Suckling Mouse Cataract Agent (SMCA)-Induced Hydrocephalus and Chronic Brain Infection in Newborn Rats (36074)

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(Introduced by M. B. Bender)

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Suckling mouse cataract agent (SMCA), originally isolated from ticks removed from dead rabbits (1), has recently been tentatively classified as a member of the biologically grouped "slow" viruses (2). Two unusual biological characteristics of this agent are its ability to induce lifelong brain infection and cataract formation when inoculated intracerebrally into newborn mice (3). In the developing chick embryo, SMCA readily multiplies to high titers in all tissues, and death of the embryo consistently occurs following a characteristic incubation period, depending on dose. Attempts to grow SMCA *in vitro* in both primary cell cultures and in established cell lines derived from man and a wide variety of other vertebrate species have been unsuccessful (1), but recently the agent has been successfully propagated and serially passed in rabbit lens organ cultures (4). As in the case of many other "slow virus" infections, the antibody response to SMCA infection is limited; significant antibody titers are detected only when immune sera are tested undiluted in a constant serum: varying virus concentration (neutralization index) type of neutralization test, assayed in intracerebrally inoculated suckling mice (5). There is no evidence that SMCA is antigenically related to any North American tick-borne virus or other mouse-lethal viruses (6).

This report describes a new characteristic of SMCA infection—a capacity to induce hydrocephalus accompanying the chronic brain infection in rats inoculated intracerebrally as newborns.

One- to 2-day-old Sprague-Dawly rats (Perfection Breeders, Douglasville, PA) were inoculated by the intracerebral route with 0.02 ml of 10^{-2} dilution of SMCA (ELD_{50}/ml 7.8 $\log_{10}$) which has been passed 12 times in the yolk sac of the developing chick embryo and once in rat brain, and which was confirmed by bacteriological tests to be free of bacteria, PPLO, and fungi. Two sets of normal 1–2-day-old rats served, respectively, as uninoculated controls and egg-yolk inoculated controls [the latter inoculated intracerebrally with 0.02 ml of normal chick embryo yolk at a dilution of 10^{-2} (log 10)]. Animals were observed daily and killed at different intervals postinoculation. Infectivity assays were carried out in RIF free 7-day-old developing chick embryos. Serial 10-fold dilutions of 10% suspension of infected brains and their corresponding uninfected controls were inoculated in 0.5 ml volume into the yolk sac. Inoculated eggs were examined daily for mortality and only deaths occurring after 48 hr postinoculation were considered significant for calculating infectivity end points by the method of Reed and Muench (7). Inoculated and control rats were killed at various intervals postinoculation; and the brains were removed for virus assay and histological study. Table I shows the infectivity titers in individual rat brains and the occurrence of hydrocephalus and cataract from 2 days to 107 weeks postinoculation. Titers (per gram of brain tissue) ranged from a high of 8.5 $\log_{10}$ at 12 to 15 days to a low of <1.0 $\log_{10}$ at 107 weeks postinoculation. The data are comparable to previously reported rat brain SMCA titers up to 26 weeks postinfection (8), and suggest that SMCA does not persist in the brains of intracerebrally inoculated rats as long as it does in mice (3). Cataract was first observed

TABLE I. Brain Infectivity Titers, Cataracts, and Hydrocephalus Incidence in SMCA-Inoculated Rats.[a]

Days postinfection	No. of rats sacrificed	Brain SMCA titers[b]	No. of rats with cataracts	No. of rats with hydrocephalus
2	2	ND	0	0
5	9	4.7	0	0
8	8	4.7	0	0
12	2	>8.5	2	0
15	4	7.6, >8.5	2	0
20	5	7.0	3	1
28	7	5.6, 5.8, 6.0, 6.7	4	1
34	8	5.5	8	5
42	3	4.1, 4.9, 5.7	3	2
49	7	4.2, 5.0, 5.2, 5.7, 6.0	7	3
77	2	4.7, 5.0	2	2
84	7	ND	7	5
112	2	4.7, 6.0	2	1
140	5	3.5, 3.7	5	3
196	1	3.5	1	1
280	1	ND	1	1
300	1	2.7	1	1
490	12	ND	12	8
560	4	<1.0, <1.0, <1.0, <1.0	4	0
651	2	<1.0, <1.0	2	0
749	2	1.3, <1.0	2	0
Total	94		68	34
%			72	36

[a] Sprague-Dawly rats inoculated intracerebrally at age 1–2 days with ca. $10^{4.1}$ ELD_{50} of SMCA.

[b] Log_{10}, ELD_{50}/g of tissue.

on day 12, (the time that suckling rat eyes are first open), coincident with virus peak titer. Animals that had not developed cataract by day 30 failed to develop it during the rest of the observation period. Of the total of 117 animals studied, 82 (70%) had cataract. Hydrocephalus was first noted in SMCA-inoculated brains at 20 days postinoculation, and frequently thereafter through 70 weeks (Fig. 1). No hydrocephalus was observed in 8 animals killed at 80 weeks and beyond. Of a total of 94 brains examined pathologically, 34 (36%) had varying degrees of hydrocephalus. This finding was made on rats independently inoculated with SMCA in two different laboratories, while uninoculated and control-inoculated rats in both laboratories remained free of hydrocephalus or cataract. At no time during the course of these studies was there evidence of any clinical disease in either SMCA-inoculated or control animals, which were housed completely separately throughout this period but otherwise handled similarly. The control animals were killed at similar ages as the infected ones, the "older" of this group consisting of 15 animals aged 61 weeks through 139 weeks.

Histologically, hydrocephalus varied from moderate to severe and was approximately symmetrical throughout the ventricular system. Increase in ventricular size varied 2- to 4-fold. There was no ventricular obstruction and no leptomeningeal reaction or thickening. The choroid plexus appeared normal. The ependyma was generally flattened and occasionally showed an apparent interruption by subependymal glial fibers. There was a corresponding decrease in periventricular white matter, especially in the cerebrum. Occasionally, a moderate isomorphic astrocyto-

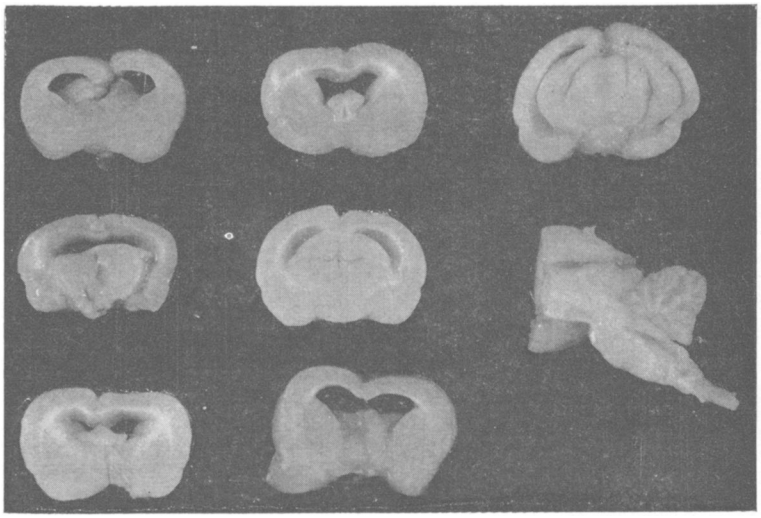

FIG. 1. Coronal and sagittal sections of brains of Sprague-Dawly rats inoculated intracerebrally at age 1–2 days with ca. $10^{4.1}$ ELD_{50} of SMCA, and killed at the following intervals postinoculation (top to bottom, left to right) (days): 20, 34, 42, 49, 112, 280, and 490; ×2.

sis was present in the white matter. The cerebral cortex and subcortical gray matter and the motor and sensory nuclei and fiber tracts of the brain stem were unremarkable. No inflammation was encountered and the parenchymal blood vessels showed no significant change (Figs. 2, 3).

Discussion. The role of viruses in the ex-

FIG. 2. Choroid plexus of lateral ventricle of rat inoculated intracerebrally at age 1 day with SMCA and killed 42 days later, appears normal, with no signs of choroiditis; hematoxylin and eosin stain, ×200.

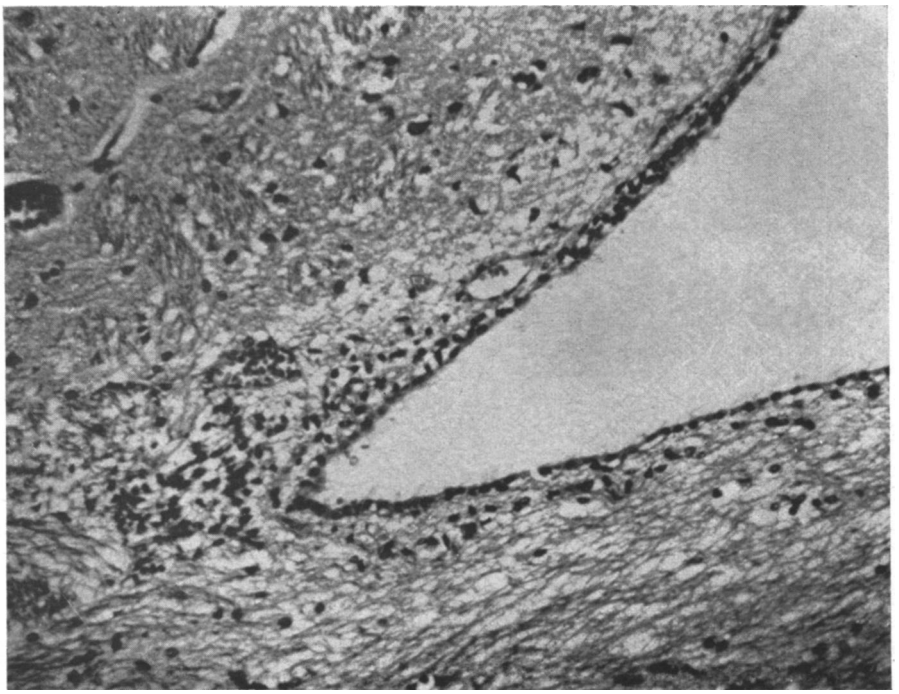

FIG. 3. Ependymal lining of lateral ventricle of rat inoculated intracerebrally at age 1 day with SMCA and killed 490 days later, is generally flattened but otherwise intact, with no signs of inflammation; hematoxylin and eosin stain, ×200.

perimental induction of developmental anomalies during the prenatal and neonatal periods has recently been reviewed (9). Huebner *et al.* (10), and Yabe *et al.* (11) have reported the chance induction of hydrocephalus in newborn hamsters inoculated intracerebrally with type 12 adenovirus, the syndrome developing 3 weeks postinoculation. Li and Jahnes (12) and Holtz *et al.* (13) have shown that a strain of polyoma virus isolated from Swiss mice bearing sarcoma-180 could produce hydrocephalus in 30% of 1-day-old Swiss white mice inoculated intracerebrally, with inflammatory response and subsequent obstruction to the CSF flow primarily at the aqueduct of Sylvius. Virus was recovered from brain tissue of hydrocephalic mice. Johnson and Johnson (14) reported an important chance finding of hydrocephalus in suckling hamsters inoculated intracerebrally with a nonneuropathic strain of mumps virus. The hydrocephalus was noted as a late sequel occurring several weeks after the acute ependymal infection, and resulted from noninflammatory aqueductal stenosis. Virus was not recoverable from brains 9 days after inoculation. Hydrocephalus was first noted at 14 to 17 days after inoculation, and at 28 days or later, 95% of the hamsters had developed clinical hydrocephalus. This report was the first demonstration that mumps, a relatively innocuous respiratory virus, unassociated with clinical disease, could cause definite central nervous system anomaly. Recently, reovirus type I, an agent ubiquitous in nature, has been reported to regularly induce obstructive hydrocephalus in suckling mice, rats, hamsters, and ferrets inoculated by intraperitoneal, intracerebral, and subcutaneous routes, as a consequence of selective attack of this agent upon ependyma (15, 16). In the hamster, hydrocephalus was noted beginning 8 to 10 days after inoculation, at which time infectious virus was still persistent in brain. Oronasal inoculation of reo I into neonatal mice (17) has also been reported to induce hydrocephalus in 9% of survivors of the acute infection from 47 to 242 days after inocula-

tion with a mean of 109 days. No infectious virus could be isolated from brains of hydrocephalic mice.

The present report on induction of hydrocephalus with SMCA is of special interest in several respects. Hydrocephalus was noted as early as 20 days postinoculation, about 1 week after peak virus titers in the brain. It was observed regularly thereafter through 70 weeks (490 days), with concurrent persistence of relatively high virus titers until about 28 weeks (196 days), and with gradual tapering off in infectivity titers to negligible concentrations at 80 weeks (560 days) and beyond. To our knowledge, this is the first time that an infectious agent continued to persist in the brain over a considerable period of time concomitant with hydrocephalus. The nature of the latter remains obscure, with no convincing histological features in the brain suggestive of any preceding, though now resolved, acute infection, such as ependymal cell loss or presence of microglial nodules in brain parenchyma.

Summary. Suckling mouse cataract agent (SMCA) inoculated intracerebrally in newborn rats results in persistence of infectious agent in the brain over a considerable period of time concomitant with hydrocephalus. The means by which the SMCA infection causes hydrocephalus remains obscure; a functional block in the cerebrospinal fluid pathways is postulated.

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