Antibodies to hepatitis C virus (anti-HCV) were quantitated in stored sera from selected groups of hemophilic children (≤18 years of age). During the period 1987 to 1989, seropositivity rates were as follows: untransfused hemophiliacs 0% (0 of 11 cases), hemophiliacs treated exclusively with vapor-heated factor VIII or IX concentrates 0% (0 of 9 cases), hemophiliacs treated only with cryoprecipitate or single donor blood products 0% (0 of 9 cases), and hemophiliacs regularly treated with unheated or dry heat-treated factor VIII or IX concentrates 95% (21 of 22 cases). Corresponding alanine aminotransferase (ALT) results were similar: values were always below the upper limit of laboratory normal (40 U/L) in untransfused hemophiliacs, hemophiliacs treated with vapor-heated factor concentrates, or those who received only cryoprecipitate or single donor blood products. By contrast ALT values were greater than 40 U/L in 82% (18 of 22 cases) of hemophili children regularly infused with unheated or dry heat-treated factor concentrates. Three conclusions are drawn from this data: (1) HCV is a major cause of chronic hepatitis in multitransfused hemophilic children, (2) unheated and dry heat-treated clotting factor concentrates carry a very high risk of transmitting HCV infection, and (3) clotting factor concentrates inactivated by vapor heating carry a very low and perhaps zero risk of transmitting HCV infection. These findings are of therapeutic significance for previously untransfused hemophiliacs susceptible to HCV infection.

CHRONIC LIVER DISEASE is an important complication of therapy with factor VIII and IX concentrates, blood products that are widely used to treat or prevent hemorrhagic episodes in patients with hemophilia A and B. The majority of hemophiliacs who have received repeated infusions of these concentrates manifest biochemical evidence of chronic hepatitis; liver biopsy of such patients shows cirrhosis in 10% to 20% of cases. These disturbing statistics relate to the use of commercial factor VIII and IX concentrates that are prepared from plasma pools that include thousands of donor units and that carry a very high risk of transmitting both the hepatitis B and non-A, non-B (NANB) hepatitis viruses. Two recent advances offer the potential to limit, and possibly eliminate, the hepatic injury so prevalent in hemophiliacs treated with commercial factor concentrates. First, a safe and effective hepatitis B vaccine is now available, and it is recommended that all newly diagnosed hemophiliacs receive this vaccine. Second, current factor VIII and IX concentrates are treated during preparation with one of a number of recently developed virus inactivation methods (super dry heat, vapor heat, pasteurization, solvent detergent) that effectively inactivate the hepatitis B and NANB hepatitis viruses.

The recent discovery of a serologic test for the hepatitis C virus (HCV), now established to be the major etiologic agent of transfusion-transmitted NANB hepatitis, is certain to provide even safer blood products to patients with bleeding disorders. In this report we summarize hepatitis C antibody (anti-HCV) test results in selected groups of hemophiliacs, some of whom have received exclusively one of the new generation virus-inactivated factor VIII or IX concentrates. The results of our studies are consistent with clinical safety studies, indicating that these newer factor VIII and IX concentrates carry an extremely low risk of transmitting NANB hepatitis.

MATERIALS AND METHODS

Patients

Six patient groups were selected from 153 hemophili children (125 factor VIII deficiency, 28 factor IX deficiency) followed in the Comprehensive Care Hemophilia Clinic at the Hospital for Sick Children, Toronto. All children were ≤18 years of age at the time of study. Individual patient groups were as follows: group I (n = 11), hemophilic children with no exposure to blood products; group II (n = 9), hemophilic children treated exclusively with vapor-heated factor VIII (Kryobulin) or IX (Bebulin) concentrates prepared by a single manufacturer (Immuno, Vienna, Austria); group III (n = 9), hemophiliacs who had received only cryoprecipitate or other single donor blood products (red blood cells, fresh frozen plasma); group IV (n = 3), hemophiliacs with single or limited exposure to dry heat-treated factor concentrates (maximum heat treatment of 68°C for 72 hours); group V (n = 11), hemophili children frequently exposed to unheated and dry heat-treated factor VIII and IX concentrates (maximum heat treatment of 68°C for 72 hours), and group VI (n = 11), hemophiliacs who have received only cryoprecipitate or single donor blood products. All available subjects in groups I and IV were included. Subgroups of children followed in our Pediatric Comprehensive Care Hemophilia Clinic (groups I, III, V, and VI) were age and disease severity matched to group II cases.

Children followed in our Comprehensive Care Hemophilia Clinic receive factor VIII or IX concentrates intermittently for clinical bleeding. Regular replacement therapy (prophylaxis) is reserved for selected children, generally those with target joint bleeding. Clinic policy is to implement home therapy as early as possible, usually at the time of school entry. Until the enhanced virus-inactivated factor VIII concentrates were available in Canada (May 1986) it was recommended that children with severe factor

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were introduced into Canada. Thereafter, our policy was to offer
vere hemophiliacs and annually for mild/ moderate hemophiliacs). 
variety of unheated and dry heat-treated factor VIII and IX
Hemophilia Clinic assessments (performed semi-annually for se-
patients so treated these enhanced virus-inactivated concentrates to all children with
30, 1989, were identified. The minimum interval between the first
factors IX concentrates at an earlier age, reflecting the lack of a
concentrates. In May 1986 and February 1988, respectively, vapor-
pared virus-inactivated concentrates to all children with
newly diagnosed severe hemophilia A or B. Patients so treated
character of all children studied is presented in Table 1. The
availability of these products in Canada.

Serum Samples
Sera for anti-HCV testing were selected from serum bank samples collected at the time of routine Comprehensive Care Hemophilia Clinic assessments (performed semi-annually for se-
and annually for mild/moderate hemophiliacs). Samples were stored at -70°C before testing. For each subject two or three sera, collected during the period January 1, 1987 to June
were included with each assay run. The plate was incubated at 37°C for 60
minutes. Following washing, 200 μL of freshly prepared o-phenylene-diamine-2 HCl in citrate-phosphate buffer was added to each well, and the reaction mixture incubated at room temperature in the dark for 30 minutes. Fifty microliters of 4 N sulfuric acid was then added to all wells to stop the reaction. Absorbance (optical density [OD]) was measured at 490 nm, with absorbance of the blank being subtracted from the test absorbance values. As
determined by the manufacturer, the cutoff was established by adding 0.4 to the mean absorbance of the negative controls. Specimens with absorbance values less than the cutoff value were
classified as nonreactive. Specimens with absorbance values equal to or greater than the cutoff value were considered initially reactive for antibody to the HCV. All initially reactive samples were retested in duplicate and, according to manufacturer’s directions, a sample considered anti-HCV positive if at least one of the two repeat tests were also reactive.

RESULTS
Antibodies to the HCV were not detected in untrans-
fused hemophilic children (group I cases), children treated exclusively with vapor-heated factor concentrates (group II cases), or those treated only with cryoprecipitate and single donor blood products (group III cases). Individual test
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results are illustrated in Fig 1. Group II cases received a median of 26 infusions (range, 1 to 148) and were exposed to a median of three different lots of vapor-heated factor VIII or IX concentrates (range, 1 to 7). Group III cases had received a median of 12 U of cryoprecipitate (range, 4 to 138). One hemophilic child treated with cryoprecipitate also received a single course of intravenous IgG as therapy for Kawasaki disease. By contrast to the negative hepatitis C serology in groups I through III cases, 95% (21 of 22) of hemophiliacs frequently treated with unheated or dry heat-treated factor concentrates (groups V and VI cases) were hepatitis C antibody positive (Fig 1).

Simultaneously obtained ALT levels paralleled the anti-HCV results (Fig 2). Values were always within normal laboratory limits (≤40 U/L) in untransfused hemophilic children, in children treated exclusively with vapor-heated factor concentrates, and in those treated with cryoprecipitate or single donor blood products alone (Fig 2); by contrast, ALT values were elevated in 82% (18 of 22) of children treated with unheated or dry heat-treated factor concentrates (Fig 2). A review of all recorded ALT results for study patients was consistent with these results: ALT values greater than 100 U/L were not observed in group I, II, and III cases, but were present in 64% (7 of 11) of group V cases and, reflecting selection criteria, in all group VI cases.

Anti-HCV test results in group IV cases are of interest. Two of three hemophilic children with only a single exposure to a dry heat-treated factor concentrate demonstrated biochemical evidence of hepatitis following therapy; both cases were anti-HCV positive and test results documented seroconversion in one case (Table 2).

DISCUSSION

The sequelae of parenterally transmitted hepatitis B and NANB hepatitis include chronic active hepatitis, cirrhosis, and hepatocellular carcinoma. In a review of liver biopsies from 155 hemophiliacs and 40 hemophiliacs on whom autopsies were performed, Aledort et al reported a 7% incidence of severe chronic active hepatitis and a 15% incidence of cirrhosis. Similar data have been reported by Spero et al for multitransfused hemophilic children; two of 13 biopsied cases had postnecrotic cirrhosis and one case chronic active hepatitis. Although the natural history of parenterally transmitted NANB hepatitis in hemophiliacs remains uncertain, it is generally accepted that chronic

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<th>Age (y)</th>
<th>Factor Deficiency</th>
<th>Factor Level (%)</th>
<th>Date (mo/yr)</th>
<th>Alt (U/L)</th>
<th>Anti-HCV</th>
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<td>364</td>
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<td>8/89</td>
<td>NT</td>
<td>-</td>
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</tbody>
</table>

*Date of infusion with clotting concentrate.

Abbreviation: NT, not tested.

Fig 2. ALT levels in hemophilic children.

Table 2. Characteristics of Hemophilic Children With Single or Limited Exposure to Dry Heat-Treated Factor Concentrates
NANB hepatitis contributes significantly to the liver disease so prevalent in this population.16

Hepatitis C infection is the major cause of posttransfusion NANB hepatitis.17,18 The anti-HCV test results reported in this study are significant; 95% of hemophilic children frequently exposed to unheated and/or dry heat-treated factor VIII or IX concentrates were seropositive. The incidence of anti-HCV positivity in our study (95%) is similar to that reported by Ludlam et al (85%) but is significantly higher than that reported by Makris et al (59%), Esteban et al (64%), Noel et al (66%), and Roggendorf et al (78%).14,19,22 The differences can be reconciled. Our data relate to children with severe hemophilia who received repeated infusions of unheated or dry heat-treated factor concentrates; HCV antibody testing was performed on more than one sample. The incidence of hepatitis C seropositivity would be expected to be lower in studies that included patients with mild/moderate hemophilia and von Willebrand's disease, and those in which testing was performed on single serum samples only.14,19,22 In contrast to hemophilic children frequently exposed to unheated and/or dry heat-treated factor VIII or IX concentrates, none of nine hemophilic children treated exclusively with vapor-heated factor VIII or IX concentrates manifested serologic evidence of hepatitis C infection.

ALT values in this study paralleled the anti-HCV results (Figs 1 and 2). Because the groups were comparable with respect to hepatitis B, CMV, and EBV serologic status (Table 1), it is reasonable to conclude that hepatitis C infection accounts for the majority of liver-related biochemical abnormalities so prevalent in frequently transfused hemophiliacs. Our laboratory findings are consistent with the results of clinical safety studies. Mannucci et al have reported that none of 28 previously untreated hemophiliacs infused with the vapor-heated factor VIII concentrate, Kryobulin, developed ALT elevations consistent with NANB hepatitis.17 Together, these findings suggest that vapor-heated factor concentrates carry little risk of transmitting NANB hepatitis, and support the recommendation of Mannucci et al17 that “vapor or wet-heated concentrates are to be preferred for previously untreated factor VIII deficient patients highly susceptible to developing hepatitis.” It is likely that factor concentrates inactivated by other methods, eg, pasteurization, solvent-detergent, or super dry heat, are also safe.11,12,23 Our observation that hepatitis C infection occurred in two of three children following a single exposure to dry-heated concentrates is consistent with the high incidence of NANB hepatitis following exposure to unheated or dry heat-treated commercial factor VIII or IX concentrates,24-26 and emphasizes the importance of exclusive use of the enhanced virus-inactivated factor concentrates in previously untreated children with severe hemophilia A or B.

The absence of hepatitis C antibodies in hemophilic children treated with cryoprecipitate alone may reflect the small number of children studied, and the fact that the incidence of HCV seropositivity in the volunteer blood donor population is approximately 1%.27 However, we caution about use of our results to support the opinion that cryoprecipitate is as safe as the enhanced virus-inactivated factor concentrates with respect to transmission of HCV infection. To determine the risk of posttransfusion hepatitis C infection following infusion of single donor blood products (cryoprecipitate, fresh frozen plasma, red blood cells), serologic studies involving larger numbers of hemophiliacs treated exclusively with these products is required. The risk of hepatitis C infection will reflect both the source of donor plasma and the amount transfused, and is certain to vary both within and between countries.27 Once adequate data are available, clinicians caring for hemophilic children will be better able to judge the risk/benefit of the new virus-inactivated factor concentrates versus single donor blood products. While the risk of both products is low, it has been well established that cryoprecipitate transmits hepatitis.2 In contrast, both theoretical, clinical, and, now, serologic grounds, it is probable that vapor-heated and wet heat factor concentrates do not transmit HCV infection.10,12,22

With improved management of hemorrhagic episodes delivered by home therapy programs, plus prevention of HIV infection by donor selection, testing, and virus inactivation, chronic liver disease has become the single most important threat to a normal lifespan for children with newly diagnosed severe hemophilia A or B. The prevention of viral hepatitis is thus a critical element in the care of such patients, and now appears to be an achievable goal with the combined use of serologic testing for the hepatitis B and C viruses and advanced techniques for the inactivation of viruses in commercial clotting factor concentrates. The importance of these measures cannot be overemphasized until recombinant factor VIII and IX concentrates become available and affordable.

ADDENDUM

Subsequent to submission of this manuscript, anti-HCV testing has been performed in a further 65 hemophilic children followed in our clinic. Three children with hemophilia had received vapor heat-treated factor concentrates exclusively; all three children were HCV antibody negative. Anti-HCV data for the remaining 62 cases were as follows: severe hemophilia A, 28 of 35 cases positive; mild/moderate hemophilia A, 4 of 12 cases positive; severe hemophilia B, 9 of 9 cases positive; mild/moderate hemophilia B, 5 of 6 cases positive. These data confirm that hemophilic children who regularly received unheated or dry heat-treated factor VIII or IX concentrates have a very high incidence of HCV seropositivity, overall 84% (37 of 44 cases). The somewhat lower incidence of hepatitis C seropositivity as compared with results reported in the main body of the manuscript (95%) may reflect loss of specific HCV antibody in some children immunosuppressed because of HIV-1 infection. Of the 62 children described in this addendum, 44% were HIV-1 antibody positive; 83% (5 of 6) of frequently transfused HCV antibody negative hemophilic children were HIV-1 antibody positive as compared with 54% (19 of 35) of HCV antibody positive children.

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