Latent virus reactivation was measured in 17 astronauts (16 male and 1 female) before, during, and after short-duration Space Shuttle missions. Blood, urine, and saliva samples were collected 2-4 months before launch, 10 days before launch (L-10), 2-3 hours after landing (R+0), 3 days after landing (R+14), and 120 days after landing (R+120). Epstein-Barr virus (EBV) DNA was measured in these samples by quantitative polymerase chain reaction. Varicella-zoster virus (VZV) DNA was measured in the 381 saliva samples and cytomegalovirus (CMV) DNA in the 66 urine samples collected from these subjects. Fourteen astronauts shed EBV DNA in 21% of their saliva samples before, during, and after flight, and 7 astronauts shed VZV in 7.4% of their samples during and after flight. It was interesting that shedding of both EBV and VZV increased during the flight phase relative to before or after flight. In the case of CMV, 32% of urine samples from 8 subjects contained DNA of this virus. In normal healthy control subjects, EBV shedding was found in 3% and VZV and CMV were found in less than 1% of the samples. The circadian rhythm of salivary cortisol measured before, during, and after space flight did not show any significant difference between flight phases. These data show that increased reactivation of latent herpes viruses may be associated with decreased immune system function, which has been reported in earlier studies as well as in these same subjects (data not reported here).