Rabbit Syncytium Virus Is a Kemerovo Serogroup Orbivirus†
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Polyacrylamide gel genome electropherotyping and negative-stain electron microscopic studies, along with
immunofluorescent staining and immune electron microscopy reactions, indicate that rabbit syncytium virus
has the morphologic, genomic, and antigenic attributes of a Kemerovo serogroup orbivirus.

Rotaviruses cause diarrhea in humans and other animals
(7), and at least five distinct rotavirus serogroups, designated
A through E, exist (18, 19). The discovery that trypsin permits group A rotavirus cell culture propagation (26)
has allowed extensive characterization of isolates belonging to
this serogroup. However, techniques for routine serial prop-
agation of non-group A rotaviruses in cell culture are lack-
ing, and investigations on these viruses are seriously re-
stricted. Consequently, cultivatable non-group A rotaviruses
are of considerable medical and veterinary importance.

Rabbit syncytium virus, isolated from a healthy cottontail
rabbit (Sylvilagus floridanus) trapped in Quinby, Va., in
1962, induces syncyta in some cell cultures (16). This virus
most closely resembled the EDIM agent (a group A rotavi-
rus) in morphology and morphogenesis, but was antigeni-
cally unrelated to it (4, 16). Syncytial cell formation, how-
ever, often occurs during group B rotaviral infections in vivo
and in vitro (30, 32, 33). Therefore, we investigated the
possibility that rabbit syncytium virus might be a cultiva-
table group B rotavirus.

Amniotic-allantoic fluid from the 12th egg passage of
rabbit syncytium virus (10 January, 1962) recovered from
rabbit 32 and the 5th egg passage of its reisolation from
rabbit 32 tissues (29 August, 1964) was kindly provided by
M. F. Bozeman, Food and Drug Administration, Depart-
ment of Health & Human Services, Bethesda, Md. Roller
tube monolayers of MA104 and BHK-21 cells, prepared as
described previously (28) but without trypsin in the mainte-
nance medium, were inoculated with egg-passageged virus
diluted 10-fold. Rabbit syncytium virus was recovered from
each egg passage and was further passaged in both cells as
described above, although only BHK-21 cells were used for
the later passages. Virus passages were stored at 4 or −20°C
in 50% glycerol.

Rabbit syncytium virus induced a cytopathic effect in both
cells which was characterized by foci of rounded cells
that gradually enlarged to destroy the monolayer. Some
MA104 cell monolayers, prepared on coverslips as described
previously (30), were infected with rabbit syncytium virus
and subsequently stained with Harris hematoxylin and eosin
after fixation in Bouin solution. Stained infected monolayers
contained many cells with eosinophilic and basophilic cyto-
plasmic inclusion bodies, but no syncytia (Fig. 1). Other
infected MA104 cell coverslip monolayers were examined by
immunofluorescence microscopy, using fluorescein-conju-
gated antibodies to bovine group A rotavirus (30) and
porcine group C rotavirus (2), or by indirect immunofluo-
rescence microscopy, using gnotobiotic pig group B rotavirus
(31) and reovirus type 3 (2) antisera. Rabbit syncytium
virus-infected cells did not contain group A rotaviral or
reoviral antigens, confirming previous results (4, 16), or
group B or C rotaviral antigens (Fig. 2).

Negatively stained lysates of rabbit syncytium virus-
infected cells, prepared by the method of Lecatsas and
Gorman (14), were examined by electron microscopy (29)
and found to contain spherical virus particles of about 60 nm
in diameter (Fig. 3). An amorphous outer layer often covered
these virions and obscured their internal structure. In con-
trast, negative staining of rabbit syncytium virus concen-
trated by ultracentrifugation from frozen and thawed in-
fected cells revealed virions lacking an outer layer and with
ringlike structures clearly evident (Fig. 3); such virions were
similar to those described earlier (4) and resembled single-
shelled rotavirus particles. Hyperimmune gnotobiotic pig
antisera to cell culture-passaged rabbit syncytium virus,

FIG. 1. Cytopathic effect of rabbit syncytium virus in MA104
cells. (a) Noninfected control monolayer. (b) Infected monolayer,
48 h postinoculation. Numerous basophilic and eosinophilic cyto-
plasmic inclusion bodies are apparent. Harris hematoxylin and eosin
stain was used. Magnification, ×400.

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prepared as described for group A rotavirus antiserum (28), was used in immune electron microscopy studies. Diluted antiserum was added to suspensions containing rabbit syncytium virus, reovirus (2), group A rotavirus (28), group B rotavirus (31), group C rotavirus (2) or group D rotavirus (20, 29), and the mixtures were examined for clumped virus as described previously (29); only homologous virus was clumped by this antiserum (Fig. 3).

Viral double-stranded RNA was extracted from infected cell lysates by CF11 cellulose chromatography (27), separated in a Laemmli 7.5% polyacrylamide slab gel, and stained with silver as described previously (3). The rabbit syncytium virus genome comprised 10 double-stranded RNA segments that produced an electropherotype distinct from those produced by the reovirus and group A rotavirus genomes (Fig. 4). These segments, with molecular weights ranging from about $2.5 \times 10^6$ to $0.4 \times 10^6$, were distributed in a pattern similar to that of the genome electropherotypes of orbiviruses belonging to the Corriparta and Kemerovo serogroups (9, 10, 17): 2 large, 4 intermediate, 3 medium, and 1 small.

Four orbiviruses, bluetongue virus serotype 8 (Bluetongue serogroup), Irituia virus (Changuinola serogroup), Corriparta virus (Corriparta serogroup), and Tribec virus (Kemerovo serogroup), from the American Type Culture Collection, Rockville, Md., were passaged in BHK-21 cells in roller tubes and then inoculated onto BHK-21 cell coverslip monolayers as described above. At 48 h postinoculation, monolayers were fixed in acetone and examined by indirect immunofluorescence microscopy, using hyperimmune anti-rabbit syncytium virus serum and fluorescein-conjugated anti-porcine immunoglobulin G antibody (ICN Biomedicals Inc., Costa Mesa, Calif.). Immunofluorescence was observed only with cells infected with rabbit syncytium or Tribec (Fig. 2) virus and consisted of small, discrete, brightly fluorescent, intracytoplasmic granules. In addition, cells infected with rabbit syncytium or bluetongue virus were examined by indirect immunofluorescence microscopy, us-
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FIG. 4. Comparison of reovirus type 3, Abney isolate (A), rabbit syncytium virus (B), and equine group A rotavirus, FI-14 isolate (C), genome electropherotypes in the same polyacrylamide gel slab. Migration is from top to bottom. Numbers to the right of each lane designate segment numbers.


