	e	2	a	b	c	d	X	a	b	Y	4	a	b	c	d	e	5	a	b	6	a	7	8	a	9	10	11	F	m	Total
ChH-Sp_1 (p 85)	2						2					1				2						2			2		2	2		2	2	1			20
	1					1	2					1		1		2						2			2		2	2		2	2	1			21
	2						2					2				2						2			2		2	2		2	2	1			21
	2						2					2				2						2			3		2	2		2	2	1			22
	2						2					1				2						2			2		2	3		2	2	2			22
	2						2					1				2						2			2		2	3		2	2	2		1	22
	2						2		1			2				2						2			1		2	2		2	2	2		1	22
	2						2					2				2						1	1		2		2	2		3	2	2			23
ChH-BP_1 (p 39)	1	1					2				1	1				1			1			2			2		2	1	1	2	2	2			22
	1	1					2			1		1				1			1	1		2			1	1	1	2		2	2	2		1	22
	1	1			1		2					1				2				1		2			1	1	1	2		2	2	2		1	22
	2						2					1			1	1	1		1			2			2		2	1	1	2	2	2			23
	2						2		1		1	1				1			1	1		2			2		2	1		2	2	2		1	23
	2			1			3					1				1					1	1		1	2		2	1		3	3	2		1	24
	2						3							1		1			1	1		2			2		2	1		2	3	2	2		25
	3						4					1			1	2			1		1	2			4		4	4		4	2	4	1		38

[a] *See* footnote to table 1.

TABLE 5.—*Tumorigenicity data for transformed cell lines*

Cell line	Primary culture	Latency, wk	No. of mice positive/No. of mice inoculated[a]
ChH-1G_1 (p 92)	II	5	6/6
ChH-1G_2 (p 102)	II	3	11/12
ChH-1G_2m (p 102)	II	8	0/12
ChH-1G_3 (p 67)	III	4	5/6
ChH-1G_4 (p 55)	III	8	0/6
ChH-1G_5 (p 57)	IV	8	0/6
ChH-1G_6 (p 56)	IV	8	0/6
ChH-Sp_1 (p 53)	II	8	0/4
ChH-BP_1 (p 32)	IV	2	6/6

[a] Inoculum of 10^6 cells/site.

Tumorigenicity studies were undertaken with all of the cell lines, and the results are presented in table 5. ChH-1G_1, ChH-1G_2, ChH-1G_3, and ChH-BP_1 cells were tumorigenic in athymic nude mice, and all gave rise to fibrosarcomas or histiocytomas with local invasion and no evidence of metastasis within 2–6 weeks. The tumors were placed in tissue culture and were karyologically similar to the original lines. Somewhat unexpectedly, ChH-1G_2m cells were not tumorigenic even though the parental ChH-1G_2 cells were tumorigenic before (*2*) and after (table 4) establishment of the ChH-1G_2m line.

DISCUSSION

The finding that 1-methylguanine could transform normal Chinese hamster embryo cells in vitro (*1, 2*) dictated the necessity for characterizing the transformed cells in detail. Chinese hamster embryo cells generally exhibit a finite lifetime in vitro, and the 1-methylguanine-transformed cell lines were selected initially for their ability to grow continuously in culture (*1*); i.e., they continued to proliferate after control cultures stopped growing. These continuous cultures were shown previously to have characteristics in vitro of transformed cells (*1, 2*). The 1-methylguanine-transformed lines were uncloned populations; thus finding a modal chromosome number of 23 in every instance was quite unexpected. This is especially true when one considers the diversity observed with ChH-BP_1 cells and that reported for Chinese hamster embryo cells transformed by the DNA tumor virus, simian virus 40 (*7, 8*).

Although the 1-methylguanine-transformed cell lines were all hyperdiploid with 23 chromosomes, detailed karyotypic analyses demonstrated two different nonrandom changes. The additional chromosome fragment in ChH-$1G_1$, ChH-$1G_2$, and ChH-$1G_2$m cells was the q-arm of chromosome #4, and in ChH-$1G_3$, ChH-$1G_4$, ChH-$1G_5$, and ChH-$1G_6$ cells there was an extra complete chromosome #6 or a fragment of it. Other chromosome changes were observed in some cell lines, especially those at later passages, but the hyperdiploidy appeared to be conferred by chromosome #4 or #6.

ChH-$1G_1$ and ChH-$1G_2$ cells grew in soft agar (*1*), were tumorigenic (table 4), and had chromosome changes associated with chromosome #4 (table 1). These findings are consistent with previous reports concerning growth characteristics and chromosome abnormalities in transformed Chinese hamster cells (*5, 6*). However, ChH-$1G_2$m cells had a karyotypic pattern similar to that of ChH-$1G_2$ cells, but they were not tumorigenic. Whether the removal of exogenous 1-methylguanine was responsible for the loss of tumorigenicity remains to be established, but contrary to the report of Bloch-Shtacher and Sachs (*6*), the presence of an additional q-arm of chromosome #4 may not be sufficient to confer malignancy on the cells. In addition, ChH-$1G_3$ cells were tumorigenic in nude mice, but they had an additional fragment of chromosome #6, not #4.

ChH-$1G_4$, ChH-$1G_5$, and ChH-$1G_6$ cells were shown previously to have biochemical and/or proliferative properties of transformed cells (*1*), and they were also changed with regard to chromosome #6. Triploidy with chromosome #6 is one of the common cytogenetic changes observed for Rous sarcomas of Chinese hamsters induced in vivo (*10*) and chemically transformed Chinese hamster cells in vitro (*11*), but the ChH-$1G_4$, ChH-$1G_5$, and ChH-$1G_6$ cell lines were not tumorigenic. However, ChH-$1G_4$, ChH-$1G_5$, and ChH-$1G_6$ cells are continuous lines; thus they may have undergone primary transformation in the absence of secondary, neoplastic transformation. Triploidy in chromosome #6 may be associated with this 1-methylguanine-induced primary event, but the results for ChH-$1G_1$ and ChH-$1G_2$ cells suggest that it is not required.

The primary culture from which the 1-methylguanine-transformed cell lines were derived may have a role in the determination of the marker chromosome obtained, but different cell lines from the same primary culture can exhibit unique properties. ChH-$1G_2$ cells were cytogenetically similar to ChH-$1G_2$m cells, but only the ChH-$1G_2$ cells were tumorigenic. ChH-$1G_3$ and ChH-$1G_4$ cells had additional marker chromosomes derived from chromosome #6, but, again, only ChH-$1G_3$ cells produced tumors in nude mice. However, ChH-$1G_3$ and ChH-$1G_4$ cells were different cytogenetically in that ChH-$1G_3$ cells were totally XY and ChH-$1G_4$ cells were XX.

Karyotypic homogeneity was observed for most of the individual 1-methylguanine-transformed cell lines. The mechanism by which chronic exposure to 1-methylguanine can induce such specific cytogenetic changes in a mixed embryo culture is under investigation.

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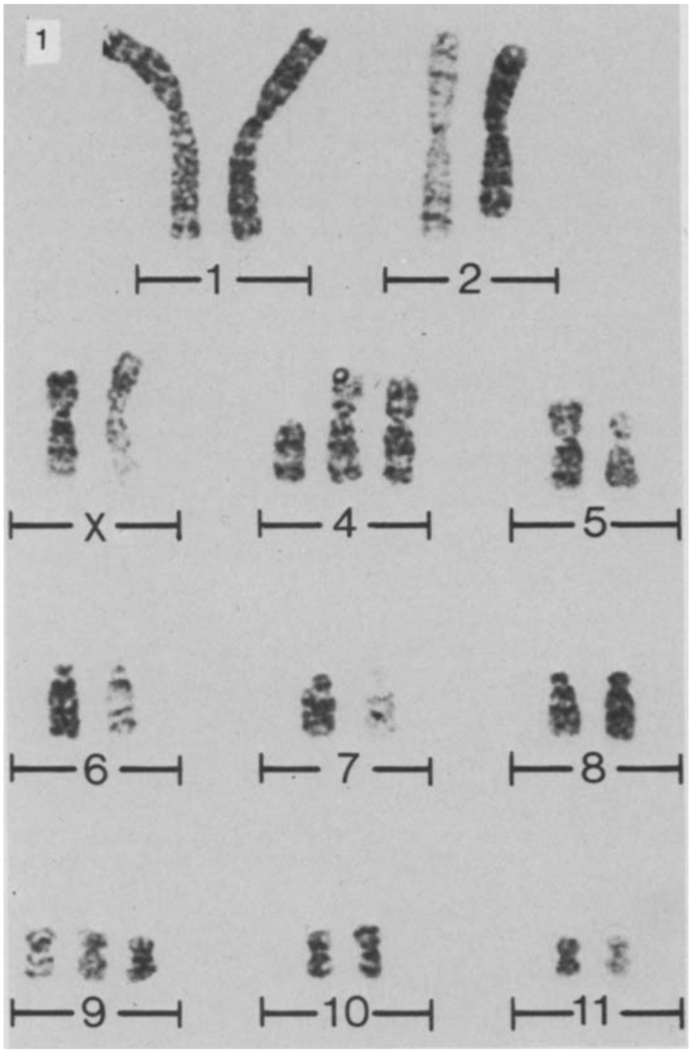

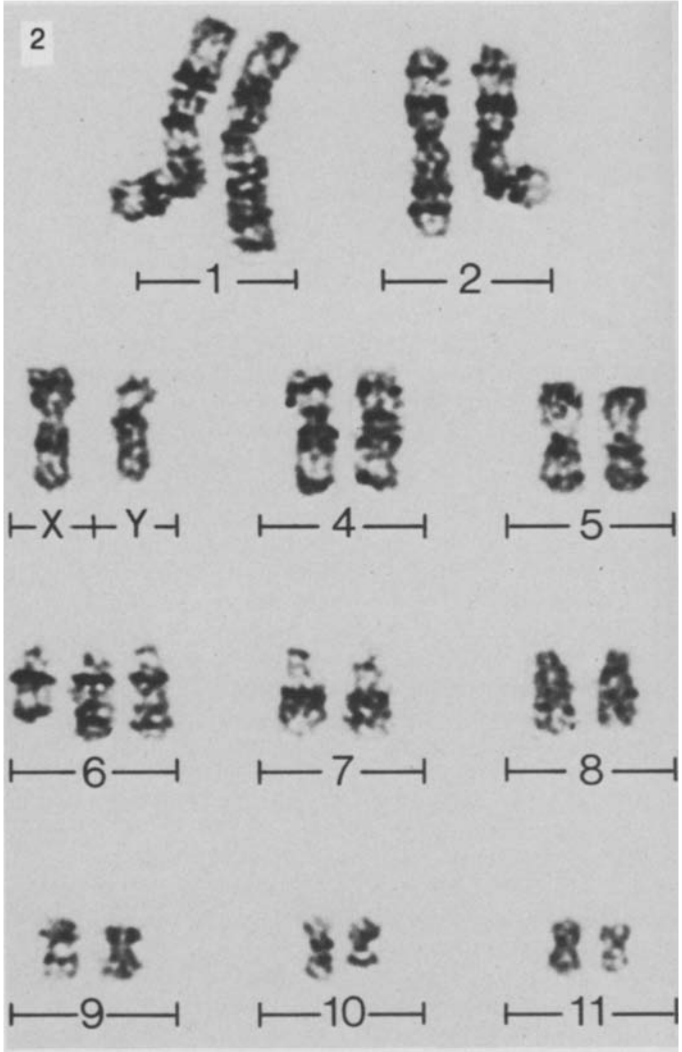

FIGURE 1.—Giemsa-banded karyotypes of ChH-1G$_1$ cell with 24 chromosomes. *Note* extra chromosome #4 (q), extra #9, and loss of portion of p-arm of #5.
FIGURE 2.—Giemsa-banded karyotype of ChH-1G$_3$ cell with 23 chromosomes. *Note* extra chromosome #6 with portion deleted.