

## Herpesvirus Type 2: Association with Carcinoma of the Cervix

**Abstract.** By utilizing the kinetics of neutralization, it was found that an antibody response to type 1 (oral) and type 2 (genital) herpesvirus infection could be independently measured and correlated with type of virus isolated from the patient. The presence of antibodies to type 1 and type 2 herpesvirus was examined in patients with carcinoma of the cervix, in matched controls, random controls, and patients with other types of malignancy. Antibodies to type 2 were found with greater frequency in patients with carcinoma of the cervix (83 percent) than in the other groups tested (0 to 20 percent). The data suggest an association of genital herpesvirus and carcinoma of the cervix.

Epidemiologic studies of carcinoma of the cervix have revealed that the factors of uncircumcised male partners (1), multiple marriages, multiple sex partners, and early age of first coitus are associated with an increased incidence of the disease (2). These studies have led to the hypothesis that an etiologic factor of carcinoma of the cervix may be venereally transmitted. In our earlier study, in which attempts were made to isolate viruses from cervical tissues showing dysplastic changes and from smegma samples, only herpesviruses were isolated from smegma samples (3). The herpesviruses isolated were found to be antigenically and biologically distinct from viruses isolated from oral lesions, an observation also reported by others (4). Oral strains are classified as type 1, genital strains as type 2. To investigate further the possible carcinogenic role of herpesvirus type 2, a study was designed to determine whether patients with carcinoma of the cervix possessed antibody to the herpesvirus type 2 at a greater frequency than persons without the disease.

Samples of serum were collected from women with histologically proven, invasive carcinoma of the cervix in the active stage or from those who had been treated for this disease within the preceding 12 months. Serum was also obtained from women of the same socioeconomic class who were matched in age, race, and geographic area with the women with carcinoma of the cervix. Women in the matched group were selected from those consulting gynecologists for diseases other than malignancy. In addition, serum was obtained from men with carcinoma, from women with carcinomas of sites other than the cervix, and from children and adults of other socioeconomic classes. Patients with oral or genital herpetic lesions, or both, diagnosed clinically or by viral isolation were also studied.

Serum was removed from clotted blood and stored at  $-20^{\circ}\text{C}$  until tested, when it was heated at  $56^{\circ}\text{C}$  for

30 minutes. Neutralization tests were performed as follows: 0.3 ml of type 1 or type 2 virus stocks containing about  $5 \times 10^5$  plaque-forming units of virus, which had been prewarmed to  $37^{\circ}\text{C}$ , was mixed with 0.3 ml of a 1:8 dilution of serum that had also been prewarmed to  $37^{\circ}\text{C}$ . The diluent used was Eagle's medium supplemented with 2 percent fetal bovine serum, penicillin (100 unit/ml), and streptomycin (100  $\mu\text{g}/\text{ml}$ ); the diluent also contained 1.5 g of sodium bicarbonate per liter. The virus-serum mixture was held for 2

minutes at  $37^{\circ}\text{C}$ ; a sample was removed, diluted 1:100 in cold diluent, and assayed for surviving virus. Simultaneous control assays of virus plus diluent were performed with each test. Neutralization of rubella virus and measles virus by selected serums was also examined for control purposes. The source of these viruses and the assay methods employed are described elsewhere (5). The test measured the rate of neutralization of the virus and the results are expressed as  $K$  values (see footnote to Table 1) without normalization (6).

Table 1 gives data obtained with serums from patients with known oral or genital herpes infections, or both. Serums from three patients who had experienced both oral and genital herpes infections rapidly neutralized both types of herpesvirus; serums from four patients with known genital infections neutralized only type 2 virus. Serums from two children in both the acute and convalescent stages of herpetic gingi-

Table 1. Neutralization of types 1 and 2 herpesvirus by serum from patients with herpetic lesions. All serum samples were obtained three or more weeks after illness except where otherwise designated for patients 8 and 9.

Patient No.	Age (yr)	Clinical illness (by examination and/or history)	Type of herpesvirus isolated*	K value†	
				Type 1	Type 2
1	41	Oral and penile	Type 2 from penis	8.7	5.5
2	38	Oral and vaginal	Type 1 from lip	8.7	4.9
3	52	Oral and vaginal	Type 1 from lip	7.4	5.0
4	25	Vaginal	Type 2 from vagina	<1	3.7
5	29	Vaginal	Type 2 from vagina	<1	5.0
6	5 mos.	Neonatal herpes	Type 2 from skin	<1	12.0
7	22	Mother of patient 6	Isolation not attempted	<1	6.6
8	2	Acute gingivostomatitis	Type 1 from mouth	<1‡ 5.4	<1 2.0
9	10	Acute gingivostomatitis	Type 1 from mouth	<1‡ 5.9	<1 1.2

\* Herpesvirus isolates typed by biologic and antigenic characteristics (Figuroa and Rawls).

$$\dagger K = \frac{\text{Dilution of antiserum (8-fold)}}{\text{Time (2 minutes)}} \cdot 2.3 \log \frac{\text{Virus titer 0 time}}{\text{Virus titer 2 minutes}}$$

‡ First serum samples obtained at onset of illness; second samples 4 weeks later.

Table 2. The occurrence of type 1 and type 2 antibodies to herpesvirus in patients with carcinoma of the cervix and other groups.

Source of serums	Number with herpesvirus type 2 antibodies*	Number without herpesvirus type 2 antibodies	Percent positive (type 2)	Percent positive (type 1)
Invasive carcinoma of the cervix†	15	3	83	85
Matched controls	9	35	20	80
Malignant tumors of noncervical sites	1	10	9	90
Random controls:				
Adults (20-59 yr)	0	10	0	100
Children (0-10 yr)	0	27	0	48

\* A  $K$  value of 4 or greater was taken as evidence of antibodies to the genital type of herpesvirus.

† Antibodies to genital herpesvirus significantly greater in this group than in matched control group. Chi square:  $P < .001$ .

vostomatitis were tested and predominantly type 1 antibodies developed, although low levels of type 2 neutralizing activity also appeared. These data suggest that antibody activity to the two types of virus could be measured independently by the kinetics of neutralization.

The variability of the test procedure was examined by repeatedly testing four serums that rapidly neutralized both types of virus. The mean *K* value of 26 determinations made with the type 1 oral isolate was 10.4, with a standard deviation of 2.8; that of 27 determinations made with the type 2 genital isolate was 9.3, standard deviation 2.7. The coefficient of variation of the 53 determinations was 28 percent.

Analysis of serums from various groups of individuals revealed a very high incidence of antibodies to the genital type of herpesvirus in patients with invasive carcinoma of the cervix (Table 2). These patients were from the lower socioeconomic class, and the incidence of type 2 antibodies was higher in women from the same class without the disease than in persons from the higher socioeconomic classes. However, the incidence of antibodies was significantly greater in the carcinoma patients than in the matched control group ( $P < .001$ ). Absence of antibodies in the serums from children and adults from the higher socioeconomic group supports the venereal mode of transmission of the genital type of herpesvirus.

Analysis of three serums, two from women with carcinoma of the cervix and one from patient number 6 (Table 1), by sucrose density gradient centrifugation revealed that the neutralizing activity against type 2 herpesvirus resided with the 7S globulins. The kinetics of neutralization of two other lipid-containing enveloped viruses, rubella and measles, was similar for serums from women with carcinoma of the cervix and for serums from their matched controls.

The results of this preliminary study indicate that women with carcinoma of the cervix have a significantly higher incidence of type 2 antibodies than women from the same segment of the population who do not have the disease. In 15 of 18 patients studied, type 2 *K* values of 4 or greater were found. Type 2 antibody activity was present in three other patients, but it was of such a low level that it may have represented cross reactivity with type 1 antibody. These three patients had systemic

metastasis of their malignancy. Type 2 herpesvirus neutralization was found to be associated with 7S globulins and was not present with a high frequency in persons with other types of carcinomas. Virus neutralization of two other lipid-containing viruses was not observed in unusual frequency with serums from the patients with carcinoma of the cervix.

The present findings are compatible with a carcinogenic or co-carcinogenic role of type 2 herpesvirus in carcinoma of the cervix. Other evidence suggesting an etiologic role of herpesvirus in carcinoma of the cervix includes the report by Naib (7) who found a 7 percent incidence of *in situ* carcinoma among women with genital herpes, while only 0.6 percent of the women without genital herpes was found to have this lesion. The epidemiologic features of carcinoma of the cervix point to a venereally transmitted agent (1, 2). Genital herpesvirus also appears to be venereally transmitted (8). An unexplained observation in the epidemiology of carcinoma of the cervix is the low frequency of the disease among women who consort with circumcised males (1). Herpes progenitalis is uncommon among circumcised males and occurs primarily in uncircumcised males (8, 9). Thus, the epidemiologic features of genital herpesvirus are in keeping with those of carcinoma of the cervix and complement the serologic data presented in this report.

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#### Program Clocks in Small Mammals

**Abstract.** *Complex patterns of time, direction, and speed of running by small nocturnal mammals in activity wheels sometimes are duplicated almost exactly from night to night. These activity pattern repetitions disclose: (i) previously unknown capabilities of biological clocks to act as sequence programmers for behavior; (ii) that animals can retain a record of the sequence and timing of their activities covering an entire night; and (iii) that the activities of one night can bias an animal toward similar behavior on subsequent nights.*

Given access to an activity wheel, small mammals spend almost all their active time running it (1, 2). In effect, this single activity substitutes for most of the locomotor and manipulatory activities of animals in the wild. If the instantaneous speed and direction of running are recorded every few seconds on a moving chart (1, 2), the activity patterns obtained rival sonographs of birdsong in their richness of individual and species detail. Performance properties, such as time spent running, speed, lengths of nonstop sessions, and directional consistency usually depend sensitively on the state of the animal and environmental variables, particularly ambient light (2). A chief finding of earlier studies is that the animals often run in the same direction all night, even though they start and stop running hundreds of times. They get their bearing for this orientation chiefly from sightings of nearby objects (2).

In locomotion studies in complex burrow-simulating mazes, white-footed mice, genus *Peromyscus*, readily learned their way back and forth along routes having hundreds of turns and blind alleys (3). Together, these studies of locomotion reveal a strong tendency toward conservatism or stereotypy of movement patterns and an impressive ability to learn complex sequences of movements and to keep track of surroundings, position, and direction. Presumably, the more conservative its movement patterns, the more readily