# Further Characterization of Suckling Mouse Cataract Agent (SMCA): A Slow, Persistent Infection of the Nervous System[1] (36855)

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(Introduced by M. B. Bender)

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Suckling mouse cataract agent (SMCA) has been shown to induce cataracts, chronic brain infection and hydrocephalus when inoculated intracerebrally into newborn mice and rats (1–3). The agent, originally isolated from ticks removed from dead rabbits (4), has since been classified as a "slow" virus (5). *In vitro* the agent can be passaged in organ cultures of rabbit lens (6) and infectivity can be assayed by death of embryonated chick eggs (4) within 7–11 days after inoculation.

In intracerebrally inoculated animals maximum virus yields per brain are obtained within 12–15 days, but can be identified in decreasing amounts for the lifetime of the animal (7, 8). The ability of SMCA to persist for long periods in the central nervous system makes it an important model for the study of a "slow" virus with its implications to chronic neurologic dysfunction.

These studies to further characterize the nature of the agent report (i) the effect of various physical and chemical drug treatments on SMCA, (ii) the size of the infectious unit, and (iii) the possible ultrastructural nature of the agent in infected tissues.

*Methods and Materials. SMCA.* The suckling mouse cataract agent (SMCA) used in these studies was originally obtained from Dr. H. Fred Clark of the Wistar Institute, Philadelphia, PA. The agent has since been passaged in yolk sac of 7-day-old developing chick embryo, and in newborn mouse and rat brains.

*Organ cultures.* Lenses were removed from rabbits and infected *in vitro* as described previously (6). Briefly, lenses from 6 to 8 wk cottontail rabbits were removed aseptically, washed and placed on wire grids in organ culture dishes in 1.0 ml growth medium (6). No antibiotics were used in lens medium. The lenses were inoculated immediately with 0.1 ml SMCA containing $10^6$ brain infectious units/ml, and incubated at 37° in $CO_2$-humidified atmosphere. At various times lenses were assayed for SMCA/lens by titration in chick eggs.

*SMCA titration in chick eggs.* Seven-day-old RIF-free chick eggs (Shamrock Poultry Co., NJ) were inoculated via the allantoic space with SMCA, the eggs were sealed and incubated at 37°. At various times, eggs were inspected for death of the embryo due to SMCA (1). Allantoic fluids were harvested and assayed for bacterial and fungal contamination which was never present.

*Physical inactivation studies.* These studies were done using preparations of SMCA from infected chick egg allantoic fluids containing $10^6$ brain infectious units/ml. The preparations were exposed to a series of deleterious treatments and then assayed for residual infectivity by death of chick embryos. The deleterious treatments employed were: ultraviolet irradiation with a GE mercury arc lamp by a method previously described (9), heating at 57° for 15 min, exposure to 0.025 *M* trypsin at 37° for 20 min, and exposure to pH 10 and 5.8 for 30 min at 37°.

*Chemical inactivation studies.* Rabbit lenses were infected *in vitro* with $10^6$ SMCA brain infectious units in the presence of medium (6) supplemented with various drugs. The growth of the agent in the lenses was assayed by the death of chick embryos after inoculation of lens homogenates. Medium was supplemented with penicillin ($10^{-2}$

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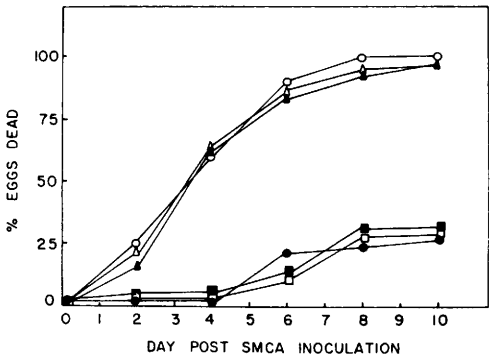


FIG. 1. Death of embryonated chick eggs upon inoculation of SMCA infected allantoic fluids which were exposed to deleterious physical treatment: (○—) no treatment; (△—) pH 5.8; (▲—) pH 10.0; (■—) 0.025 *M* trypsin; (●—) uV irradiation; (□—) heating. For details of treatments, see text.

units/ml), streptomycin (0.1 μg/ml), kanamycin (100 μg/ml), rifampin (Ciba, 80 μg/ml), hydroxyurea ($5 \times 10^{-3}$ *M*), actinomycin D (10 μg/ml) and 5-bromodeoxyuridine (BUdR) (15 μg/ml).

*Size studies.* Allantoic fluids containing $10^7$ SMCA brain infectious units/ml were filtered through a graded series of sterile Millipore filters (pore size 50–10,000 nm). Residual infectivity in filtrates was assayed by death of embryonated chick eggs.

*Electron microscopy.* High-speed pellets of allantoic fluids from SMCA and virally infected and uninfected eggs, and infected and uninfected rabbit lenses and mouse brains were processed for electron microscopy as previously described (10). Thin sections were examined in a Hitachi HU 11E electron microscope.

*Viruses.* Embryonated chick eggs were inoculated in the allantoic space with appropriate amounts of a series of RNA and DNA viruses for comparative studies. Herpes simplex and vaccinia viruses were grown and titered as previously described (10, 11). Mumps, rubella and reoviruses were kindly provided by various sources at the Mount Sinai Hospital, New York.

*Results.* Figure 1 shows the kinetics of killing of chick embryos by SMCA infected allantoic fluids. These fluids were exposed to a series of deleterious physical agents. As shown, heat (57° for 15 min), ultraviolet irradiation and 0.025 *M* trypsin (37° for 30 min) reduced the killing titer by at least 75%: These agents inactivate SMCA. pH 5.8 and 10 (37° for 30 min) did not inactivate the agent.

The inhibition of the growth of SMCA by drugs in lenses is shown in Fig. 2. These studies show that penicillin and kanamycin have little effect on reducing the growth of SMCA as assayed in chick eggs. Streptomycin also had no effect upon SMCA. Hydroxyurea, actinomycin D and 5-bromodeoxyuridine (BUdR) all greatly reduce the infectivity of SMCA in rabbit lenses. These drugs are metabolic inhibitors of DNA and RNA synthesis.

The size of the infectious SMCA unit was determined by experiments summarized in Fig. 3. Infected allantoic fluids were filtered through a graded series of Millipore filters and filtrates were assayed for infectivity in chick eggs. Pore sizes of 200 nm and below in diameter held back almost all the infectivity while pore sizes 500 nm and above permitted most infectivity through: The infectious particle of SMCA in allantoic fluids is between 200 and 500 nm in size.

Embryonated chick eggs were inoculated

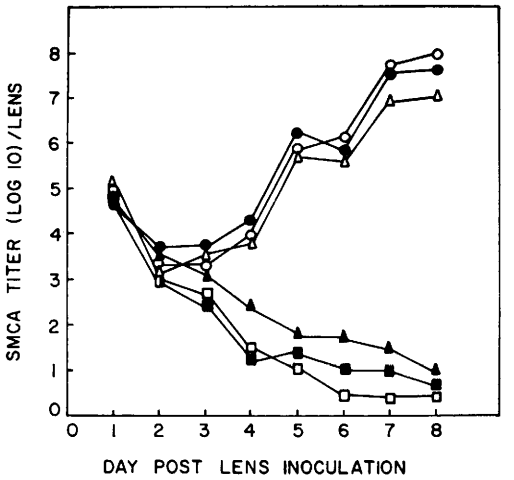


FIG. 2. Growth of SMCA *in vitro* in rabbit lenses incubated in medium containing drugs (○—) no drug; (●—) kanamycin; (△—) penicillin; (▲—) hydroxyurea; (□—) actinomycin D; (■—) BUdR. For drug doses, see text.

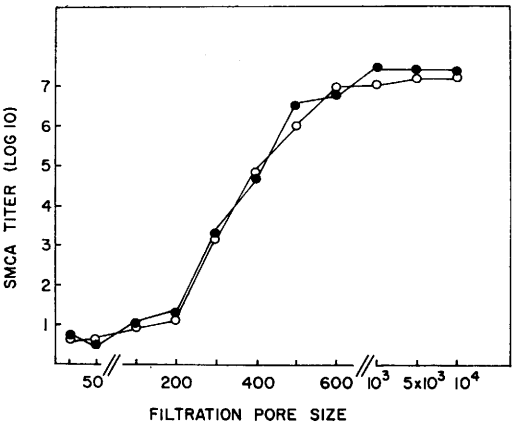


FIG. 3. SMCA titer in filtrates of infected allantoic fluids passed through Milipore filters of graded pore size. The results of two experiments are shown.

with a series of RNA and DNA viruses in the allantoic space. Upon death of the embryo (or day 5 if no death occurred) allantoic fluids were harvested, pelleted at high speed and examined by electron microscopy. Table I summarizes the ultrastructural findings of such experiments. Only viruses which were inoculated were found in the corresponding pellets. Each pellet contained red blood cells, yolk and filamentous, extracellular material as well as cells containing aggregates of ribosomes (12). Only eggs receiving SMCA had structures shown in Fig. 4 in their allantoic fluids. Figure 4A shows particles surrounding a cell containing many microtubule-like profiles concentrated at its surface. The particles (arrows) are 30 × 400 nm. Figure 4B shows such a cell and the particles near electron-dense aggregates of granular material. These dense aggregates were sometimes seen in allantoic fluids infected with viruses. Figure 4C and D shows particles at higher magnification, one of which appears to be branched (arrow).

Figure 4B shows two intracellular vacuoles (arrows) surrounded by microtubule-like profiles. The number of these vacuoles, which may appear to be empty or contain granular material, varies from cell to cell. Microtubule-like profiles containing unique, densely staining material are concentrated around vacuoles and at the cell surface.

Electron microscopy of SMCA infected and uninfected mouse brains and rabbit lenses were negative for any structures recognizable as infectious agents.

*Discussion.* The persistence for long periods of time of infectious agents in the brains of animals and man has given rise to a family of agents known as the "slow" viruses, termed slow because of the long duration of the infection, and often because of the slow

TABLE I. Features[a] of Allantoic Fluids from Inoculated Chick Eggs.

| | Uninoculated control | Herpes simplex | Mumps virus | Reovirus | Vaccinia | Rubella | SMCA |
|---|---|---|---|---|---|---|---|
| Red blood cells | +[b] | + | + | + | + | + | + |
| Filaments, extracellular 20 × 400 nm | + | + | + | + | + | + | + |
| Dense granular aggregates | — | + | + | — | — | — | + |
| Rough precipitate; yolk? | + | + | + | + | + | + | + |
| Virus particles | — | + | + | + | + | + | ?[c] |
| Agent-like particles | — | — | — | — | — | — | + |
| Cells with aggregates of ribosomes[d] | + | + | + | + | + | + | + |
| Cells with microtubule-like profiles at surface | — | — | — | — | — | — | + |

[a] Features as seen in the electron microscope.

[b] + = seen; — = not seen.

[c] The only extracellular particles seen are represented by Fig. 4.

[d] See Ref. (12).

progression of the associated disease (13). This family includes the infectious agent of scrapie in sheep; the agent of Kuru in man; measles virus, which has been implicated as the causative agent of subacute sclerosing panencephalitis, a neurological degenerative disease of man (14); the herpesviruses (15); and other viruses that only under certain

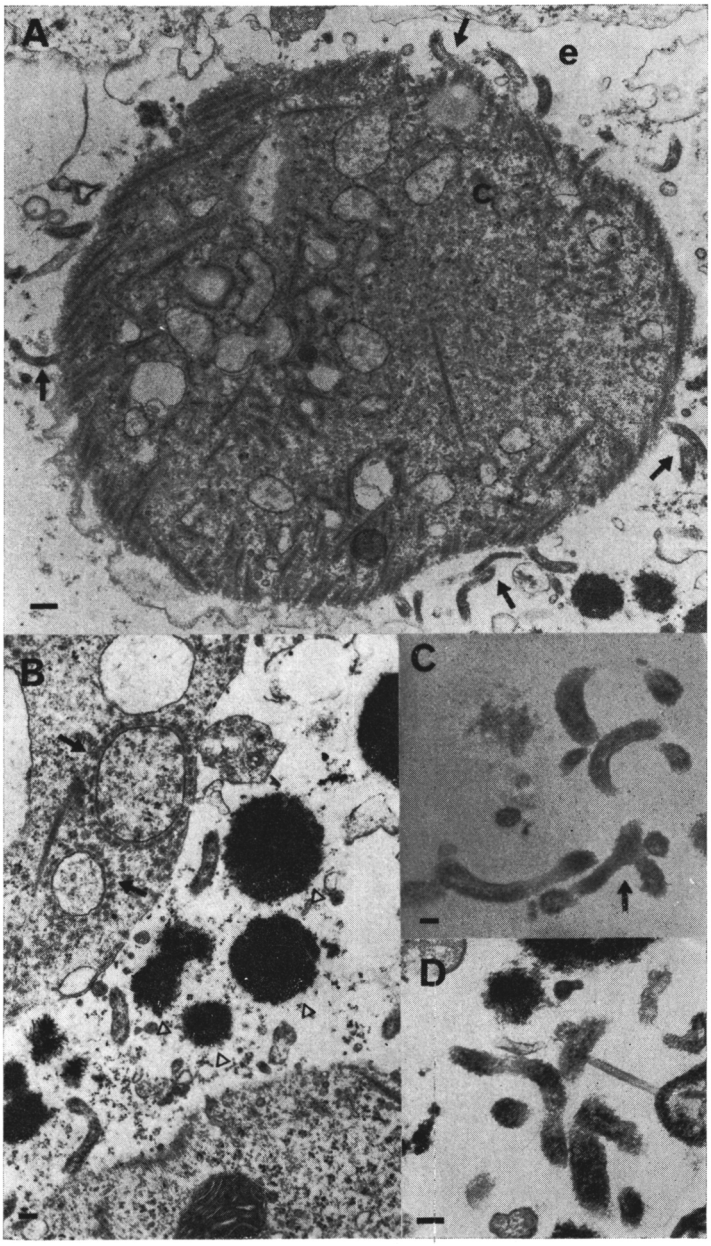


FIG. 4. Electron micrograph of high-speed pellet of allantoic fluid from SMCA infected egg. (A) Cell containing microtubule-like profiles with particles (arrows) at its surface. The relationship between particles and cell is unclear. Bar = 90 nm; c = cell; e = extracellular space. (B) Microtubule-like profiles surrounding 2 intracellular vacuoles (arrows). Dense extracellular aggregates (△) are seen in uninfected pellets. Bar = 40 nm. (C and D) Higher magnification of particles. (C) Arrow points to branched particle. Bars = 30 nm.

conditions slowly infect cells.

The definition of the infectious agent associated with scrapie and Kuru has been difficult mainly because of the lack of an *in vitro* system to study and because of the protracted course of the infection *in vivo*.

SMCA is unlike the agent of scrapie in that it is thermosensitive, inactivated by uv irradiation and of larger size. It is similar to scrapie in that in both infections no infectious agent is recognizable at the site of the disease, *i.e.*, the brain (16). Preliminary experiments indicating that the size of the SMCA infectious agent from brains is smaller than that in the *in vitro* system are reminiscent of the scrapie agent (H. F. Clark, personal communication).

SMCA replication is resistant to kanamycin, penicillin, streptomycin and rifampin which implies that the agent is not bacterial or mycoplasma. The extreme sensitivity of the agent to inhibitors of both RNA and DNA metabolism means that the agent undergoes a complex replicative pathway utilizing both types of nucleic acid.

Ultrastructurally, the particles seen in SMCA infected allantoic fluids resemble small mycoplasmas (17). This possibility cannot be ruled out at this time other than by the fact that the agent is (i) resistant to kanamycin and (ii) not seen in infected brains, which surely mycoplasma would be. The relationship between the cells containing microtuble-like profiles and the particles seen in Fig. 4, is not clear. Electron microscopy upon allantoic fluids of similar preparationss in other laboratories have shown similar particles (R. F. Zeigel and H. F. Clark, personal communication) outside of cells.

At this time inactivation experiments, drug analyses and electron microscopy make it probable that the agent is of a viral nature. Further attempts at characterization by purfication and localization of specific infected sites within affected brains are now under way.

*Summary.* Experiments were designed to further characterize the nature of suckling mouse cataract agent (SMCA). The agent is sensitive to heat, trypsin and ultraviolet inactivation, but is stable over the pH range 5.8 to 10. Penicillin, kanamycin, streptomycin and rifampin have no effect on the replication of the agent while BUdR, actinomycin D and hydroxyurea are inhibitory. By filtration the size of the infectious particle was determined to be between 200 and 500 nm. Ultrastructural studies upon infected allantoic fluids reveal unique particles not present in fluids infected with a series of RNA and DNA viruses.

The response of the agent to drugs indicates that it is not a bacterial agent and is probably viral. Filtration experiments and ultrastructural studies favor a viral nature.

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