International Forum

Vox Sang. *38:* 106–119 (1980)

What is the Importance of the 'Small Pool Concept' in the Preparation of Fraction I and Cryoprecipitates for the Prevention of Post-transfusion Hepatitis?

L. F. Barker. The administration of a single small pool, i.e. from 5 to 10 donors, of cryoprecipitate containing antihemophilic factor (AHF) and fibrinogen (fraction I) would clearly carry a lower risk of transmitting hepatitis than the administration of AHF concentrate or fibrinogen prepared from a large pool of plasma collected from several thousand donors. On the other hand, the 'small pool concept' is less important with regard to prevention of posttransfusion hepatitis for chronic recipients, such as severe hemophilics, than for one-time fibrinogen recipients, since the number of donors to which the severe hemophilic will be exposed will eventually approach the number of donors contributing to large pools.

Therefore, the 'small pool concept' would seem to be most important for the patient with mild hemophilia who requires only infrequent treatments for special situations such as surgical or dental procedures. Although the medical indications for fibrinogen are limited [1], small pools of cryoprecipitate as a source of fibrinogen would be preferred on the same basis, that is, when the use is expected to be infrequent or one time only. In either situation, small pools would be expected to carry the same risk of transmitting hepatitis as the same number of units of whole blood or any other blood component.

There are no prospective studies establishing the comparative hepatitis risks following the transfusion of small pools of cryoprecipitate versus the transfusion of AHF concentrates or fibrinogen derived from large plasma pools. However, a retrospective analysis of patients with severe hemophilia treated with small pools of cryoprecipitate showed a similar prevalence of hepatitis B markers and elevated levels of SGPT when compared with patients treated with AHF concentrate [2]. As predicted, then, prevention of posttransfusion hepatitis does not appear to be feasible by use of the 'small pool concept' for severe hemophilics requiring multiple treatments over many years.

References

1 Bove, J. R.: Fibrinogen – Is the benefit worth the risk? Transfusion *18:* 129–136 (1978).
2 Counts, R. B.: Serum transaminase and HBsAg antibody in hemophiliacs treated exclusively with cryoprecipitate. Proc. Conf. on Unsolved Problems in Hemophilia, DHEW Publ. No. (NIH) 77–1089, 1976, p. 77.

L. F. Barker, MD,
Vice President,
Blood Services,
American Red Cross,
National Headquarters,
Washington, DC 20006 (USA)

Joseph R. Bove. Recent trends in the therapy of hemophilia, especially prophylactic treatment and the use of Factor VIII concentrate, appear to be associated with an increased incidence of liver disease [1, 2]. After long-term therapy the incidence

of abnormal liver function in such patients is approximately 50% and liver disease has become an important cause of death in them. Because of the belief that the incidence of liver disease is directly related to the number of donor exposures at least one group has suggested that 'single donor products should be the preferred mode of treatment for mild hemophiliacs who require only infrequent therapy' [2]. An extension of this reasoning suggests that Factor VIII concentrates prepared from pool sizes smaller than that currently used (approximately 5,000 donors) might have a lower hepatitis risk. The importance of this 'small pool concept' in the prevention of posttransfusion hepatitis depends upon, (1) a realistic definition of a small pool, (2) an accurate knowledge of the incidence of hepatitis in patients treated exclusively with material from small or large pools, (3) the pool size that one HBsAg-negative *but infectious* donor will render infectious, and (4) an estimate of the acceptable benefit to risk ratio. Since neither the data nor the definitions are available I will attempt to answer the question with some speculation.

Tests for HBsAg have reduced, but not eliminated, the risk of posttransfusion hepatitis. Data from blood transfusion studies suggest that the infectivity rate for HBsAg-negative commercial donors may be as high as 108 per 1,000 [3]. It is well known that risk of hepatitis is related to the concentration of HBsAg in the pool and that some serums maintain infectivity (for HB) at dilutions of 10^{-5} to 10^{-7} [4]. If similar considerations apply to the agent or agents associated with non-A, non-B disease it is obvious that one donor can easily infect a pool. Furthermore, if about 10% of donors are carriers [3], pool sizes of as few as 100 donors will be infectious. It is possible that some intermediate size will be large enough to dilute all but the most infectious starting plasma but small enough to limit the remaining high titer donors to relatively few pools. The experiments to test this hypothesis are almost impossible since infectivity in primates is the only suitable test system. In my view, attempts to limit infectivity by restricting pool size will be impractical until there is a sensitive *in vitro* test for infectivity.

The benefits of modern therapy have worked a minor miracle in the lives of many hemophiliacs. One price, unfortunately, seems to be an increase in the incidence of liver disease. Smaller pool sizes, although an attractive possibility, will probably not be the solution.

References

1 Hilgartner, M. W. and Giardina, P.: Liver dysfunction in patients with hemophilia A, B, and von Willebrand's disease. Transfusion *17:* 495 (1977).
2 Hasiba, U. W.; Spero, J. A., and Lewis, J. H.: Chronic liver dysfunction in multitransfused hemophiliacs. Transfusion *17:* 490 (1977).
3 Goldfield, M.: quoted by Barker, L.: How frequent is posttransfusion hepatitis after the introduction of 3rd generation donor screening for hepatitis B? What is its probable nature? Vox Sang. *32:* 346 (1977).
4 Barker, L. F.; Shulman, R.; Murray, R.; Hirschman, R. J.; Ratner, F.; Diefenbach, W. C. L., and Geller, H. M.: Transmission of serum hepatitis. J. Am. med. Ass. *211:* 2509 (1970).

Joseph R. Bove, MD,
Blood Bank,
Yale-New Haven Hospital,
789 Howard Avenue,
New Haven, CT 06504 (USA)

R. Bütler. Everybody will agree that all prophylactic measures should be applied which contribute efficiently to prevent viral hepatitis due to the administration of blood or blood products. The question raised by the Editor of the International Forum refers to fibrinogen (Cohn Fraction I) and AHF. The hepatitis risk of these two products will be considered separately.

Despite the fact that there are very few indications for the administration of fibrinogen, this product is still often used in the clinic. Since these cases concern regularly episodic bleeding events, it is obvious that the risk of transmitting hepatitis is much less if small pool instead of large pool products are used. For this reason our institute has produced fraction I units of only two donations from the beginning of its activity. It appears that this concept has now been adopted worldwide.

The hepatitis problem is more complex regarding patients treated with AHF products. It must be kept in mind that the main prophylactic measure concerning posttransfusion hepatitis in these patients is presently the systematic screening for hepatitis B_s antigen (with RIA or similarly sensitive methods) in blood donations used for the production of AHF preparations. Numerous studies have clearly shown that about 20% of the hemophiliacs develop an acute clinical hepatitis in the course of their lives, in spite of this procedure. Furthermore, at least 80% of the patients show signs of hepatitis B virus exposure. Finally, a not yet well-defined proportion of the hemophiliacs – estimations go from 20 to 50% – appear to suffer from (mostly asymptomatic) chronic liver disease [3, 4]. The question is now, whether a strict adoption of the so-called 'small pool concept' would lead to a notable change of this situation. Since no comprehensive comparative studies of the hepatitis incidence in patients receiving exclusively small pool or large pool AHF products respectively, have been performed to my knowledge, the problem can again only be considered from a theoretical point of view.

Since the exposure rate to hepatitis viruses increases with the number of infectious blood units used (whether for the preparation of small or large pool AHF products), the hepatitis risk for hemophiliacs will depend upon the frequency of treatments and the total amount of administrated AHF preparations. In analogy to the conclusion drawn for the application of fibrinogen, it appears reasonable to use small pool AHF preparations for the occasional substitution of patients with mild hemophilia A as well as for the majority of the von Willebrand patients. The argumentation is different for patients with severe hemophilia A who frequently receive AHF preparations. It also appears that a growing number of patients with hemophilia A of so-called intermediate severity receive more and more often AHF substitution due to the increasing medical care which is offered to these patients. Whether this practice is adequate is another question, but it seems unrealistic that it could be influenced by the producers of AHF preparations. All these patients will almost inevitably be exposed to the hepatitis B virus (probably also to the viruses of non-A/non-B-hepatitis) and a relatively high proportion will develop either acute or chronic hepatitis as confirmed by the epidemiological data outlined above. It cannot be denied that AHF concentrates have some advantages (standardized AHF content, small volume to be infused, easy handling, etc.) in frequent AHF substitution therapy and especially in home treatment. Therefore, AHF concentrates are frequently asked for by clinicians. From the standpoint of the hepatitis risk, there is no convincing reason to renounce to use AHF concentrates of large pool origin for the treatment of these patients.

If we accept that the pool size *per se* of AHF preparations is of minor importance for the prophylaxis of posttransfusion hepatitis in frequently substituted hemophiliacs, this is probably not true for the source of plasma. In fact, a number of studies performed in the USA have shown that the hepatitis risk of commercial blood is markedly higher as compared to that of blood from unpaid donors [1, 2, 5, 6]. This is mainly due to the elevated hepatitis prevalence in this donor population which is often composed of persons from lower social classes, drug addicts, etc. One can assume that AHF products prepared from blood of such origin which is supposed to contain higher virus quantities will induce more frequently and more severe posttransfusion hepatitis in the recipients of such AHF preparations, whereby even the preexisting immunological barrier might be broken through. Consequently, one can expect some prophylactic effect when AHF from all volunteer blood donors is used instead of AHF from blood of commercial origin or from blood of donors living in countries with a high hepatitis incidence. In addition to this, efforts should be focussed on more efficient blood donor screenings for serological hepatitis virus markers which correlate with the infectiousity of hepatitis B virus as well as that of the non-A/non-B agents.

The main lines of the standpoint outlined in this paper are in accordance with the recommendations issued in January 1978 by the Medical Council of the Swiss Society of Hemophilia [7].

References

1 Allen, J. G.: Commercially obtained blood and serum hepatitis. Surgery Gynec. Obstet. *131:* 277–281 (1970).

2 Alter, H. J.; Hollan, P. V.; Purcell, R. H.; Lander, J. J.; Feinstone, St. M.; Morrow, A. G.; and Schmidt, P. J.: Posttransfusion hepatitis after exclusion of commercial and hepatitis-B antigen-positive donors. Ann. intern. Med. *77:* 691–699 (1972).
3 Bütler, R.: L'hépatite virale chez les hémophiles. Méd. Hyg., Genève *36:* 1289–1290 (1978).
4 Bütler, R. and Stampfli, K.: Hepatitis epidemiology in Swiss haemophiliacs. Schweiz. med. Wschr. (to be published, 1979).
5 Grady, G. F. and Bennett, A. J. E.: Risk of posttransfusion hepatitis in the United States. A prospective cooperative study. J. Am. med. Ass. *220:* 692–701 (1972).
6 Walsh, J. H.; Purcell, R. H.; Morrow, A. G.; Chanock, R. M., and Schmidt, P. J.: Posttransfusion hepatitis after open-heart operations. J. Am. med. Ass. *211:* 261–265 (1970).
7 Wiesmann, E.: Die Hepatitisgefährdung von Hämophilie-Patienten durch Verabreichung von Gerinnungspräparaten menschlicher Herkunft. Bull. Eidg. Gesundheitsamtes, No. 16, pp. 221–224 (1978).

Prof. R. Bütler,
Blutspendedienst SRK,
Zentrallaboratorium,
Wankdorfstrasse 10,
CH–3000 Bern 22 (Switzerland)

P. M. Mannucci. This commentary is written from the viewpoint of a hemophilia center director who follows, on a regular basis, more than 300 patients with hemophilia and allied coagulation disorders. Hemophiliacs are the group of multitransfused patients exposed most frequently and for the longest period of time to agents implicated in posttransfusion hepatitis. This risk has most probably increased since the adoption of intensive home and hospital replacement therapy in the attempt to prevent disability related to joint and muscle bleeding; and since such treatment is carried out in many countries with freeze-dried concentrates prepared from large plasma pools. Despite these premises, the incidence of *clinical* illness associated with jaundice is surprisingly low in hemophiliacs [1–4] and the problem of liver disease was overlooked until 1975, when a study of 91 patients with severe hemophilia A and B reported a high incidence of abnormal function tests unaccompanied by over liver disease [4]. Following confirmation of these results in other series of patients [5, 6], an important step forward in understanding the significance of such abnormalities has recently been made through the availability of liver biopsies, carried out by four groups of investigators in hemophiliacs presenting with persistently abnormal transaminases [7–10]. The great majority of biopsies provided histological evidence of active liver disease, ranging from chronic hepatitis to cirrhosis. These data suggest that function test abnormalities frequently encountered in hemophiliacs are not an aspecific and benign consequence of repeated exposure to plasma derivatives, but the serological expression of chronic liver disease.

Since the importance and magnitude of the problem has become apparent, hematologists and manufacturers of clotting factor concentrates have been faced with searching for an acceptable solution. Needless to say, withdrawal or limitation of the present strategy of aggressive replacement therapy is not justified. Not only because this change would be accompanied by a consistent deterioration in the present pattern of life of hemophiliacs, but also because it would not give the expected results. These views are supported by the findings of *Hilgartner and Giardina* [6], who have related the incidence of abnormal liver function tests to the amount of concentrates given to their patients. Elevation of serum enzymes and the occurrence of HB_sAg and/or Ab were not remarkably different in patients on prophylactic or episodic treatment, although the amounts of concentrates transfused in the latter group was significantly lower [6].

The observation of *Hasiba et al.* [5] that abnormal liver function was more frequent in patients treated with commercial concentrates, prepared from large pools rather than in those given only blood bank cryoprecipitate clearly shows a way by which prevention could be attempted. Cryoprecipitate, however, is a difficult material to handle, it shows variable potency from bag to bag and needs to be stored at –20 °C. In contrast, freeze-dried concentrates are characterized by known potency, stability at 4 °C, ease in reconstitution and low risk of allergic reactions. These advantages make them essential in home treatment which is now

considered by hemophiliacs and their doctors the most effective form of management of which the withdrawal would not be acceptable. The adoption of the 'small pool concept' in the preparation of such freeze-dried concentrates would be undoubtedly a significant step forward in the prevention of posttransfusion hepatitis and liver disease in hemophiliacs. This approach appears realistic also for commercial manufacturers, as demonstrated by the experience gained in Sweden. In this country, the fraction most widely used in the management of hemophilia A and von Willebrand's disease is a commercially available fraction I–0, whereas the management of hemophilia B is carried out with a prothrombin complex concentrate made from a small pool of donors from Scandinavian countries characterized by a low incidence of hepatitis [11–13]. Since the factor VIII concentrate is not sufficient to cope entirely with the national demand, a commercial freeze-dried concentrate made from a large pool of donors is being used for surgical cases or patients with hemophilia A needing large doses of factor VIII [14]. Strict adherence to the 'small pool concept' in the treatment of patients with hemophilia B has resulted in a very low incidence of posttransfusion hepatitis [15], supporting the usefulness of this approach in the prevention of liver disease.

It must be realized, however, that in many countries hemophilia care is almost exclusively based on commercial concentrates from large plasma pools. Leaving aside the complex ethical problem involved in this situation and the responsibilities of those countries who have not even attempted to organize a national blood program, any solution to the prevention of posttransfusion liver disease in hemophiliacs should take into account this reality, as well as the fact that a deterioration of the present pattern of life now assured by commercial concentrates would be unacceptable to hemophiliacs. Therefore, any chauvinistic attitude to ban commercial concentrates from these countries would be unjustified, unless a national program of blood transfusion capable of meeting the demand is soon developed. Health authorities, however, have the right and the duty to establish guidelines for blood products used in their countries. They should encourage manufacturers to make available as part of the imported concentrates also a number of preparations made from small pool of donors at low risk of transmitting hepatitis. These might be used electively in children or in patients who, having received a small number of transfusions, are not actively immunized against the agents of posttransfusion hepatitis and hence at higher risk of developing this complication [1–3]. After multiple transfusions, such risk is likely to be much lower [1–3], and patients could be switched to treatment with concentrates made from larger donor pools. National health authorities should of course be prepared to meet the additional cost of these special fractions since the additional cost is certainly outweighed by benefits.

References

1 Kasper, C. K. and Kipnis, S. A.: Hepatitis and clotting factor concentrates. J. Am. med. Ass. *221:* 510 (1972).

2 Lewis, J. H.; Maxwell, N. G., and Buanden, J. M.: Jaundice and hepatitis B antigen/antibody in hemophilia. Transfusion *14:* 203–211 (1974).

3 Biggs, R.: Jaundice and antibodies directed against factor VIII and IX in patients treated for hemophilia and Christmas disease in the United Kingdom. Br. J. Haemat. *26:* 313–329 (1974).

4 Mannucci, P. M.; Capitanio, A.; Del Ninno, E.; Colombo, M.; Pareti, F., and Ruggeri, Z. M.: Asymptomatic liver disease in haemophiliacs.

5 Hasiba, U.; Spero, H. A., and Lewis, J. H.: Chronic hepatitis in haemophilia. Scand. J. Haematol., suppl. 30, pp. 27–32 (1977).

6 Hilgartner, M. W. and Giardina, P.: Liver dysfunction in patients with haemophilia. Scand. J. Haematol., suppl. 30, pp. 6–10 (1977).

7 Lesesne, H. R.; Morgan, J. E.; Blatt, P. M.; Wedster, W. P., and Roberts, H. R.: Liver biopsy in hemophilia A. Ann. intern. Med. *86:* 703–707 (1977).

8 Mannucci, A. M.; Ronchi, G.; Rota, L.; and Colombo, M.: A clinicopathological study of liver disease in haemophiliacs. J. clin. Path. *31:* 779–783 (1978).

9 Preston, F. E.; Triger, D. R.; Underwood, J. C. E.; Bardhan, G.; Mitchell, V. E.; Stewart, R. M., and Blackburn, E. K.: Percutaneous liver biopsy and chronic liver disease in haemophiliacs. Lancet *i:* 592–594 (1978).

10 Spero, J. A.; Lewis, J. H.; Van Thiel, D. H.; Hasiba, U., and Rabin, B. S.: Asymptomatic structural liver disease in hemophilia. New Engl. J. Med. *298:* 1373–1378 (1978).
11 Helske, T.: Carriers of hepatitis B antigen and transfusion hepatitis in Finland. Scand. J. Haematol., suppl. 22 (1974).
12 Reinicke, V.: Posttransfusion hepatitis. Scand. J. infect Dis. *6:* 285–290 (1974).
13 Iwarson, S.; Hermodsson, S.; Lindholm, A., and Magnius, L.: Hepatitis B antigen and prevention of post-transfusion hepatitis B. Vox Sang. *28:* 278–284 (1975).
14 Nilsson, I. M. and Hedner, U.: Characteristics of various factor VIII concentrates used in the treatment of hemophilia A. Br. J. Haemat. *37:* 543–557 (1977).
15 Iwarson, S.; Kjellman, H., and Teger-Nilsson, A. C.: Incidence of viral hepatitis after administration of factor IX concentrates. Vox Sang. *31:* 136–140 (1976).

P. M. Mannucci, MD,
Hemophilia and Thrombosis Centre,
Angelo Bianchi Bonomi,
International Training Centre of the World Federation of Hemophilia,
Via Pace 15,
I–20122 Milano (Italy)

John E. Mercer. The continued relevance of the 'small pool concept' (pools of 10 donor units or less) for the preparation of Fraction I and cryoprecipitates has been reaffirmed by the action of the Bureau of Biologics of FDA in the form of a ban on the production, distribution, and use of Dried Fibrinogen (Human) in the United States as well as the production of said product for export. This action by FDA has forced clinicians in the United States to use single unit cryoprecipitate preparations in place of Dried Fibrinogen (Human) normally prepared from large donor pools. The full impact of this change will only be measured with time and experience with this change in medical practice. This decision was based on the risk: benefit ratio of the continued use of this product favoring the apparent risk. The basis for this decision has been reviewed in a recent article by *Bove* [2].

The real question is not the risk:benefit ratio of this one banished product; it is in fact a question of the availability of relatively safe therapy for a group of individuals at risk of repeated exposure to products that may contain HBV (hepatitis B virus) infectivity.

The initiation of screening all donor blood for HB_sAg (hepatitis B surface antigen) has reduced the risk of posttransfusion hepatitis in the majority of the patient population receiving whole blood, components and derivatives. This risk is markedly lower for recipients of volunteer blood as compared to commercial blood [1, 3].

Screening for HB_sAg or other markers of HBV will not, however, assure elimination of posttransfusion hepatitis in that it does not address the problem of possible transmission of nonspecific hepatitis (non-A non-B hepatitis). Developing technology on the identification and characterization of the non-A non-B virion(s) may be helpful in solution of this problem. Based on present knowledge, it would appear that hepatitis A virus is not a significant contributor to posttransfusion hepatitis and as such will probably not require a screening test [3].

Current evidence indicates that, as a group, patients suffering from coagulation disorders and primarily those with deficiencies of either antihemophilic factor (AHF, Factor VIII) or Factor IX are still at high risk of developing hepatitis in spite of HB_sAg screening of the donor population [5]. This risk is the result of several contributing factors, including: (1) the increased use of concentrates prepared from large donor pools increases the potential of including infective plasma in the pool; (2) hepatitis infectivity may be selectively concentrated in coagulation factor concentrates [6]; (3) screening of donors of HB_sAg alone may not detect and eliminate all HBV infective plasma [3, 4]; (4) the major supplies of AHF and Factor IX concentrates are derived from the commercial donor population and this may contribute to chronic HBV infection and asymptomatic structural liver disease [5].

In the face of this evidence it would appear that there is still a role for the 'small pool concept' in the treatment of these patients. This concept may be of value in the treatment of individual cases depending on local circumstances. It would be, however, naive to expect that we should return

to the exclusive use of the 'small pool concept' to meet the ongoing therapeutic needs of the hemophiliac population *in lieu* of the more effective concentrates. Such a move would indeed be regressive. An effort to improve the relative safety of the existing concentrates would be more meaningful. To improve on the rapid gains made in recent years to identify and effectively screen out infective blood from the donor population would be the more reasonable approach. *Hoofnagle et al.* [4] have suggested the use of anti-HBc (antibody to hepatitis B core antigen) as a possible additional indicator of HBV infectivity. Should further studies substantiate the efficacy of anti-HBc as a reliable indicator of infectivity, it would appear to be the next logical step to more effective screening of donor population.

Grady [3] has suggested that even with more extensive screening of commercial blood, the infectivity of this source could not be reduced to the level extant in volunteer blood. If such is in fact the case, it would appear that reduction of dependence on commercial blood as a source for the preparation of coagulation factor concentrates would significantly reduce the infectivity of these products. This approach to the altruistic all-volunteer donor base has been espoused by and is the ultimate goal of the American Blood Commission. This ultimate move to volunteerism does not, however, solve the immediate problem of the high hepatitis risk of the hemophiliac receiving concentrates.

Several alternatives could be suggested that may improve the relative safety of these products including: (1) in addition to testing the products for the absence of HBsAg, the products could be tested for the presence or absence of other markers of HBV infectivity including anti-HBc and eAg (soluble antigen associated with the virus core) to screen out products that would be potentially infective; (2) equally important would be the elimination of the use of Cohn Fr. III as a source for the preparation of prothrombin complex (Factor IX) concentrates. This particular Cohn fraction has been shown to be a rich source of most of the presently known markers of HBV infectivity including HB_sAg, eAg, DNA polymerase and Dane particles [6]. The elimination of this potentially infective source would have a beneficial effect on reducing the risk of hepatitis for both Factor IX and AHF deficient patients as well as other patients in which prothrombin complex preparations are used as part of the therapeutic regimen.

In conclusion, it appears that the small pool concept will still play a role, albeit a minor role, in replacement therapy. The role of this concept in the United States will be major in the case of therapy requiring fibrinogen. However, it has already been relegated to only minor importance in the treatment of the major coagulation defects so dependent on the source of available coagulation factor concentrates for effective long-term management in home care programs.

References

1 Alter, H. J.; Holland, P. V.; Purcell, R. H., *et al.:* Posttransfusion hepatitis after exclusion of commercial and hepatitis B antigen-positive donors. Ann. intern. Med. *77:* 691–699 (1972).
2 Bove, J. R.: Fibrinogen – Is the benefit worth the risk? Transfusion *18:* 129–136 (1978).
3 Grady, G. F.: Transfusions and hepatitis: update in '78. New Engl. J. Med. *298:* 1413–1415 (1978).
4 Hoofnagle, J. B.; Seeff, L. B.; Bales, Z. B., *et al.:* Type B hepatitis after transfusion with blood containing antibody to hepatitis B core antigen. New Engl. J. Med. *298:* 1379–1383 (1978).
5 Spero, J. A.; Lewis, J. H.; Van Thiel, D. H., *et al.:* Asymptomatic structural liver disease in hemophilia. New Engl. J. Med. *298:* 1373–1378 (1978).
6 Trepo, C.; Hanlz, O.; Jacquier, M. F., *et al.:* Different fates of hepatitis B virus markers during plasma fractionation. Vox Sang. *35:* 143–148 (1978).

John E. Mercer, PhD, Chief,
Blood Derivatives Section,
Division of Biologic Products,
Bureau of Disease Control and
Laboratory Services,
Michigan Department of Public Health,
3500 N. Logan St., PO Box 30035,
Lansing, MI, 48909 (USA)

J. P. Soulier. To discuss usefully if Factor VIII concentrates and eventually fibrinogen may be prepared in large pools or should be prepared in small pools, we need to take into account objective considerations which are focussed on the transmission of viral hepatitis.

(1) The risk of hepatitis B transmission must be distinguished from that of transmission of non-A, non-B hepatitis, since the detection of dangerous donors by serological means can only, for the time being, be made on HB. If the detection is done by sensitive methods (Third Generation Test) on each blood donation and on the final product, the risk of transmitting hepatitis is extremely slight.

(2) The epidemiologic state of the recipients has also to be considered. Most of the hemophiliac recipients are already immunized against HBV: in a recent study done by Mme *Couroucé* in the Medical Boarding School at La Queue-les-Yvelines, 92% of the 80 hemophiliacs already had contact with HBV, possessing a marker of infection such as anti-HBc or anti-HBs. Of course this data may vary in the future through the better selection of nondangerous donors.

(3) For non-A, non-B hepatitis we have no immunological markers, but we know from American statistics that paid donors in the USA are ten times more prone to transmitting hepatitis than unpaid donors. Thus, discussion on whether to choose small or large pools has to take into account the fact that fractionated plasma comes from remunerated or unremunerated donors.

(4) It is obvious that standardization is only possible with large batches of Factor VIII-containing fractions and is not applicable to small pools.

Considering these four points, in France we use large pools for the preparation of cryoprecipitates, fibrinogen and factor VIII concentrates, since more than 9 out of 10 French hemophiliac recipients are already infected or immunized by HBV, and we feel that it is important to give the utilizer a well-defined fraction, having a constant amount of antihemophilic activity (5 units ml for cryo, and 25 units ml for the concentrates with a relatively low fibrinogen content). Also, the level of isohemagglutinins can only be checked on large pools, allowing the injection of products not containing an excess of anti-A hemagglutinin to blood group A hemophiliacs.

Of course, we are fully aware of the fact that we do not control the risk of transmitting non-A, non-B hepatitis, which is increased by the large pools. In France, we lack statistics which would let us appreciate the frequency of non-A, non-B virus carriers. We believe it is much less than in some other countries, due to the fact that we only use unpaid donors, but this situation could evolve in the future, as it is hoped that non-A, non-B virus will become detectable by nonimmunological means and of course it would be a great progress to be able to exclude healthy carriers of such a virus.

Prof. J. P. Soulier,
Centre National de Transfusion Sanguine,
53, Boulevard Diderot,
F-75571 Paris Cédex 12 (France)

Kenneth R. Woods and Bernard Horowitz. Despite the advent of highly sensitive tests for antigenic markers of hepatitis B virus, a small though significant number of infectious blood donations and plasma products derived from them are transfused. Tests for non-A/non-B hepatitis virus markers are not generally available nor applicable to testing donor blood, and the risk of transmission of this form of hepatitis by transfusion of blood or blood products continues to be a complication in antihemophilic factor replacement therapy.

It is apparent that if patients receive plasma coagulation factor concentrates from a sufficient number of potentially infectious sources, they will inevitably be exposed to hepatitis virus no matter how low the infectivity rate of the donor source. Incident and cumulative risks can be estimated over the course of several years of treatment by simple probability calculations from knowledge about therapeutic requirements and hepatitis transmission rates from randomly selected donors. Models of revised strategies designed to provide equivalent replacement therapy while reducing the incidence of posttransfusion hepatitis can then be evaluated and compared to current practice with regard to reduction in risk expected in relation to the effort required and feasibility of modifying practice to conform to proposed models.

Two schools of practice now exist; advocates of the 'small pool' concept prefer the use of single donor cryoprecipitates or AHF concentrates made from the plasma of not more than eight donors in order to reduce the risk of posttransfusion hepatitis, while advocates of the 'large pool' concept prefer the use of AHF concentrates made from fresh frozen plasma pooled from large numbers of donors to permit increased process and quality control of purity, potency, sterility and safety and other efficiencies related to scale.

It is difficult to estimate the probability of hepatitis virus inclusion in commercial coagulation products prepared from large volumes of plasma obtained by plasmapheresis, though it has been shown in the past that such products carry a high risk of hepatitis transmission [1–3]. Each lot would be likely to consist of plasma from considerably fewer individual donors than would be apparent from the batch volume (one US manufacturer has indicated that a given lot of its factor VIII concentrate is derived from not more than 1,200 donors); a given manufacturer's successive production lots would engender additional exposure risk only to the extent of the added risk due to indroduction of new plasmapheresis participants and also new infections among them; and estimates of hepatitis risk from commercial plasma pools are complicated by the effect of dilution of the virus on its infectivity.

The risk of the large donor pool, whatever it may be, must be compared to the aggregate risk of infection from administration of single-unit cryoprecipitates or concentrates prepared from serial, small donor pools, keeping in mind that replacement of a congenitally deficient coagulation factor at frequent intervals becomes a lifetime regimen. Given a typical voluntary donor base the following assumptions would appear to be a valid basis for probability estimates of hepatitis transmission on transfusion of single-donor cryoprecipitates: (1) a person treated for severe hemophilia A uses 20,000 IU AHF/year; (2) the infection rate per blood donation for hepatitis B is 0.05% and for hepatitis non-A/non-B is 3.3% [4]; (3) 100 IU AHF are recovered in the cryoprecipitate from each donor; (4) probability of infection = $1-[(1-\text{risk per donor})^{\text{number of donors}}]$.

Calculations based on the above assumptions indicate that transmission of type B hepatitis to hemophiliacs would appear to be delayed significantly by adoption of a small pool strategy as contrasted with the use of concentrates prepared from large pools of plasma (table I). However, substantial risk remains; after only 10 years of treatment 63% of those treated would be expected to become infected with hepatitis B. Delay of infection with hepatitis is not altogether satisfying when one considers that hepatitis B could be a more fulminating and disabling disease if contracted later rather than earlier in life [5]. Episodes of traumatic injury, dental extractions, or surgical intervention, requiring very high dosages of Factor VIII during this interval, or dependence on a blood donor population having a higher prevalence of hepatitis carriers would further increase the probability of infection. The transmission of type non-A/non-B hepatitis to hemophiliacs appears to be highly probable even after only 1 year of treatment.

Table I. Probability of transfusion-related hepatitis: 1. donors randomly assigned to recipient

Years treatment	HB	Non-A/ non-B
1	0.10	1.00
5	0.39	1.00
10	0.63	1.00
15	0.78	1.00
20	0.86	1.00
30	0.95	1.00

The relatively high risk of hepatitis B transmission which would appear to remain despite exclusive use of single donor cryoprecipitates is a consequence of exposure to donors whose number increases linearly with time (table II, column 1). In a typical voluntary blood donor system the transmission rate could probably be reduced if the plasma for Factor VIII were derived from a restricted number of sets of repeat whole blood or plasmapheresis donors, each set dedicated to the needs of an individual patient. The following assumptions seem reasonable for the operation of such a system: (1) assigned blood donors contribute 2 U of whole blood per year; (2) assigned plasmapheresis donors contribute 2 liters of plasma

Table II. Number of donors to which hemophiliac is exposed

Years treatment	Donor: Assignment:	Whole blood Random	Whole blood Assigned	Plasmapheresis Assigned
1		200	100	25
5		1,000	180	45
10		2,000	280	70
15		3,000	380	95
20		4,000	480	120
30		6,000	680	170

Table III. Probability of transfusion-related hepatitis type B

Years treatment	Donor: Assignment:	Whole blood Random	Whole blood Assigned	Plasmapheresis Assigned
1		0.10	0.05	0.01
5		0.39	0.09	0.02
10		0.63	0.13	0.03
15		0.78	0.17	0.05
20		0.86	0.21	0.06
30		0.95	0.29	0.08

per year; (3) the yearly turnover rate in the assigned donor population is 20%.

Successful implementation of this strategy would result in a sharp reduction in the number of donors to which an individual hemophiliac is exposed, for donors contributing whole blood or plasma only (table II). Reduced exposure to individual donors would be predicted to cause a substantial decline in the probability of transmission of type B (table III), but not non-A/non-B, hepatitis.

The validity of the foregoing estimates of hepatitis transmission rests principally on the accuracy and practicality of the assumptions. A brief examination of the impact of alternate assumptions on the probability of transmission of hepatitis B during 10 years treatment is provided. On the one hand, all strategies of donor organization benefit from reduced annual usage of AHF, from reduced estimates of the per donor risk, and less so from improved process yields of AHF (table IV A). Of these three factors the one with largest impact and the one most amenable to change and subject to challenge is the per donor risk rate. The best information available today suggests that 0.05% accurately reflects the current escape rate of volunteer blood infectious for hepatitis B in the United States when whole blood or components are infused [4]. As hepatitis B virus appears to be concentrated by cryoprecipitation, it seems reasonable to assume that AHF carries a similar risk of infection. Improved screening techniques might be expected to cause a reduction in the escape rate, though it is not currently known how great an increase in sensitivity would be needed to substantially reduce the rate of transmission. Since dilution studies suggest that as few as 10^5 particles can transmit hepatitis B, and routine screening methods detect HBsAg only when its concentration is greater than 10^8 particles/ml, and the number of particles in a single cryoprecipitate probably reflects the number in more than 100 ml of plasma, it seems unlikely that improved routine screening methods would reduce the transmission rate substantially.

On the other hand, the apparently large impact of assigning donors to recipients on the transmission of hepatitis B relies on a reliable donor source and good management techniques. The transmission of hepatitis B to hemophilics would appear to remain substantially reduced even when only a single donation is given per year, or when the annual turnover in the donor group is as high as 50%, or both (table IV B). Use of AHF from other than the assigned donor group or assignment of a donor group to more than 2 or 3 hemophiliacs would cause a substantial increase in transmission of hepatitis B. In contrast, as few as 4 donors giving 15 liters of plasma per year could provide the average requisite plasma for treatment of 1 patient with cryoprecipitate.

The probability of hepatitis transmission on single exposure to a blood derivative made from plasma pools of different sizes can be predicted by similar analytical methods, assuming that inclusion of a single infectious unit makes the pool infective. The probability of transmission of type B hepatitis remains relatively low ($< 5\%$) when fewer than 100 donors contribute to the pool; however, with products made from large pools, the

Table IV. Probability of HB infection with 10 years usage modified assumptions

Change	Donor: Assignment:	Whole blood Random	Whole blood Assigned	Plasmapheresis Assigned
	Initial P:	0.63	0.13	0.03
A 1 Reducing annual usage of AHF to				
10,000 IU		0.39	0.07	0.02
5,000 IU		0.22	0.03	0.01
2 Reducing the per donor risk to				
0.0250%		0.39	0.07	0.02
0.0125%		0.22	0.03	0.01
0.0050%		0.10	0.01	0.00
3 Reducing annual usage of AHF to 10,000 IU and reducing per donor risk to				
0.0250%		0.22	0.03	0.01
0.0125%		0.12	0.02	0.00
4 Yield of AHF from each donor increased to				
120 IU		0.57	0.11	0.03
135 IU		0.53	0.10	0.03
150 IU		0.49	0.07	0.02
B 5 Decrease in frequency of donation by an individual donor to once per year		–	0.24	0.13
6 Increase in annual turnover in assigned donor population to				
30% turnover		–	0.17	0.05
40% turnover		–	0.21	0.06
50% turnover		–	0.24	0.07
7 Decrease in frequency of donation by an individual donor to once per year and increase in annual turnover in assigned donor population to				
30% turnover		–	0.31	0.17
40% turnover		–	0.37	0.21
50% turnover		–	0.42	0.24
8 Instead of linkage of donor group to one hemophiliac linkage to				
2 hemophiliacs		–	0.24	0.07
3 hemophiliacs		–	0.34	0.10
5 hemophiliacs		–	0.50	0.16

Table V. Probability of hepatitis infection with a single administration of a blood derivative

Number of donors contributing to pool	Probability	
	HB	Non-A/ non-B
1	5×10^{-4}	0.03
4	2×10^{-3}	0.13
8	4×10^{-3}	0.24
10	5×10^{-3}	0.29
15	0.01	0.40
20	0.01	0.49
40	0.02	0.74
100	0.05	0.97
500	0.22	1.00
1,200	0.45	1.00
2,000	0.63	1.00
5,000	0.92	1.00

probability of hepatitis B infection is quite high (table V). The risk of transmission of type non-A/ non-B hepatitis on single exposure to a blood derivative would appear to be sufficiently high to suggest the use of derivatives made from a single donor whenever possible.

Active elimination of viral infectivity of plasma pools, whatever the size, would appear to be a promising alternative approach toward assurance that recipients of plasma products will be protected from exposure to virulent agents. Fortunately, it was recognized years ago that this could be accomplished by thermal inactivation of viral agents in albumin solutions, but the clinically important coagulation factors tend to lose biological activity when solutions are subjected to elevated temperatures. Other means of virus inactivation should be critically evaluated [6, 7], or new means must be devised if the complications of viral diseases are to be avoided in the treatment of hemophilia with transfusions of human plasma derivatives.

Implementation of the practices suggested by the above calculations will require considerable planning and modified fractionation and donor management techniques. Nonetheless, the potential benefits in reducing the transmission of hepatitis would appear to justify the effort.

References

1 Craske, J.; Dilling, N., and Stern, D.: An outbreak of hepatitis associated with intravenous injection of factor VIII concentrate. Lancet *ii:* 221–223 (1975).

2 Dane, D. S. and Cameron, C. H.: Factor VIII concentrate and hepatitis. Lancet *ii:* 328–329 (1975).

3 Aronson, D. L.: Factor IX concentrates; in Sandberg, Proc. Int. Wkshop on Technology for Protein Separation and Improvement of Blood Plasma Fractionation, DHEW Publ. No. NIH 78–1422, 1978, pp. 345–357.

4 Aach, R. D.; Lander, J. J.; Sherman, L. A., et al.: Transfusion-transmitted viruses: interim analysis of hepatitis among transfused and non-transfused patients; in Girish, Vyas, Cohen and Schmid, Viral hepatitis, pp. 383–396 (Franklin Inst. Press, Philadelphia 1978).

5 Bryan, J. A. and Gregg, M. B.: Viral hepatitis in the United States, 1970–1973: viral hepatitis in the United States, 1970–1973. An analysis of morbidity trends and the impact of HBsAg testing on surveillance and epidemiology. Am. J. med. Sci. *270:* 271 (1975).

6 Lo Grippo, G. A. and Hayaski, H.: Efficacy of betaprone with ultraviolet irradiation on hepatitis antigen in human plasma pools. Henry Ford Hosp. med. J. *21:* No. 2 (1973).

7 Stephan, W. U. und Kotitschke, R.: Prothombinkomplex (PPSB) aus kaltsterilisiertem Plasma. Forsch. Transfusion Immunohäm. *4:* 72 (1977).

Kenneth R. Woods, PhD, and B. Horowitz,
The New York Blood Center,
310 East 67th Street,
New York, NY 10021 (USA)

A. J. Zuckerman. The classification in 1965 by *Mosley* [1] of blood and blood derivatives according to the risk of post-transfusion hepatitis in recipients as 'average-risk' materials, which include fresh blood and single donor plasma; 'high-risk' products, which include pooled plasma; fibrinogen and antihaemophilic factors and 'safe' derivatives such as immunoglobulin, has withstood the test of time. At first sight, this may appear as a somewhat rash statement. However, while the applica-

tion of sensitive techniques such as radioimmunoassay and enzyme immunoassay for the detection of hepatitis B surface antigen in individual units of blood has reduced dramatically the risk of hepatitis B following transfusion, infectious units of blood have not been completely eliminated [2]. Inevitably, therefore, the pooling of a large number of units of plasma for the preparation of plasma derivatives will increase the risk of introducing an infectious unit into the pool, even though an additional measure of safety is introduced if every single unit of plasma is pretested for hepatitis B surface antigen by a very sensitive technique and only non-reactive plasma enters the pool [2]. In addition, the problem of non-A:non-B hepatitis emerged when hepatitis A, hepatitis B and other viruses such as cytomegalovirus and Epstein-Barr virus which may infect the liver, have been excluded from donor blood.

Although many well-documented reports have been published on hepatitis B caused by high-risk plasma components, it was not clear until recently whether these products can also induce the new form(s) of non-A:non-B hepatitis. A retrospective survey of transfusion hepatitis associated with a brand of commercially prepared factor VIII carried out in 1974–1975 in 24 centres for the treatment of haemophilia in the United Kingdom revealed two types of hepatitis [3]. One type of infection was a short incubation non-B hepatitis with an incubation period of 8–60 days, clinically identical with hepatitis A but not associated with secondary cases and unlikely to be caused by this virus, and hepatitis B, with incubation periods of 50–185 days. 78 episodes of hepatitis affecting 66 out of 371 patients transfused with one brand of factor VIII were identified. Of these, 48 were non-B hepatitis and 30 were hepatitis B. 12 patients contracted two attacks of hepatitis, non-B hepatitis followed by hepatitis B. Of the 48 cases of non-B hepatitis, 7 were anicteric and most were clinically mild. 11 of the 30 cases of hepatitis B were asymptomatic. 1 patient died and 5 out of the 30 patients became persistent carriers of the surface antigen for at least 1 year. The original concentrates of 4 of the 6 batches associated with cases of hepatitis B were found to contain hepatitis B surface antigen by radioimmunoassay. It was noted that the reported incidence of hepatitis in patients with haemophilia in the United Kingdom before the introduction of commercial factor VIII prepared from large plasma pools obtained by plasmapheresis from paid donors was about 1.8%, whereas treatment of such patients with commercial factor VIII was associated with one or more attacks of hepatitis in 66 (17.7% out of a total of 371 patients transfused. In 1978, *Craske et al.* [4, tabulated the evidence for the existence of at least two types of factor VIII-associated non-B transfusion hepatitis in the United Kingdom. Direct evidence of a transmissible agent in a plasma component now comes from the experimental transmission of non-A:non-B hepatitis by factor IX concentrates to 2 chimpanzees [5]. *Wyke et al.* [5] described 6 cases of non-A:non-B hepatitis in London which followed the administration of 4 different batches of factor IX from commercial and non-commercial sources. Of 17 patients who received factor IX on account of chronic liver disease, 4 developed hepatitis, and in 3 of these the illness proved fatal. The incubation periods ranged from 42 to 103 days with a mean of 65 days. 3 young male chimpanzees were inoculated, one with the same batch of factor IX used for the treatment of the above patients, a second chimpanzee with another commercially prepared batch of factor IX upon which there were no reported adverse reactions, and the third chimpanzee received a documented infective non-A:non-B plasma. All 3 chimpanzees developed acute hepatitis after 10 weeks incubation. As in the patients, viral markers for hepatitis A and B, cytomegalovirus and Epstein-Barr virus were unchanged. *Bradley et al.* [6] transmitted non-A:non-B hepatitis to 4 chimpanzees by the infusion of 3 batches of factor VIII implicated in hepatitis in 2 human recipients. Acute phase plasma from 1 of the infected chimpanzees induced hepatitis in 2 other chimpanzees after a relatively short incubation period of 3 weeks. Inoculation of a purified preparation of virus particles obtained from an open liver wedge biopsy resulted in hepatitis in 2 other chimpanzees after a shorter incubation period of about 15 days. Cross-challenge experiments in chimpanzees with other implicated blood products should indicate whether one or more viruses are responsible for non-A:non-B hepatitis.

Since the accumulated evidence [7] concerning non-A:non-B hepatitis suggests firstly, a carrier state in apparently healthy donors, and secondly that the infection must be common, the prepara-

tion of plasma components from large pools of plasma must carry a higher risk than products fractionated from small pools. The highest priority for the prevention of post-transfusion hepatitis must now be directed to the development of specific detection techniques for the virus(s) of non-A: non-B hepatitis or developing methods for inactivation of these agents or their effective removal from blood or plasma.

References

1 Mosley, J. W.: The surveillance of transfusion-associated viral hepatitis. J. Am. med. Ass. *193:* 1007 (1965).

2 World Health Organization: Advances in viral hepatitis. Report of the WHO Expert Committee on Viral Hepatitis. Tech. Rep. Ser. No. 602 (WHO, Geneva 1977).

3 Craske, J.; Kirke, P.; Cohen, B., and Vandervelde, E. M.: Commercial factor VIII associated hepatitis, 1974–75 in the United Kingdom: a retrospective survey. J. Hyg., Camb. *80:* 327 (1978).

4 Craske, J.; Spooner, R. J. D., and Vandervelde, E. M.: Evidence for existence of at least two types of factor VIII-associated non-B transfusion hepatitis. Lancet *ii:* 1051 (1978).

5 Wyke, R. J.; Tsiquaye, K. N.; Thornton, A.; White, Y.; Portman, B.; Das, P. K.; Zuckerman, A. J., and Williams, R.: Transmission of non-A:non-B hepatitis to chimpanzees by factor IX concentrates after fatal complications in patients with chronic liver disease. Lancet *i:* 520 (1979).

6 Bradley, D. W.; Cook, E. H.; Maynard, J. E.; McCaustland, K. A.; Ebert, J. W.; Dolana, G. H.; Petzel, R. A.; Kantor, R. J.; Heilbrunn, A.; Fields, H. A., and Murphy, B. L.: Experimental infection of chimpanzees with antihemophilic (Factor VIII' materials: recovery of virus-like particles associated with non-A:non-B hepatitis. J. med. Virol. (in press, 1978).

7 Zuckerman, A. J.: The three types of viral hepatitis. Bull. Wld Hlth Org. *58:* 1 (1978).

Arie J. Zuckerman, MD, DSc, MRCP, FRCPath, Professor of Microbiology, Director of the Department of Medical Microbiology and WHO Collaborating Centre for Reference and Research on Viral Hepatitis, London School of Hygiene and Tropical Medicine, London, WC1E 7HT (England)