ers.” This is not necessarily correct. Percy et al. (1) have reported that the detection rate (the proportion of hospital diagnoses of NPC in which the death certificate reflects the same diagnosis) and confirmation rate (the proportion of NPC deaths on death certificates that are also specified as NPC in hospital records) were poor. These rates for NPC (65.5% and 78.6%, respectively) are low compared with some other cancers, such as breast and lung cancers, where both rates are over 90% and indicate that, contrary to the implication by Lucas, relying upon death certificates to identify NPC may actually result in slightly fewer cases of NPC than from hospital-based diagnoses. This is because 34.5% of the NPC cases in hospital records are missed on death certificates, but only 21.4% of the death certificates that list NPC as the cause of death are inappropriately coded.

The major error made by Lucas, however, is that she corrects only the death certificate diagnoses in the cohort and ignores the comparison population, which, in the NPC analysis of the National Cancer Institute’s formaldehyde study (2), was the U.S. general population. The guiding principle in disease assessment in cohort studies is that diagnostic procedures applied to the cohort of interest must be the same as those applied to the referent population. The approach proposed by Lucas, i.e., removal of causes of death inaccurately classified as NPC in the cohort only, would actually bias the relative risk estimate downward because of the failure to correct for similar misclassification in the general U.S. population statistics.

We agree that more accurate diagnosis of disease in cohort studies is desirable, but the suggestion of Lucas that investigators confirm all NPC cases in studies of formaldehyde is inappropriate, unless this procedure is applied to the comparison population as well.

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Molecular Variant Analysis as an Epidemiological Tool to Study Persistence of Cervical Human Papillomavirus Infection

There is now strong evidence that the human papillomavirus (HPV) is the sexually transmissible agent responsible for the initiating steps in cervical carcinogenesis (1). The risk seems to be considerably increased if a woman has persistent cervical HPV infection (2). To address this question, we used DNA sequencing techniques to detect molecular variants of HPV in a follow-up study of a group of 56 asymptomatic female participants in a survey in northeastern Brazil (3). These variants are identified on the basis of mutational patterns in the DNA, thus allowing interpretation of persistence on firmer grounds. All subjects in the study provided their written consent after being informed about the investigation. The study was approved by an institutional review board.

HPV detection and typing in cervical cell specimens were done by a consensus-primer polymerase chain reaction (PCR) technique followed by hybridization of the PCR product with specific probes for 25 individual HPV types (1,4,5). If a woman had the same HPV type during follow-up, we cloned and sequenced segments from the long-control region (LCR) or from the L1 gene. For HPV 16, we used a PCR sequencing method with primers flanking a 364 base pair (bp) LCR segment (nucleotide positions 7478 to 7841), a hypervariable genomic segment of HPV (6). These primers have 5' extended ends representing target sites for restriction endonucleases, Asp 718 and Xba I, that permit cloning into the corresponding sites of the plasmid pUC19 (6). The amplified fragment was separated by gel electrophoresis, digested with KpnI and XbaI ligated to pUC18/19, restricted with the same enzymes, and introduced into Escherichia coli XL Blue. Recombinant plasmid DNAs were isolated from two positive clones and sequenced (sequenase 2.0 DNA sequencing kit; U.S. Biochemical Col., Cleveland, Ohio). For types other than HPV 16, we compared isolates from the same patient by sequencing the 450 bp L1 region amplified in the PCR protocol described above to detect HPV (4).

The 56 women returned one or more times for a total of 66 additional visits over a period of 21 months for follow-up cervical specimens. Of 40 assessable subjects, 10 women were positive for HPV 16 in the first or subsequent visits. Only three women had two consecutive positive specimens. Of these, one subject had different HPV types, one had the same type (HPV 16) in both visits, and, in the other, both specimens tested positive for an unidentified type. Different variants were present in the only presumably persistent HPV 16 case (Fig. 1). The isolate obtained at her first visit had the LCR mutation pattern of a common Brazilian variant, whereas the second isolate, obtained 7 months later, was identical to the HPV 16 prototype, which is also common in Brazil. These two isolates differed in nine nucleotide positions (7483, 7487, 7519, 7667, 7687, 7727, 7741, 7762, and 7784; the latter six nucleotide positions are shown in Fig. 1). No sequence differences were found between the two isolates from the other case with the unidentified HPV type.

Although based on a small sample of women, our findings suggest that the occurrence of persistent HPV infections (2.5%; 95% confidence interval = 0.1%-11.7%) may be in fact lower than that measured in studies searching for viral types only (5,7-9). Epidemiologic studies of molecular variants of HPV may contribute to our understanding of viral infectivity and oncogenicity. Al-
though laborious, the sequencing technique we used provides a finer level of detail than single-strand conformation polymorphism (10) and restriction fragment length polymorphism analyses. Two important issues that need to be investigated are the significance of persistent infections by individual variants concerning risk of cervical neoplasia and the determinants for their acquisition. We are currently addressing these questions in ongoing cohort studies in São Paulo (Brazil) and Montreal (Canada).

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