Suckling Mouse Cataract Agent (SMCA) in Tissue Culture (35200)

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Suckling mouse cataract agent (SMCA) was originally isolated by Clark in 1961 (1) from ticks removed from dead rabbits. It has since been classified as a "slow" virus (2). Intracerebral inoculation of SMCA into newborn mice and rats not only produced cataract with severe chorioretinitis, but also signs of mild encephalitis with chronic brain infection. In animals so inoculated virus has been isolated from the brain up to 827 days postinoculation (3). We have also observed that these animals develop hydrocephalus, a condition which has not been previously reported for this virus. In addition, SMCA grows readily in the 7-day-old developing chick embryo, which usually dies from the infection. SMCA, as a "slow" virus, has not been previously successfully adapted to propagate in any tissue culture.

The ability of SMCA to persist indefinitely in the central nervous system makes it an important experimental model for the study of a "slow" virus with its implication to chronic neurologic dysfunction. An accurate and inexpensive assay system is obviously desirable. This paper presents data in the propagation and adaptation of SMCA in tissue culture.

Materials and Methods. Virus. The suckling mouse cataract agent (SMCA) used in these studies was obtained from Dr. H. Fred Clark of the Wistar Institute, Philadelphia, Pennsylvania. The agent has had 12 passages in the yolk sac of a 7-day-old developing chick embryo, and a passage in the newborn rat brain.

Cell cultures and media. Primary cultures of chick embryo, normal adult rat brain, 30-day-SMCA-infected rat brains, co-culture of infected rat brain and normal chick embryo, SMCA-infected chick embryo and organ culture of rabbit lens were used.

Preparation of primary tissue culture. Normal RIF-free 12-day-old chick embryos (from Shamrock Farms, North Brunswick, New Jersey) with heads, legs, and viscera removed, or rat brain were minced and digested in a medium consisting of 500 ml of Eagles basal medium in Hanks' salt solution, 100 ml of 2.5% trypsin in salt solution, 10 ml inactivated newborn calf serum (for mild digestion of tissue), 5 ml of 200 m*M* L-glutamine, 100 units/ml of penicillin, and 100 μg/ml streptomycin. Treatment continued for 1 hr. At 15-min intervals cells suspended in the trypsin medium were poured through a 4 $\times$ 4 (12 ply) sterile gauze and the remaining pieces of tissue retreated with fresh medium. Pooled cells were centrifuged in the cold, washed three times and resuspended in growth medium, and counted. Three ml of the suspended cells (containing a total of 300,000 cells) were used to seed each 30 ml of plastic "Falcon" bottle with screw cap. Growth medium consisted of 500 ml of Eagles minimal essential medium, 50 ml of inactivated newborn calf serum, 5 ml of 200 m*M* L-glutamine, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. Maintenance medium was the same as growth medium except that the serum content was lowered to 20 ml. Bottles of cells were incubated at 37°. Normal uninfected confluent tissue culture cells were inoculated with 0.5 ml/bottle of undiluted egg yolk adapted SMCA. These cultures were usually maintained for 28–35 days before being discarded.

Infected chick embryo tissue culture. Seven-day-old RIF-free chick embryos are inoculated via the yolk sac with 0.5 ml of 10^{-3} dilution of SMCA. Four days later, the embryos are examined and only those remaining viable are treated with trypsin medium as

described above. These cells are also maintained for 28–35 days. Some of the cells in suspension are exposed to ultraviolet irradiation in an attempt to induce CPE when planted. Irradiation is accomplished with a GE mercury arc lamp by a previously described method (4). Cells are planted at the same concentration as the unirradiated ones.

Whole rabbit lens organ culture. Whole lenses from normal 6- to 8-week-old cottontail rabbits are removed aseptically, washed, and placed on wire grids in organ culture petri dishes containing 1 ml of medium (described above). The lenses are inoculated immediately with 0.1ml of SMCA. The initial adaptation in rabbit lens organ culture is initiated with an egg yolk adapted SMCA which had had 12 passages in egg yolk, one in rat brain and two alternate rabbit lens-egg yolk passages. Rabbit lens cultures are kept at 37° in a CO_2 incubator. At 48-hr postinoculation the overlay fluid is removed and replaced with fresh medium. Rabbit lens cultures are usually kept for 5 days.

Infectivity assays. At intervals all tissue cultures are sampled for infectivity. Samples, overlay fluid, 10% suspensions of homogenized tissue culture cells or organs, are titrated in 7-day-old chick embryos. Serial 10-fold dilutions (in 0.5-ml vol) are inoculated via the yolk sac; the eggs are examined daily for deaths (except that deaths occurring within the first 48 hr are not included in calculating the end points). Infectivity titer end points are calculated by the method of Reed and Muench (5).

Neutralization test. Rat anti-SMCA serum pool was inactivated at 56° for 30 min. Rabbit lens adapted SMCA in the fourth passage was used. Block neutralization test (6), employing equal mixtures of serum and virus with variable dilutions of both, were incubated at 37° for 1 hr before being inoculated into yolk sacs of 7-day-old developing chick embryos. Deaths of embryos were recorded daily, and end points were calculated by the method of Reed and Muench (5).

Results and Discussion. No significant levels of SMCA were obtained in the following primary tissue culture systems: infected chick embryo, normal adult rat brain, culture

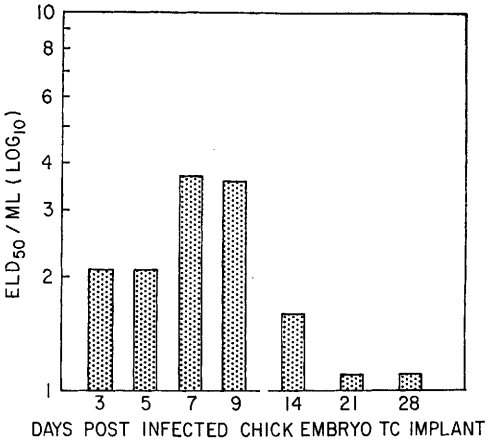

FIG. 1. SMCA in tissue cultures prepared from infected chick embryos. (Titers are those of intracellular virus only.)

(explant) of SMCA infected rat brain, and co-culture of SMCA-infected rat brain and normal chick embryo. No evidence of cell degeneration due to SMCA was observed in any of the above tissue cultures during the observation period of 28–35 days.

Figure 1 shows the results of infectivity titrations on tissue culture cells prepared from SMCA-infected chick embryos. These cells continue to produce infectious SMCA to appreciable titers through day 14 postimplantation. A peak was reached between days 7 and 9. The cells gradually lose their ability to grow more SMCA, however, after prolonged maintenance. During the period of observation (28–35 days) no degeneration due to SMCA is observed. Serial passages of SMCA could not be made from these cultures. Ultraviolet irradiation of these cells and of normal uninfected chick embryo cells in suspension in an attempt to induce CPE reveals that the cells are very sensitive to as little as 60-sec exposure to UV. These cells (both control and infected) fail to grow.

A growth curve of SMCA in rabbit lens organ cultures is shown in Fig. 2. Peak titers are obtained by the fifth day postinoculation. These organ cultures are not kept beyond the sixth day of cultivation, since the lens tends to autolyze at this time. Results of infectivity titers from serial passages of SMCA in rabbit lens organ cultures are shown in Table I. The data indicate that

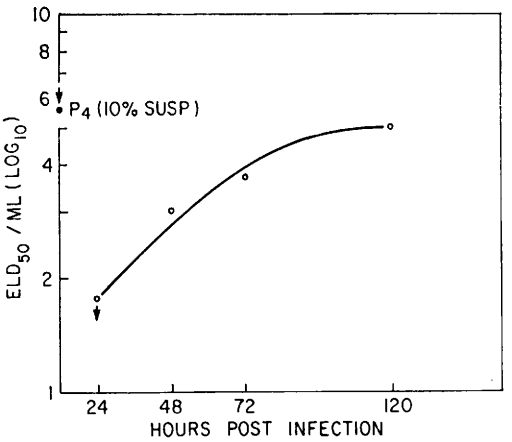

FIG. 2. Growth curve of SMCA in rabbit lens organ culture. (Titers are those of intracellular virus only.)

SMCA propagates readily in rabbit organ tissue culture. The infectivity titer at each passage level is reasonably high and ranges from a low of 3.5 to a high of 6.8 $\log_{10}$. It must be noted that the overlay tissue culture fluid containing the original inoculum in each culture of SMCA is usually removed and replaced with fresh medium at 48 hr postinoculation. The dilution factor of the virus (inoculum) in such a change is equivalent to 1:100.

TABLE I. Propagation of SMCA in Rabbit Lens Organ Cultures.

Passage level	Infectivity titers[a] (ELD_{50})/ml ($\log_{10}$)	
	Tissue culture fluid	10% Lens suspension
1	ND[b]	ND
2 (P_1)-1	5.3[c]	ND
2 (P_1)-2	5.6	6.0
3 (P_2)	ND	4.8
4 (P_3)-1	5.8	6.8
4 (P_3)-2	4.3	6.0
5 (P_4)-1	ND	3.5
5 (P_4)-2	ND	5.0
6 (P_5)	ND	4.8
7 (P_6)	ND	ND
8 (P_7)	ND	4.8

[a] Titers 5 days after inoculation and 3 days after complete change and replacement of old medium with fresh one.

[b] ND = not done.

[c] Fluid 48 hr postinoculation.

Limited serial passages without appreciable fall in infectivity titer is another indication of the ability of rabbit lens organ culture to support the growth of SMCA.

The specificity of SMCA serially passed in rabbit lens organ cultures was verified by two tests: (a) Induction of cataract in newborn rats with fourth passaged rabbit lens SMCA using 0.02 ml/rat by intracerebral inoculation. 10–20% of inoculated animals show either unilateral or bilateral cataracts 30 days postinoculation; in addition SMCA was isolated from brains of all animals inoculated. These results were consistent with SMCA characteristics. The rate of cataract induction, however, was lower than when egg yolk or rat brain adapted SMCA was used; this could be explained by the level of infectious SMCA in the inocula (4.5 $\log_{10}$ in organ culture, versus 7 to 9 $\log_{10}$ in egg yolk or rat brain). Clark and Karzon (3) have shown that the rate of cataract induction was influenced by the level of infectious virus in the inoculum; the higher the virus titer, the higher the rate of cataract induced. The second test, a neutralization test of the rabbit lens adapted SMCA, showed a neutralization index of 1.3 $\log_{10}$. This result was similar to what Clark and Karzon reported for SMCA neutralization test in 7-day-old chick embryos using mouse and rabbit anti-SMCA sera and egg yolk adapted SMCA (7).

The apparent ease of cultivation and serial passage of SMCA in rabbit lens organ culture may be due to a number of factors one of which is that the lens by itself is essentially a pure "culture." The lens has a simple and pure cell population, since it is made up of a single epithelial cell type in a single layer which covers its upper surface. These cells are surrounded by a noncellular laminated capsule and therefore free of contamination by other tissue elements. The other factor is that SMCA has an apparent predilection for eye tissues with resultant ocular lesions (8) and high infectivity titers in individual compartments of the eye of newborn C5781/6Ha mice inoculated intracerebrally with the virus (7, 9).

SMCA has not been previously successfully propagated or adapted to grow in any tissue culture system. This is the first time

that this virus has been propagated and serially passed to significant titers in tissue culture. Earlier attempts by Clark (1) to grow SMCA in primary rabbit, guinea pig, monkey, fetal human kidney, chick embryo, human amnion, and in HeLa, KB, FL cells, human fetal lung continuous tissue culture lines were not successful. No CPE was observed in any of these systems.

Summary. Suckling mouse cataract agent, a new addition to the "slow" virus group, has shown appreciable growth in explant tissue cultures of SMCA-infected chick embryos. SMCA readily propagated for the first time in significant titers in organ cultures of rabbit lens. In addition, several serial passages of SMCA in rabbit lens organ cultures were made with no significant loss in infectivity titers. The data presented should be of considerable value in further studies on the nature of this agent and its pathogenesis in chronically infected brain.

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