

## Replication of Murine Coronaviruses in Somatic Cell Hybrids between Murine Fibroblasts and Rat Schwannoma Cells<sup>1</sup>

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The replication of the murine coronaviruses MHV<sub>3</sub> and JHM has been studied in somatic cell hybrids formed between murine fibroblast L2 cells which support lytic infections with both these agents, and rat RN2 Schwannoma cells which support the replication of JHM in a temperature-sensitive, persistent manner but are restrictive to the replication of MHV<sub>3</sub>. The results described in this report indicate that the totally permissive state is dominant over the persistent or restricted state since the hybrid cells permit the replication of both these viral agents in a lytic manner.

### INTRODUCTION

Murine hepatitis viruses are members of the group of RNA-containing enveloped viruses termed Coronaviruses (Tyrrell *et al.*, 1978). The agents cause a wide variety of diseases in rodents including hepatitis, enteritis, and encephalomyelitis, the disease type varying with the strain of virus, and age and genetic background of the host (Wege *et al.*, 1982). In some virus-host combinations, persistent infections occur resulting in chronic hepatitis or chronic demyelination of the central nervous systems (Wege *et al.*, 1982). This later observation, centered mainly on studies with the JHM strain of the virus (Cheever *et al.*, 1949; Weiner, 1973; Sorensen *et al.*, 1980) or a temperature-sensitive mutant of it (Knobler *et al.*, 1982), has received considerable interest as a potential model relevant for the study of slowly degenerative neuropathies (Weiner, 1973; Weiner *et al.*, 1973; Sorensen *et al.*, 1980).

These agents can also readily establish persistent infections *in vitro* in both neural and nonneural cell lines (Lucas *et al.*, 1977, 1978; Stohلمان and Weiner, 1978), without requirements of viral modifications or en-

vironmental manipulations such as the presence of viral antibody or interferon. Our previous results have indicated that when the infection was of the persistent type, virus replication was almost invariably thermosensitive and that this was due to unknown factors under host control, since the progeny virions themselves were not temperature sensitive (Lucas *et al.*, 1977, 1978). In addition, one cell line, the RN2 Schwannoma, had the unique ability to discriminate between the MHV<sub>3</sub> strain and the more neurotropic strain JHM (Lucas *et al.*, 1977). JHM was replicated persistently in this line, whereas, MHV<sub>3</sub> replication was aborted. At present, it is unclear as to the mechanism of persistence or restriction in this cell line. These observations coupled with others (Stohلمان and Weiner, 1978; Holmes and Behnke, 1982) strongly imply that the host cell has a profound influence in regulating the replication of these agents. As a further approach to analyzing the host functions involved in viral persistence and restriction, somatic cell hybrids have been formed between mouse L2 cells, a cell line totally permissive for both MHV<sub>3</sub> and JHM infection, and the rat RN2 cells. The results described in this report indicate that the L-cell functions appear to be dominant over

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the RN2 controlled ones since both viral agents replicated lytically in the somatic cell hybrids.

#### MATERIALS AND METHODS

*Cells and virus.* The sources and routine propagation of the L2 and RN2-2 cell lines, and the MHV<sub>3</sub> and JHM strains of mouse hepatitis virus were as previously described (Lucas *et al.*, 1977) except that alpha medium (Stanners *et al.*, 1971) was used in place of Eagle's minimal essential medium.

Virus production was monitored by a plaque assay on L2-cell monolayers as previously described (Lucas *et al.*, 1977). Yields are expressed as PFU/ml (plaque-forming units/milliliter). To determine the fraction of cells able to release virus, an infectious center assay was performed (Lucas *et al.*, 1978).

*Selection of genetically marked L-2 cells.* L2 cells were treated for 3 hr in the presence of 0.2  $\mu\text{g/ml}$  *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine at 34°, washed, and resuspended in fresh medium. Survival was usually about 50%. The cells were allowed to grow 6 days to allow for expression of putative mutations before selections were carried out. The basic procedure for mutant selections is described elsewhere (Flintoff *et al.*, 1976). Cells, at  $5 \times 10^5/100\text{-mm}$  tissue culture dish, were exposed to 0.2  $\mu\text{g/ml}$  6-thioguanine (TG) (Sigma Chemical Co.) for 8 days at 34° with replacement of drug and medium every 2 days. Colonies surviving at a frequency of  $4 \times 10^{-6}$  were picked, cloned by limit dilution, and tested for resistance. One clone, L2 TG<sup>R7</sup>, was resistant to at least 50  $\mu\text{g/ml}$  TG, a concentration  $10^3$  higher than was cytotoxic for the wild-type cells, and contained <0.1% of the wild-type hypoxanthine phosphoribosyl transferase activity as determined by the assay described by Chasin and Ur-laub (1976).

The L2 TG<sup>R7</sup> cells were exposed to 3 mM ouabain (Oua) to select for Oua-resistant cells (Baker *et al.*, 1974). Colonies surviving at a frequency of  $10^{-5}$  were isolated, cloned, and shown to be resistant to at least 3 mM Oua. Wild-type cells were unable to grow

at concentrations above 0.5 mM. One doubly marked clone, L2 TG<sup>R</sup>Oua<sup>R7</sup>-1, was used in the hybridization experiments.

Neither resistance to TG, nor Oua, nor the presence of both of these markers affected the ability of the cells to support the replication of either the MHV<sub>3</sub> or JHM strains of mouse hepatitis virus.

*Cell-cell hybridization.* Somatic cell hybrids were formed between the L2 TG<sup>R</sup>Oua<sup>R7</sup>-1 and RN2-2 cells by exposure to polyethylene glycol (PEG) 6000 (British Drug House) for 1 min using the procedure described by Pontecorvo (1975). Cells were plated in complete medium for 1 day to allow recovery from the fusion process prior to the addition of HAT + Oua selection medium ( $7 \times 10^{-5}$  M hypoxanthine,  $2 \times 10^{-8}$  M methotrexate,  $4 \times 10^{-5}$  M thymidine,  $2 \times 10^{-3}$  M Oua). Using these selective conditions, neither parental cell line survives. The mouse L2 cells are unable to survive in the HAT medium and the rat RN2-2 are killed by the Oua. Table 1 summarizes the hybridization frequencies obtained in two separate experiments. After 8 to 10 days incubation at 34° surviving colonies were picked, expanded, maintained in normal medium, and used for study. Subclones of some of these isolates were obtained by limit dilution. There were no differences in the responses of these subclones and the original isolates to virus infection.

*Karyotypic analyses.* Exponentially growing cells were incubated with 0.25  $\mu\text{g/ml}$  colcemid (Grand Island Biological Co.) for 1.5 hr at 34°. The cells were washed with hypotonic and fixing solutions and chromosome spreads prepared (Worton and Duff, 1979). Preparations were either treated with 0.05% trypsin for varying periods of time and stained with Giemsa or the centromeres were stained using the procedure described by Marshall (1975). For this latter procedure, slides were baked for 48 hr at 55°, treated for 1 min with 0.05% trypsin in 0.15 M NaCl, treated with formamide-SCC (95 ml neutralized formamide and 5 ml 20× SSC (1× SSC is 0.15 M NaCl, 1.5 mM Na citrate) at 65° for 20 min, rinsed with distilled H<sub>2</sub>O, stained with 10% Giemsa in Gurr's buffer, pH 6.8, and

TABLE 1  
HYBRIDIZATION FREQUENCIES<sup>a</sup>

Cross	Hybridization frequencies		
	PEG	Experiment	
		1	2
L2 TG <sup>R</sup> Oua <sup>R</sup> 7-1 × L2 TG <sup>R</sup> Oua <sup>R</sup> 7-1	+	<6 × 10 <sup>-7</sup>	<10 <sup>-6</sup>
RN2-2 × RN2-2	+	<1 × 10 <sup>-7</sup>	<10 <sup>-6</sup>
RN2-2 × L2 TG <sup>R</sup> Oua <sup>R</sup> 7-1	-	6 × 10 <sup>-6</sup>	1 × 10 <sup>-4</sup>
RN2-2 × L2 TG <sup>R</sup> Oua <sup>R</sup> 7-1	+	4 × 10 <sup>-5</sup>	6 × 10 <sup>-4</sup>

<sup>a</sup> Hybrids were formed and selected by growth in HAT medium containing 2 mM Oua as described under Materials and Methods.

visualized. From 5 to 10 chromosome spreads were examined for each cell line.

**Lactate dehydrogenase assay.** The preparation of cell extracts and the assay for lactate dehydrogenase were essentially as described by Weiss and Ephrussi (1966). Briefly, exponentially growing cells were harvested, washed two times in phosphate-buffered saline, resuspended in 10 mM Tris-Cl, pH 7.0, quick frozen and thawed two times, and homogenized. The homogenates were centrifuged for 1 hr at 30,000 *g* in a Sorval SS34 rotor at 4° and the supernatants used as the enzyme source.

*In vitro* hybridizations were carried out as described by Markert (1963) in the presence of 1 M NaCl and 70 mM Na phosphate, pH 8.0. The preparations were stored at -20° overnight.

For electrophoresis, 3- $\mu$ l samples were applied with a Gelman stainless-steel wire applicator to Sephaphore III cellulose acetate strips (Gelman Instrument Co.) that had been soaked for 30 min in electrophoresis buffer (0.3 M sodium borate, pH 8.6) and blotted dry. Electrophoresis was for 100 min at 200 V.

To detect lactate dehydrogenase, the strips were stained for 30 min at 37° in the dark with 0.025 M Tris-Cl, pH 7.4, 0.1 M *d* + *l* lactate, 0.005 KCN, 0.001 M NAD, 50  $\mu$ g/ml nitroblue tetrazolium, and 20  $\mu$ g/ml phenazine methosulfate. The strips were fixed for 10 min in 50% methanol, 10% acetic acid, 40% H<sub>2</sub>O, dried at room temperature, and cleared in glycerol at 70°.

**$\beta$ -Glucuronidase assay.** The preparation of cell extracts and assay for  $\beta$ -glucuronidase was essentially as described by Weiss and Ephrussi (1966) and by Herrup and Mullen (1977). Briefly, cells were harvested, washed two times in phosphate-buffered saline, resuspended in H<sub>2</sub>O, and homogenized in 0.2% Triton X-100. The homogenates were centrifuged for 10 min at 1470 *g* in a Sorval SS34 rotor at 4°. The supernatants were used as the enzyme sources.

$\beta$ -Glucuronidase assays were carried out in 2 ml reaction volumes consisting of 0.1 M Na acetate, pH 4.6, 0.2 mM *p*-nitrophenol- $\beta$ -D glucuronic acid, 0.2% Triton X-100, and various amounts of enzyme extract equivalent to 2 × 10<sup>5</sup> to 10<sup>6</sup> cells. Incubations were at 37° for 2 hr. Under such conditions, enzyme activity was linear with respect to both extract level and time. Reactions were stopped by the addition of 0.8 ml of 17.5% trichloroacetic acid, and centrifuged at 600 *g* for 10 min to remove the protein precipitate. The clear supernatants were added to tubes containing 1.2 ml of 4 N NaOH to develop the color, and the optical density read at 415 nM. All assays were done in duplicate and reagent blanks containing no enzyme were subtracted from the readings.

For heat inactivation, samples were incubated in glass tubes at 62° in 0.1 M Na acetate, pH 4.6. At various intervals, duplicate samples were removed, and chilled on ice. Substrate solution was then added to the tubes and the standard assay per-

formed. Results are expressed as the percentage of the unheated controls.

## RESULTS

*Properties of hybrid cells.* Somatic cell hybrids were formed between the genetically marked mouse L2 cells and the rat RN2 cells by fusing with polyethylene glycol and selection in HAT + Oua medium. Although the frequency of hybrid formation was greater than the survival of either the mouse or rat parental cell lines under these selective conditions (Table 1), it was conceivable that parental cells might have survived the selection scheme. Thus, it was important to distinguish between authentic cell hybrids and parental survivors. Hybrid cells formed between these mouse and rat cells could be readily distinguished from the parental cells on the basis of their chromosome content and on the production of species-specific gene products.

As shown in Table 2, the mouse-rat hybrid cells had average chromosome numbers that were much greater than those of either the mouse or rat parental cells used to form them. A majority of the hybrid cells contained almost the entire complement of both mouse and rat chromosomes

(Table 2), as determined by their characteristic trypsin Giemsa or centromeric banding patterns determined as described under Materials and Methods. Although there was variation in the chromosome composition among the hybrids, there was no apparent consistent pattern in the nature and type of chromosomes that were missing. Figure 1 illustrates the chromosomes present in the parental and two hybrid cell lines.

Since rat and mouse cells produce different isozymes of lactate dehydrogenase (Weiss and Ephrussi, 1966), it was of interest to determine whether these mouse-rat cell hybrids produced both isozymes. As shown in Fig. 2, the rat RN2 cells produced a lactate dehydrogenase enzyme that migrated more cathodally than similar enzyme from mouse L2 cells. When a mixture of rat and mouse lactate dehydrogenases was dissociated and reassembled *in vitro*, four major bands and a faint fifth band of enzymatic activity were obtained (Fig. 2, lane D). Such a pattern was absent in a mixture of parental extracts (Fig. 2, lane C). When extracts of hybrid cells were assayed for lactate dehydrogenase activity (Fig. 2, lanes E, F) an isozyme pattern similar to that of the *in vitro* assembled iso-

TABLE 2  
CHROMOSOME CONTENT OF HYBRIDS

Cell line	Chromosome number <sup>a</sup>	Mouse chromosomes <sup>b</sup>	Rat chromosomes <sup>b</sup>
Parental			
L2 TG <sup>R</sup> Oua <sup>R7-1</sup>	43 ± 3	43 ± 3	—
RN2-2	41 ± 2	—	41 ± 2
Hybrids			
1	83 ± 5	42 ± 3	40 ± 3
2	85 ± 6	45 ± 7	40 ± 4
3	83 ± 10	48 ± 9	37 ± 4
4	70 ± 6	34 ± 4	37 ± 5
5	75 ± 3	43 ± 2	38 ± 5
6	60 ± 6	27 ± 5	35 ± 4
7	73 ± 7	38 ± 8	36 ± 7
8	76 ± 4	36 ± 5	38 ± 5

<sup>a</sup> Average chromosome number based on 5 to 10 spreads with the indicated standard deviation.

<sup>b</sup> Determined by standard trypsin-Giemsa banding and the centromere staining procedure of Marshall (1975).

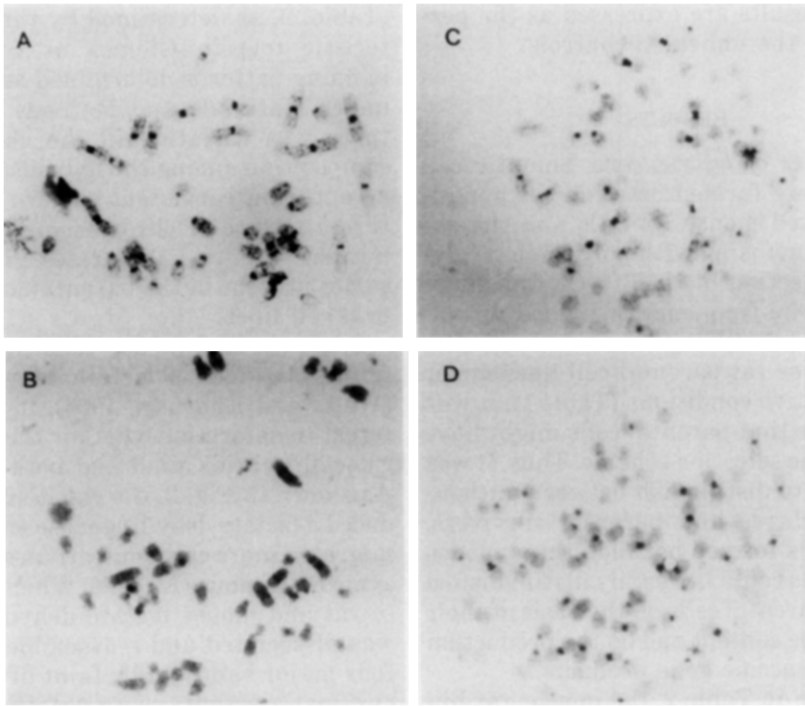


FIG. 1. Chromosomes of selected cell lines. Chromosomes were prepared and treated with trypsin and formamide and stained with Giemsa as described under Materials and Methods. Chromosomes from (A) L2 TG<sup>R</sup>Oua<sup>R7-1</sup>, (B) RN2-2, (C) Hybrid 6, and (D) Hybrid 2 cells.

zymes was obtained. In some cases the more cathodally migrating fifth band corresponding to the rat parental isozyme was very pale and only visible when the strips were overstained. This appears to be a

common property for these isozymes in mouse-rat hybrids (Weiss and Ephrussi, 1966). These results are consistent with the presence and association of both mouse and rat forms of lactate dehydrogenase in the hybrid cells.

Another function that is readily distinguished between rat and mouse cells is  $\beta$ -glucuronidase (Weiss and Ephrussi, 1966). Rat  $\beta$ -glucuronidase is more sensitive to heat inactivation than the mouse enzyme. To examine whether both mouse and rat forms of the enzyme were being produced in the hybrid cells, cell extracts were prepared from the cell lines, heated at 62° for various periods of time, and assayed for  $\beta$ -glucuronidase activity. As shown in Fig. 3, the enzymatic activity produced in the hybrid cells was inactivated by heat at a rate intermediate between the mouse and rat enzymes. This is consistent with the presence in the hybrids of both mouse and rat  $\beta$ -glucuronidases since the heat inactivation was similar to

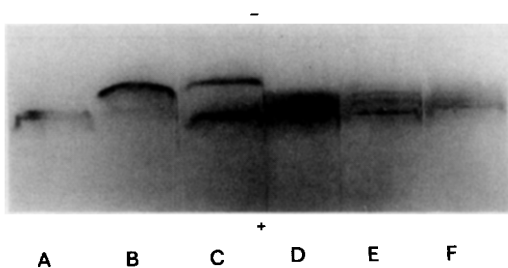


FIG. 2. Lactate dehydrogenase isozymes in hybrid cells. Lactate dehydrogenase was assayed as described under Materials and Methods. Lactate dehydrogenase from (A) L2 TG<sup>R</sup>Oua<sup>R7-1</sup> extract. (B) RN2-2 extract; (C) a 1:1 mixture of L2 TG<sup>R</sup>Oua<sup>R7-1</sup> and RN2-2 extracts; (D) a 1:1 mixture of L2 TG<sup>R</sup>Oua<sup>R7-1</sup> and RN2-2 extracts assembled *in vitro*; (E) Hybrid 6 extract; and (F) Hybrid 2 extract.

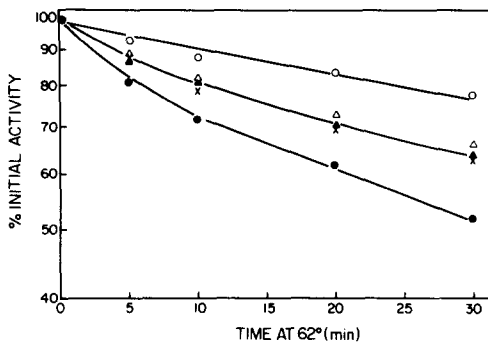


FIG. 3.  $\beta$ -Glucuronidase in hybrid cells. Cell extracts were prepared, heated to 62° for various periods of time, and assayed for  $\beta$ -glucuronidase activity as described under Materials and Methods.  $\beta$ -glucuronidase in L2 TG<sup>R</sup>Oua<sup>R</sup>7-1, O; RN2-2, ●; Hybrid 2,  $\Delta$ ; Hybrid 6,  $\times$ , and 1:1 mixture of L2 TG<sup>R</sup>Oua<sup>R</sup>7-1 and RN2-2 extracts,  $\blacktriangle$ .

that of a 1-to-1 mixture of cell extracts from the two parental lines.

Taken together the above results indicate that these cells are authentic mouse-rat hybrids. Morphologically, these hybrid cells did not differ dramatically from the two parental lines. Some hybrids contained cells displaying the flattened irregularly shaped morphology of the RN2-2 cell line, whereas, others contained cells roughly triangular in shape with well-defined margins characteristic of the L2 cell line. Still others contained cells with more variable morphologies, some of which had well-defined but ragged edges.

*Replication of JHM and MHV<sub>3</sub> in mouse-rat cell hybrids.* Previous results indicated that mouse L2 cells supported the replication of both the JHM and MHV<sub>3</sub> virus strains in a lytic fashion involving extensive cell destruction through syncytial formation. When RN2 cells were used as host, JHM replicated persistently with restricted cytopathology in a temperature-sensitive manner. MHV<sub>3</sub> replication was totally restricted in the RN2 cells (Lucas *et al.*, 1977, 1978). The availability of somatic cell hybrids between these two cell lines permitted an examination of which host cell type dominantly affected the virus replication process.

Confluent monolayer cultures of several independently selected hybrid cells were

infected at a multiplicity of infection (m.o.i.) of 0.05 with either JHM or MHV<sub>3</sub>, maintained at either 32 or 39°, and virus production determined after 24 hr. As shown in Table 3, the hybrid cells could replicate both JHM and MHV<sub>3</sub> at both temperatures. The virus yields for the most part were similar to those obtained with the L2 TG<sup>R</sup>Oua<sup>R</sup>7-1 cell line as host and considerably higher than those obtained with the RN2 cell. Accompanying these high levels of virus production was an extensive cytopathic effect (cpe) resulting from syncytial formation. By 24 hr at 39°, essentially all the cells in the monolayer were involved and total destruction and cell lifting had occurred. A similar cpe was observed at 32°, however, total destruction was delayed until 30 to 36 hr postinfection. These effects were apparent with the L2 TG<sup>R</sup>Oua<sup>R</sup>7-1, and hybrid lines 1, 2, 3, 4, 5, 7, and 8. Hybrid 6 showed some differences. This hybrid, which produced lower yields of virus than the other hybrid lines at 24 hr (Table 3), showed very little, if any, cpe, at both temperatures with JHM virus. If a cpe was present it was restricted to less than 10% of the cells in the population. Similar results were also obtained with MHV<sub>3</sub> infections, although the cpe was somewhat more extensive, perhaps involving 20 to 30% of the cells in the population. The cpe observed with either virus in this line appeared to be restricted since longer incubation periods up to 7 days did not result in a more extensive cpe even though 30–80% of the cells scored as infectious centers. The extent of the cpe was also not affected by increasing the m.o.i. to 5 even though the number of cells scoring as infectious centers was 80%. Initially hybrid 6 cells could produce either JHM or MHV<sub>3</sub> virus at 39°. However, after about 1 week in culture these infected cells lost the ability to shed virus at 39° even though they continued to produce virus in a cyclical manner at 32°. At this time, if cells shedding virus at 32° were shifted to 39° there was a cessation of virus production. The properties of cyclical release of virus and restricted virus replication at the elevated temperature is reminiscent of the JHM infection of RN2 cells in which from 0.1 to

TABLE 3  
 REPLICATION OF JHM AND MHV<sub>3</sub> IN PARENTAL AND HYBRID CELL LINES<sup>a</sup>

Cell Line	Virus yield (PFU/ml)			
	JHM		MHV <sub>3</sub>	
	32°	39.5°	32°	39.5°
L2 TG <sup>R</sup> Oua <sup>R</sup> 7-1	4 × 10 <sup>5</sup>	2 × 10 <sup>5</sup>	5 × 10 <sup>5</sup>	4 × 10 <sup>5</sup>
RN2-2	4.5 × 10 <sup>2</sup>	5	50	5
Hybrid				
1	5 × 10 <sup>5</sup>	4 × 10 <sup>5</sup>	1 × 10 <sup>5</sup>	1.5 × 10 <sup>5</sup>
2	3 × 10 <sup>5</sup>	2 × 10 <sup>5</sup>	5 × 10 <sup>5</sup>	3 × 10 <sup>5</sup>
3	2 × 10 <sup>5</sup>	1.5 × 10 <sup>5</sup>	1.8 × 10 <sup>5</sup>	1.1 × 10 <sup>5</sup>
4	5 × 10 <sup>5</sup>	3 × 10 <sup>5</sup>	5 × 10 <sup>5</sup>	3 × 10 <sup>5</sup>
5	2 × 10 <sup>5</sup>	3 × 10 <sup>5</sup>	5 × 10 <sup>5</sup>	7 × 10 <sup>4</sup>
6	6.5 × 10 <sup>3</sup>	2.9 × 10 <sup>3</sup>	7.5 × 10 <sup>4</sup>	3.3 × 10 <sup>4</sup>
7	2 × 10 <sup>5</sup>	2 × 10 <sup>5</sup>	5 × 10 <sup>5</sup>	3.6 × 10 <sup>5</sup>
8	4 × 10 <sup>5</sup>	3 × 10 <sup>5</sup>	3 × 10 <sup>5</sup>	1 × 10 <sup>5</sup>

<sup>a</sup> Confluent monolayers of the various cell lines were infected at a m.o.i. of 0.05 with either JHM or MHV<sub>3</sub> at either 32 or 39°. After 1 hr to allow for virus adsorption, the infected cells were washed two times with phosphate-buffered saline, fed fresh medium, and incubated at the appropriate temperature. Virus released into the medium was assayed at 24 hr postinfection by the plaque assay at 32° on L2 monolayers as described under Materials and Methods.

10% of the cells are infected (Lucas *et al.*, 1977, 1978). In the case with hybrid 6 cells, however, both JHM and MHV<sub>3</sub> were replicated and the number of infected cells was from 50 to 80%. Ten subclones of the hybrid 6 cells behaved similarly when challenged with virus.

To examine more closely the early phases of virus production, monolayers of either L2 TG<sup>R</sup>Oua<sup>R</sup>7-1, RN2-2, hybrid 2, or hybrid 6 cells were infected with JHM virus at a m.o.i. of 0.05 at 32 and 39°, and virus released into the medium determined at various times.

Virus production was similar for JHM infections of L2 TG<sup>R</sup>Oua<sup>R</sup>7-1 and hybrid 2 cells (Fig. 4). The cpe was evident at 6-8 and 3-6 hr postinfection for infections initiated at 32 and 39°, respectively. Virus production from hybrid 6 cells showed slightly different kinetics in that virus replication appeared to lag behind that of both the L2 TG<sup>R</sup>Oua<sup>R</sup>7-1 and hybrid 2 cells. This was clearly evident for the infection at 32° and less so for that at 39°. Although virus yields reached levels comparable to

those from the L2 TG<sup>R</sup>Oua<sup>R</sup>7-1 and hybrid 2 infections, the cpe was never very extensive involving at the most approximately 10-20% of the monolayer. Virus replication in the RN2-2 cells progressed at a much slower rate than in the other cell lines and a few small syncytia were apparent at 70 hr postinfection. Similar results were obtained for infections with MHV<sub>3</sub> at the same m.o.i. (data not shown), except that the cpe was somewhat more extensive in the hybrid 6 line involving 50-80% of the cells, and that MHV<sub>3</sub> showed no evidence for replication in the RN2 cells.

#### DISCUSSION

The availability of cell lines which respond differently when challenged with the coronaviruses JHM and MHV<sub>3</sub> has provided the opportunity to examine which host cell functions dominate in the determination of the outcome of the virus infection. This was accomplished by the formation of somatic cell hybrids between the mouse L2 and rat RN2 cells. Such fused

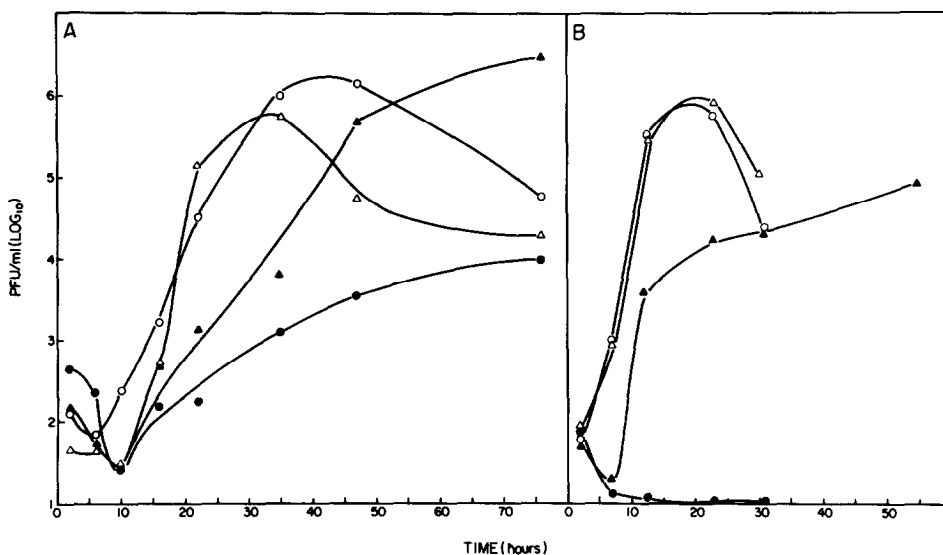


FIG. 4. Replication of JHM in selected cells lines. Confluent monolayers of the various cell lines were infected with JHM at a m.o.i. of 0.05 at either 32° (A) or 39° (B). After 1 hr to allow for adsorption, unadsorbed virus was removed and the monolayers were washed two times with phosphate-buffered saline. Fresh medium was added and the virus present determined by the plaque assay on L-2 cell monolayers at 32° as described under Materials and Methods. Virus production from L2 TG<sup>R</sup>Oua<sup>R</sup>7-1, ○; RN2-2, ●; Hybrid 2, △; and Hybrid 6, ▲ cells.

cells are authentic cell-cell hybrids since they contained both mouse and rat chromosomes and expressed both mouse and rat forms of lactate dehydrogenase and  $\beta$ -glucuronidase. The results indicate that the permissive or lytic state of coronavirus infection characteristic of the infected mouse L2 cells was dominant over the persistent or restrictive host state of the infected rat RN2 cells. This conclusion is based on the demonstration that the hybrids support the replication in a lytic manner of both JHM and MHV<sub>3</sub> at 32 and 39°. The features of these infections are similar to those of L2 cells with a comparable time course, virus yields, and cytopathic effects. Such features are not characteristic of the infections of RN2-2 cells (Lucas *et al.*, 1977, 1978). It is conceivable, however, that one of the rat chromosomes codes for a dominantly acting factor which is responsible for virus resistance or persistence. Since chromosome loss does occur in cell-cell hybrids (Francke and Francke, 1981), perhaps this chromosome is frequently lost which would

then lead to virus susceptibility in the hybrids. This seems unlikely, however, since although there was variability among the rat chromosomes present in the hybrid cells, there was no apparent preference for the loss of specific rat chromosomes.

One hybrid, hybrid 6, and its subclones differed in the response to virus infection. These cells could initially replicate both virus strains at 32 and 39° without extensive syncytial formation. After about 1 week in culture such cells lost the ability to produce virus at 39° but continued to produce virus at 32°. The reasons for the difference between this hybrid and the others is at present unclear. It may be related in some way to the chromosome content of this hybrid cell since its chromosome number is lower than that of the others (Table 2).

Once established these hybrids appear to be phenotypically stable. Several of the lines have been kept in continuous culture for up to 8 months and periodically screened for susceptibility to lytic virus infection. To date, all lines after prolonged



culture behaved similarly in their responses to infection as they did shortly after isolation.

To my knowledge, this is the first study with cell hybrids formed between a cell line that is totally permissive for virus production and one that supports either a persistent infection or is restrictive to infection. The result that the totally permissive state (i.e., the L cell) is dominant over the restrictive or persistent state (i.e., the RN2 cell) is not unlike other host-virus systems where it has been demonstrated that permissive host functions are dominant over nonpermissive ones (Miller *et al.*, 1974; Garver, *et al.*, 1980; Lemons *et al.*, 1980).

At present it is unclear as to the nature of the host cell functions that are involved in coronavirus persistence. Recent evidence suggests that in one system the ability of the host cell to resist infection and withstand cytopathic effects may be important parameters (Mizzen *et al.*, 1983). It is thus unclear as to the nature and role that the L2 functions play in overcoming the RN2-2 controlled persistent, and thermosensitive replication of JHM, and the restriction of MHV<sub>3</sub>. Knowledge of these L-cell functions might prove useful in an understanding of both coronavirus persistence and restriction. It is of interest to note that although MHV<sub>3</sub> replication is restricted in RN2 cells, if it is permitted to initiate replication as it does in hybrid 6 then after about 1 week in culture a persistent infection not unlike that of the JHM infection of RN2-2 cells can be obtained. Since there are available several different cell lines that can become persistently infected with the JHM and MHV<sub>3</sub> virus strains (Lucas *et al.*, 1978), it will be of interest to determine whether these cell lines behave in a manner similar to the RN2 cells when somatic cell hybrids are formed with the mouse L2 cells. Such studies are currently in progress.

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