Urinary Virus Excretion in Pregnancy

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SUMMARY
In a series of 774 women with pregnancies ranging from 7 weeks to term, virus particles representing five different groups have been detected in urine specimens by negative staining electron microscopy. Of the virus-positive women, 0.39% excreted papillomavirus (in the absence of genital warts), 3.1% polyomavirus, 0.26% adenovirus type II, 0.65% an antibody-coated unidentified virus particle and 7.7% a membrane-associated virus-like particle. The implications of these findings are discussed in the light of the apparently increased susceptibility of pregnant women to virus infection.


The excretion of human polyomavirus in urine in a variety of immunodeficiencies and following organ transplantation is well documented and has stimulated a search for these and other viruses during pregnancy, when susceptibility to virus infections appears to be increased. Coleman et al. reported the presence of JC polyomavirus, an agent normally associated with neurological disease, in the urine of a pregnant woman and we have recently detected polyomavirus in approximately 3.3% of pregnant women in the H. F. Verwoerd Maternity Hospital, Pretoria. Rubella and cytomegalovirus (CMV) are associated with neonatal abnormalities. The present study was undertaken to ascertain the spectrum and possible significance of viral agents detectable in pregnancy by negative-staining electron microscopy of urine sediments.

SUBJECTS AND METHODS
Urine specimens were taken from Black and White women attending the antenatal clinic for the first time. The stage of pregnancy varied from 7 weeks to term. Both multiparous and nulliparous women were screened. As far as possible, urine specimens were processed soon after receipt to minimize contamination, although this does not materially affect virus identification. Urine specimens taken from 50 randomly selected non-pregnant maternity hospital nursing staff and patients attending the family planning clinic served as controls. Ten millilitre quantities of urine were centrifuged at 100 000 g for 2 hours on a Damon IEC B-60 ultracentrifuge. The resultant pellet was resuspended in distilled water and centrifuged again. The washed pellet was then dissolved in a small quantity of distilled water and a drop of this suspension was mixed with a drop of 3% phosphotungstic acid. A drop of this mixture was then applied to a Formvar carbon-coated copper grid, the excess removed with filter paper, and the dry grid examined using a Philips EM 300 electron microscope at an instrumental magnification of 42 000 and an accelerating voltage of 60 kV. Where possible, repeat specimens of urine of patients who were found to excrete virus or virus-like particles were examined. The pregnancies and pregnancy outcome of virus-positive patients were evaluated.

RESULTS

Virus Identification
The series of pregnant patients included 724 Whites and 50 Blacks of whom a total of 83 were found to excrete one or more viruses or virus-like particles. The spectrum of viruses detected in this investigation represents five virus groups including papillomavirus, polyomavirus, adenovirus type II, an antibody-coated unidentified virus particle with an enterovirus morphology and size, and a newly recognized particle with a distinct virus morphology which we have provisionally designated 'praegnavirus' as a result of its high incidence in pregnancy urine, in comparison with certain disorders in immunity in which it occasionally occurs. A detailed account of the structure of this particle will appear elsewhere. No virus was found in controls. Table I represents the distribution of the different virus groups while Fig. 1 represents the virus forms detected. All the papillomavirus positive specimens were from White women who had no genital warts although they excreted the virus in quantities readily detectable by electron microscopy. All have since given birth to normal offspring. Polyomavirus occurred in a total of 24 women (3.1%), while adenovirus type II was found in 1 White patient and in 1 Black patient who excreted four different virus particles simultaneously, including polyomavirus and 'praegnavirus'. The antibody-coated particle, which indicated seroconversion in the mothers, was excreted by 3 White and 2 Black women (0.65%). The size and morphology of the latter particle resembled those of the enterovirus group. The most commonly excreted particle, 'praegnavirus', was detected in 55 White and 5 Black women (7.7%). Efforts to culture the particle in fungal medium were unsuccessful, making it unlikely that it is a fungal agent while its structure and membrane association are characteristic of animal virus particles. Its size, structure and association with membranous vesicles rule out the possibility of it being a bacteriophage. In addition 'praegnavirus'-positive urine specimens failed to produce significant bacterial growth.
### TABLE I. VIRUS FORMS DETECTED BY ELECTRON MICROSCOPY (PREGNANCY URINE SEDIMENTS)

<table>
<thead>
<tr>
<th></th>
<th>Papillomavirus</th>
<th>Polyomavirus</th>
<th>Adenovirus type II</th>
<th>Antibody-coated virus</th>
<th>'Praegnavirus'</th>
<th>Total* virus-positive women</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>3</td>
<td>21</td>
<td>1</td>
<td>3</td>
<td>55</td>
<td>75 (10.4%)</td>
</tr>
<tr>
<td>Black</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>8 (16.0%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3</strong></td>
<td><strong>24</strong></td>
<td><strong>2</strong></td>
<td><strong>5</strong></td>
<td></td>
<td><strong>83 (10.6%)</strong></td>
</tr>
<tr>
<td>% positive (W + B)</td>
<td><strong>0.39</strong></td>
<td><strong>3.1</strong></td>
<td><strong>0.26</strong></td>
<td><strong>0.65</strong></td>
<td></td>
<td><strong>7.7</strong></td>
</tr>
</tbody>
</table>

*Total number of women investigated: 724 White, 50 Black. Some women excreted more than one virus type.

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**Pregnancy and Pregnancy Outcome**

No significant difference between the virus-positive group and the rest of the hospital patient population was found in respect of age, parity, blood group distribution or rubella titre. No case of severe pre-eclampsia or antepartum haemorrhage occurred in the virus-positive women. The incidence of other complications of pregnancy including mild pre-eclampsia, polyhydramnios, anaemia and nonspecific vaginal discharge in the two groups was remarkably similar, as was the sex ratio and birth weights of the infants. There was one set of twins. No perinatal deaths occurred. Except for one case each of familial aniridia, undescended testis and anal fistula, no congenital abnormalities were noted. There were two cases of ABO incompatibility, one of which required exchange transfusion.

**DISCUSSION**

The absence of any virus particle in our age-matched non-pregnant controls suggests that the incidence of virus excretion in urine during pregnancy is significant. The presence of papillomavirus in urine in the absence of genital warts has to our knowledge not been previously...
reported and suggests that this virus may play a role in lesions of tissues other than the skin and genital and laryngeal mucosa, where it induces papillomas. Zur Hausen has recently drawn attention to the biochemical and serological differences existing between different human wart viruses and the possible role these might play in cervical cancer. He has suggested that more human papillomavirus serotypes exist, a contention borne out in this work. The 3% incidence of human polyomavirus in our study and the 5% incidence reported by Gardner et al. are worthy of note. These newly recognized human viruses, particularly JC polyomavirus, are highly oncogenic in animals and capable of transforming human cells in culture. The association of JC polyomavirus with neurological disease, its presence in disorders of the immune system, after kidney transplantation, and in pregnancy urine may be of significance. These oncogenic viruses in the presence of an immune disorder suggest a possible relationship with tumour incidence. Since JC polyomavirus shows a specific affinity for nervous tissue for its growth and BK polyomavirus shows a similar affinity for the urothelium, some relationship may exist between these viruses and conditions such as neuroblastoma and Wilms' tumour. Adenovirus type II has been associated with haemorrhagic cystitis and we have reported a similar association in patients after renal transplantation, although no such association was evident in the present study. However, adenovirus type II has recently been classified as having oncogenic potential, while other studies indicate that one particular subtype is associated with haemorrhagic cystitis while others, including the prototype, are not.

The antibody-coated particle, although unidentified owing to unsuccessful efforts at culturing the virus, indicates seroconversion in the mothers and consequently the virus must be labelled a human agent. Since it resembles the enteroviruses in size and structure, its presence in urine suggests that it may represent an unidentified serotype. Efforts to grow this agent in the laboratory are continuing.

We have recently detected the 'praegnavirus' particle in the urine of 1 case of pernicious anaemia, 1 case of Fanconi's syndrome and in 3 renal transplant recipients. Its structure and size suggest that it is viral in nature and its relatively high incidence in pregnancy urine makes its further characterization essential. Several women who excreted the particle remained positive for up to 1 month after the initial detection of the particle, suggesting that its appearance is not a chance finding. Most virus-positive women have shown a transient appearance of this virus particle. Polyomavirus, adenovirus and herpesvirus in renal transplant recipients also appear transiently in urine. It is noteworthy that papillomavirus, polyomavirus and adenovirus type II are latent viruses capable of integration into host cell genomes and activated in immunosuppressed states, particularly after organ transplantation. All three viruses are DNA viruses and it is an open question whether the other viruses are capable of integration into host cell genomes. The vertical transmission of these viruses from mother to offspring is possible, since viruses are known to pass the placental barrier. Antibodies to polyomavirus have been demonstrated in a high proportion of children from the age of 1 year. The presence of these viruses in the urine of pregnant women supports the contention that pregnancy renders the mother more susceptible to virus infection, particularly as the latent viruses detected are those found in inherent or induced immunosuppressed states. Approximately 10% of human births are associated with one or more abnormalities of the newborn. The viruses of rubella and CMV play a significant role in this incidence. That other viruses do so cannot be ruled out. Our failure to detect CMV in the present study and its common occurrence in urine after renal transplantation suggests a difference in immune response in the two states. Similarly, papillomavirus which has not been detected in urine after renal transplantation, has not been detected in at least 3 pregnant patients. Conversely, adenovirus type II and polyomavirus are found in both pregnancy and renal transplantation. Although the particles detected in our study may prove to be 'orphan' viruses, having no apparent effect on the host, practically all human viruses are involved in some disease process, either as a result of the lytic cycle of replication or via cell transformation. This is particularly so when the activation of latent viruses is associated with one or other inherent immunodeficiency. Furthermore, fetal tissue, particularly the kidney, is an ideal site for virus establishment. Low concentrations of virus particles would be missed by our techniques, and consequently the incidences of the various viruses are probably somewhat higher than our survey suggests. In addition, the transient nature of virus excretion would also tend to lower the incidence, since patients were not continually monitored. No effect of the virus on the pregnancy or its outcome could be detected, although long-term effects cannot be ruled out.

REFERENCES