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Salivary Excretion of Coxsackie B-1 Virus in Rabbits

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ABSTRACT

MADONIA, JOHN V. (Northwestern University, Chicago, Ill.), ARTHUR N. BAHN, AND JOSEPH C. CALANDRA. Salivary excretion of Coxsackie B-1 virus in rabbits. Appl. Microbiol. 14:394–396. 1966.—Coxsackie B-1 virus was injected into the ear vein of albino doe rabbits. Saliva and blood samples were taken before the injection of virus and at specific times thereafter. Virus was recovered in the whole saliva when the blood titer was approximately 10^4 $TCID_{50}$ per 0.1 ml or greater. The virus could be detected in the saliva as early as 2 min after the initiation of the viremia. The recovered virus was shown to be the same as the injected virus by serological identification of the recovered virus with neutralizing antibody for Coxsackie B-1 virus. These results suggest that virus may be transmitted to other animals in the saliva of animals who are in the viremic phase of infection without infection of the oropharyngeal tissues.

An excretory function has been attributed to the salivary glands for a number of years. The composition of saliva has been shown to reflect the presence of certain substances in the blood, such as antibodies, blood-grouping substances, inorganic ions, and serum proteins. Recently, Schein and Tung (5) reported the passage of foreign proteins from the circulating blood to the whole saliva. Radioactive labeled human albumin was injected intravenously into either rabbits or humans, and after 3 hr the labeled protein could be recovered in the whole saliva of the subjects. The albumin crossed the blood barrier and passed into the saliva. The limits of this excretory function of saliva have never been outlined, and the size of a particle that can be excreted into the saliva has not yet been determined. Viruses, on the other hand, have been shown to pass a number of tissue barriers such as skin (2), placenta (7), and through the kidney into the urine (3, 6). This investigation describes the passage of a virus from the blood stream into whole saliva as a result of an artificially produced viremia.

MATERIALS AND METHODS

Unstimulated saliva. Coxsackie B-1 virus, suspended in tissue culture maintenance medium consisting of Eagle's basal medium (BME) in Hank's balanced salt solution with 2% calf serum and fortified with 200 units per ml of penicillin, 200 μg/ml of streptomycin, and 5 μg/ml of amphotericin B, was injected into the marginal ear vein of albino doe rabbits weighing approximately 4 kg. The inoculum varied in concentration from 10^3 to 10^9 $TCID_{50}$ depending on the experiment. Saliva and blood samples were taken before initiating the viremia and at varying intervals thereafter. Unstimulated saliva samples were taken by swabbing the mouth of the animal with a cotton-tipped applicator. The applicator was then washed in 1 ml of maintenance medium. Blood samples from rabbits were taken from the marginal ear vein opposite to the ear used for inoculation. The blood was allowed to clot, and the serum fraction was collected by centrifugation. The serum and maintenance medium were then frozen and stored at −70 C for virus isolation.

Stimulated saliva. To quantitate the excretion of virus, it was necessary to collect at least 1 ml of saliva. Saliva was collected by using pilocarpine nitrate as a salivary gland stimulant. In all experiments, a dosage of 1.5 mg per kg of body weight was administered intravenously. Whole saliva was aseptically collected in a clean petri dish as it flowed directly from the mouth. Blood specimens were collected in the same manner as in the experiments with unstimulated saliva. All specimens were stored in the frozen state at −70 C.

Virus isolation. All viral isolations were carried out with the use of human epidermoid carcinoma (HEp-2) tissue culture monolayers with a maintenance medium of 2% calf serum in BME with Hank's balanced salt solution and fortified with 200 units per ml of penicillin, 200 μg/ml of streptomycin, and 5 μg/ml of amphotericin B. Each of two tubes of HEp-2 monolayers was inoculated with 0.1 ml of serum. Maintenance medium used in washing the saliva-

TABLE 1. *Isolation of Coxsackie B-1 virus from unstimulated saliva of rabbits with a viremia*

Inoculum ($TCID_{50}$)	Blood*					Saliva*					Neutralization of recovered salivary virus with Coxsackie B-1 antisera
	0 min	30 min	60 min	120 min	180 min	0 min	30 min	60 min	120 min	180 min	
10^4	—	—	—	—	—	—	—	—	—	—	—†
	—	—	2+	—	—	—	—	—	—	—	
10^5	—	4+	4+	2+	2+	—	—	—	—	—	—†
	—	4+	4+	4+	4+	—	—	—	—	—	
10^6	—	4+	4+	4+	4+	—	—	—	—	—	—†
	—	4+	4+	4+	4+	—	—	—	—	—	
10^7	—	4+	4+	4+	4+	—	4+	4+	4+	—	+
	—	4+	4+	4+	4+	—	4+	4+	4+	—	
10^8	—	4+	4+	4+	4+	—	4+	4+	4+	4+	+
	—	4+	4+	4+	4+	—	4+	4+	4+	4+	
10^9	—	4+	4+	4+	4+	—	4+	4+	4+	4+	+
	—	4+	4+	4+	4+	—	4+	4+	4+	4+	

* Symbols: 4+ = complete destruction of tissue culture monolayer; 2+ = 50% destruction of monolayer; — = no observable cytopathic effect in the monolayer.

† No salivary recovered virus.

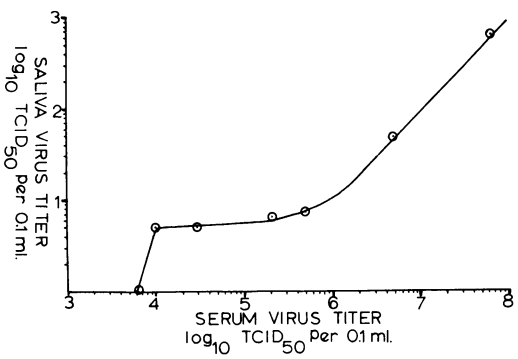

FIG. 1. *Relationship between the serum and salivary recovered virus. Saliva was stimulated with 1.5 mg of pilocarpine nitrate per kg of body weight.*

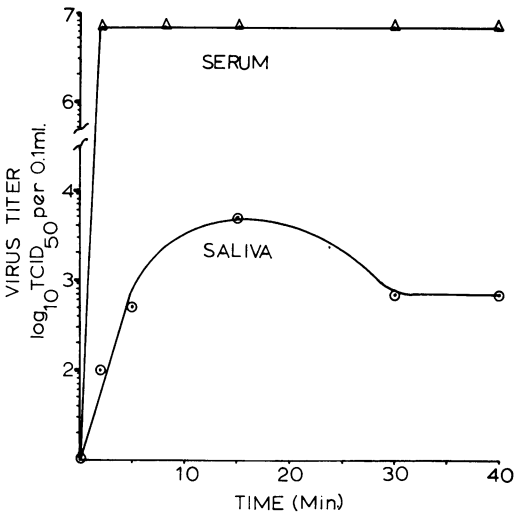

FIG. 2. *Titer of virus in both serum and saliva during 40 min after the establishment of viremia.*

soaked swabs was placed into two more monolayer tubes replacing the previous maintenance medium. All tubes were observed after 72 hr for a cytopathic effect typical of Coxsackie B-1 virus. When quantitation of the specimen was desired, the specimen was serially diluted, and the tissue culture infective dose 50% end point ($TCID_{50}$) was determined by the Reed-Muench method (4).

In the initial experiments, the virus recovered from the saliva was identified as the same virus injected intravenously by characterization with specific antisera against Coxsackie B-1. The antisera were prepared from rabbits inoculated with the same strain of virus used to initiate the viremias.

RESULTS

Table 1 shows the recovery of Coxsackie B-1 virus from the saliva of rabbits at times from 0 to 180 min and at an inoculum dose of 10^4 to 10^9 $TCID_{50}$. An inoculum of at least 10^4 $TCID_{50}$ was required before a viremia could be detected at 60 min. An inoculum of 10^7 $TCID_{50}$ was required before virus could be detected in the saliva at 30 min. All viruses recovered from the saliva were neutralized by antisera against Coxsackie B-1. It was previously determined that an inoculum of 10^7 $TCID_{50}$ will give a blood titer of approximately $10^{4.5}$ $TCID_{50}$ per 0.1 ml after 30 min. Therefore, it can be approximated that virus was excreted into the saliva when the blood titer was at least $10^{4.5}$ $TCID_{50}$ per 0.1 ml. An accurate determination of the relationship between the blood titer and the salivary titer can be seen in Fig. 1. The salivary titer increased with the blood

titer. Between 10^4 $TCID_{50}$ per 0.1 ml and $10^{5.2}$ $TCID_{50}$ per 0.1 ml, the saliva titer increased only slightly as compared with the increase in serum titer. After $10^{5.5}$ $TCID_{50}$ per 0.1 ml, the increase in salivary titer was more pronounced. A blood titer of between $10^{3.6}$ and 10^4 $TCID_{50}$ per 0.1 ml was required to recover virus in the saliva. A smaller blood titer was necessary to detect the presence of virus in the saliva than was observed in the unstimulated saliva experiments. This may be because the quantity of saliva assayed with stimulated saliva was greater and the chances for viral isolation were increased. Figure 2 shows the time course of blood and saliva titers. The blood titer remained constant throughout the 40-min period. Virus was recovered in the saliva as early as 2 min after injection of the virus into the ear vein of the rabbit. The salivary titer rises to a peak at about 16 min and then falls.

DISCUSSION

These results have shown that blood-borne Coxsackie B-1 virus in the rabbit is excreted into the saliva in less than 2 min after the establishment of a viremia of approximately 10^4 $TCID_{50}$ per 0.1 ml. Coxsackie B-1 virus is not infective for the rabbit, and the virus need only be present in the blood for less than 2 min. There appears to be a physical passage of the virus from the blood to the saliva, and the virus is not present in the saliva as a result of an infective process. In the present study, all viral isolations were made from the whole saliva. Two possible methods of transmission of the virus from the blood to the saliva exist: the virus may pass from the blood through the salivary glands into the oral cavity, and the virus may pass from the blood stream across other structures in the oral cavity such as the oral mucosa or the periodontal membrane. Both of these methods of transmission are being investigated.

Larkin and Schultz (3) reported the recovery of Coxsackie B-1 virus from the urine of dogs whenever there was a serum titer of at least 10^2 $TCID_{50}$ per 0.1 ml. A urine titer of $10^{1.5}$ $TCID_{50}$ per 0.1 ml was recovered when there existed a viremia of $10^{3.5}$ $TCID_{50}$ per 0.1 ml. The recovery of virus was accomplished as early as 3 min after the initiation of the viremia. The results of the present study with saliva are in agreement with this passage of virus to the urine, except that more virus was recovered from the urine by Larkin and Schultz, and a higher serum titer was required before virus could be detected in the saliva in the present study. These differences may be due to the homogeneous nature of urine as compared with the heterogeneous nature of whole saliva. Whole saliva is a mixture of a number of different capacities for virus excretion.

It seems logical that this process must occur in the infected animal since we have demonstrated that it occurs in the uninfected animal. A virus, if it is similar to Coxsackie B-1, may pass from the blood stream into the saliva during the viremic phase of an infection. Experimental infections in monkeys with virulent poliovirus have yielded viremias with viral titers as high as 10^7 $TCID_{50}$ per ml (1). Poliovirus is related to Coxsackie virus in size and other infective characteristics. This titer of viremia is well within the minimal range for the recovery of virus in the saliva. Saliva is a bacterial and viral vectoring medium in the transmission of viral infection by aerosol or through contamination of inanimate objects. Since the viremic phase of virus infection occurs primarily prior to overt clinical symptoms, the patient and physician are not aware of illness and therefore do not attempt to control the spread of infection. In view of these findings saliva may play a more important role in the epidemiology of virus infection.

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