Chronic Subclinical Infection with Mouse Salivary Gland Virus. (24451)

ISADORE BRODSKY AND WALLACE P. ROWE (Introduced by K. Habel)
U. S. Dept. of Health, P.H.S., Nat. Inst. of Allergy and Infectious Diseases, Bethesda, Md.

Studies conducted by Rowe *et al.*(1) demonstrated persistence of human salivary gland virus in some children for at least 15-24 months. Similarly, on the basis of cross-sectional studies, it has been assumed that salivary gland viruses of rodents produce long term chronic infection of the salivary gland, but there has been little experimental basis for this assertion and no estimate of duration of infection. This report describes a year's study of subclinical mouse salivary gland virus (MSGV) infection. In view of marked biological and physical similarities between mouse and human salivary gland viruses(2), it was believed that a study of chronicity with MSGV would serve as experimental model for human salivary gland virus.

Materials and methods. Virus was obtained from Drs. Margaret G. Smith and Richard S. Lee, St. Louis, and maintained by passage of submaxillary salivary gland suspensions inoculated subcutaneously into the region of submaxillary glands of NIH "General Purpose" Swiss mice, a strain free of spontaneous salivary gland virus infection(2). Forty male "General Purpose" mice 3 weeks old were inoculated intraperitoneally with 0.2 ml of 10^{-3} dilution of infected mouse salivary gland suspension of the 30th mouse passage representing approximately 10^2 to 10^3 $TCID_{50}$ doses. Mice were maintained for a year during which time, at given intervals, 2 to 4 mice were removed for study. Mouth swabs, urine and submaxillary glands were obtained from individual mice. Trypsin dispersed mouse embryo tissue culture tubes were used for all virus isolations. Cultures were prepared from whole 14-16 day mouse embryos, grown in 10% human serum in Eagle's basal medium, and maintained in 5% horse serum in Eagle's medium; media contained penicillin and streptomycin. The mouth swab was rinsed in a vial containing 2 ml of Hanks' medium, and 0.5 ml transferred to each of two tissue culture tubes. Urine was diluted with one ml of Hanks' medium and 0.1 ml inoculated into each of 2 tubes. Ten percent suspensions of the salivary glands were prepared and 0.1 ml of 10-fold serial dilutions inoculated into each of 2 culture tubes. Cultures were observed for at least 2 weeks. Virus isolations were identified by the characteristic pattern of cytopathic effects and typical intranuclear inclusions(3). Virus titers are expressed as number of tissue culture doses/0.1 ml of 10% gland suspension. Glands were fixed in Zenker's-formalin and stained with hematoxylin-eosin. Slides were carefully examined for intranuclear inclusions by at least two observers.

Results. No evidence of overt disease was seen in any of the 40 mice. Results of virus recovery and pathologic studies are summarized in Table I. The first isolation of MSGV from the submaxillary gland, on eighth day after inoculation, coincided with appearance of virus in mouth swabs and of inclusions in the submaxillary gland. Maximum titer was obtained on 12th day; this was the only time at which virus was recovered from urine. After one year the submaxillary glands of 3 mice were still positive for MSGV and of the 2 mice tested both were excreting virus in the saliva.

Inclusion bodies were most numerous during early stages of infection. After 60th day, no inclusions could be demonstrated in submaxillary glands of any of the 9 mice studied although all had virus in the glands in titers of 10^2 to 10^5. There appeared to be no definite correlation between virus titer and number of inclusions. However, further studies would be needed to establish this point.

Discussion. Large doses (approximately 10^6 $TCID_{50}$) of MSGV are required to produce fatal infection of adult mice after intraperitoneal or intracerebral inoculation, but subclinical infection is established regularly with as little as 1 to 10 $TCID_{50}$(2). The dose used in the experiment reported here produced no overt disease, but produced chronic subclinical infection of submaxillary salivary

TABLE I. Virus Isolation Attempts and Presence of Inclusion Bodies in Individual Mice Sacrificed at Intervals after Intraperitoneal MSGV Inoculation as Weanlings.

Days after inoculation	Virus titer* in submaxillary gland, Mouse No. 1	2	3	4	Inclusion bodies in submaxillary gland, Mouse No. 1	2	3	4	Virus in mouth swab, Mouse No. 1	2	Virus in urine, Mouse No. 1	2
1	$<10^0$	$<10^0$			0	0			0	0	0	0
4	"	"			0	0			0	0	0	0
6	"	"			0	0			0	0	0	0
8	10^4	10^4			+	+			+	+	0	0
12	$\geq 10^6$	$\geq 10^6$			++	++			+	+	+	+
18	10^4	10^4			+++	+++			+	+	0	0
36	10^5	10^3			+++	0			+	+	0	0
60	10^3	$<10^0$			++	0			0	0	0	0
120	"	10^2	10^5	10^4	0	0	0	0				
240	"	10^3			0	0						
365	10^2	"	10^2		0	0	0		+	+	0	0

* Titer expressed as No. of tissue culture doses/0.1 ml of 10% gland suspension.

glands of at least one year's duration. On the basis of inclusion bodies, one might have concluded that the infection disappeared between the 60th and 120th days, but virus was present in moderate titer from 120th to 365th day, and at least some mice were still excreting virus in the saliva. This finding implies that absence of inclusion bodies in adult human salivary glands should not *per se* be considered proof of the absence of virus; however, attempts to recover virus from saliva and parotid glands of a small number of adult humans have been unsuccessful(1, and unpublished). Failure to recover virus from urine of mice after the acute phase of infection is in marked contrast to the high frequency of recovery of human salivary gland virus from urine of children months after initial infection. This may be related to failure of the mouse virus to produce lesions in the renal tubules(4), as opposed to their frequent presence in infected children(5).

Our data indicate that MSGV would provide a rather unique experimental model for study of a localized, subclinical virus infection. Of particular interest would be a study of experimental conditions that might activate the virus to produce disseminated disease. Such a study might have bearing upon human cytomegalic inclusion disease since dissemination of the human salivary gland virus is often noted in the presence of stress conditions such as prematurity, pertussis, and malignant disease(5).

Summary. Inoculation of weanling mice with a sublethal dose of MSGV produced chronic, subclinical infection of submaxillary gland of at least one year's duration. Virus was isolated from urine during the acute stage of infection and from saliva during both acute and chronic stages. Inclusion bodies in the salivary gland were not seen after the 60th day of infection.

1. Rowe, W. P., Hartley, J. W., Cramblett, H. G., Mastrota, F. M., *Am. J. Hyg.*, 1958, v67, 57.

2. Hartley, J. W., Doctoral Dissertation, George Washington Univ., Oct. 19, 1957.

3. Smith, M. G., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 435.

4. McCordock, H. A., Smith, M. G., *J. Exp. Med.*, 1936, v63, 303.

5. Smith, M. G., Vellios, F., *A.M.A. Arch Path.*, 1950, v50, 862.

Received September 12, 1958. P.S.E.B.M., 1958, v99.