Hydrocephalus in Mice Inoculated Neonatally by the Oronasal Route with Reovirus Type 1

Abstract. *Mice inoculated neonatally by the oronasal route with reovirus type 1 or 2 developed typical acute disease. Fifteen percent of the mice recovered from the acute infection. No further disease was noted in mice infected with reovirus 2, but 9 percent of the survivors of reovirus 1 infections developed hydrocephalus at a mean time of 109 days after inoculation. Infectious virus could not be isolated from hydrocephalic mice.*

Hydrocephalus has been induced in hamsters inoculated intracerebrally with adenovirus (*1, 2*) and various myxoviruses (*3, 4*), and in rats inoculated by various routes with polyoma virus (*5*). In mice, hydrocephalus has resulted from intracerebral inoculation with lymphogranuloma venereum agent (LGV) (*6, 7*), mycoplasma in conjunction with lymphocytic choriomeningitis virus (*8*), polyoma virus (*9, 10*), and myxoviruses (*4, 11*). Recently, reovirus type 1 has been reported to cause hydrocephalus in suckling mice, rats, hamsters, and ferrets inoculated by intraperitoneal, intracerebral, and subcutaneous routes (*12*).

Stanley and co-workers, in their studies of reovirus infections of newborn mice (*13–15*), have used the oronasal route of inoculation as an approximation of one natural route of infection. In mice inoculated at birth by this route, we have noted the development of hydrocephalus in mice infected with reovirus 1 but not in those infected with reovirus 2 or reovirus 3. Experiments comparing the outcome of inoculation with reovirus 1 and 2 are reported below.

Mice of the Prince Henry (PH) strain (*13*) were inoculated by the oronasal route, no later than 24 hours after birth, with reovirus type 1 (strain Lang) or reovirus type 2 (strain D5). Each mouse received about 10 LD_{50} units of virus (titrated in newborn PH mice by the oronasal route) in a volume of 0.01 ml. Typical acute infections (*15*) were thereby established with both virus types.

Virus infectivity was tested on monolayers of L cells. Clarified 5 to 10 percent extracts in Hanks balanced salt solution were prepared from various tissues from affected mice. Leukocytes and plasma were obtained from blood from the retroorbital plexus by a modification of the method of Jack and Grutzner (*16*). Explant cultures were established from pieces of hydrocephalic brain tissue 1 to 2 mm^3 in size. Subcultures were made by removing the explants to fresh culture vessels. Cocultures were set up by seeding brain cultures with L cells, stable monkey embryo kidney cells, or human or mouse primary embryo fibroblasts.

A total of 1361 mice were inoculated with reovirus type 2. Of these, 217 (16 percent) survived to weaning age (21 days). Hydrocephalus was not observed in any reovirus 2–inoculated mice up to termination of the experiments 236 to 585 days after inoculation.

In the course of previous experiments, more than 100,000 newborn PH mice received reovirus 3 oronasally, and the survivors of the acute disease were observed for as long as 2 years. In not one instance was hydrocephalus observed.

Fig. 1. Clinical hydrocephalus in a 210-day-old mouse (upper) inoculated at birth with reovirus type 1. The lower mouse is an apparently normal littermate.

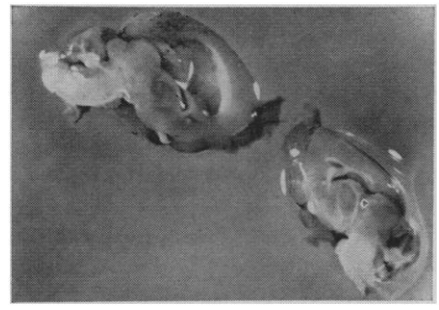

Fig. 2. Midline sagittal sections of normal (right) and hydrocephalic (left) mouse brains. The latter brain demonstrates the marked dilatation of the lateral ventricles.

Reovirus type 1 was inoculated into 1545 newborn mice, of which 236 (15 percent) survived to weaning age. Hydrocephalus developed in 21 (9 percent) of the mice that recovered from the acute neonatal infection with reovirus 1. The time of onset of the disease varied between 47 and 242 days after inoculation, with a mean of 109 days. The disease was diagnosed by an enlargement of the skull in dorsal and lateral directions (Fig. 1). Midline sagittal sections of brains dissected from hydrocephalic mice revealed gross distension of the third and lateral ventricles (Fig. 2) with cerebrospinal fluid. The fourth ventricle was normal in size, and the amount of cerebrospinal fluid in the subarachnoid space was not excessive.

Extracts from brain, eye, thymus, heart, lung, liver, spleen, pancreas, and kidney from four hydrocephalic mice were found to contain no infectious reovirus when tested on L cells. Explant cultures were established from the brains of five other hydrocephalic mice, subcultured, and used for cocultivation tests. Reovirus cytopathic effects were not observed in any cultures containing hydrocephalic brain cells, nor could infectious virus be demonstrated in supernatant fluids from such cultures. Leukocytes and plasma from seven mice with hydrocephalus were found to be negative for infectious reovirus.

The results suggest that the disease is a noncommunicating hydrocephalus. Although reovirus could readily be demonstrated in infected mice up to weaning age, it was not demonstrated in brains or various other tissues of hydrocephalic mice at the time of clinical manifestation of the disease. A similar temporal relationship between isolation of causative virus and clinical evidence of disease has been reported previously for reovirus 3 infection of mice, in connection with virus-induced biliary obstruction (*17*), as well as the late induction of runting and lymphomas (*18*). Inability to isolate the hydrocephalus-inducing agent at the time of presentation of the disease has been demonstrated for myxovirus infections of hamsters and mice (*3, 4*) and polyoma infection of rats (*5*). In LGV-induced hydrocephalus of mice (*6*), the agent was isolated from the brains of some mice but not from others.

Reovirus 1–induced hydrocephalus of mice has been noted by Kilham and Margolis (*12*) in a study carried out principally in hamsters. Details of the pathology, virology, morbidity rate, and time taken to clinical onset of the disease in mice were not specifically mentioned. Hamsters inoculated neonatally with reovirus 1 have been found to develop noncommunicating hydrocephalus (*12, 19*) beginning 8 to 10 days after inoculation. At this time infectious virus was still persistent in brain and liver. The pathogenesis of the disease involved the ependymal cells lining the aqueduct of Sylvius (*20*).

The mean time span of 109 days between inoculation of the virus and manifestation of hydrocephalus is a noteworthy feature of the present series of experiments. It is comparable with that observed for LGV-infected mice (*6*) and greater than the maximum time recorded for myxovirus- (*4*) and polyoma virus–induced hydrocephalus (*5, 10*).

Induction of hydrocephalus in animals inoculated by routes other than the intracerebral one is known only for polyoma virus (*5*) and reovirus (*12*). To our knowledge, the disease has not been reported in any experimental animal after oronasal infection with virus.

The induction, via the oronasal route, of hydrocephalus in mice by such a ubiquitous virus as the mammalian reovirus means that similar induction could easily occur under natural conditions in many species, including man. The search for such a cause is complicated by the long latent period before clinical disease and the associated disappearance of infectious virus (phenomena now increasingly recognized as the outcome of virus-host interaction). It may be an important clue that this capacity for inducing hydrocephalus is restricted to only one of the three reovirus types.

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20. Pathological studies being carried out in our own series by Dr. Byron Kakulas will be reported in a separate communication.
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Human Cystinosis: Intracellular Deposition of Cystine

Abstract. *Membrane-limited inclusions were found in the cytoplasm of cells of the rectal mucosa, leukocytes, and cultured fibroblasts from two humans with cystinosis. Most of the inclusions contained amorphous material, presumably cystine. In cells of the rectal mucosa the material appeared frequently crystallized. This was rarely seen in leukocytes, and never in cultured fibroblasts. The fact that acid phosphatase could be demonstrated consistently in the organelles responsible for sequestration of cystine indicates that they are lysosomes.*

Cystinosis, a heritable disease of childhood, is characterized by cellular accumulation of cystine (*1*). In some cells, primarily those of the reticuloendothelial system, this accumulation leads to crystal formation which is readily visible with either the light or electron microscope. In other cells, such as leukocytes or fibroblastic cell lines derived from cystinotic children, no crystal formation has been observed with light microscopy although biochemical analysis shows that both types of cells have a high amount of cystine (*2, 3*). Indirect evidence has suggested that the lysosome is the organelle of storage in cystinotic fibroblasts (*3*). Direct evidence of this has been presented for crystals in reticuloendothelial cells where the limiting membranes showed acid phosphatase activity (*4*).

Recently, differential centrifugation of homogenates of leukocytes from cystinotic patients has revealed the cystine to be associated with the cell fractions high in lysosomal enzyme activity (*5*). Calculations of specific gravity from the density gradients used suggested that the cystine was very likely not crystalline but in an amorphous form. We now present electromicroscopic evidence that in leukocytes, fibroblasts, and other cells of cystinotic subjects that do not reveal crystal formation, cystine is stored in amorphous form compartmentalized in cell organelles consistent with lysosomes.

Cells of the lamina propria of the rectum (Fig. 1) in cystinotic children are particularly rich in stored cystine

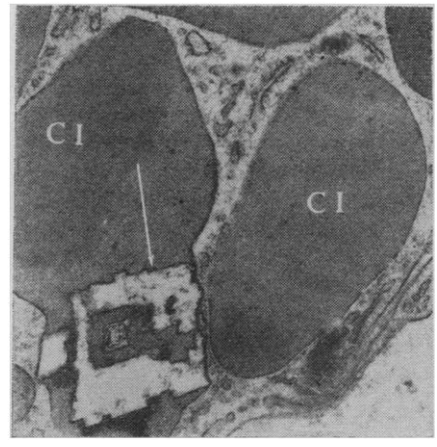

Fig. 1. Rectal biopsy from patient J.E. The tissue was fixed in 1 percent buffered osmic acid, dehydrated in ethanol, and embedded in epoxy resin. Sections were doubly stained with uranyl acetate and lead citrate. The cytoplasm of the cell of the lamina propria contains large inclusions (*CI*). Some of the material has crystallized (arrow), whereas in other areas it appears amorphous (× 36,750).