Journal of the Arkansas Academy of Science

Volume 21 Article 7

1967

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Arkansas Academy of Science Proceedings, Vol. 21, 1967

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INTRODUCTION

In the 25 years since Gregg reported that congenital lens cataracts may develop in infants following rubella (German measles) virus infection in the mother during pregnancy (4), only a few viruses have been associated with the production of lens cataracts in experimental animals These include the Enders strain of mumps virus and the suckling mouse cataract agent. When the Enders strain of mumps virus was injected into the blastoderm of the chick embryo prior to the separation of the lens vesicle from the surface ectoderm, the secondary lens fibers were damaged and white cataracts resulted in a high percentage of the surviving animals (7, 9). Intracerebral injection of the suckling mouse cataract agent into mice during the first day of life when the secondary lens fibers are beginning to form resulted in white cataracts in 44% of the animals within a few months (1, 6).

The human embryonic lens is susceptible to viral invasion when the mother becomes infected with rubella virus from the 4th through the 7th week of pregnancy (4). This period of susceptibility for lens cataract formation from rubella virus corresponds to the time just before the lens vesicle separates from the surface ectoderm until the secondary lens fibers begin to form (5). In the rubella-infected embryo at 8 weeks of pregnancy, the cataract has been found to be a dense white body, the lens consisted mainly of degenerative primary fibers at the equator (8) and the cataractous lens had a high titer of rubella virus (10).

In our laboratory, we have been concerned with the maze-learning ability of white rats which have recovered from clinical encephalitis following the inoculation of various of the arthropod-borne encephalitis viruses. These have included St. Louis encephalitis virus which was first isolated in 1933 during an epidemic in St. Louis, Missouri, and more recently was shown to be responsible for the outbreak of encephalitis in Texas.

In 1942, Duffy and Sabin reported the reason that St. Louis encephalitis virus had been thought to be non-pathogenic for rats was that young enough animals had not been used (2). The series of events which follow intracerebral inoculation of St. Louis encephalitis virus in white laboratory rats are dependent on the age of the animals at the time of inoculation. If injected during the first week of life, all become acutely ill and uniformly succumb to a fatal encephalitis. If injected at 21 days of age or older, none of the animals develop clinical symptoms and no deaths occur. If animals are injected at 12 days of

age, all develop severe clinical encephalitis but approximately 33% will survive the infection.

In preliminary studies using infectious ribonucleic acid (RNA) extracted from St. Louis encephalitis virus (SLE), a similar age susceptibility pattern was demonstrated. However, to have a 25-30% recovery rate after all animals had developed encephalitis, it was necessary to inoculate rats at 4 days of age.

MATERIALS AND METHODS

Ten percent suspensions (W/V) of SLE-infected mouse brains were prepared by homogenizing the brain tissue in 0.1 phosphate buffer at pH 8.7 — 8.9 with 5×10^{-4} M versene. Following centrifugation, the virus-containing supernatant fluids had LD_{50} titers of $10^{-7.6}$ to $10^{-8.4}$ when tested by intracerebral inoculation of ten-fold dilutions into 3-week-old Rockland Swiss mice.

A portion of each virus suspension was extracted 3 times with equal volumes of liquefied phenol which had been washed 3 times with 0.1 M phosphate buffer. The 3 phenol extractions were followed by 3 extractions with anhydrous ether and then nitrogen aeration for 10 minutes (3). All steps were carried out in the cold over a period of 2½ hours. The water-clear liquid which was left was considered to be a 10^{-1} preparation of infectious RNA and LD_{50} titers were between $10^{-3.5}$ and $10^{-4.1}$ when tested in mice. The infectious RNA gave a negative Biuret test, and, after treatment with crystalline RNase for 1 minute at room temperature, it had lost all infectivity for mice and 4-day-old rats by the intracerebral route.

Part of each litter of Sprague-Dawley rats was inoculated intracerebrally into the right hemisphere with 0.03 ml. of the infectious RNA. The remainder of each litter was inoculated in the same manner with infectious RNA which had been treated with RNase. The RNase rats were used as control animals throughout this study. All young were sexed, numbered and weighed the day of inoculation. All animals were examined daily for signs of central nervous system involvement and weighed at least every 5 days.

RESULTS

All young inoculated with infectious RNA developed encephalitis with signs of CNS involvement usually seen beginning on the 5th or 6th post-inoculation day. They were smaller and weaker than their control litter-mates. In addition, they were hyperexcitable and had tremors of the head. Approximately 74% of the test animals died. After recovery, the 76 surviving test rats continued to show nervous symptoms and stunting of growth. Data in Table 1 show that the control males consistently weighed about 100 grams more than the test males after approximately 4 months of age. The weight difference between control females and test females was not as great until both groups reached about 12 months of age. The test rats never reached the weight attained by the control rats even by 15 months of age.

In this experiment, 37 test males and 39 test females survived and were kept with 74 control rats. Data in Table 2 show the cumulative number of cataracts which devoloped in the test rats. A total of 36 rats or 47% of the test animals developed unilateral cataracts, and a total of 29 or 39% developed bilateral cataracts. Only 11 rats or 14% of the test animals had both lenses clear at 22 months, and examination of their lenses with a slit lamp showed no developing white cataracts. None of the control rats developed cataracts.

All test rats with bilateral cataracts and 6 male and 6 female control rats were killed at 16 months of age. All surviving rats were killed at 22 months of age. The equatorial cornea and lens diameters of all the rats were measured. The mean values of the range of sizes in millimeters are given in Table 3. Each group represents a total of 12 rats, 6 male and 6 female, except the group of rats at 22 months with clear lenses. This group was made up of the 11 rats of the 76 test animals which did not develop cataracts. On comparing the lens and cornea diameters of the test rats with those of the control rats, it can be seen that the earlier the cataract formed, the greater the degree of microcornea and microlens. Abnormal smallness of the entire globe paralleled the degree of microcornea.

In a subsequent study, the eyes of control and test rats were repeatedly examined with a slit lamp. It was found that there was an initial posterior opacity as the lens cataracts began to form and that about 6 weeks was required for the cataract to be complete. In addition to arrested growth of the lens, cornea and globe, the lens capsule was wrinkled. No abnormalities were noted in the retinae of the eyes. In this experiment, 8 rats were killed at varying intervals from 3 to 7 days after inoculation and their eyes removed. St. Louis encephalitis virus was shown to be present in the globes by means of neutralization tests in mice using specific SLE antiserum.

DISCUSSION

Lens cataracts which resulted in rats injected intracerebrally at 4 days of age with infectious ribonucleic acid extracted from St. Louis encephalitis virus resembled in many respects those induced in the human embryo by rubella virus. The cataract is white with an initial posterior opacity, and marked microphthalmus, microcornea and microlens may develop. In addition, the virus can be demonstrated in the eye.

The critical period for the development of lens cataracts in rats may be around 4 days of age. During this embryonic time of lens development, the secondary lens fibers are rapidly forming. However, the critical period may be the result of factors other than the stage of lens development. Apparently the virus must invade the eye and the ease with which the virus can invade the eye from the rest of the brain may change with age. Certainly the infectivity of the infectious ribonucleic acid was changed or decreased in some fashion since intra-

cerebral inoculation of intact SLE particles would have killed all test animals before any changes could have been seen in the eyes.

Table 1

Approximate Weight in Grams of Test and Control Rats

	4 months of age	12 months of age	15 months of age
Control Males	450	550	650
Test Males	350	450	550
Control Females	250	350	400
Test Females	200	290	300

Table 2

Cataract Development in Rats Following Intracerebral Inoculation with RNA Extracted from St. Louis Encephalitis Virus

Total Number of Test Rats	76	
Number with Unilateral Cataracts	36 (47%)	14 males, 22 females
Number with Bilateral Cataracts	29 (39%)	19 males, 10 females
Number with Clear Lenses	11 (14%)	

Table 3

Lens and Cornea Diameters of Eyes from Rats Injected at 4 Days of Age

	Lens*	Cornea*
Controls	4.9	6.4
Rats at 22 months with clear lenses	4.5	6.3
Rats which developed cataracts between 15 and 22 months of age	3.5	5.8
Rats which developed cataracts between 5 and 14 months of age	3.3	5.5
Rats which developed cataracts between 0 and 4 months of age	2.8	5.3

*Mean values in millimeters.

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