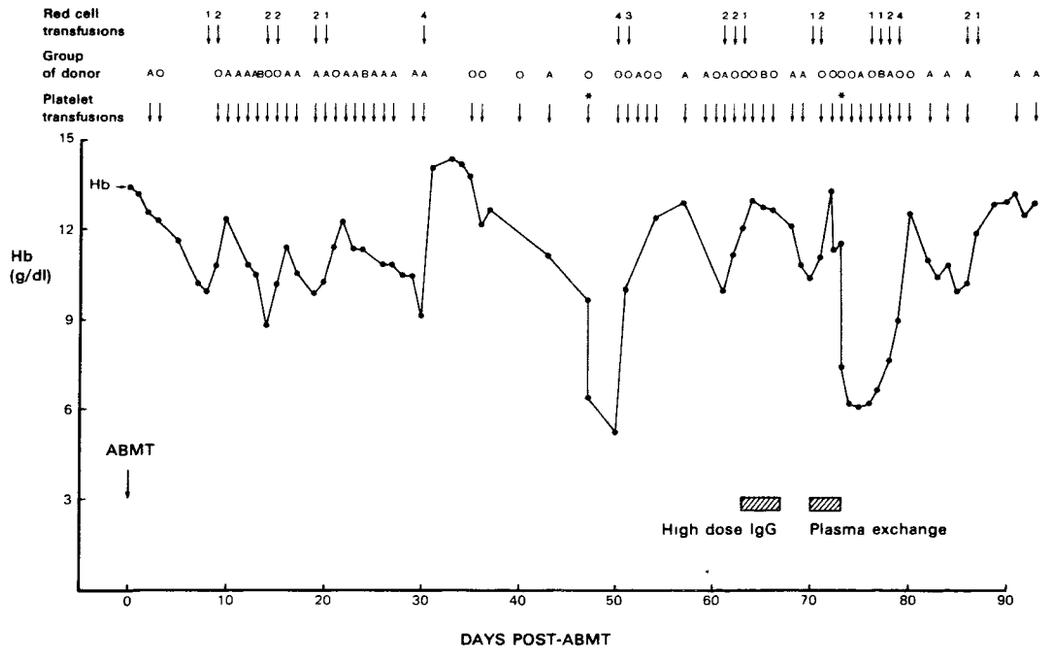

Red cell and platelet transfusions and patient's haemoglobin levels.

has not been routine practice to test ABO-incompatible donors for high-titre anti-A and anti-B. This is unlikely to cause any problem with platelet transfusions in the form of multiple standard platelet concentrates, but haemolysis is much more likely with platelet concentrates prepared by plateletpheresis of a single high-risk donor. We report a severe haemolytic reaction in a group A_1 patient given a single donor platelet concentrate with high-titre anti-A.

A 30-year-old woman with acute myeloblastic leukaemia was blood group A_1 Rh(D) negative. She achieved a complete remission after the second of four cycles of chemotherapy. She was subsequently given cyclophosphamide and total body irradiation supported by autologous bone-marrow transplantation (ABMT). Post-ABMT the platelet count did not recover—it remained below 10 000/μl until day 85 and below 20 000/μl until day 115—and she did not respond to platelet transfusions. She had persistent bleeding in her mouth and skin and frequent epistaxes. She had multispecific HLA antibodies, and the responses to platelet transfusions from unrelated and related HLA-matched and platelet crossmatch-compatible donors were unsatisfactory, and not improved by either intravenous gammaglobulin (0·4 g/kg for 4 days) or plasma exchange (5 litres per day for 3 days). She received 58 platelet transfusions in the first 90 days after ABMT; 48 were single donor platelet concentrates (volume 200–450 ml) and 25 were from group O donors (figure).

On day 72 post-ABMT she felt very unwell after plasma exchange with 4·5% albumin and a platelet transfusion from a group O HLA-matched donor. On the following morning she was pale and icteric and her haemoglobin had fallen from 11·4 to 6·0 g/dl; spherocytes were present on the blood film. The direct antiglobulin test was strongly positive (anti-IgG and C3d) and anti-A was eluted from her red cells. The donor had an anti-A titre of 256 by saline agglutination, and 1024 by the indirect antiglobulin test which was not reduced by treating the serum with dithiothreitol. The haemolysin test (one volume of a 1 in 4 dilution of fresh serum from the donor incubated with one volume of a 10% suspension of test cells) was positive with A_1 cells (+ + +), with A_2 cells (+ +), and with B cells (+), and negative with O cells. Despite acute renal failure (creatinine clearance 6 ml/min) dialysis was not required and her renal function returned to normal.

At day 47 post-ABMT there had been a similar episode of anaemia after a platelet transfusion from the same donor, which had gone unnoticed. Stored serum from the donor, taken at the time of the first episode, had an anti-A titre of 512 by saline agglutination and 2048 by the indirect antiglobulin test. The donor was male and had no history of transfusion or recent inoculations or infection that might have boosted his IgG anti-A titre. The greater severity of the later episode was probably related to the greater volume of plasma infused (448 ml *vs* 255 ml).

You recommend that group O donors on plateletpheresis panels should be tested for high titre anti-A and anti-B. However, precise guidelines have not been set, and there is no generally agreed discriminatory test. It is most important to avoid transfusing high titre immune anti-A to an A_1 recipient (as in our case). Group A patients should therefore receive platelets from group A donors wherever possible, and attention should be given to reducing the volume of donor plasma in single-donor platelet concentrates.

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Lyme borreliosis and Raynaud's syndrome

SIR,—We believe that some cases of Raynaud's syndrome[1] —usually those that need unpleasant and expensive investigations and treatment—are caused by *Borrelia burgdorferi*. These cases might represent a particular type of Lyme borreliosis. We report such a case.

In June, 1989—an unusual time for the appearance of Raynaud's syndrome—a 38-year-old woman with serious symptoms of Raynaud's syndrome was referred to our department. Her disease had begun suddenly, a short time earlier. Her fingertips were erythematous and painful. She had two pin-head ulcers on the first and second fingers of her left hand. Digital arteries were doppler positive only after warming her hand in water. All laboratory indices were normal, apart from the presence of serum antibodies to *Borrelia burgdorferi* (titre 3200) detected by the passive haemagglutination method (PHA; 'Lymag', Diagast, France). An absorbent (Diagast) was used to remove cross-reacting antibodies from the sample before titration. PHA titres above 200 were judged of diagnostic importance (manufacturer's recommendation).

After 8 weeks' treatment with doxycycline 300 mg daily, methisoprinol 3 g daily, and multivitamins, the hand ulcers healed. Fingertip erythema disappeared and an algid attack could be provoked only be very cold water or ice. After treatment the serum antibody titre was 1600. The patient has remained symptom-free up to December, 1989, and the antibody titre has decreased to 400.

Treatment of Lyme borreliosis is expensive since high doses of various antibiotics and other drugs are necessary. But our case suggests that Raynaud's syndrome caused by *Borrelia burgdorferi* can also be cured. On the basis of our observations we think it advisable to look for Lyme borreliosis in Raynaud's syndrome, even if there is no indication of such infection and/or the onset of the disease is unusual.

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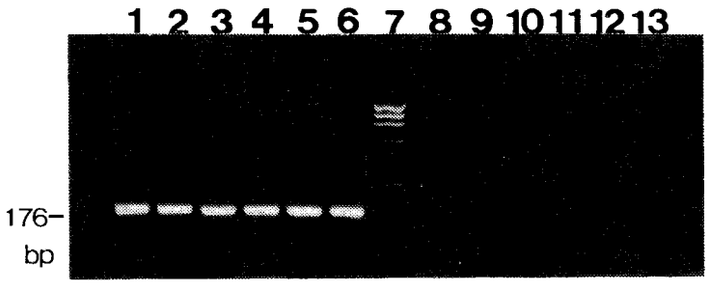
Detection of serum hepatitis C virus RNA

SIR,—Dr Weiner and colleagues (Jan 6, p 1) describe the detection of hepatitis C viral (HCV) sequences in individuals with post-transfusion non-A, non-B hepatitis. However, direct analysis of sera for HCV genome is still a research tool because the titre of circulating HCV is usually low, the hybridisation analysis is not sensitive enough to detect HCV RNA, HCV is very heterogeneous in respect of its RNA nucleotide sequences, and some HCV genomes are not detected. We have developed a sensitive procedure based on the polymerase chain reaction (PCR) technique.

To select efficient and specific primers one HCV cDNA clone, 282 base pair (bp) in size, was isolated from seven samples of liver from Japanese patients with chronic non-A, non-B hepatitis, and their sequences were compared with that of the Chiron clone. (The nucleotide sequences can be obtained from S. K.) The nucleotide sequences showed 76–77% homology with the Chiron HCV. However, two sets of primer pair sequences were highly conserved. Primer JK3779 (5'-GAGTGCGCCTCACACCTTCCTTACA-TCGAA-3') begins at map position 3779 of the Chiron HCV genome; primer JK3966R, from the reverse (R) strand (5'-ATCC-CGCTGATGAAGTTCCACATGTGCTTC-3') begins at position 3966; primer JK3794-E (5'-GACGAATTCCTTCCT-TACATCGAACAAGGA-3') begins at position 3794 and was synthesised to contain an *Eco*RI recognition cleavage site at its 5' end; and primer JK3951R-B (5'-ATGGAATTCTTCCACAT-GTGCTTCGCCCAG-3') begins at position 3951 and was synthesised to contain a *Bam*HI recognition site at its 5' end. The primers were used to test for HCV RNA in the sera of six chronic non-A, non-B hepatitis patients who were positive for HCV antibody (Ortho Diagnostic Systems enzyme immunoassay). After one round of amplification, the PCR-amplified sample was reamplified with a second pair of primers internal to the original pair (PCR-PCR[1]).



HCV RNA in serum of patients positive for antibody to HCV.

Serum from six patients was analysed for HCV RNA by PCR-PCR. RNA was extracted from 800 µl of serum, cDNA was synthesised with reverse transcriptase, and amplified by PCR-PCR using two sets of primer pairs—namely JK3779/JK3966R and JK3794-E/JK3951R-B in lanes 1–6 and primers C3751/C3930R and C3766-E/C3915R-B in lanes 8–13. A sample from the reaction mixture was subjected to agarose-gel electrophoresis, and nucleic acid was visualised under ultraviolet light after staining with ethidium bromide. Lane 7 = molecular weight standard φX174 (*Hae*III digest).

In all six samples an HCV cDNA band of expected size (176 bp) was produced on ethidium bromide fluorescence (figure, lanes 1–6). On the other hand, on PCR-PCR with two successive sets of primer pairs derived from the Chiron HCV sequence[2] (primers C3751/C3930R and C3766-E/C3915R-B) none of the six sera was positive for HCV RNA (figure, lanes 8–13). Another ten sets of primer pairs from other regions of the Chiron genome also produced unsatisfactory results.

HCV isolates showed extensive sequence diversity and a PCR technique based on conserved sequences may revolutionise assays for detecting HCV RNA and yield valuable information on the biology of HCV.

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Metronidazole resistance in *Helicobacter pylori*

SIR,—Dr Becx and colleagues (March 3, p 539) report metronidazole resistance in *Helicobacter pylori* and its association with previous administration of metronidazole or tinidazole for unrelated reasons. We have evaluated the frequency of metronidazole resistance in *H pylori* isolated from patients differing in age, race, and geographical setting, and present further data indicating that resistance is related to earlier nitroimidazole use and that resistance may vary from population to population. We have also correlated the rate of resistance in *H pylori* before treatment with the clinical efficacy of a nitroimidazole plus amoxycillin regimen.

H pylori was isolated from 206 unselected patients with *H pylori* antral gastritis alone or associated with peptic ulcer disease referred to our hospitals in Brussels and from 32 black African patients from rural eastern Zaire. *H pylori* was cultured from gastric mucosa and sensitivity to nitroimidazoles before treatment was determined by a disc diffusion with later retesting by agar dilution with metronidazole 8 mg/l. Patients with biopsy evidence of gastritis associated with *H pylori* were included in open trials of amoxycillin 4 × 500 mg capsules plus tinidazole 2 × 500 mg tablets daily for 7 days. Eradication of *H pylori* was evaluated by endoscopy with biopsy and/or a ^{14}C-urea breath test at least 4 weeks after the end of treatment.

In Brussels the rate of resistance to metronidazole was 27% (56/206). There was no difference in resistance between males (29/28) and females (27/109). There was a significant difference in resistance among native-born Belgians (18/108, 16·6%) compared with immigrants from Mediterranean countries (Morocco, Greece, Turkey, Spain, and Italy) ($p < 0{\cdot}001$). Resistance was more common in those above age 20 (51/161, 32%) than in younger patients (6/45, 13%) and was highest in patients aged 30–40 years (16/27, 59%). However, multivariate analysis revealed that ethnic origin was the only factor significantly predicting resistance ($p = 0{\cdot}0009$); sex and age did not contribute significantly. Of the children and adolescents investigated in Brussels, 5 out of 6 with resistant organisms were not Belgian born and could have acquired