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CULTIVATION OF MEASLES VIRUS IN SHEEP KIDNEY CELLS (*)

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Since the first isolation of measles virus in human and monkey renal cells by Enders and Peebles (1), the virus has been grown in primary cultures of human amnion (5, 6), chick embryo (3), dog kidney (2), bovine kidney (4, 8), and guinea pig kidney (9). Some of these tissues have now even been employed for the production of measles vaccine. This report presents our data indicating the high susceptibility of primary sheep kidney cell cultures to measles virus, and some preliminary results regarding the development of attenuated live measles virus vaccine by serial passage in this host system.

Dispersed cells obtained by trypsinization from kidney tissue of a young sheep were grown in rubber-stoppered culture bottles at 37 C using Hanks solution containing 0.5% lactalbumin hydrolysate, 10% calf serum, penicilin 100 u/ml and streptomycin 100 μg/ml. The Edmonston strain, kindly supplied by Dr. J. F. Enders, was used. The strain had been passaged 24 times in primary cultures of human kidney cells and 39 times in primary cultures of human amnion cells when we received it, and was used in the present study after one passage in primary cultures of human amnion cells. After inoculation with 10^{-4} TCID50/cell (as determined in Vero cell culture (7)), sheep kidney cultures were incubated at 36 C and 33 C using Eagle's minimum essential medium (Earle base) supplemented with 3% calf serum and antibiotics. Uninoculated cultures were included as controls.

On the 20th day after inoculation, cytopathic changes were first observed in the inoculated cultures. The changes consisted of syncytial formation, and increased in number and area as the incubation was continued. On the 21st day, the fluid from the cultures incubated at 36 C had a titer of $10^{5.0}$TCID50/ml, 5000 times greater than the initial seeding, while that attained at 33 C was 1250 times. The uninoculated control cultures were maintained in excellent condition over 2

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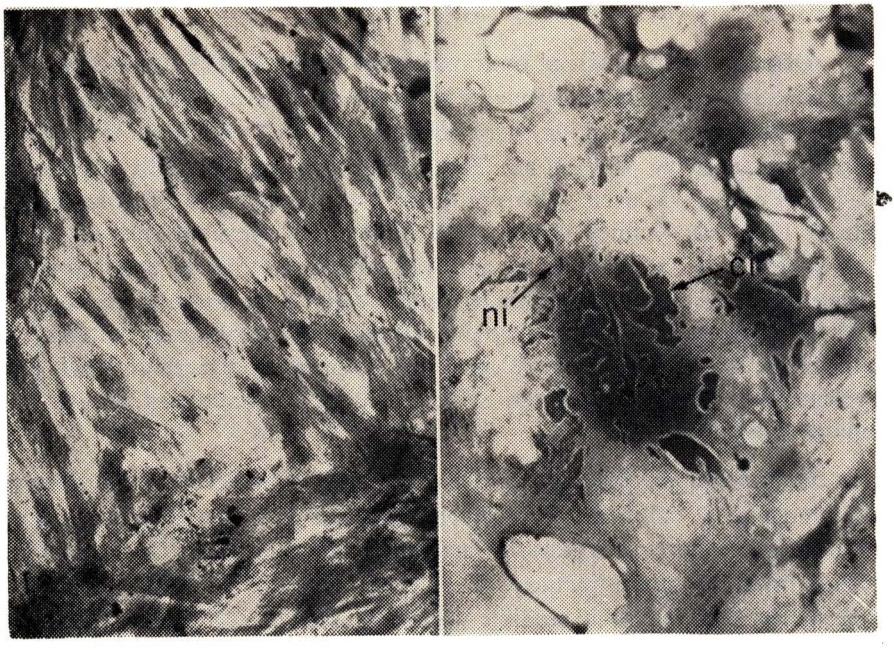
months of incubation. Serial passages were readily accomplished at 33 C and 36 C (Table 1). The same cytopathic changes appeared in each passage, and eosino-

Table 1. Virus titers found in fluids of serial passage of Edmonston virus in sheep kidney tiuess cultures

Passage	36C passage		33C passage
	Titer* log (TCID50/ml)		Titer* log (TCID50/ml)
	at 36C	at 36C	at 33C
1	5.0 (21)	4.4	3.4 (21)
2	3.4 (15)	4.2	3.4 (16)
3	5.5 (37)	5.2	4.7 (26)
4	4.2 (7)	6.4	6.1 (7)
5	4.7 (8)	(Plaque cloning)	
6	5.7 (10)	5.2	5.7 (26)
7	7.0 (10)	5.2	6.7 (21)
8	6.7 (10)	6.0	6.7 (20)

* The titer was determined in Vero cell cultures with the culture fluid harvested after days of incubation indicated in parentheses.



a. b.

Fig. 1. a. Uninfected sheep kidney cells. H & E stain. Orig. mag. 100×. b. Syncytial formation containing intranuclear (ni) and cytoplasmic inclusion bodies (ci) in sheep kidney cell culture 20 days after inoculation of Edmonston measles virus at the 8th passage level (at 33C). H & E stain. Orig. mag. 100×.

philic inclusion bodies were observed in the nucleus and cytoplasm in stained preparations (Fig. 1). The neutrali zation test (7) confirmed the identity of the passaged virus (Table 2).

Table 2. Serological confirmation of the passaged Edmonston strain viruses by neutralization test

Source of virus	Human sera *b)*				Rabbit sera *c)*	
	Case 1		Case 2		No. 1	No. 2
	Acute	Conv.	Acute	Conv.		
1. Sheep kidney cell, 36C-line: P3*a)*	<2	512	—	—	—	512
2. Sheep kidney cell, 33C-line: P3	<2	512	—	—	512	512
3. Sheep kidney cell, 33C-line: P30	—	—	<2	256	1024	512
4. Vero cell: P2	<2	512	<2	128	1024	512

NT titer is expressed by the reciprocal of the highest dilution of serum against 300 TCID50.

—: not tested.

a) Virus from the 3rd passage in sheep kidney cells (virus line of 36C-culture).

b) Acute and convalescent sera from measles patients.

c) Antisera from rabbits immunized with the Edmonston strain adapted to FL cells.

Three green monkeys, without preexisting measles neutralizing antibodies, were inoculated subcutaneously with 3000 TCID50 of the 8th passage-virus of the 33 C line. They developed no clinical symptoms, but significant antibody responses were shown by the neutralization, hemagglutination-inhibition and complement fixation tests of their sera.

The original Edmonston strain and that passaged in sheep kidney cells produced clear plaques with sharp boundaries, 0.5 to 1 mm in diameter on sheep kidney cell monolayers in rubber-stoppered 2-oz bottle. The procedure previously described for MS cells (7) was used with minor modifications. The overlay medium was Eagle's minimum essential medium (Earle base, without phenol red) containing 3% calf serum and 1% agar. The overlayed cultures were incubated at 33 C, received a second agar overlay on the 7th day, and stained with 0.2 ml of 0.1% neutral red in PBS at 33 C overnight on the 13th day.

This cell system has permitted propagation of measles strains with different passage histories: the Tanabe strain adapted to green monkey renal cells, the Edmonston strain adapted to HeLa, FL, or Vero cells, and the attenuated Sugiyama strain adapted to bovine renal cells.

We found that goat kidney cells were also highly susceptible to measles virus, but uninoculated cultures also developed cytopathic effects frequently. On

the other hand, such cytological changes were never observed in kidney cell cultures prepared from the 8 sheep in Japan which we tested.

Sheep kidney cell monolayers can easily be maintained in excellent condition for about 7 days in Eagle's minimum essential medium without serum at 33 C, and readily stored for as long as 3 to 5 months at 25 C or 29 C in Eagle's medium with 3% calf serum with medium changes at appropriate intervals. The properties of sheep kidney cells, in addition to their high susceptibility to measles virus, emphasize the availability of this cell culture in the development of live vaccine seed and its production. We have easily established 4 cold variants from the virulent Edmonston strain by plaque cloning at 33, 29, 27, and 25 C, and field trials with some vaccines prepared with these variants are in progress. The results of these experiments and field trials will be published later.

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