*HYBRIDIZATION OF DWARF HAMSTER CELLS BY UV-INACTIVATED SENDAI VIRUS**

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Communicated by John F. Enders, March 2, 1966

The phenomenon of virus-induced polykaryocytosis[1] was first postulated by Ephrussi and Sorieul[2] as one mechanism which could lead to an elevation in the incidence of somatic cell hybridizations. Recently, Harris and Watkins observed a high degree of induced heterokaryons and mononucleated hybrid cells following exposure of HeLa and Ehrlich ascites cells to UV-inactivated Sendai virus.[3] The agglutinating property of myxoviruses,[4] in general, is expected to encourage cytological and physiological studies on a variety of hybrid combinations between rapidly proliferating and differentiated cells.[5]

Although HeLa and Ehrlich ascites cells proliferate rapidly, *in vitro* and *in vivo*, respectively, mononucleated homosynkaryons (intraspecific) and heterosynkaryons (interspecific) of these two cells failed to survive beyond 15 days *in vitro.*[3] Some hybrid derivatives are, however, capable of cell division soon after their induction. Fusions involving one HeLa and one Ehrlich ascites cell have chromosome numbers ranging from 111 to 172, with an expected mode of 133.[6] The brief survival period of these hybrids is considered by Harris *et al.* to reflect the inability of Ehrlich ascites cells to proliferate *in vitro.*

Earlier attempts to hybridize untreated somatic derivatives of the Armenian hamster, *Cricetulus migratorius* ($2n = 22$), and the Chinese hamster, *Cricetulus griseus* ($2n = 22$), were unsuccessful. By employing procedures outlined by Harris and Watkins, a relatively high number of rapidly proliferating, continuous hybrid clonal lines have now been isolated, using UV-inactivated Sendai virus.

In sharp contrast to the expected uniformity of F_1 hybrid offspring following the fusion of normal gametes, somatic hybrid derivatives may vary considerably in cellular and clonal morphology. Distinct phenotypes, probably due to the dominance of individual genomes, are superimposed upon minor variations resulting from slight differences in chromosome number. Upon cloning, hybrid derivatives may be placed into one of three major morphological categories: (*a*) type-A cells and clones having exaggerated features of the Armenian hamster parent cell, (*b*) type-B cells and clones combining features of both parent cell types, and (*c*) type-C cells and clones having features associated with Chinese hamster parent cells. Intermediate types may be further divided into subclasses, such as A/B, and B/C. The importance of these observations is that more than one stable cell type may be derived following the fusion of near-diploid cultured cells.

Materials and Methods.—Although normal, malignant, and virus-transformed euploid cell lines of the Chinese hamster are on hand, past experience with balanced tetraploids influenced our decision not to employ a normal euploid at this time.[7] In addition, the relatively low plating efficiency of normal euploid cells (10% or less) could have altered these results, particularly when seeding low numbers of cells in Petri dishes.

Characteristics of cell types: (*a*) The Chinese hamster 12E cell line was derived from an 18-day-old female fetus and exposed to human adenovirus 7 at the first passage. Some ten subcultivations later (*ca.* 30–35 days), clones and confluent cultures exhibited extensive multilayering and a corre-

sponding increase in cell density while retaining euploidy. During the 24th to 30th passages, an aneuploid subline (trisomy for chromosome 8) gradually replaced the euploid population. Trisomy of chromosome 8 is reflected by an increase in cytoplasmic volume, separation of chromatids, and clarity of secondary constrictions on the X chromosomes. Similar features were noted earlier among clones having partial and whole trisomies for chromosome 3. Other autosomal trisomies are generally accompanied by an increase in chromosome spiralization which hinders immediate identification of X chromosomes. Clones of 12E cells are irregular in outline with juxtaposed cells having the tendency to multilayer centrally after 5 days of growth (Fig. 1). Viable cells rarely lift off to form smaller colonies, even when the medium is exhausted. When seeding 500–1000 cells per 60-mm Petri dish, plating efficiency is approximately 20%.

(*b*) The Armenian hamster parent cell, 6B, was derived from an embryonic culture transformed by human adenovirus, type 18.[8] It is essentially euploid (Table 1) except for a prominent secondary constriction near the centromere of one member of chromosome pair 1. This feature is seen in approximately 50% of the metaphases and serves as a marker to distinguish chromosome 1 from chromosome 2 of the Chinese hamster in hybrid cells. Clones of 6B consist of well-spaced angular cells with cytoplasmic extensions crisscrossing to form a loose network by the seventh day of growth (Fig. 2). When seeding 500–1000 cells in a 60-mm Petri dish, plating efficiency is approximately 16%.

Thus, the Chinese hamster 12E cells are predominantly aneuploids (23 chromosomes), non-oncogenic, exhibit multilayering, and lack contact inhibition, whereas 6B cells of the Armenian hamster are essentially euploid, moderately oncogenic, and differ in cell and clonal morphology. Both have extremely low frequencies of spontaneous chromosome aberrations.

Handling of cells: (*a*) Equal numbers of untreated (control) parent cells were pipetted into various types of culture vessels. Chromosome analyses were repeated over the course of 3–4 weeks, or some seven transfer generations; (*b*) equal numbers of trypsinized parent cells were placed in a suspension of UV-inactivated Sendai virus, following the steps outlined by Harris and Watkins.[3] More precisely, a virus suspension with a titer of 2400 hemagglutinating units (obtained from Dr. John F. Enders) was placed in a Petri dish and exposed for 3 min (at a distance of 3 in.) to ultraviolet light emanating from a 15-watt G.E. germicidal lamp (type G14T8). Cells were trypsinized and concentrated to approximately 10^6 cells/ml. Half a milliliter of each cell suspension was pipetted into a chilled tube, along with 1.0 ml of the inactivated virus suspension. This mixture was kept at 4°C for 15 min, and then agitated at 37°C for 20 min. Following this, the suspension was adjusted to a concentration of 5×10^4 cells/ml and pipetted into a series of Yerganian cytology tubes for chromosome preparations.[9] Petri dishes were seeded with 1000 cells for clonal isolations. The remainder of the virus-parent cell suspensions were placed in larger culture vessels for routine propagation.

Focal and clonal isolations: Disposable Pasteur pipettes, inserted in a latex tube adapted for oral manipulation, were employed to loosen individual colonies from the glass surface. Each was then drawn into a pipette by gentle suction and transferred to a Yerganian tube. Cloning operations were repeated several times to assure stability in chromosome number and cell morphology.

Results.—After three passages of untreated mixed cultures (1:1), only one spontaneous hybrid cell with 66 chromosomes was noted. The complement consisted of 44 Chinese and 22 Armenian hamster chromosomes. Henceforth, discussion will be confined to features of hybrid cells induced by UV-inactivated Sendai virus.

Initial chromosome studies: Two days after exposure to inactivated virus, 18 hybrid metaphases were recorded among 3000–4000 metaphases in each of two preparations seeded with approximately 5×10^4 cells. Chromosome numbers ranged from 44 to 46 and included both parent complements in the absence of new or marker chromosomes. Minor differences were due primarily to variations in the number of small metacentrics (chromosomes 8 and/or 9) of the Chinese hamster. The two metaphase complements were markedly "out of phase" with respect to spiralization or chromosome contraction, causing one to appear more darkly stained (Chinese hamster) while the other appeared loosely spiraled, with prominent major coils.

The second chromosome sampling was done 7 days after virus exposure. Numerous mitoses were arrested by a 4-hr colchicine pretreatment, but the incidence of hybrid cells was similar to that observed above. However, by this time, the two complements were fully synchronized, i.e., all chromosomes spiraled at the same rate, and identification of individual chromosome types depended entirely upon arm ratios and centromeric positions. Telomeric associations involving specific chromosome ends of both complements indicated nucleoli were being formed and/or shared by five Chinese and eight Armenian hamster chromosome types.[10] Several hybrid cells were noted bearing the identical chromosome number, plus a rare marker chromosome. This indicated that some hybrid cells had undergone at least one or more mitoses since their induction. Chromosome numbers now ranged from 36 to 48, with an occasional aneuploid having 23–26 chromosomes. The latter cells were noted only during this period of observation and were, presumably, not viable and derived from sporadic tetrapolar divisions.

Clonal identification of hybrid derivatives: Dividing hybrid cells were noticeably infrequent in cultures and samples 2 and 7 days after seeding with 5×10^4 cells. This was puzzling since two-cell associations were abundant in unsquashed preparations initially seeded with 1×10^3 cells. This discrepancy was partially explained when 60-mm Petri dishes, also seeded sparsely with $1–2 \times 10^3$ cells, were observed to contain a moderately high number of focal proliferations which bore no resemblance to colonies of either parent cell type. Seven to ten days after virus exposure, approximately 55 per cent of the foci (200–300) were judged to be parental in origin. The remainder were suspected of being composed primarily of tetraploid cells. Aside from increased cellular dimensions, the latter cells exhibited exaggerated focal and clonal morphologies, ranging from dispersed networks (a result of extensive migration of daughter cells) to dense colonies with tendencies to multilayer. Intermediate types were also present but were difficult to identify when adjacent either to parent or to the above-mentioned colonies. The new forms of growth were much larger by the seventh day, and the medium acidified rapidly. When the medium was not replenished, parent clones remained adherent, in contrast to the new cell types which rounded up with some lifting off the glass surface. Subsequent clonings and chromosome studies revealed three major categories of clones, i.e., "dispersed" (type A), "intermediate" (type B), and "dense" (type C) to represent hybrid cells (Figs. 3–6). Consequently, delays in changing the medium accentuate hybrid proliferations and, in turn, facilitate focal isolations.

Initially, 13 foci of suspected hybrid cells were isolated from among 200–300 colonies formed in sparsely seeded Petri dishes. Seven were selected as representing "dispersed" and "intermediate" cells (type A and type B), and the remainder represented "dense" or type-C cells. Only one focal line, 4X, was predominantly composed of a mutant Chinese hamster parent cell which cloned in a dispersed fashion. It featured an X-chromosome mutation (short arm of X_1:long arm of chromosome 2).

Seventeen additional clones were isolated from sparse secondary cultures. Focal and clonal lines were recloned during the course of the third to fifth passages. Representative hybrid cell types were selected for subculturing, and the remainder were preserved in a liquid nitrogen cell bank. By the fourth to sixth passage, 6B cells were noticeably absent in both focal lines and mixed cultures. Periodic clon-

ings of mixed cultures, during the course of 10 passages, continued to yield the three basic hybrid cell types.

Hybrid cells proliferate very rapidly. In fact, "hybrid vigor" describes the situation quite well, and there is little or no lag phase following each trypsinization of the type-B and type-C cells. A typical chromosome squash preparation is illustrated in Figure 7. Seventeen of the 22 chromosome types, including the sex elements, are readily identified. Apart from minor features which serve to construct idiograms, the five remaining medium-sized metacentrics resemble one another. Their identification is even more difficult when one attempts to assign marker chromosomes that form after hybridization. For example, the centrally located chromosome 4 of Figure 7 has a medial deletion of one arm.

Absence of a prominent chromosome modal class during the 60-day period of monitoring indicates nondisjunction to be quite extensive (Table 1). Cells with 41–48 intact chromosomes are equally viable. For the most part, numerical variations are confined to unequal distribution of the smaller metacentrics (chromosomes 8 and/or 9) of the Chinese hamster.

TABLE 1

DISTRIBUTION OF CHROMOSOMES IN SOMATIC HYBRIDS OF 6B × 12E CELLS*

Clones	<39	40	41	42	43	44	45	46	47	48	49	<50	No. of cells
Type A:													
7XC-7	1	3	*13*	8	5	8	6	5	...	1	...	...	50
A10XC-2	...	...	2	...	5	14	*26*	14	4	4	...	...	69
B10XC-1	...	1	2	4	4	10	*16*	4	...	...	...	...	41
Type B:													
24XC-3	3	...	2	3	1	5	*31*	2	...	1	2	...	50
Type C:													
A8XC-2	2	...	3	2	2	5	10	12	*35*	*30*	3	4	108
O8XC-2	...	...	1	2	3	6	*30*	*31*	6	1	...	...	80
O8XC-7	...	2	...	3	...	*16*	*18*	9	1	...	...	1	50
Total:	6	6	23	22	20	64	*137*	77	46	37	5	5	448

* 12 E: $2n = 22 + 1$ (66%: 299/439). 6B: $2n = 22$ (87%: 392/452).

Hybrid cells: Type-A cells fail to form clones in which two or more cells remain juxtaposed or stationary for any length of time. Instead, each cell division is followed by extensive migration or wandering of daughter cells (Figs. 3 and 4). Numerous cytoplasmic bridges extend between daughter cells over considerable distances. Loss of these strands to the supernate tends to deplete the cytoplasm. Clonal patterns appear to be exaggerations of features noted for the 6B Armenian hamster parent cell (Fig. 2). Loss of cytoplasm is peculiar only to type-A cells.

Plating efficiencies of 10XC, A10XC, and B10XC cells are 23.0, 9.0, and 60.0 per cent, respectively. Although differing in chromosome distribution patterns, clonal lines 7XC and 10XC migrate extensively when cloned (Fig. 3).

Clone 24X is an intermediate or type-B hybrid cell which initially forms clones resembling those of type C. After 5 days of rapid proliferation and several changes of medium, centrally located cells lift off and resettle nearby to form secondary colonies. Peripheral cells are also suspected of migrating to form new colonies (Fig. 5). Secondary proliferations were omitted in determining a plating efficiency of 55.0 per cent. Some intermediate types tend to be more Armenian hamster-

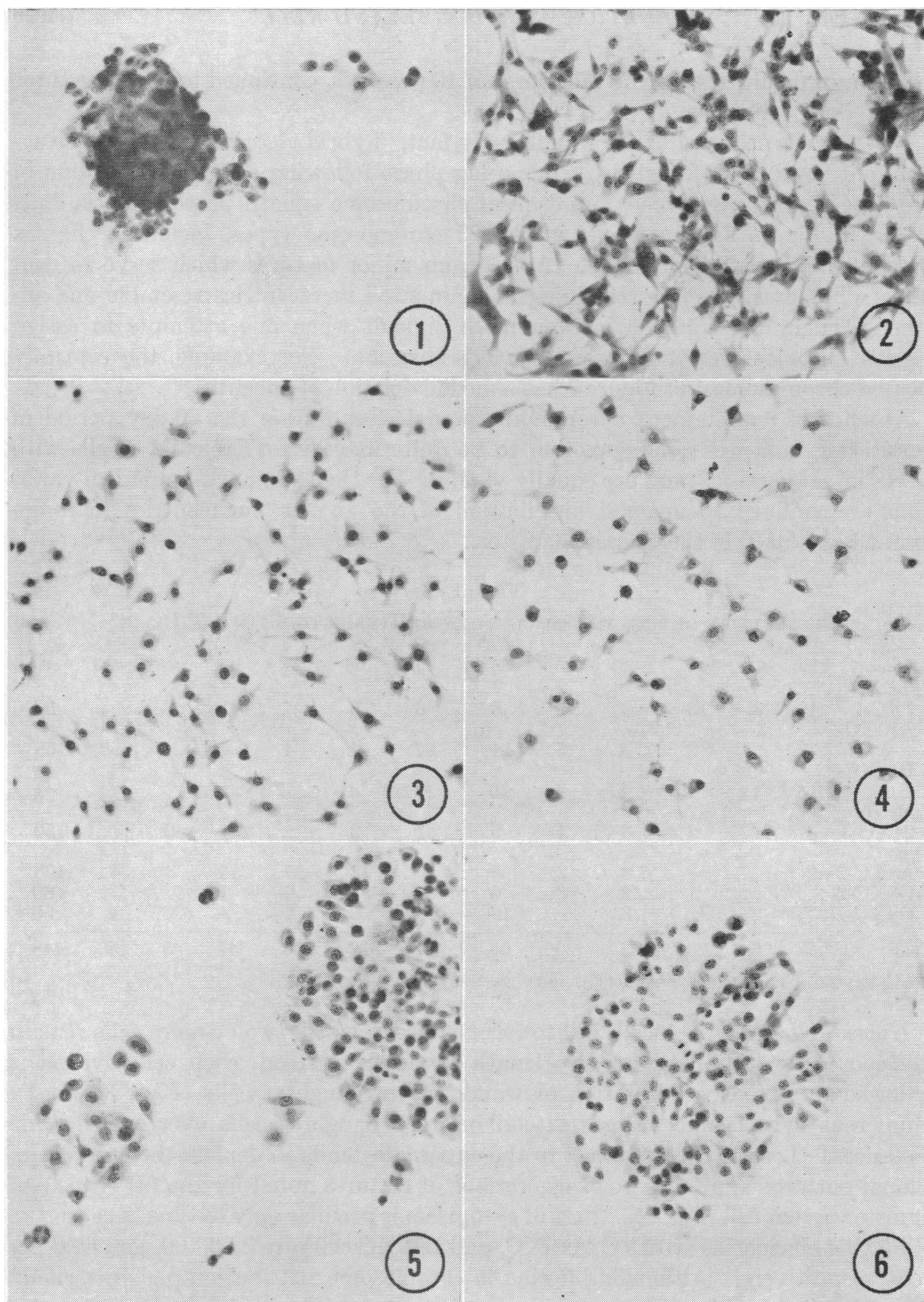

FIG. 1.—Five-day clone of 12E Chinese hamster parent cell. Note extensive secondary layering and possible abortive clone to the right.

FIG. 2.—Central portion of a 7-day clone of 6B Armenian hamster parent cell.

FIG. 3.—Central portion of 7-day clone of type-A hybrid derivative, A10XC, illustrating extensive migration and similarity to Armenian hamster parent cell type.

FIG. 4.—Central portion of 7-day clone of another type-A hybrid derivative, 27XC, having more cytoplasmic adherence than A10XC cells.

FIG. 5.—Five-day clone of an intermediate or type-B hybrid derivative 24XC. Note the formation of secondary colonies from cells migrating from main clone (*upper right*).

FIG. 6.—Five-day clone of type-C hybrid derivative 08XC.

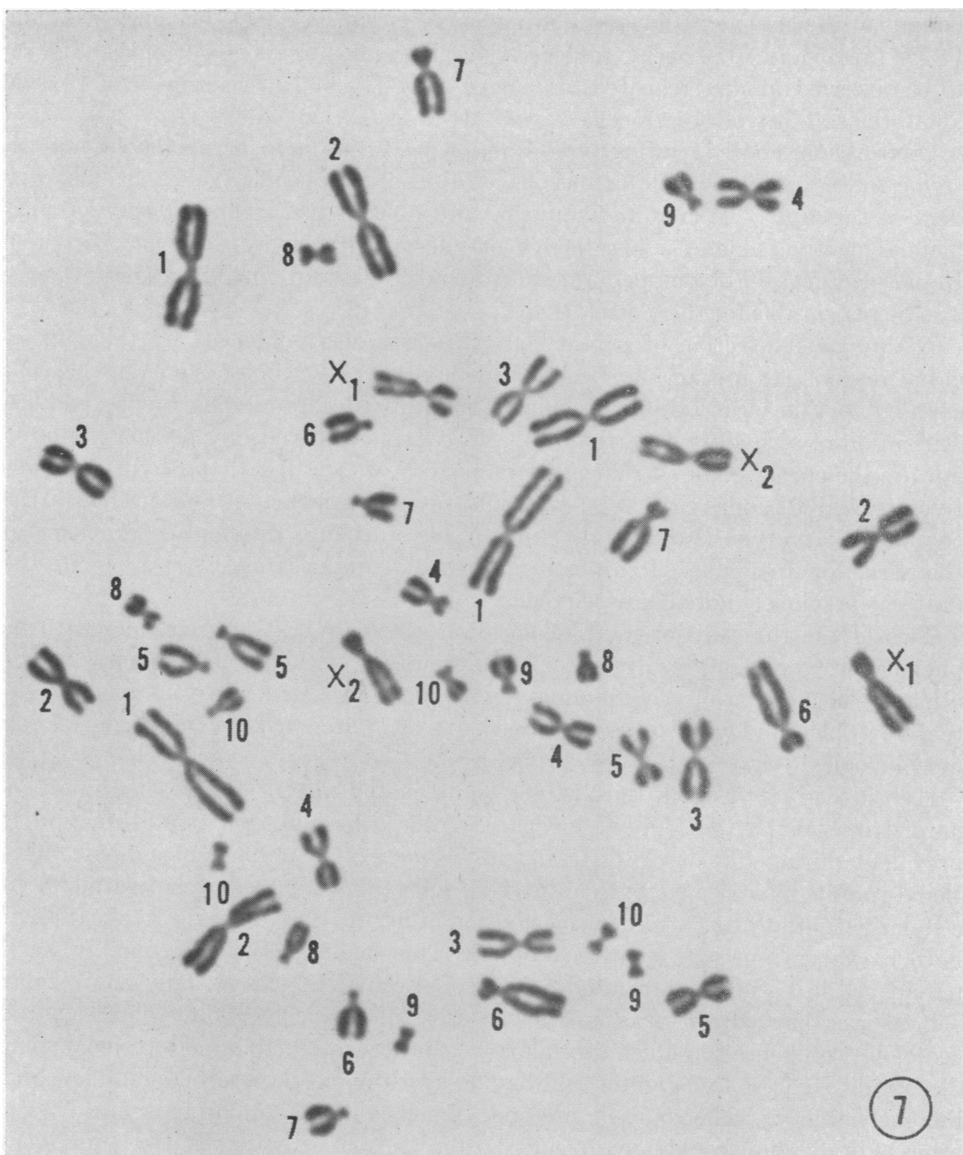

FIG. 7.—Squash preparation of hybrid metaphase with 44 chromosomes. Chromosomes with numbers placed at ends designate Chinese hamster origin, while Armenian hamster chromosomes have numbers placed alongside or near centromeres. Centrally located chromosome *4* is modified.

like, or type A, in that they form clones composed of wider and more elongated cells with the long axis tapering to fine cytoplasmic extensions. Slight variations in chromosome number fail to alter intermediate clonal patterns toward the extremes in migration and cytoplasmic loss noted for type-A cells.

Clones derived from single type-C hybrid cells have bimodal chromosome distributions (Table 1), proliferate rapidly, and are composed of denser elements with little or no cytoplasmic extensions (Fig. 6). The more centrally located cells remain stationary, with a tendency to multilayer, while peripheral cells extend the

bounds of the compact colony. Plating efficiencies of typical subclones 08XC and P8XC are 55 and 70 per cent, respectively. Dispersed or type-A colonies have yet to be observed among repeated clonings of type-C cells. The same can be said about the stability of type-A cells.

On occasion, poorly growing type-A and type-B cells have large vacuoles and extreme forms of cytoplasmic extensions. Minute cytoplasmic bodies are also evident at this time. It is quite likely that the stock virus suspension was not completely inactivated and a low degree of infection or carrier-state has persisted. Rapid acidification of the medium and prolonged exposure of cells at pH 6.4 may also be responsible for these alterations.

Discussion.—Selection of parent cell types was also influenced by their differential response to mutagenic effects of adenovirus 12. Chinese hamster 12E cells exposed to this virus exhibit specificity or localized chromosome breaks and exaggeration of secondary constriction.[11] In contrast, 6B cells are neither inhibited nor are the chromosomes significantly damaged. It was felt that a hybrid derivative exposed to adenovirus type 12 would provide a means of determining if the combined karyotype is totally resistant or susceptible to the mutagenic effects of this virus, or if each set of chromosomes responds independently to duplicate the patterns previously noted in parent cells.

Two of the three major morphological classes of hybrids are considered to be phenotypic expressions resulting from the dominance of one parent genome over the other. Clarification of this unique sequence must await the outcome of more extensive trials employing other precloned euploid parent cells having contrasting morphologies.

Hybrid derivatives lack the distinct and elevated modal chromosome numbers or distribution patterns noted for parent cells. In addition, nondisjunction is prevalent during the course of bipolar divisions. The presence of a single functional spindle may also serve as an over-all reflection of immediate adjustments in cellular activities prior to the initial division of hybrid derivatives. Replication of both karyotypes occurred within days after fusion of nuclei. Presumably, each parent cell in G_1 also contributed a functional centriole and yet only one bipolar spindle was normally formed in the majority of viable hybrids. This suggests centriolar compensation must also take place, otherwise; double and tetrapolar spindles would give rise to daughter cells having near-diploid chromosome numbers and limited viability. The sporadic presence of cells with 23–26 chromosomes 7 days after hybridization could have resulted from the participation of two functional centrioles. The noticeable absence of tetraploids and endoreduplicants during this period lends some support for considering early aneuploids to have resulted from spindle anomalies. Since fusion of centrioles is structurally limited and elimination of one centriole equally difficult to demonstrate for the moment, present considerations have led us to hypothesize that both centrioles may function by undergoing a temporary cessation of replication during their orientation to share in the formation of a common bipolar spindle. Synchronization of events leading to replication, spiralization, and movement of chromosomes must be accompanied by a series of immediate compensations in centriolar activity.

Recent trials, using UV-inactivated parainfluenza type-3 virus, led to the induction of both intra- and interspecific hybrids,[12] in contrast to the interspecific types

described herein for Sendai virus. It is still too early to consider the possibility that the direction of hybridization may be preferentially influenced by the use of different strains of myxoviruses and cell types.

Repeated attempts to obtain viable species hybrids have, thus far, failed. Natural matings have been observed sporadically between opposite sexes reared together with no apparent signs of pregnancy, and artificial inseminations terminate in the formation of defective 2-32-cell embryos.[13] Since both species are members of the same genus, one may refer to their somatic cell hybrids as *intrageneric* forms. This leaves the use of *interspecific* to denote other somatic hybrids having wider phylogenetic origins, such as man and mouse,[3] and rat and mouse.[14] Also, their close taxonomic relationship may have been conducive to the early formation of vigorous and fully synchronized hybrids, aside from the use of near-diploid parent cells.

Summary.—Intrageneric hybridizations of near-diploid cultured cells of dwarf species of hamsters are readily induced by UV-inactivated Sendai virus. Hybrid clonal isolates may have stable morphological features of either parent cell, or of one that is intermediate in form. Seventeen of the 22 parent chromosome types are readily identified in rapidly proliferating hybrid derivatives.

This paper is dedicated to Prof. Sajiro Makino, Zoological Institute, Hokkaido University, Sapporo, Japan, in honor of his sixtieth birthday, June 21, 1966.

We wish to express our sincere appreciation to Professor Boris Ephrussi for his critical review of the manuscript. We wish to acknowledge the excellent assistance of Mr. M. B. Furlong.

* Supported by grants from the National Science Foundation (GB-4558), Damon Runyon Memorial Fund (293), National Institutes of Health, USPHS (CA-08378 and C-6516).

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