*CELLULAR ALTERATIONS IN THE MCH LINE OF CHINESE HAMSTER CELLS FOLLOWING INFECTION WITH HERPES SIMPLEX VIRUS**

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We previously reported the isolation of the aneuploid MCH line of Chinese hamster cells from a normal line originally derived by Dr. George Yerganian from female adult fibroblast derivatives which formed a short-term carrier state with herpes simplex virus.[1] We also reported the occurrence of chromosomal aberrations during the first postinfection division with this virus, and subsequently the presence of persistent chromosomal aberrations in clonal sublines derived from infected cells.[2] This paper is concerned with further studies of the effects of viral infection upon chromosomes of MCH cells.

Materials and Methods.—Cell line: The MCH line and the composition of the medium employed were previously described.[1] All sera used were heated to 56° for 40 min before use.

Virus: The SAE strain of herpes simplex virus used was previously described.[1] Virus was harvested from cultures of HeLa cells inoculated 24 hr earlier with 10^8 $TCID_{50}$ of virus. The cells were collected, washed 5–6 times with Hanks' balanced salt solution, and resuspended in 0.2 *M* PO_4 buffer containing 0.1% bovine serum albumin (Armour fraction V) at pH 7.0. The cells were then disrupted by 3 cycles of rapid freeze-thawing in a dry ice-alcohol mixture, followed by 4 min of sonication at maximum output in a 10 kc Raytheon sonic oscillator. Following disruption of cells, the suspension was centrifuged at 4°, the supernatant collected, and then filtered through a millipore filter (MF type HA) in a Swinny hypodermic adaptor before use.

Virus titrations were performed in six HeLa tubes per dilution, and the $TCID_{50}$ calculated by the method of Reed and Muench.[3] This strain of herpes simplex virus causes mainly a nonproliferative type of cytopathic effect in HeLa cells as described by Gray *et al.*[4]

Infection of cells: A stock bottle of MCH cells was trypsinized (0.25% Bactotrypsin-Difco) and centrifuged at room temperature. The resultant pellet was resuspended in medium, and the number of cells determined by direct count in a hemocytometer chamber. Three cc of a cell suspension containing 2×10^5 cells plus 0.1–0.2 ml of virus suspension or buffer was added to a 25 ml sterile plastic flask (Falcon Plastics). The cell-virus mixture was then placed on a rotating shaker and shaken (370 rpm) for 1 hr at 37°C. The suspension was either diluted directly for seeding of plates or first centrifuged and the cells washed to remove the bulk of residual virus. For cloning and plating efficiency studies, approximately 500 cells were seeded in Petri dishes. Within 24 hr the location of single attached cells was noted. These cells were observed during the next 7 days, and individual clones which were well isolated were removed with a capillary pipette and seeded in separate 25 ml plastic flasks. The flasks were incubated at 37° in an atmosphere of 5% CO_2 in air and refed every 2–3 days until a full cell sheet had formed. The cells were then trypsinized and passaged normally.

Virus antiserum: Antiserum was prepared in rabbits by intravenous inoculation of 10^8 $TCID_{50}$ of virus for six consecutive weeks. Neutralizing antibody was assayed in HeLa cells by a constant virus-varying serum dilution method following incubation at room temperature for one-half hour. Serum from a single animal having a titer of 1:130 for 10^5 $TCID_{50}$ virus was used throughout. Preinoculation bleeding from the same animal was used as control. All sera were heated to 56° for 40 min before use.

Chromosome preparations: Approximately 2×10^5 cells in 3 ml medium were seeded in glass Petri dishes containing 2 coverslips. After 48–72 hr, colchicine was added to yield a final concentration of 0.5 μg/ml. Plates were reincubated for an additional 2–4 hr. The medium was made hypotonic by adding $^1/_2$ volume of prewarmed deionized water, and the plates were incubated

for 7 min. Cells were fixed in Carnoy's 3:1 at room temperature for 2–4 hr. The coverslips were stained with aceto-carmine and mounted on slides using a squash technique.

Cells were studied under the phase microscope at 250× magnification, and the location of intact cells with well-dispersed chromosomes was noted. These same cells were then studied under the oil immersion lens. Any cell whose chromosomal morphology was doubtful was photographed and an enlarged 8 × 10 print was made to facilitate study.

Results.—The MCH line has a consistent plating efficiency of 35–45 per cent. Even at viral multiplicities greater than 10^4 per cell, no decrease in plating efficiency was noted when virus was added either at the time cultures were initiated or subsequent to cell attachment. However, when the virus-cell suspension was shaken for 1 hr at 37°, cell killing as measured by a decrease in plating efficiency was observed. The linearity of the relation between cell survival and the amount of virus added (as shown in Fig. 1) indicates single-hit kinetics. Shaking by itself caused no decrease in the plating efficiency of control cells.

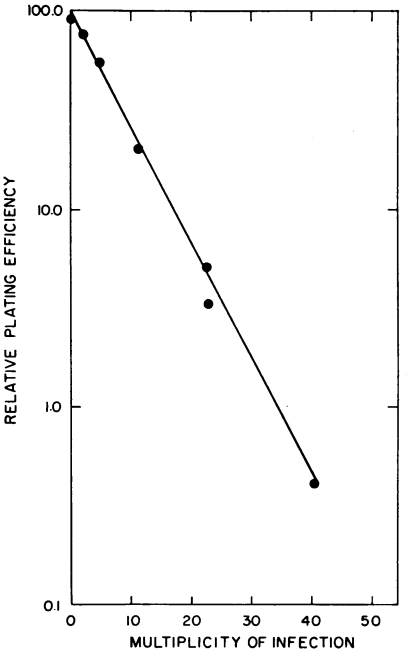

FIG. 1.—Cell survival following shaking of MCH cells with herpes simplex virus at various multiplicities.

Virus neutralized with specific antiserum or inactivated at 56° for 30 min did not cause a decrease in plating efficiency. Neither normal rabbit serum nor antiserum to virus had any effect on the plating efficiency of uninfected cells. Normal rabbit serum did not affect virus killing. Inoculation of control cells with phosphate buffer containing bovine serum albumin or extracts from uninfected HeLa cells had no effect. No cell killing was evident when cells were shaken alone and then virus added. The role of shaking in the production of the chromosomal abnormalities to be described is not understood.

The chromosomes of the MCH line are similar to those reported in other Chinese hamster lines with two exceptions. The first is the appearance of a distinctive marker (M) chromosome, apparently unique to the MCH line, and the second is a modification in the X_2-chromosome seen in over 70 per cent of the cells, where a deletion has occurred in the short arm. This latter alteration has been seen in other Chinese hamster lines.[5] The origin of the M-chromosome is not known, as the MCH line appeared spontaneously in a bottle seeded with a normal aneuploid (24–26 chromosomes) line of adult female Chinese hamster cells. The stemline number in the MCH line has remained at the 24 chromosome level with no tendency toward heteroploidy. There is, however, no regularity in the karyotype of these cells as regards the number of each chromosome type present. More than one M-chromosome per cell is rarely encountered. No variations in the number of sex chromosomes have been observed. We have never seen an uninfected MCH or clonal subline cell which did not contain a marker (M) chromosome.

Figure 2 shows comparative idiograms of an MCH cell and a diploid Chinese

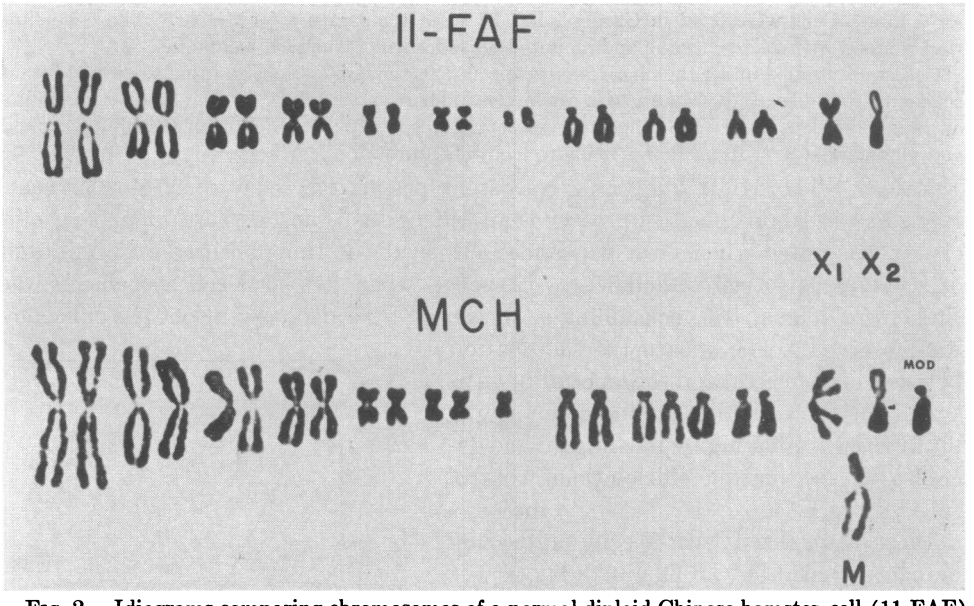

FIG. 2.—Idiograms comparing chromosomes of a normal diploid Chinese hamster cell (11-FAF) and an MCH cell. Both a normal and a modified X_2 are included for comparison. The marker (M) is seen only in the MCH line.

hamster cell from a line kindly supplied by Dr. Yerganian.[6] Except for the M and modified X_2, the chromosome arm ratios are very similar to those seen in other Chinese hamster cells.[7-9] The M-chromosome has an interarm ratio of 1.84 and the modified X_2, 4.0.

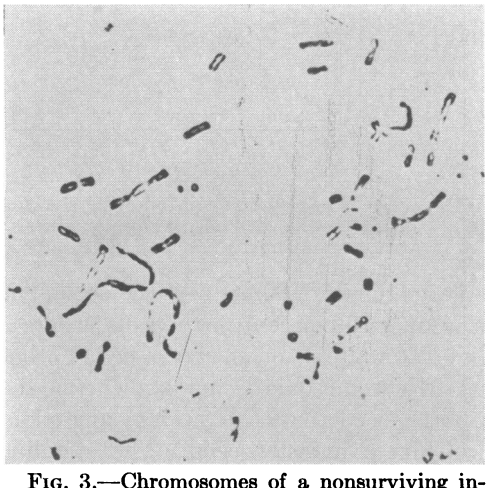

FIG. 3.—Chromosomes of a nonsurviving infected cell during the first postinfection division after shaking.

The chromosomes of infected shaken cells were studied during the period of their first cell division. Depending on the multiplicity of infection, severe chromosomal aberrations manifested by multiple breakages occurred during the first 3 days after infection of cultures as shown in Figure 3. The incidence of the aberrations paralleled the rate of cellular killing as determined by the decrease in plating efficiency. The aberration rate returned to the control level of 1–3 per cent by the fifth postinfection day, and remained so during subsequent passages. When the cells of later passages were studied with respect to possible chromosomal alterations, no differences from normal were evident. Strikingly different results were obtained when infected shaken cells were plated at low concentration immediately after infection as for plating efficiency studies, and individual clones isolated and propagated separately after 7 days.

TABLE 1

CHROMOSOME DELETIONS IN INFECTED AND UNINFECTED CLONES*

	No. deletions/cell	Percent of Total Deletions — Chromosomes No. 1	2	3	4	X_1
Control	0.025† (0.02–0.03)‡	25	25	47	3	0
Infected	1.9 (1–2.5)	16	52	22	5	5

* Represents data from 50 cells per clone.
† Average from all clones.
‡ Range among clones.

We have thus far studied 12 such infected and 17 control clones in detail. No degenerative changes appeared in any of the infected clones during the subsequent two-month period, nor were there any changes from normal in plating efficiency.

Chromosomes of all infected clonal lines studied 5–6 weeks after infection showed a number of alterations, many of which could be identified as surviving chromosome or chromatin deletions of specific segments on chromosomes Nos. 1, 2, and 3, following loss of the acentric fragments. The aberration rate in these cells varied from 1–3 per cent. Table 1 shows the number and involved chromosomes with stable changes having the major deletions that were found among infected and control clones. These do not include asymmetrical aberrations such as chromatid lesions or gaps, dicentrics, rings, and translocations. Deletions occurred at a frequency of 80–100 per cent in all infected clones, while in controls the maximum frequency was 3 per cent. Figure 4 shows the frequency of the major deletions at the various loci on chromosomes Nos. 1, 2, and 3. The numbers below chromosomes Nos. 1 and 2 represent the loci designated by Somers and Hsu[10] as involved in aberrations following treatment of Chinese hamster cells with 5-bromodeoxyuridine, hydroxylamine and X irradiation. We have seen no deletions on these chromosomes occur-

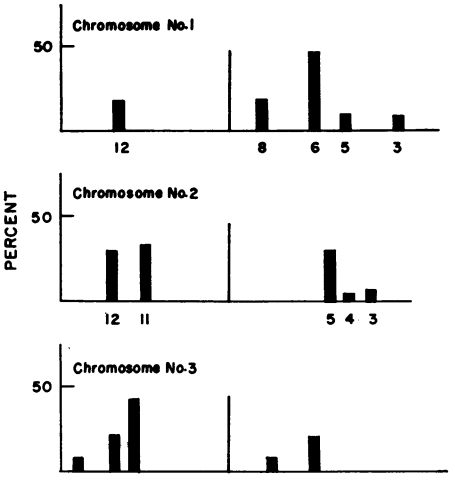

FIG. 4.—Diagrammatic representation of chromosomes Nos. 1, 2, and 3 showing the relative frequency of deletions at various loci in infected cells. The numbers below chromosomes Nos. 1 and 2 are taken from Somers and Hsu.[10]

TABLE 2

ANALYSIS OF 20 CELLS FROM TWO INFECTED CLONES SHOWING ALTERED CHROMOSOMES

	Cell No.	Chromosome affected		Cell No.	Chromosome affected	
Clone 4	1	2	Clone 8	1	2,3	
	2	2,3		2	2	X_1
	3	2		3	2	X_1
	4	1,2		4	2	
	5	2		5	3	
	6	2 4		6	2 4	
	7	1		7	2 4	
	8	2		8	2,3	
	9	2		9	2,3	X_1
	10	2		10	1,2,3	

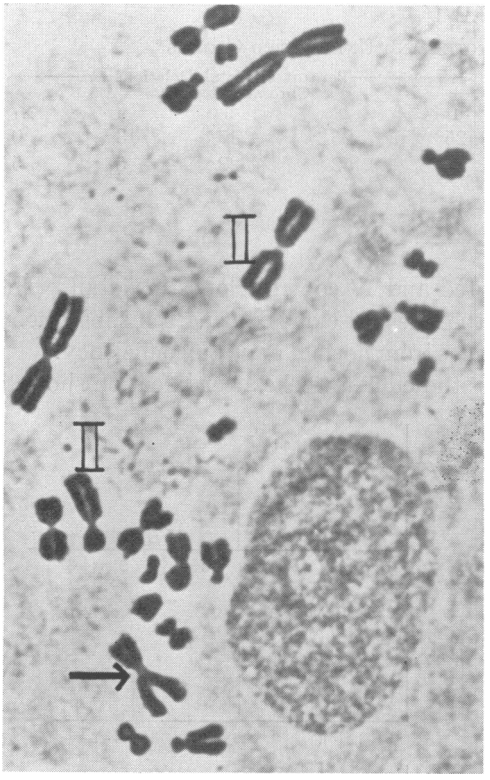

FIG. 5.—Chromosomes of an infected MCH cell showing one unidentifiable chromosome (arrow). One chromosome No. 2 has a deletion at position 11. No marker chromosome is evident.

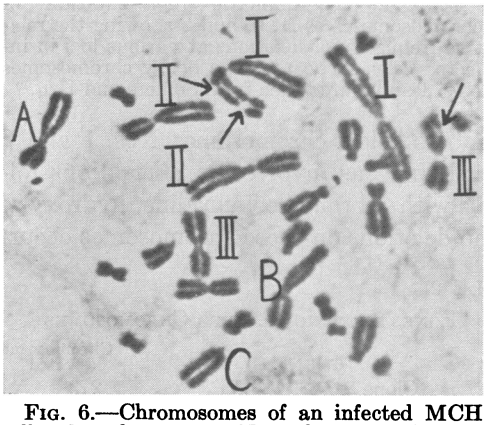

FIG. 6.—Chromosomes of an infected MCH cell. One chromosome No. 1 shows two chromatid breaks (arrows). One chromosome No. 3 shows a chromatid break (arrow). The chromosome labeled (B) is probably the X_1 with an elongated long arm. Chromosomes labeled (A) and (C) are unidentified. No marker chromosome is evident. One chromosome No. 2 has a deletion at position 11.

ring at loci differing morphologically from those reported by these authors. Deletions in control cells occurred at the same loci on chromosomes Nos. 1, 2, and 3.

While the relative distribution of affected chromosomes within each clone generally followed the scheme described in Table 1, there was always some variation within cells of a given clone as to which chromosomes were involved. Table 2 shows the analysis of 20 cells selected at random from 2 infected clonal sublines. Frequently a cell showed deletions in more than one chromosome. Rarely were both chromosomes of a pair involved within a single cell. These changes are apparently compatible with cell survival, as shown by the maintenance of normal plating efficiency. Deletions in control cells occurred at the same loci on chromosomes Nos. 1, 2, and 3.

Chromosome alterations other than deletions were also evident. In at least 3 of the infected clones, over 50 per cent of the cells showed no recognizable marker chromosome (Figs. 5 and 6). In two infected clones, almost every cell had at least one new chromosome which would not be identified, and was morphologically different from cell to cell (Figs. 5 and 6). Changes were also seen in the sex chromosomes; however, due to the similarity of the X-chromosomes to autosomes, we have not been able to make a systematic study of the frequency or morphology of these changes. Those changes which could be identified occurred mainly in the X_1-chromosome (Figs. 6–8). Some of these changes were reported to occur in both spontaneous and polyoma-induced tumor cells of Chinese hamsters.[7]

Discussion. — The MCH system shows arrested mitosis when killing

occurs following shaking of cells with virus. Killing is manifested by multiple chromosomal aberrations in colchicine blocked metaphase cells. The severity of the aberrations indicates that cell death results from the cells' inability to complete division. Apparently, this represents a special type of cytopathic effect for this virus. Stoker and Newton[11] and Roizman[12] reported arrested mitosis in herpes-infected cells, but no chromosomal aberrations were noted. Cell killing concomitant with multiple aberrations was seen in the first generation after X irradiation of Chinese hamster cells by Greenblatt.[13]

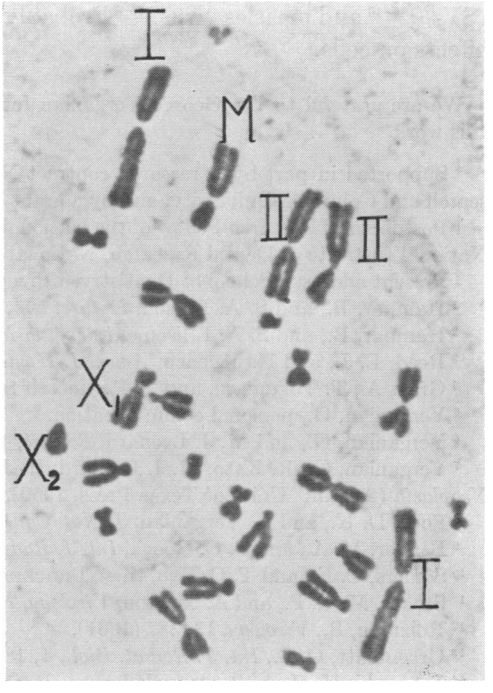

FIG. 7. Chromosomes of an infected MCH cell. One chromosome No. 2 has a deletion at position 11. The X_1-chromosome has a deletion in its short arm. The X_2-chromosome has a centromeric break with only one arm evident.

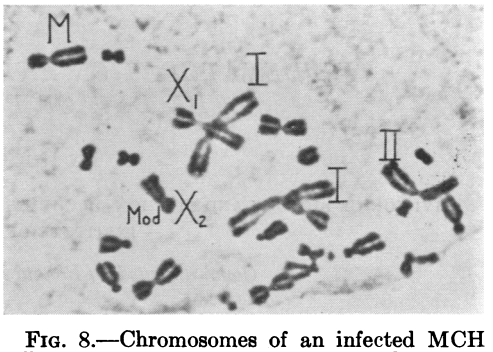

FIG. 8.—Chromosomes of an infected MCH cell. The X_1-chromosome shows an elongation of its long arm.

The reason for the apparent absence of permanent chromosomal alterations when infected shaken cells are seeded in large numbers (2×10^5), in contrast to cells cloned after infection, is not clear at the moment. It is possible that cell density influences the appearance of the alterations, and that the alterations are produced in cell lineages which have a selective disadvantage in mass cultures. The presence of alterations at high frequency in all infected clones is especially interesting owing to two characteristics of the process. The first characteristic is the different distribution of breaks in the cells of the same clone. This difference shows that breaks were formed in different cells during the growth of the clone. The observations would suggest that new breaks appear for several cell generations after infection. Thus, an agent causing breaks may persist in the cells for some time. The other characteristic is the location of the breaks. They are located at loci morphologically undistinguishable from those at which breaks occur following exposure of the cells to 5-bromodeoxyuridine, hydroxylamine, and X-irradiation treatment. This suggests that the location of the breaks is a property of the cell and not of the inducing agent.

Subsequent to our initial findings, a number of reports have appeared of chromosomal involvement following infection of cells with simian vacuolating virus

(SV_{40})[14-16] and measles virus.[17] Probably other viruses will be found to have similar effects on cells.

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*THE DENATURATION AND THE RENATURATION OF THE DNA OF POLYOMA VIRUS**

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Polyoma virus induces neoplastic growths in some rodents and causes cytopathic effects in mouse embryo tissue cultures.[1] It contains DNA corresponding to a molecular weight of 7.5×10^6 per intact virus particle.[2] The genetic information for the replication of the virus and for the initiation of tumors in hamsters is contained in a subviral infective agent (SIA) which can be extracted from the virus[3, 4] with phenol. The SIA consists essentially of the viral DNA which is mainly base-paired.[5] From the buoyant density (1.709 gm cm^{-3}) in CsCl gradients and from the melting profile it can be estimated that the DNA of polyoma virus should contain about 49 mol % of guanine-cytosine. The presence of small numbers of unusual nucleotides or of small amounts of constituents other than DNA could not be excluded. Sedimentation velocity studies of phenol extracts from purified