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Growth Curve Studies of the Suckling Mouse Cataract Agent in Individual Compartments of the Eye* (33954)

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The suckling mouse cataract agent (SM CA) is an egg-lethal virus isolated originally from rabbit ticks (*Haemaphysalis leporis-palustris*) in Georgia (1). The SMCA consistently causes a high incidence of cataracts, appearing between 15 and 30 days of age, in mice inoculated intracerebrally as newborns. A particularly high cataract rate, approximately 85%, has been observed in mice of strain C57Bl/6Ha (2).

The histopathological and clinical appearance of eye disease in C57Bl mice is described elsewhere (3–4). Briefly, retinal inflammation began on the fourth day, and increased in severity through day 11 when severe inflammation of the choroid was also noted. During the third week retinal atrophy began. By 60 days the retina was reduced to a membrane 2–3 cells thick and choroid inflammation had also subsided. Early changes in the anterior lens epithelium were first noted at 4 days, with marked changes of the lens fibers appearing at 11–13 days. Lens disease subsequently progressed to dissolution and calcification. Mild iridocyclitis sometimes followed posterior uveal disease.

Clinical observations revealed vitreous exudate and anterior vacuolation of the lens appearing by 15–20 days of age. In many cases pathologic changes in the lens subsequently increased until total opacity was observed at 25–40 days. Anterior synechia indicative of late anterior uveitis were often observed several months after the onset of clinical eye disease.

Studies of SMCA titer in the various compartments of the eye were conducted in parallel with the pathological and clinical studies

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reviewed above. The results of this intraocular SMCA growth study are herein reported; data on the viral content of brain, liver–spleen, and blood are presented for comparative purposes.

Materials and Methods. The C57Bl/6Ha mice were obtained from the Roswell Park Memorial Institute. Newborn mice (less than 24 hr old) were inoculated intracerebrally with $10^{5.0}$ to $10^{6.6}$ egg lethal doses (ELD_{50}) of allantoic fluid origin SMCA in a volume of 0.01 ml. The SMCA used represented the seventh and eighth passage level of a strain with a history of passage in embryonated eggs only. At intervals from 1 hr to 180 days after inoculation, mice were killed by dislocation of the neck. Two mice from different litters were killed at each age. Mice were bled from severed axillary vessels, the brain was harvested and the liver and spleen were harvested and pooled. In two individual mice a number of other visceral organs were harvested.

Eyes were dissected by a technique suggested by Dr. Werner Noell, Department of Physiology, School of Medicine, State University of New York at Buffalo. With the mouse pinned on one side, the uppermost eye was prolapsed with curved iris forceps, and the cornea was incised with a sterile razor blade. Pressure applied to the back of the eye with a second pair of iris forceps forced the lens and then the retina and vitreous body out through the incision. (Lenses of older mice were washed several times in buffered saline; the fluid consistency of lenses of mice less than 7 days old made this impossible.) The eye was then enucleated, cleaned of adnexa, flattened on a piece of filter paper, and the cornea was severed from the sclera. The separation of the mouse eye into the 4 named segments: (i) lens, (ii) retina and vitreous, (iii) sclera and choroid, and (iv) cornea and anterior uvea, was confirmed by histological examination of eye parts dissected by this method.

Each eye part was triturated with alundum in a mortar and pestle in 3.0 ml of 0.75% bovine albumin in phosphate buffered saline (BAPS). Pooled liver and spleen, brain, and occasionally other organs were triturated as 10% (w/v) suspensions in BAPS. The total volume of blood obtained from each animal was suspended in 1.0 ml BAPS. All tissues were frozen at —70° until tested.

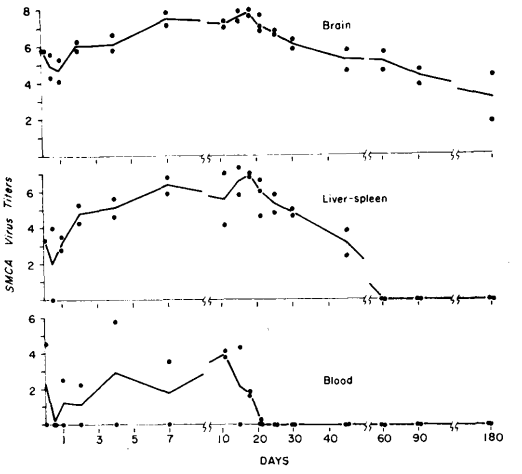

FIG. 1. Recovery of SMCA from the blood and viscera of C57/Bl mice inoculated ic with ca. $10^{6.0}$ egg lethal doses (ELD_{50}) on the first day of life: titers are ELD_{50} (log_{10}) per gram or milliliter of tissue; negative points are $<10^{0.8}$.

Content of SMCA in tissues was determined by titration for lethal end point in 7-day embryonated eggs as previously described (*2*). Titers of eye parts represent the total SMCA content in ELD_{50} recovered from each sample. Titers of visceral organs and blood are expressed as ELD_{50} per gram or milliliter of original tissue.

Results. Virus recovery from the brain, liver-spleen, blood, and eye parts is shown in Fig. 1 and 2. SMCA in the brain reached peak concentrations of $\geqq 10^{7.0}$ by 7 days after inoculation. Infection persisted at this level through 21 days, after which gradual decline in titers occurred. Virus in low titer then persisted in the brain throughout the 180-day observation period. Liver-spleen titers peaked concurrently with brain titers, but diminished rapidly thereafter. No virus was recovered from these tissues after 45 days. Viremia titers were inconsistent. SMCA was recovered from the blood of 5 of 12 mice sacrificed between 1 hr and 7 days and from 5 of 6 mice sacrificed between 11 and

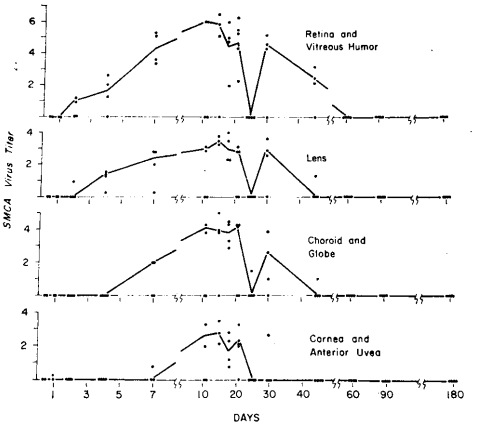

FIG. 2. Recovery of SMCA from the separate eye tissues of C57/Bl mice. Titers represent the total SMCA content of each tissue, *i.e.*, concentration of virus in tissue suspension multiplied by the total volume of that suspension.

18 days after infection. No viremia was detected after age 21 days. Blood concentrations were usually considerably lower than those observed in brain and liver-spleen.

Trace amounts of virus were detected in the whole eye as early as 12 hr after inoculation (eyes of this age were not dissected). Small amounts of virus were detected sporadically in the cornea, retina, and lens at 24 and 48 hr. At 96 hr, virus first appeared consistently in eyes, both in the retina-vitreous and in the lens. Retina-vitreous and lens virus titers reached peak levels at about day 11, remained high until day 21 and then diminished. Virus did not appear consistently in the choroid-globe and cornea until day 11. Virus persisted in these tissues until day 21 and then diminished concurrently with the decline in other eye tissues. The highest virus titers were found in the retina-vitreous tissues. Average choroid-globe titers were 10- to 100-fold lower and lens titers were 100- to 1000-fold lower. Titers in the cornea and anterior uvea were the lowest observed in the ocular tissues.

Titers in the eye could not be accounted for by circulating SMCA in the blood stream. Individual mice sacrificed 1 hr, 1 and 11 days after inoculation yielded SMCA from the blood but none from either eye. On the other hand, many mice, sacrificed at intervals from 12 hr to 45 days after inoculation, exhibited eye infection in the absence of detectable viremia.

The virus titers in a number of visceral organs of 2 mice in the same experiment, sacrificed 7 and 15 days after inoculation, are shown in Table I. Although small amounts of virus were detected in the lungs of each animal, lung titers were much lower than those found in the brains, liver-spleen tissues and eyes of the same mice (Table I). No other visceral organs tested contained significant titers of SMCA.

Discussion. The SMCA content of the several tissues of the mouse eye could be readily correlated with the histopathological appearance of disease. The initial consistent appearance of virus in the retina at 4 days coincided with the initial appearance of retinal inflammation observed histologically. Peak retinal titers reached at 11 days were concurrent with the appearance of severe inflammation seen in the posterior layers of the retina. The gradual decline of virus titer in the retina coincided with atrophic retinal degeneration.

The observation that virus appeared later in the choroid than in the retina is in agreement with the observation that inflammation in the choroid appeared several days after

TABLE I. Recovery of SMCA from Visceral Organs of C57Bl/6Ha Mice.[a]

Mouse	Age (days)	Heart	Lung	Testes	Duodenum	Kidney	Thymus	Pancreas	Bladder and urine
CV227	7	<0.8	3.5	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
CV217	15	<0.8	Trace (<1.0)	<0.8	<0.8	Trace (<1.0)	<0.8	<0.8	nd[b]

[a] Concentration of SMCA recovered ($\log_{10}$) from mice inoculated intracerebrally with ca. $10^{6.0}$ egg lethal doses (ELD_{50}) at age <24 hr; titers are ELD_{50} per gram of tissue.

[b] nd = not determined.

retinitis. However, high titers were attained rapidly by day 11, when severe choroid inflammation developed.

The initial consistent appearance of virus in the lens at 4 days of age corresponded to the time of appearance of the earliest pathological changes seen in the anterior epithelium of the lens. It should be noted that virus in the lens declined concomitantly with the resolution of inflammation in the other eye tissues despite the fact that pathological changes in the lens continued to progress. The low titers of virus in the cornea and anterior uvea corresponded to a low incidence and late onset of disease observed in these tissues.

Virus was isolated from 29 of 34 mouse eyes harvested between 4 and 30 days after inoculation. This rate of isolation (86%) was identical to the rate of eyes observed to have clinical disease in SMCA-inoculated C57Bl mice. The close correlation of SMCA titer with disease in the retina and posterior uvea suggests that pathologic changes in the posterior eye may be caused directly by cytocidal infection of affected cells. The fact that peak SMCA titers in the lens preceded the development of maximum lens changes suggests that severe lens damage is secondary either to early viral damage of lens epithelium or to the severe inflammation in the posterior eye. The possible role of an immune or hypersensitive response in the induction of cataract cannot be ruled out. Maximal levels of antibody to SMCA appear by 20 days after infection, at which time the cataractogenesis is often progressing (5). However, antibody directed to lens antigens has not been detected in SMCA-infected mice (Bash, J., and Milgrom, F., unpublished).

Rubella, cytomegalovirus, herpes simplex, herpes zoster and the virus of Behcet's disease have been demonstrated to cause eye disease in man (6–12). The manner in which these viruses reach the eye and cause ocular pathology is poorly understood, partly because of the lack of suitable animal models of eye infection. The consistency with which SMCA can be recovered from the several tissues of the infected mouse eye suggests that this system might serve as a useful model for further study of the basic pathogenesis of virus-induced eye disease.

Summary. The suckling mouse cataract agent (SMCA) consistently causes cataracts, retinitis, and posterior uveitis in intracerebrally inoculated suckling mice. Sequential studies were performed of the level of virus infection in several compartments of eyes dissected at regular intervals following inoculation. The time of appearance of infection in the lens, retina, and posterior uveal tissues was closely correlated with the time of onset of pathologic changes. Peak levels of infection coincided with maximum inflammation in the retina and uvea. The decline of infection in these tissues was also correlated with the resolution of inflammation. However, virus content of the lens declined at a time when lens disease continued to progress. The incidence of eye infection detected by viral studies was identical to that observed by clinical and histopathological studies. The SMCA system appears to provide a useful model for the study of intraocular infection.

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