Enhancement of Cytopathic Effects of Reoviruses in Rolled Cultures of Rhesus Kidney.* (27632)

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Judging from the frequency with which hemagglutinin-inhibiting antibodies for reoviruses have been demonstrated in sera of patients at this hospital(1), infections with these viruses must be very common; yet there are few reports of isolation of reoviruses in animals(2-4) and even fewer from humans(5-8). The data presented here indicate that the cytopathic effects (CPE) of all 3 types of reoviruses may be markedly increased in rolled rhesus kidney tissue cultures (MKTC) as compared with stationary cultures of the same kind, thus permitting more rapid and efficient isolation and identification of these viruses.

Materials and methods. The same inoculum of reovirus types 1, 2 and 3(9) was introduced into 60 tubes of MKTC§ for each type; one-half of them were incubated in stationary racks and the rest on a roller drum making 15 revolutions per hour (rph). All the cultures were kept in the same incubator at 37°C. The reoviruses used were: types 1 (Lang) and 3 (Abney), obtained from Dr. Leon Rosen, and type 2 (strain 988) isolated in this laboratory(1). Cultures were observed for CPE and fluids from 5 tubes of each set were harvested daily, pooled, and kept frozen at –20°C until they were titrated simultaneously for infectivity and hemagglutinins. Human adult "O, Rh-negative" erythrocytes were used for hemagglutination (HA). The technics are described elsewhere (10). In other experiments, stocks of these 3 viruses and another type 2 strain (D_5), also obtained from Dr. Rosen, were incubated at 37°C; infectivity and hemagglutinins were assayed at intervals over a period of 42 days. Reoviruses type 1 and 2 (988) were also assayed before and after incubation at 56°C for 45 minutes. Infectivity was assayed in roller tube cultures, 5 tubes per dilution, in which CPE was roughly quantitated over a period of 7 days. Blind passages were not done. The maintenance medium contained 0.2% bovine plasma albumin, 0.2% yeast extract, and 2% chicken serum in Earle's balanced salt solution plus antibiotics (250 units of penicillin, 250 μg of streptomycin and 100 units of nystatin per ml).

Results and discussion. CPE (Table I)

TABLE I. CPE* and Lysis of Rolled (R) and Stationary (S) MKTC by Reoviruses.

Days at 37°C	Type 1		Type 2		Type 3	
	R	S	R	S	R	S
1	0	0	0	0	0	0
2	0	0	0	0	++	0
3	++	0	0	0	++++,P	+
4	++++,P	0	0	0	++++,C	++
5	++++,C	0	++,+++	0		++++,P
6		++,+++	++++,C	0		

* Cytopathic effect: +, <25%; ++, 26-50%; +++, 51-75%; ++++, >75% tissue cells damaged. P, partial lysis; C, complete lysis of cells.

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§ Obtained from Microbiological Associates, Inc., Bethesda, Md.

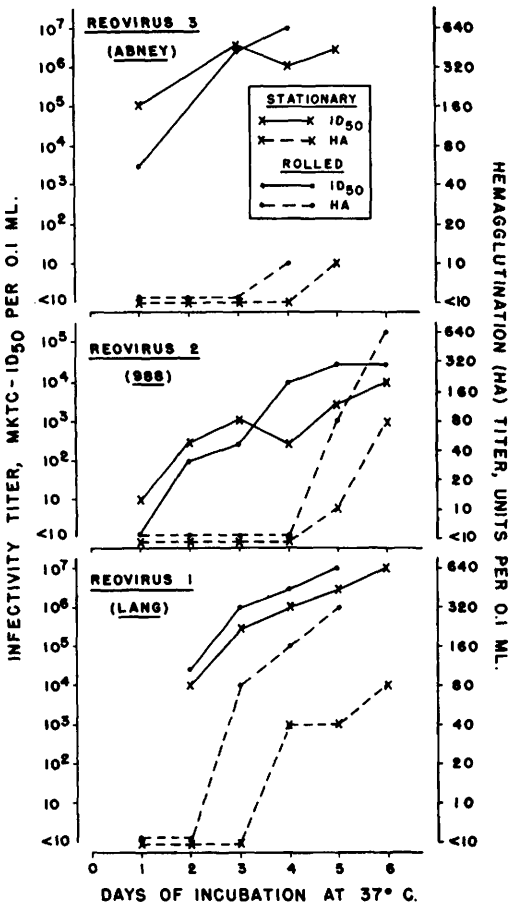

FIG. 1. Infectivity and hemagglutinating activity of reoviruses in stationary and rolled cultures.

appeared 2-3 days earlier in the rolled cultures for all 3 types of reoviruses. Fig. 1 shows the results of titrations for infectious virus and for hemagglutinins in rolled and stationary cultures of reoviruses. Generally, infectivity and HA titers were somewhat higher in pools of fluids from rolled cultures, but the striking effect was the enhancement of CPE with the roller technic; the latter was reflected in the higher HA titers, which appeared earlier in the rolled tubes. Fig. 2 shows complete destruction of a typical roller culture of type 3 virus on day 3 after inoculation, and a barely detectable focus of infected cells in a parallel stationary culture.

Obviously much infectious virus could be missed in microscopic observations of stationary cultures of reoviruses. In addition, blind passages, which had been used for detection of reoviruses(3,7,8) were no longer necessary when the roller technic was employed.

Inactivation of infectivity and of the hemagglutinating activity of the viruses at 37°C are shown in Fig. 3. Complete degradation of infectivity varied from 30 days for reo-

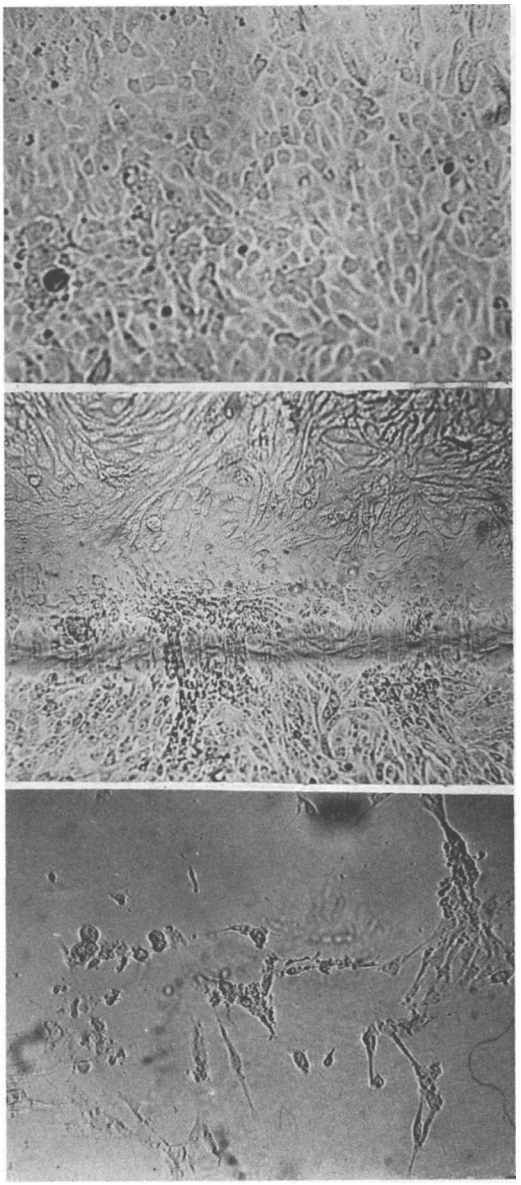

FIG. 2. Microscopic appearance of MKTC of reovirus type 3 (Abney) after 3 days at 37°C. Top: Uninoculated, stationary culture. Middle: Inoculated, stationary culture. Bottom: Inoculated culture rolled at 15 rph.

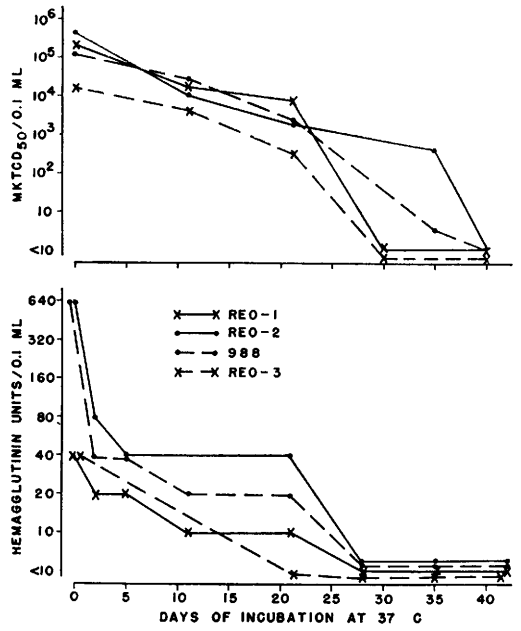

FIG. 3. Inactivation of infectivity and of hemagglutinating activity of reoviruses in MKTC at 37°C.

viruses types 1 and 3 to 42 days for reovirus type 2 (D5 and 988). Hemagglutinins declined and disappeared earlier, since 10^3-10^4 TCD_{50} (tissue culture infecting dose causing CPE in 50% of inoculated cultures) were necessary per HA unit. Similarly, incubation of reoviruses types 1 and 2 (988) for 45 minutes at 56°C did not completely destroy the infectious virus; the titers of type 1 virus before and after heating at this temperature were $10^{5.5}$ and $10^{1.8}$ TCD_{50} per ml, respectively, and the corresponding values for type 2 (988) were $10^{4.5}$ and $10^{1.5}$. In contrast to these results, Rhim, Smith and Melnick(11) using stationary plaque cultures, found that reovirus type 1 (strain 716) was inactivated in less than 17 days at 37°C and within 45 minutes at 56°C.

Summary. Cytopathic effects of reoviruses in rhesus kidney tube cultures were markedly enhanced by incubation on a roller drum. Higher hemagglutinin and infectivity titers were attained after a shorter incubation period in rolled cultures, but the striking effect is in the increased cytocidal nature of reovirus replication under these conditions. Also, when cultivated in rolled monkey kidney culture tubes, infectious reovirus was still detected after incubation for 3-5 weeks at 37°C and after 45 minutes at 56°C.

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