Posttransfusion Hepatitis After Exclusion of Commercial and Hepatitis-B Antigen-Positive Donors

HARVEY J. ALTER, M.D., PAUL V. HOLLAND, M.D., ROBERT H. PURCELL, M.D., JERROLD J. LANDER, M.D., STEPHEN M. FEINSTONE, M.D., ANDREW G. MORROW, M.D., and PAUL J. SCHMIDT, M.D., Bethesda, Maryland

In a prospective study the exclusion of commercial blood donors and donors positive for hepatitis-B antigen (HBAg) resulted in a hepatitis frequency of only 3.7 cases/1000 units transfused. Residual posttransfusion hepatitis was caused by at least two viruses. Four of 10 cases were short incubation, HBAg negative; six cases were long incubation, HBAg positive. One patient had both diseases in sequence. Some cases of HBAg-positive hepatitis occurred despite the fact that patients received only blood that was HBAg negative by counterelectrophoresis; current tests for HBAg, including radioimmunoassay, cannot detect all donors capable of transmitting HBAg-positive hepatitis. The presence of anti-HBAg in donor blood was common and did not correlate with posttransfusion hepatitis; donors with anti-HBAg should not be excluded.

THE FIRST of three prospective studies on posttransfusion hepatitis in open-heart surgery patients at the National Institutes of Health (NIH) was initiated in 1965. That first study (1) demonstrated the risk of commercial blood; 50% of multiply transfused patients who received primarily commercial blood developed anicteric or icteric hepatitis. The second study (2), begun in 1968, showed the risk of receiving blood contaminated with hepatitis-B antigen (HBAg, Au·SH antigen); 69% of patients receiving at least one unit of HBAg-positive blood developed hepatitis, of which 50% was icteric. In that second study, donor units were tested retrospectively for the presence of HBAg, whereas patients were followed prospectively for the development of hepatitis.

The current study was begun in early 1970, when it became feasible to fulfill blood requirements solely from voluntary sources and to screen donor blood prospectively for HBAg. We report on the effect of the combined exclusion of commercial and HBAg-positive donors on the frequency and severity of posttransfusion hepatitis, from a study of 126 patients.

Patients and Methods

DESIGN OF STUDY

Study design was similar to that previously reported (1, 2). Consecutive patients undergoing open-heart surgery between February 1970 and March 1971 were entered into the study if they were over 21 years of age and lived in the continental United States. One hundred and fifty-five patients fulfilled these criteria, of whom 126 completed a 6-month prospective follow-up for the development of icteric or anicteric hepatitis. One patient was excluded from the study because hepatic enzyme elevations present before transfusion made it impossible to evaluate posttransfusion enzyme abnormalities. Two patients refused to participate in the study, and two were lost to follow-up. Twenty-four patients died before the study was completed. Twenty of the 24 deaths occurred in the days or weeks immediately after surgery, and only 4 deaths occurred in those who recovered sufficiently to be discharged from the hospital. None of the deaths were related to hepatitis. Excluding deaths, 98% of those entered in the study completed 6 months of clinical observation as defined below. Eligible patients were divided into two groups according to their geographic location. The first group (45 patients) resided in areas where NIH personnel could obtain blood samples preoperatively, at least biweekly for the first 12 postoperative weeks and monthly thereafter until 6 months after operation. Preoperative and a minimum of 9 postoperative blood samples were obtained; the average collection per patient was 13.5 samples. Serum samples were tested for HBAg, antibody to HBAg

► From the Blood Bank Department, Clinical Center; Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases; and the Surgery Branch, National Heart and Lung Institute; National Institutes of Health, Bethesda, Md.

(anti-HBAg), serum glutamic-oxalacetic and -pyruvic transaminases (SGOT, SGPT), and, where indicated, bilirubin.

The second group (81 patients) lived at a great distance from NIH and were followed by their referring physicians. Measurements of SGOT and SGPT were performed by local laboratories preoperatively, biweekly during the first 3 postoperative months, and monthly during the subsequent 3 months. Results were mailed to NIH. All of these patients had at least the minimum of 10 transaminase tests performed; the average number of tests per patient was 12. Serum samples for serologic testing were only obtained preoperatively, 6 months postoperatively (in 70%), and if transaminase determinations indicated the onset of hepatitis.

A patient was considered to have posttransfusion hepatitis when, between the 2nd and 26th week after transfusion, the SGPT level was greater than 100 Karmen units/ml or its equivalent (two and a half times the upper limit of normal) and a repeat sample, one or more weeks thereafter, was greater than 80 Karmen units/ml. Icteric hepatitis was diagnosed when the total bilirubin was greater than 2.0 mg/100 ml or jaundice was noted by a physician. Histories were reviewed and patients examined before the diagnosis of hepatitis was established, to exclude other causes of enzyme elevations, such as congestive failure and drug- or anesthesia-induced hepatitis. No evidence was found in any patient to suggest that the hepatitis was contracted from infectious sources other than transfusion.

Donor units were screened for HBAg by agar gel diffusion during the first 7 months of the study and by counterelectrophoresis during the last 8 months. Positive units were not transfused. Serum samples from pilot tubes of donor units were stored at −20 °C. Seventy percent of the serums initially screened by agar gel diffusion were subsequently retested by counterelectrophoresis, and 77% of those initially screened by agar gel diffusion or counterelectrophoresis were subsequently retested by complement fixation. Donor units that were given to a patient who developed posttransfusion hepatitis were, in addition, tested for HBAg by hemagglutination inhibition and solid-phase radioimmunoassay. They were also tested for anti-HBAg by radioimmunoprecipitation. Donor serums to 51 (40%) of the patients were tested for SGOT and SGPT at the time of donation.

TEST METHODS

Agar gel diffusion was performed in 0.9% agarose by the method of Prince (3), except that protamine was not added. Wells were 2 mm in diameter and were separated by 3 mm edge to edge.

Counterelectrophoresis was performed by the method of Alter, Holland, and Purcell (4) with 1% agarose, a discontinuous buffer system, 5-mm (25-μlitre) wells, and a constant current of 30 mA (2.5 V/cm) for 90 minutes.

Complement fixation was performed as previously described (5), with 4 to 8 units of antibody and 1.7 exact units of complement and by overnight fixation at 4 °C.

Hemagglutination inhibition was done by the method of Vyas and Shulman (6), using a microtiter system. Purified HBAg was coupled to cells with chromic chloride*, and inhibition at a titer of 1:2 or greater was considered positive.

Solid-phase radioimmunoassay was performed by a test system (AusRIA) developed by Abbot Laboratories, North Chicago, Ill. Unknown serums or positive control serums (0.1 ml) were added to polypropylene tubes coated with guinea pig immunoglobulin that contained anti-HBAg and were incubated for a minimum of 16 hours. After incubation, tubes were washed five times with tris buffer and dried by aspiration. Anti-HBAg (0.1 ml) labeled with ^{125}I was then added and the tubes incubated for an additional 90 minutes. After an additional wash, tubes were counted in a gamma counter†. A negative control pool was tested 10 times in each run. The mean and standard deviations were established for these 10 measurements and were used to define a positive and negative limit. Unknowns that on at least two measurements exceeded the control mean by five standard deviations were considered positive. Most of these tests were performed for us by Abbott Laboratories, but some were performed in our laboratory, using Abbott products on an investigational basis.

Radioimmune precipitation test for anti-HBAg was performed by the double antibody microtiter technique of Lander, Alter, and Purcell (7). Serums to be tested were first incubated with ^{125}I-labeled HBAg for 4 days, followed by addition of anti-human gamma globulin and reincubation overnight. Both supernate and precipitate were counted, and results were expressed as percent of radioactive antigen bound. Binding of greater than 15% on duplicate analysis was considered positive.

The tests for SGOT and SGPT levels on donor serums were performed by a commercial laboratory by the Trans Ac automated system and a modified Reitman-Frankel method, respectively. Both are colorometric procedures. Normal values for SGOT are 9 to 43 International units and for SGPT, 5 to 30 Dade units.

Transaminase tests on recipients were done by a kinetic assay, with an upper normal limit of 40 Karmen units for SGOT and 35 Karmen units for SGPT.

Results

METHODS OF DONOR SCREENING

A total of 13 995 donor bloods were processed at the NIH Blood Bank during this study; 17% of them were administered to patients in the study. The first 7766 were tested for HBAg by agar gel diffusion. Nine (0.12%) were found positive. When counterelectrophoresis was introduced later, 8 of 6229 subsequent donors (0.13%) were found to be HBAg positive. The rate of detection was thus the same with agar gel diffusion and counterelectrophoresis; the overall frequency of HBAg in 13 995 voluntary donors was 0.12%. None of these HBAg-positive units were transfused.

Of the blood units screened initially by agar gel diffusion and transfused to patients in this study, 70% and 65% were subsequently retested by coun-

* Prepared by Electro-Nucleonics, Inc., Bethesda, Md.
† Nuclear-Chicago Corp., Des Plaines, Ill.

terelectrophoresis and complement fixation, respectively. Counterelectrophoresis did not detect any HBAg-positive donors who had been missed by agar gel diffusion. Complement fixation did detect one HBAg-positive unit that had been missed by agar gel diffusion and counterelectrophoresis; this blood had been given to a patient who developed posttransfusion hepatitis (*see* below). The results of additional tests on donors whose blood was given to patients who contracted hepatitis or to patients whose antibody responses suggested exposure to HBAg are described below.

FREQUENCY OF HEPATITIS: CLINICAL AND SEROLOGIC ANALYSIS OF HEPATITIS CASES

Nine of 126 patients (7.1%) developed hepatitis; 3 of the 9 cases were icteric. Based on an average transfusion of 19 units of blood per patient, this represents a hepatitis risk of 0.37% per unit (3.7 cases/1000 units) and an icteric hepatitis risk of 0.13% per unit.

Table 1 depicts the occurrence of 10 episodes of hepatitis in these 9 patients. Patient J.K. had two distinct episodes of hepatitis occurring in sequence; one was short incubation, HBAg negative, and one was long incubation, HBAg positive. Overall, 4 of the 10 episodes had a short incubation period. None of these recipients showed HBAg in their acute-phase serum by counterelectrophoresis, complement fixation, hemagglutination inhibition, or solid-phase radioimmunoassay, and none developed antibody to HBAg that was detectable by the radioimmune precipitation test. All of the donors to these patients were negative for HBAg by agar gel diffusion, counterelectrophoresis, complement fixation, and hemagglutination. Eighty of the 82 donors were available for retest by solid-phase radioimmunoassay; none were positive for HBAg.

In contrast, six episodes of hepatitis had a long incubation period. In all of these patients the acute-phase serum showed HBAg. In two of these six the acute-phase serum was negative by agar gel diffusion, counterelectrophoresis, and hemagglutination and equivocal by complement fixation but was reproducibly positive by solid-phase radioimmunoassay. Five of these six patients, including the two whose HBAg was detected only by solid-phase radioimmunoassay, subsequently developed anti-HBAg, as demonstrated by radioimmune precipitation. The sixth became a chronic HBAg carrier and has not had detectable antibody.

Pre- and post-transfusion samples from the four patients with short-incubation hepatitis were tested for complement-fixing antibody to the AD-169 strain of cytomegalovirus. All four had preexisting antibody titers ranging from 1:8 to 1:64, and in no case did posttransfusion samples show an increase in titer. Antibody to Epstein-Barr virus was not measured.

Table 1. Serologic Analysis of 10 Episodes of Posttransfusion Hepatitis

Recipient	Incubation Period	Presence of HBAg	Anti-HBAg (Seroconversion)
	days		
W.A.	25	No	No
J.C.	28	No	No
M.K.	39	No	No
J.K.*	14	No	No
J.K.*	89	Yes	Yes
J.S.	95	Yes	Yes
M.W.	105	Yes	Yes
V.F.	75	Yes	Yes
A.T.	89	Yes	Yes
J.G.	106	Yes	No†

* Same patient with two distinct episodes of posttransfusion hepatitis.
† Became chronic carrier of HBAg.

Of the four episodes of short-incubation hepatitis unrelated to HBAg, three were anicteric and one icteric. Of the six episodes of long-incubation, HBAg-related hepatitis, four were anicteric and two icteric. Transaminase values returned to normal in all nine patients who developed hepatitis. Recovery, as timed by normal transaminase values, occurred in from 2 to 10 weeks in eight of these patients. One patient's transaminase level was still abnormal 13 weeks after the onset of hepatitis but had returned to normal when next tested 1 year later. This patient became a persistent carrier of HBAg and continued to have the antigen even when hepatic enzyme levels had become normal.

ANALYSIS OF NONHEPATITIS CASES

Serial samples (average of 12) were available for serologic testing from 43 of the 117 patients who did not develop hepatitis. None of these 43 had HBAg in posttransfusion samples, by counterelectrophoresis and complement fixation. Only one patient seroconverted; radioimmune-precipitation-detectable antibody was present in a 6-month posttransfusion sample but not in a pretransfusion sample. This patient had maximum elevations of serum glutamic-oxalacetic and -pyruvic transaminases (SGOT, SGPT) of 92 and 51 Karmen units/ml, respectively, 8 weeks after transfusion. These enzyme abnormalities may have represented hepatitis, but the magnitude of elevation was not sufficient to meet the criteria of our study. This solitary seroconversion without definite hepatitis contrasts with the 45% seroconversion rate in those who developed hepatitis

and the 83% rate in those who developed HBAg-positive hepatitis and suggests that inapparent infection with the hepatitis-B virus occurred infrequently in this study.

Although 6-month serial samples for serologic testing were available from only 43 of the 117 non-hepatitis patients, samples taken during the first 2 postoperative weeks were available from all of these patients. During this period 10 patients had anamnestic antibody responses to HBAg; antibody, detectable preoperatively only by the very sensitive radioimmune precipitation test, became readily detectable postoperatively by agar gel diffusion and counterelectrophoresis. Within the limitations of our sampling, antibody detectable by gel techniques first appeared from 4 to 13 days after transfusion (average, 10 days). Passive transfer of anti-HBAg was ruled out in all cases by an earlier postoperative sample that was negative by agar gel diffusion and counterelectrophoresis (six cases) or by the pattern and duration of antibody responses (four cases).

From seroconversion and anamnestic antibody responses, it is thus probable that 11 of 117 patients who did not develop hepatitis by our criteria were exposed to HBAg at the time of transfusion (*see* below).

PREEXISTING ANTI-HBAg IN THE RECIPIENT, AND SUSCEPTIBILITY TO HEPATITIS

In 29 of 126 patients (23%) anti-HBAg was detectable in the pretransfusion sample by radioimmune precipitation (Table 2). One of the 29 (3.4%) developed hepatitis (HBAg negative), as compared with 9 of 97 patients (9.3%) who did not have preexisting anti-HBAg (6 cases HBAg positive). Chi-square analysis with Yates correction did not show a significant relationship ($P > 0.3$) between the presence of preexisting anti-HBAg and the subsequent overall occurrence of posttransfusion hepatitis or the specific occurrence of HBAg-positive hepatitis. However, no patient with preexisting anti-HBAg developed HBAg-positive hepatitis, including 10 patients who had anamnestic rises in anti-HBAg, suggesting exposure to HBAg at the time of surgery.

Table 2. Preexisting Anti-HBAg in the Recipient and Susceptibility to Posttransfusion Hepatitis

	Number	HBAg-Positive Hepatitis	HBAg-Negative Hepatitis	Total Cases of Hepatitis
Preexisting anti-HBAg	29*	0	1	1
No preexisting anti-HBAg	97	6	3	9

* Includes 10 patients who had anamnestic antibody response, suggesting exposure to HBAg at surgery.

RETROSPECTIVE SEROLOGIC ANALYSIS OF SUSPECT DONORS

Serum samples from donors involved in a case of posttransfusion hepatitis or in a case where seroconversion or an anamnestic antibody response to HBAg occurred were tested by complement fixation, hemagglutination inhibition, and solid-phase radioimmunoassay, in addition to the initial tests by agar gel diffusion and counterelectrophoresis. This testing is summarized in Table 3. Only those cases where most of the donor serums were available for retest are tabulated.

Serums from 95% of the donors involved in the four episodes of short-incubation, HBAg-negative hepatitis were available for retest; all were negative for HBAg by complement fixation, hemagglutination inhibition, and solid-phase radioimmunoassay. Analysis of donors to the six cases of long-incubation, HBAg-positive hepatitis showed the following: [1] In one case (Patient V.F.), a single donor was positive by complement fixation. This complement-fixation-positive reaction was confirmed on retesting, but insufficient serum was available for test by hemagglutination inhibition or solid-phase radioimmunoassay. [2] In two cases most of donor serums were lost or not present in sufficient quantity to make retrospective testing meaningful. All available serums were negative by all the listed tests. [3] In three cases (Patients J.K., J.S., and M.W.) 94% of donor serums were available for retest; all were negative for HBAg by counterelectrophoresis, complement fixation, hemagglutination inhibition, and solid-phase radioimmunoassay.

Most (83%) of the serums from donors to 6 of 11 patients who had seroconversion or anamnestic antibody responses were available for retest by solid-phase radioimmunoassay. One antigen-positive donor was found in each of two cases. These 2 serums were reproducibly positive by solid-phase radioimmunoassay, exceeding the mean of the negative controls by more than 10 standard deviations, but were negative by agar gel diffusion, counterelectrophoresis, complement fixation, and hemagglutination inhibition.

ANTI-HBAg IN THE DONOR AND HEPATITIS IN THE RECIPIENT

The donors to 54 patients were tested for the presence of anti-HBAg by radioimmune precipitation. Donor serums were not tested for anti-HBAg if the recipient had had anti-HBAg before transfusion

Table 3. Retrospective HBAg Testing of Suspect Donors

Recipient Status	Total Donors (All Negative by CEP or AGD, or Both)*	Number Tested by CF, HAI*	Number Positive by CF, HAI	Number Tested by SPRIA*	Number Positive by SPIRA
HBAg-negative hepatitis					
W.A.	13	13	0	13	0
J.C.	18	18	0	18	0
M.K.	35	33	0	33	0
J.K.†	16	16	0	14	0
HBAg-positive hepatitis					
J.K.†	16	16	0	14	0
J.S.	13	13	0	12	0
M.W.	22	22	0	22	0
V.F.	23	14	1	13	0‡
Seroconversion without hepatitis					
J.L.	14	14	0	14	0
Anamnestic antibody response					
W.B.	16	14	0	14	1§
L.H.	10	10	0	10	0
G.P.	13	10	0	10	0
O.W.	23	23	0	16	1‖
E.J.	19	19	0	15	0

* CEP = counterelectrophoresis; AGD = agar gel diffusion; CF = complement fixation; HAI = hemagglutination inhibition; SPRIA = solid-phase radioimmunoassay.
† Same patient developed two episodes of posttransfusion hepatitis.
‡ The CF positive unit given this patient was not available for SPRIA determination.
§ Eighteen standard deviations from the mean.
‖ Eleven standard deviations from the mean.

or was shown to have received a unit of HBAg-positive blood or to have seroconverted or if less than 80% of donor serums were available for retest by radioimmune precipitation. A total of 827 donor serums were tested, of which 73 (8.8%) had anti-HBAg.

Three of five patients who developed hepatitis received at least one unit of blood containing anti-HBAg, but so did 35 of 49 patients who did not develop hepatitis (Table 4). Chi-square analysis did not show a significant relationship ($P > 0.3$) between donor anti-HBAg and recipient hepatitis.

Despite the fact that three patients who received anti-HBAg developed hepatitis, in two the hepatitis was HBAg negative and was not followed by the development of anti-HBAg. Indeed, one of these patients received eight units of blood containing anti-HBAg without showing HBAg or anti-HBAg himself.

The 35 patients in the nonhepatitis group received a total of 61 units of blood containing anti-HBAg; 19 received 2 or more units. None of these 35 patients had anti-HBAg when tested 6 months postoperatively.

Donor serums were not available for a sufficient number of recipients to calculate the absolute number of anti-HBAg units received by each. However, extrapolating from the 8.8% frequency of anti-HBAg in this donor population, it can be estimated that 141 units of blood containing anti-HBAg were given to 89 presumably susceptible patients (that is, those without anti-HBAg before transfusion) who did not develop hepatitis.

ABNORMALITIES OF DONOR TRANSAMINASE AND HEPATITIS IN THE RECIPIENT

Measurements of SGOT and SGPT levels were done on samples of donor units administered to 51 patients. All the donor serums were available in 28 of these cases and greater than 85% in the rest. An abnormal transaminase level was arbitrarily established as one that exceeded the upper limit of laboratory normal by more than 10 units. By this criterion, of 944 serums tested, 48 (5%) had an abnormal SGPT level, 79 (8%) had an abnormal SGOT, and 110 (11%) had an abnormal SGOT or SGPT, or both. Overall, 80% of the 51 recipients received 1 or more units of blood with an elevated transaminase level; only 1 recipient developed hepatitis.

Table 4. Anti-HBAg in the Donor and Hepatitis in the Recipient

	Number of Patients	Cases of Hepatitis	No Hepatitis
Received anti-HBAg*	38	3†	35
No anti-HBAg‡	16	2	14
Total	54	5	49

* Received at least one unit of blood containing anti-HBAg.
† Hepatitis HBAg negative in two cases.
‡ None of blood received contained anti-HBAg.

Because SGPT levels more specifically reflect hepatic dysfunction, the following relates only to data derived from this test. Only 1 of 26 patients who received at least 1 unit of blood with an elevated SGPT level developed hepatitis (Table 5). Five of these patients received 3 or more units of blood with an elevated level. Twenty-five patients received blood only from donors with a normal SGPT level; one developed hepatitis. Chi-square analysis with Yates correction did not show a significant association ($P > 0.3$) between abnormalities of donor SGPT and subsequent recipient hepatitis.

Eleven patients received at least 1 unit of blood in which the SGPT level was more than twice the upper limit of laboratory normal; four received 2 such units. None of these 11 patients developed hepatitis.

The SGPT level was measured in the donor units administered to two patients with short-incubation, HBAg-negative hepatitis. All the donors to 1 patient and 31 of 35 to the other were tested. Only one donor had an abnormal SGPT level, and this was minimally elevated (41 Dade units).

Table 5. Donor Levels of Serum Glutamic-Pyruvic Transaminase (SGPT) and Recipient Hepatitis

SGPT Level	Number of Patients	Cases of Hepatitis	No Hepatitis
Normal*	25	1	24
Elevated†	26	1	25
Total	51	2	49

* All donors to each patient had normal SGPT levels.
† At least one donor to each patient had an SGPT level of more than 10 Dade units/ml above the upper limit of laboratory normal. Includes five patients who received blood from three or more such donors.

Discussion

A prospective study to determine the effectiveness of the combined exclusion of commercial and HBAg-positive blood donors was a logical outgrowth of posttransfusion hepatitis studies in this and other laboratories (1, 2, 8-10). The relative importance of each of these variables (transfusion of commercial and of HBAg-positive blood) can be estimated by an analysis of an earlier, identically structured study (2) of the same patient population. In that earlier study (1967 to 1969) the overall frequency of posttransfusion hepatitis was 33% (20 cases/1000 units), representing the net result of some patients who received only commercial blood and some who received only voluntary blood, some in each donor class who received HBAg-positive blood and the majority who received only HBAg-negative blood. It can be estimated from that study that if HBAg-positive blood had been excluded and HBAg-negative blood substituted, while maintaining donor sources constant, the hepatitis rate would have decreased from 33% to 25%, a reduction of 25%. If, on the other hand, commercial donors had been excluded but HBAg-positive voluntary donors retained, the rate would have decreased from 33% to 10%, a reduction of 70%. Lastly, if all commercial and HBAg-positive donors had been excluded and only HBAg-negative voluntary blood transfused, the predicted hepatitis rate would have been 5%.

In our study the simultaneous exclusion of commercial and HBAg-positive donors resulted in a hepatitis rate remarkably close to that predicted. Nine (7.1%) of 126 patients developed hepatitis (3 cases icteric). Based on an average transfusion number of 19 units per patient, this represents a hepatitis risk per unit of 0.37% (3.7 cases/1000 units) and an icteric risk of 0.13% (1.3 cases/1000 units). Although it is impossible to sort out totally the relative importance of each variable altered, it would appear that the exclusion of the commercial donor was the most significant determinant in the marked decrease (82%) in the hepatitis rate that was achieved.

It had previously been estimated that exclusion of HBAg-positive blood donors, detected by agar gel diffusion and counterelectrophoresis, would decrease the rate of posttransfusion hepatitis by approximately 25% (11). This is consistent with the prediction made from our earlier study as well as with the data obtained in this study. The frequency of HBAg, as detected by counterelectrophoresis, in our donor population is 1.3/1000 donors. Since approximately 70% of HBAg-positive units result in hepatitis (2), the expected frequency of HBAg-related hepatitis would be 0.9 cases/1000 units transfused. In our study 3.7 cases occurred per 1000 units transfused, despite the fact that donors positive by counterelectrophoresis were excluded. Based on the above calculations, an additional 0.9 cases/1000 units would have been expected if blood positive by counterelectrophoresis had been transfused, for a total of 4.6 cases/1000 units. The exclusion of counterelectrophoresis-positive donors thus prevented an estimated 20% (0.9/4.6) of posttransfusion hepatitis. Somewhat in contrast is the study of Senior and associates (12), which suggests that the exclusion of HBAg-positive donors plays a greater role in the reduction of posttransfusion hepatitis than we have indicated. In that study posttransfusion hepatitis was reduced from 18% to 3.3% solely by exclusion of HBAg-positive donors.

The reasons for residual cases of posttransfusion hepatitis after exclusion of these two major risk

factors are of considerable interest and can be explained, in part, by analysis of the 10 episodes of hepatitis that occurred in 9 patients in this study. That their posttransfusion hepatitis was the result of at least two different viruses was demonstrated by Patient J.K., who first developed short-incubation, HBAg-negative hepatitis, unassociated with the development of anti-HBAg, and who later developed long-incubation, HBAg-positive hepatitis with subsequent seroconversion. Overall (Table 1), four episodes were clearly of short incubation and unrelated to HBAg, as demonstrated serologically by testing for antigen and antibody by all the methods used in this study. These cases were presumably caused by hepatitis-A virus, but the lack of a marker for this virus precludes a specific diagnosis. Antibody titers to cytomegalovirus did not implicate this virus in any of these four episodes.

Most striking was the fact that six episodes of hepatitis were HBAg positive despite the fact that all donors were HBAg negative by current screening methods. The donor serums to two of these six cases were not available for retrospective testing. In one case an HBAg-positive donor missed by counterelectrophoresis was detected by complement fixation. In one case, where 100% of donor serums were available for retest, and in two others, where greater than 85% were available, donors were negative by all methods, including radioimmunoassay. The possibility that HBAg-positive hepatitis was coincidentally transmitted by a nonparenteral route cannot be excluded absolutely, but there was nothing to suggest this in the clinical histories, and the onset of hepatitis in each case was typical of that for posttransfusion hepatitis (incubation period, 11 to 15 weeks). Evidently, there is a discrepancy between the level of detection of HBAg and the level of infectivity of this agent. This is consistent with the studies of Barker and colleagues (13), which showed that a plasma pool containing a low titer of HBAg (complement fixation, 1:10) could be diluted 1:10-000 and still produce icteric hepatitis in recipient volunteers and could be diluted 1:10 000 000 and still produce antigenemia in these volunteers. These infectivity levels are beyond the range of any currently available method. A significantly more sensitive test for the detection of HBAg is thus needed.

The solid-phase radioimmunoassay test, although not sufficiently sensitive to detect all infectious samples, was more sensitive than other methods tested. Although it failed to implicate a specific donor in three cases of HBAg-positive hepatitis, it detected an HBAg-positive donor in two of six nonhepatitis cases tested, in which the recipient either seroconverted or had an anamnestic antibody response to HBAg (these donors were negative by agar gel diffusion, counterelectrophoresis, complement fixation, and hemagglutination inhibition). It also showed HBAg in the serums of two hepatitis patients despite negative or equivocal results by all other tests.

Although serums were not available for retrospective testing of all donors, analysis of these 10 episodes of hepatitis suggests that a proportion of posttransfusion hepatitis is unrelated to HBAg and cannot be prevented by current screening methods for HBAg; that a proportion of posttransfusion hepatitis is HBAg positive despite prescreening of donors for HBAg by current methods; and that a proportion of HBAg-positive donors cannot be detected by any currently available method, but that the radioimmunoassay detects some implicated donors missed by other methods.

The data in this study provide information on the significance of the presence of anti-HBAg in both recipients and donors. Twenty-nine patients had anti-HBAg at the time of transfusion; only one developed hepatitis, and this hepatitis was HBAg negative. The other nine episodes of hepatitis occurred in patients who did not have preexisting antibody; six episodes were HBAg positive. Although chi-square analysis did not show a significant association between the presence of preexisting anti-HBAg and the subsequent development of hepatitis, this may reflect the relatively small number of hepatitis cases involved. It is noteworthy that no patient with preexisting anti-HBAg developed HBAg-positive hepatitis despite the fact that 10 of these patients developed anamnestic antibody responses after transfusion, suggesting specific exposure. Such exposure to HBAg was documented in two of these cases tested retrospectively by solid-phase radioimmunoassay. Based on our second transfusion study (2), where 69% of patients receiving at least 1 unit of HBAg-positive blood developed hepatitis, one would have anticipated 7 hepatitis cases among the 10 patients who developed anamnestic responses if the presence of preexisting antibody did not confer immunologic protection. One could argue that the failure of these patients to develop hepatitis was caused by the small inoculum of HBAg, since donor HBAg was detected either only by radioimmunoassay or not at all. This, however, does not seem tenable, since five other patients developed HBAg-positive hepatitis despite the fact that their donors had HBAg in quantities too small to be detected by counterelectrophoresis. The protective effect of anti-HBAg is also indicated in the studies at Willowbrook State School by Lander and co-workers (14), which

showed that children exposed to the MS-2 pool who developed anti-HBAg were then protected from subsequent hepatitis when rechallenged with the same MS-2 pool. Greenberg and Gocke (15) also showed that HBAg-positive hepatitis did not occur in patients who had anti-HBAg. A previous report (16) by Holland and associates cites two cases of post-transfusion hepatitis occurring despite the presence of high-titer anti-HBAg. However, both of their cases were HBAg negative, and the hepatitis was probably caused by an agent serologically distinct from HBAg. Prince, Brotman, and Cherubin (17) reported the occurrence of hepatitis in five patients who developed an anamnestic rise in anti-HBAg after transfusion, but, in these also, the hepatitis was HBAg negative. On the other hand, Barker and Peterson (18) have shown the sequential development of an anamnestic antibody response to HBAg, followed by HBAg-positive hepatitis. It may be that, in these cases, the virus dose was large enough to overcome the protective effect of antibody or that the anti-HBAg was directed against an HBAg subtype different from that responsible for the hepatitis.

It has been proposed that all blood donors be tested for anti-HBAg and that positive donors be excluded. This is based on the possibility that donors who have antibody may also have immune complexes of HBAg that may, directly or by dissociation, cause hepatitis. Theoretically, this is possible, but available clinical evidence rules against the infectivity of blood containing anti-HBAg. In our study, where 72% of recipients received at least 1 unit containing anti-HBAg, and in a similar prospective study by Gocke (19) there was no correlation between anti-HBAg in the donor and hepatitis in the recipient. Furthermore, 8.8% of donors in our study had anti-HBAg detectable by radioimmune precipitation, a carrier rate far greater than the hepatitis risk per unit (0.37%). Other studies, using radioimmune precipitation (20) or hemagglutination (21), have also indicated that 10% to 20% of normal donors have anti-HBAg. The exclusion of these donors would be a staggering blow to blood procurement programs at a time when donor sources are already threatened by the well-justified attempt to exclude the commercial donor.

The transaminase test has not proved to be a practical method of screening donors for hepatitis (22). However, we investigated the possibility that it could be used as an adjunct to HBAg testing, particularly to implicate donors transmitting hepatitis-A virus. No correlation was found between levels of SGOT or SGPT, or both, and recipient hepatitis. Donor bloods with the highest transaminase levels were transfused to patients who did not develop hepatitis. The exclusion of donors with an abnormal transaminase levels would probably produce a prohibitive donor loss without a concomitant decrease in posttransfusion hepatitis. However, donor SGPT levels were measured in only two cases of short-incubation, HBAg-negative hepatitis. Although no significant abnormalities were detected in these donors, the small number of cases tested does not permit a definitive statement regarding the efficacy of transaminase testing for the prevention of posttransfusion hepatitis-A.

The exclusion of commercial and HBAg-positive blood donors has resulted in a marked reduction in posttransfusion hepatitis. Further decrease in the hepatitis rate will depend on discovery of a marker for hepatitis virus A, a more sensitive detection system for HBAg, or development of a practical method of inactivation or removal of hepatitis viruses from blood and blood products.

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► Requests for reprints should be addressed to Harvey J. Alter, M.D., Blood Bank Department, Clinical Center, National Institutes of Health, Bethesda, Md.

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