the carcinomas and of adjacent non-neoplastic oesophageal epithelium were examined histologically. Binucleation and/or perinuclear clearing similar to koilocytosis was noted in epithelium adjacent to carcinoma tissue in about 25% of the cases. However, distinctive changes identifiable as definite HPV effect were not seen. The complex of appearances attributable to HPV in the cervix—koilocytosis with nuclear atypia, multinucleation, papillomatosis, and basal cell hyperplasia—was not present in any case. On examination of the tissue for genus-specific HPV capsid antigen by the avidin-biotin immunoperoxidase method (Vector Laboratories, Burlingame, California) no case was positive, either in the tumour or in adjacent squamous epithelium.

DNA hybridisation studies were done in 10 cases of oesophageal carcinoma using a method developed for paraffin-embedded material, the accuracy being verified by one of us (J. K.) using tissue and smears of cervical HPV infections. The method involves a combination of extraction of nuclei from tissue and filter in-situ hybridisation (FISH). Hybridisation was done under stringent conditions, using a mixed probe of HPV types 11, 13, 16, and 18 obtained from Dr H. zur Hausen and Dr L. Gissmann (Deutsche KrebsforschungsZentrum, Heidelberg, West Germany). In 5 cases oesophageal carcinoma tissue was positive. 1 case was graded 3+ positive (figure, a), 1 as 2+ positive (c), and 2 cases showed only minor positivity (b and d). The DNA hybridisation method used here is being further evaluated for sensitivity and specificity. However, from these preliminary observations it seems that HPV DNA is present in some oesophageal carcinomas in a low-risk area and that HPV is worthy of further investigation as a possible aetiological factor in some oesophageal carcinomas.

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HLA PHENOTYPES OF DR2-NEGATIVE NARCOLEPSY PATIENTS

<table>
<thead>
<tr>
<th>Patient</th>
<th>HLA</th>
<th>DQ2-6 band</th>
<th>Cataplexy</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>1, 24, 55</td>
<td>4, 5</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>60, 61</td>
<td>1, 5</td>
</tr>
<tr>
<td>DR</td>
<td>52, 53</td>
<td>3</td>
<td>-</td>
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<tr>
<td>DRg</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>DQ</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DQw3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
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</tbody>
</table>

2 of our 4 patients had the full picture of narcolepsy, including cataplexy, and cannot be distinguished clinically from DR2-positive cases. However, the other 2 did not have cataplexy. 6 out of 6 additional patients from Prague with narcolepsy after organic brain damage (encephalitis or brain trauma) were positive for DR2 and all had cataplexy. Perhaps a genetic predisposition to narcolepsy existed before the organic brain damage, which may or may not have contributed to the onset of narcolepsy.
We have not identified any DR-β polymorphism which correlates with a subtype of DR2 in narcolepsy. However, with an EcoRI digest of genomic DNA and a DQ-β probe we have identified a 2.7 kb band, correlating with DR2 and DRw13 (a subtype of DRw6)\(^\dagger\), which is almost certainly identical to DQR2 -6\(^\dagger\) (figure). We have found this DQR2-6 band in all 27 DR2-positive patients tested at the DNA level so far, which confirms the data of Marcadet et al.\(^\dagger\) In the DR2-negative patients, however, we do not find this band (figure), except in the patient with DR4, DRw6, where it would be expected (on the DRw6 haplotype). In DR2-negative healthy controls we found the DQR2-6 band in 19 of 21 cases—i.e., much more frequently than in Marcadet’s controls. However, our DR2-positive healthy panel contained a high proportion of DR2 individuals who carried HLA-B7 (11 of 21), a combination strongly associated with DQR2-6.

We conclude that narcolepsy cannot be excluded just because the patient is negative for DR2 and that the DQR2-6 subtype of DQwl, which has been found to be highly associated with narcolepsy, is negative in the DR2-negative patients. It remains to be seen whether DR2-negative narcolepsy patients have any HLA antigens in common.

We thank Prof Per Peterson, Uppsala, for the DRβ and DQβ probes; Dr D. Cohen, Ms M.-P. Font, Dr E. Weiss, and Prof H. Wolf, Munich, for their help in the establishment of the technique; and Ms Annette Grooms for technical assistance. Rabbit complement was donated by Behring-Werke, Munich. Supported by DFG Sonderforschungsbereich 217, project A2.

**AMINOACID LOSSES ON HAEMOFILTRATION**

SIR—Intravenous aminoacid solutions are often given to patients with acute renal failure who cannot be fed enterally. Aminoacid losses on conventional haemodialysis are small\(^\dagger\) but little is known about losses during haemofiltration because the molecular weight cut-off for the filter (30 000 or more) is much greater than the molecular weights of individual aminoacids.

Continuous veno-venous haemofiltration (CVVHF) was used in the treatment of a 29-year-old woman in acute renal failure due to an exacerbation of Lyme disease and who was also unconscious due to vasogenic cerebral oedema. 24 litres of haemofiltrate were collected daily for 7 days via a Gambro FHSS haemofilter, the rate then being reduced to 12 litres per day for a further 7 days. During this period she received no oral feeding, remained anuric, and passed no faeces. She was parenterally fed, whilst on CVVHF, with 500 ml of 50% dextrose with insulin and added vitamins, 1 litre of ‘Vamin 9’ by continuous infusion over 24 h, and 290 ml of 20%. ‘Intralipid’ daily. Haemofiltrates were collected for each 24 hour period with a corresponding timed serum sample and assayed for aminoacid content (‘Chromaspek’; Rank Hilger, Margate). Samples were analysed daily for each week at the two different filtration rates, and the results are expressed as mean (and standard error) for 7 days. A

**SPIROCHAETES, LYME DISEASE, AND MULTIPLE SCLEROSIS**

SIR,—Dr Muhlemann and colleagues (May 10, p 1097) and Dr Fumara (Sept 6, p 575) state that we have proposed an association between multiple sclerosis (MS) and *Borrelia burgdorferi*, the causative organism of Lyme disease. This is not so. Lyme disease, being exclusively a tick-borne infection with characteristic epidemiological features (localised epidemics, restricted to persons in areas where Lyme disease is endemic, who have been labelled as “possible MS”, will ultimately be shown to have Lyme demyelinating encephalopathy. A search for these patients should be undertaken in endemic areas.

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