Propagation of Measles Virus in a Strain of Human Epidermoid Cancer Cells (Hep-2).* (22677)

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In 1954 Enders and Peebles(1) reported the isolation of a cytopathogenic agent from patients with measles. Human and monkey kidney cultures were used in their study, but they reported that uninoculated monkey cultures may contain transmissible agents which cause effects at times difficult to distinguish from those of measles virus. The infectivity titer of human kidney cultures, including fluid and disrupted cells, was only $10^{3.5}$ TCD_{50} per ml. The purpose of this note is to report the adaptation of measles virus to a human cancer cell strain, Hep-2(2), and certain characteristics of the virus grown in this cell strain. Hep-2 is easily grown in the absence of human serum, has thus far proved free of latent agents, and can yield titers of measles virus greater than 10^6 TCD_{50} per ml.

Methods. Adaptation to Hep-2 cells. The 23rd human kidney passage of the Edmonston strain of measles virus† was inoculated into cultures of several cell strains, including Hep-2.† All cultures were kept in Eagle basal medium for HeLa cells(3) supplemented with 10% calf serum. The medium was changed every 4-7 days. No cytopathic (CP) changes characteristic of measles (multinucleate giant cells with intranuclear inclusions) were observed, but one HeLa culture degenerated in a nonspecific manner after 38 days incubation. When fluid from this culture was passaged into fresh HeLa and Hep-2 cultures, a non-specific type of degeneration again occurred in the HeLa culture but giant syncytial cells were found in Hep-2. Characteristic CP effects have been noted in later HeLa passages but they have been easier to recognize in Hep-2 cultures where the controls can be maintained in excellent condition for weeks in the medium free of human serum. CP changes have been observed in Hep-2 cultures 2 days after infection when large inocula were used but with small inocula 3-4 weeks might pass before recognizable changes appeared. Another measles virus line has been established in Hep-2 without HeLa passage, but after 2 passages in monkey kidney culture and with similarly prolonged initial incubation periods.

Results. Only 6 passages in Hep-2 cells have been made to date but multiplication has been established by the facts that, including changes, a total dilution of about 10^{46} of our original seed material has been involved and the titer of the last harvest was 100 times greater than that of the initial seed. The identity of the virus has been established by its characteristic CP effect and by a neutralization test. The typical CP effect was best manifest when the cultures were grown in Enders biological medium containing 35% Hanks salt solution, 35% beef amniotic fluid, 25% heated horse or calf serum and 5% beef embryo extract, or in a synthetic medium lacking glutamine(4). The neutralization test (Table I) was carried out by mixing 1/10 acute or convalescent serum from patients with measles, with serial virus dilutions and incubating one hour at room temperature before inoculation of Hep-2 cultures. Dr. John F. Enders had found that these patients had developed a marked rise in measles antibodies and kindly made aliquots of the sera available to us.

Growth of Virus in Hep-2 Cells. Cultures of Hep-2 cells in Eagle medium were inoculated with 10^5 TCD_{50} of adapted measles virus and washed 2 hours later. New virus was first detectable 18 hours after inoculation when the cells were ruptured by freezing and

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TABLE I. Neutralization of Hep-2 Passage Measles by Human Serum.

Serum	Virus titer (neg. log.)	Logs virus neutralized
None	7.0	
Acute	7.2	0
Convalescent	2.5	4.5

thawing. Free virus first appeared in the fluid about 12 hours later (Table II). Peak titers were achieved in 2½ days. The maximum complement-fixation titers have been ⅛. Enders and Peebles(1) reported that their human kidney grown material provided a usable antigen without concentration. It is thus apparent that Hep-2 cultures do not offer, in comparison with human kidney, proportionally as great increases in C-F antigens as of infective virus.

Infection of monkeys. Enders(5) has found the rhesus and cynomolgus macaques may possess naturally acquired antibodies against measles virus. In an attempt to determine how widespread this phenomenon might be, sera from 10 monkeys of various species have been tested. Six of these, including rhesus (*Macaca mulatta*), mangabey (*Cercocebus torquatus lunulatus*), green (*Cercopithecus aethiops sabeus*) and African white (*C. a. tantalus*), possessed virus inhibitors in their serum. The same sera, as well as 4 specimens from the African red grass monkey (*Erythrocebus patas*), gave positive C-F reactions with measles antigen. Eight monkeys, including 4 rhesus without pre-existing neutralizing antibodies, were inoculated intramuscularly or intravenously with 1 ml of Hep-2 passage virus. One of those with preantibodies had an elevated temperature and bouts of shivering for 4 days, beginning 12 days after injection. It is questionable whether this illness was associated with the inoculation. The only sign of illness in the other monkeys was a marginal temperature elevation in one monkey without antibodies 13 days after inoculation. Sera from 4 monkeys with antibodies and 2 without were checked for viremia. Positive results were obtained 7 and 13 days after inoculation from those monkeys without prior antibodies but not from the others. The virus that was isolated from the monkeys' blood did not cause recognizable CP changes in Hep-2 cells until after 30 to 56 days of incubation. It seems possible that the single monkey passage had partially reversed the adaptation of the virus to Hep-2 cultures.

TABLE II. Growth of Measles in Hep-2 Culture.

Hr after inoculation	Virus titer (neg. log.) Frozen cells	Supernatant fluid
12	0	0
18	3.0	0
30	3.7	1.2
57	6.0	5.2

Summary. Measles virus has been adapted to a cancer cell strain derived from a human epithelioma. The Hep-2 cell strain provides a particularly useful system for the study of measles because of its growth characteristics in media free of human serum, its freedom from latent cytopathogenic agents, and the high yields of virus that may be rapidly obtained. After adaptation to Hep-2 the virus was still capable of infecting monkeys but caused little or no illness.

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