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A Prospective Study of Posttransfusion Hepatitis

The Role of Australia Antigen

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Transfusion of blood containing Australia antigen (Au) was followed by development of hepatitis, Au antigenemia, or both in 52% of recipients. Another 23% of Au recipients exhibited an immune response. However, transfusion of Au-*negative* blood was associated with anticteric hepatitis in 16% and icteric hepatitis in 2% of the recipients. The typical Au recipient with hepatitis was Au positive during the acute phase and had a severe clinical illness. In the recipient of Au-negative blood, hepatitis was characterized by Au-negative tests in the acute phase and a mild illness. On the basis of these data, estimates of the relation of Au to the overall problem of posttransfusion hepatitis suggest that Au accounts for approximately 25% of icteric cases. Twenty percent of patients who developed Au antigenemia following transfusion became carriers of the antigen. Patients who became Au carriers tended to have mild or anicteric hepatitis, while those with severe, icteric attacks reverted to the Au-negative state. The frequency of Au antigen in commercial donor blood was 13-fold greater than in volunteer blood.

The development of hepatitis following blood transfusion is a long-standing and continuing medical problem. The discovery of the Australia (Au, HAA, SH) antigen and early reports indicating a correlation with "serum hepatitis" gave rise to new hope that posttransfusion hepatitis could be prevented.[1,2] The studies described here were undertaken in a prospective fashion to assess the role of Au in posttransfusion hepatitis. A positive correlation between transfusion of blood containing Au and the development of hepatitis in the recipients was suggested early in the course of the study, and preliminary reports of this finding have appeared.[3,4] This article further documents the hazard of transfusing Au-positive blood in a more substantial number of patients. In addition, evidence is presented that posttransfusion hepatitis is not a single entity, and the clinical characteristics of Au-positive and Au-negative posttransfusion hepatitis are described. These observations provide new perspective on the overall problem of posttransfusion hepatitis and the relative contribution of Au.

Subjects and Methods

The patients in this study underwent transfusion at the Columbia-Presbyterian Medical Center in New York City from December 1968 to January 1971. During this period all donor blood entering the Blood Bank was tested for Au. The recipients of all Au-positive blood were identified and followed up. A control group composed of the recipient of every tenth unit of Au-negative blood was followed up in the same manner as the Au-positive recipients. Contrary to our preliminary report on the early phases of this study,[4] there was no difference in age, sex, race, or medical diagnosis between the Au-positive and Au-negative recipient groups described here. The average number of transfusions was 6.7 in the Au-positive recipients and 6.3 in the Au-negative recipients.

The protocol for prospective follow up consisted of observation of the recipients for signs and symptoms of hepatitis every one to two weeks for a period of six months following transfusion. Serum glutamic oxaloacetic transaminase (SGOT) and Au levels were determined regardless of whether the patient was symptomatic. In most instances, patients were seen and tests done at the Columbia-Presbyterian Medical Center. Occasionally, follow up was accomplished with the aid of outside physicians and laboratories, with specimens sent to this laboratory for Au testing. As a measure of the efficiency of the follow-up program, the average number of contacts per patient was 10.9 in the positive recipient group and 12.1 in the negative recipient group out of a scheduled 12 visits per patient over the six month period of observation. Hepatitis was defined in this study as an SGOT elevation of greater than 100 international units (IU) on two consecutive occasions without other evident cause. Icteric hepatitis was characterized by clinically detectable jaundice and a serum bilirubin level in excess of 4 mg/100 ml.

In the midst of the study it became obvious that recipients of Au-positive blood were manifesting hepatitis with a much greater frequency than recipients of negative blood. This evidence was so compelling that in 1970 transfusion of known positive units was no longer permitted, although in

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Table 1.—Observations in 283 Recipients of Au-Positive or Au-Negative Blood Transfusions

	Blood Infused	
	Au Positive	Au Negative
Died or lost to follow up	33	71
Survivors	84	94
Hepatitis and Au antigen	21	2 (2%)
Hepatitis only	14 (52%)	17 (18%)
Au antigen	9	0
Au antibody	19 (23%)	1 (1%)
Well	21 (25%)	74 (79%)

Table 2.—Severity of Au-Positive and Au-Negative Hepatitis

Data	Au Antigen Positive	Au Antigen Negative
Recipents	84	94
No. with hepatitis	35	17
No. icteric	28	2
No. hospitalized	23	1
Average maximum bilirubin level (mg/100 ml)*	9.0	1.7
Average maximum SGOT level (IU)†	1,159	414

* $P < .005$.
† $P < .025$.

Table 3.—Au-Negative Recipients Alleged to Have Hepatitis

Condition	No. of Recipients
Definite hepatitis, Au-negative	50
Hepatitis with Au antigen	17
Other liver diseases	26
Definitely not liver disease	10
	103

emergency situations occasional units slipped through before the Au test result was available.

Australia antigen was detected by the two-dimensional immunodiffusion method (ID) in the initial phases of the study.[5] Subsequently, the more sensitive and rapid counterimmunoelectrophoretic (CEP) method was developed in this laboratory and gradually phased into the routine screening process.[6] The effect on the study of this change in methods will be discussed. Hemagglutination (HA) and complement fixation techniques (CF) were employed as more sensitive methods for retrospective examination of selected specimens. Hemagglutination tests were performed by the method of Vyas and Shulman[7] and the purified Au antigen for sensitization of erythrocytes was prepared by repeated density gradient centrifugation. Complement fixation was carried out by a standard microtiter procedure employing 2 exact units of complement and 4 to 8 units of antibody with an 18-hour fixation step.[8] The specificity of standard serum containing Australia antigen and antibody have been compared with standard reagents in the laboratories of Blumberg, Prince, and Holland. Titers are expressed as the greatest dilution of the patient's serum giving a definite positive reaction, and are based on the ID technique unless otherwise specified.

Results

Transfusion of Blood Containing Au.—Table 1 summarizes observations in 117 recipients of blood transfusions containing Au. A number of patients were lost to the study either because of death or inadequate follow up. Of 84 survivors who were adequately followed up, hepatitis with Au antigenemia was seen in 21, hepatitis without antigen in 14, and antigenemia in nine. In all, hepatitis, antigenemia or both occurred in 44 (52%) of the 84 surviving recipients of positive blood. The nine patients listed as having only Au antigenemia also had abnormal liver chemical values, but are categorized separately because they had other diseases which may have affected the liver. Acute-phase serum samples from 14 patients with hepatitis in whom Au was *not* detected by ID were also tested by the more sensitive CEP, HA, and CF techniques and still found negative. The majority of the 35 patients with clear attacks of acute hepatitis were clinically jaundiced, and this will be described further below. In addition, 19 (23%) of the recipients of Au-positive blood developed antibody to Au which was not detectable in pretransfusion specimens. Overall, 63 (75%) of the recipients exhibited a response to the transfusion of Au either by the development of hepatitis antigenemia or by an immune response.

Transfusion of Au-Negative Blood.—Table 1 also shows the findings in a control group of recipients of Au-negative blood. In this group, 94 survivors were successfully followed up for a minimum of six months. Au-negative hepatitis was seen in 17 individuals (18%). Only two of these patients were icteric. The remainder had mild, anicteric hepatitis that could easily have been missed had the patient not been followed up closely (see below). Both acute-phase serum samples from these 17 patients and serum samples from their respective donor specimens were retested for Au by CEP, HAI, and CF, but were uniformly antigen negative. It is significant that two cases of hepatitis with Au antigenemia and one individual who developed antibody to Au were seen in this group of presumably negative recipients. Retesting of the donors of the individual who developed antibody revealed that a unit of blood containing a small amount of antigen had originally been missed by ID. Retesting of the donors of the two patients who developed hepatitis with antigenemia failed to disclose a missed positive by the more sensitive techniques.

Patterns of Au-Positive Hepatitis and the Carrier State.—Figure 1 is a schematic illustration of the course of a single patient who received a transfusion of blood containing Au. It will be noted that the antigen was first detected in the patient's serum 17 days after the transfusion and the titer rose rapidly while the patient remained asymptomatic. Thirty-two days following transfusion a definite elevation of SGOT level was noted. On the 42nd day the patient presented with symptoms of hepatitis and was clinically jaundiced. By the time the patient came to the physi-

cian, the serum titer of Au had begun to decline and it disappeared coincident with the patient's recovery from the clinical signs and symptoms of the illness.

This pattern, in which the antigen titer peaks during the incubation period, declines as the patient becomes asymptomatic, and disappears with recovery, was the most common course observed in patients with Au-positive hepatitis following blood transfusion. The antigen was detected in one recipient as early as three days after transfusion (with documented absence before and the first day after transfusion) and appeared as late as 96 days in another, but in most cases Au was first found between one and four weeks after transfusion. Au was shown to be present from 5 to 35 days before appearance of laboratory abnormalities, but once enzyme changes appeared, the onset of clinical symptoms and signs usually followed within 7 to 14 days. In general, the antigen titer declined and disappeared slightly in advance of the resolution of other signs and symptoms, and in some the antigen was undetectable by the time the patient was admitted to the hospital. Thus, the chance of detecting Au was best early in the course of the illness.

Figure 2 illustrates a pattern of disease seen in patients who became carriers of Au. It will be noted that this patient had only mild SGOT elevations following the positive transfusion and never had jaundice or symptoms of hepatitis. However, Au was detected in the serum 28 days following the positive transfusion and rose rapidly to high titers. The antigen has persisted in the patient's serum for more then two years without physical signs or symptoms of hepatitis at any time. Six of the 30 patients who developed Australia antigenemia following a positive blood transfusion progressed to a carrier state in from 17 to 29 months. Four of the six individuals who became carriers had an anicteric course similar to that illustrated in Fig 2, and the others had mild episodes of icteric hepatitis. None of the carriers were receiving renal dialysis or had apparent deficiencies of immune mechanisms.

Comparison of Au-Positive and Au-Negative Hepatitis.—Table 2 compares the severity of the clinical illness seen in the Au-positive and Au-negative recipient groups. In the 84 patients who were Au-positive recipients, 35 cases of hepatitis occurred. Twenty-eight of these 35 were icteric, ie, had visible jaundice and a serum bilirubin level greater than 4 mg/100 ml. Twenty-three patients were sick enough to be hospitalized. In contrast, of the 17 cases of hepatitis in the Au-negative recipient group only two were jaundiced and only one was hospitalized. In addition, the average maximum serum bilirubin concentration was 9.0 mg/100 ml in those with Au-positive hepatitis and 1.7 mg/100 ml in those with Au-negative hepatitis. The average maximum SGOT level was 1,159 IU in the Au-positive patients and 414 IU in the Au-negative patients. These chemical differences are significant at the 0.005 and 0.025 levels, respectively.[9] One death occurred in an Au-positive recipient. Thus, the illness seen in Au-positive recipients was clearly more severe with a higher incidence of jaundice and hospitalization than in Au-negative recipients.

Table 4.—Prevalence and Source of Au-Positive Donor Blood*

Data	1969	1970
Units tested	15,932	17,058
Percent commercial	74	58
Au-Positive units		
Commercial	59	81
Volunteer	0	11
Frequency of antigen		
Commercial	1/83	1/121
Volunteer	0	1/657

*Commercial signifies blood purchased from commercial blood suppliers; volunteer blood was obtained from patients' families, staff, or volunteer community blood banks.

The incubation periods seen in Au-positive and Au-negative patients varied greatly. For the purposes of this study the incubation period was considered to be the time from transfusion to the detection of the first abnormal SGOT value. Clinical symptoms were already present or occurred within a few days following the first SGOT abnormality in most patients, but the onset of symptoms was more variable and difficult to document. Expressed in this way, the average incubation period in the 21 recipients with Au-positive hepatitis was 63 days (range, 30 to 150). In contrast, the average incubation period in the 17 patients with Au-negative hepatitis in the Au-negative recipient group was 45 days (range, 27 to 94). The possibility was considered that the patients with Au-negative hepatitis in the positive recipient group actually had the Au-negative type of disease. However, the mean incubation period in these 14 patients was 66 days. While there may have been a tendency for the Au-positive hepatitis to have a longer incubation period, these differences were not statistically significant. There was no evidence that the dose of virus, as reflected in the Au titer of the donor unit, affected the incubation period, the severity, or the antigen positivity of the illness in these patients.

Hepatitis in Recipients Not Followed in Protocol.—During the period of our study, 6,274 patients received transfusions in this hospital (corrected for patients observed and for deaths). All units of donor blood were tested for Au, and all recipients of Au-positive units were identified and followed. Thus, the large group of patients who were not prospectively followed up was considered to be composed of Au-negative recipients. Many of these patients did continue under the care of a physician and some were eventually reported to have developed posttransfusion hepatitis (Table 3). When the clinical findings in these patients were reviewed, it was concluded that ten of the 103 patients said to have posttransfusion hepatitis had no liver disease at all. Instead, hemolysis, pulmonary disease, or some other explanation was found for their jaundice or enzyme elevations or both. In addition, 26 patients had good clinical evidence of some liver disease other than viral hepatitis, such as halothane hepatitis or metastatic carcinoma. Thus, about one third of the total group of patients who were alleged to have "serum hepatitis" did not really have convincing evidence of a viral form of hepatitis on closer examination. Nevertheless, a significant number of patients did have what appeared to be viral hepatitis on the basis of clinical criteria. Seventeen were Au-positive and 50 were Au-negative while acutely ill.

The group with Au-positive hepatitis during the acute phase was of special interest. It was possible to re-

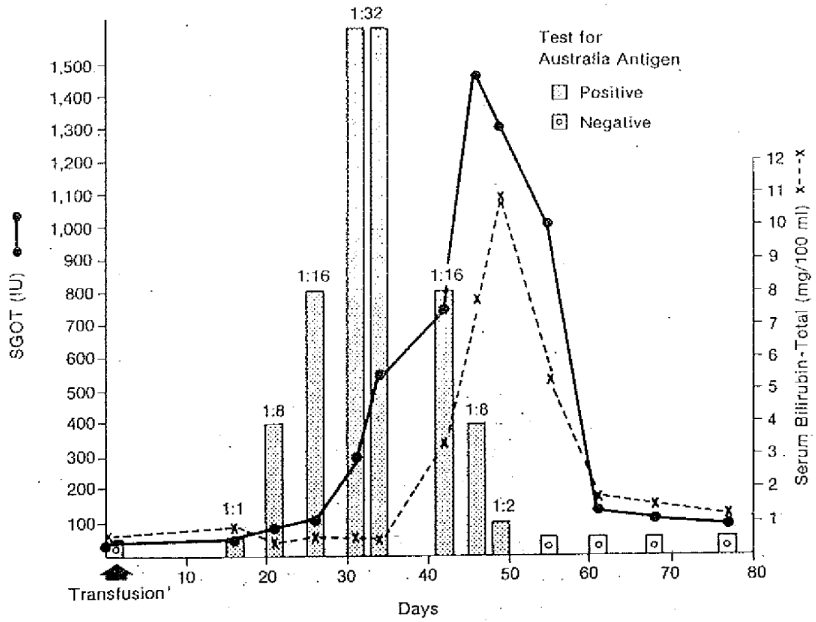
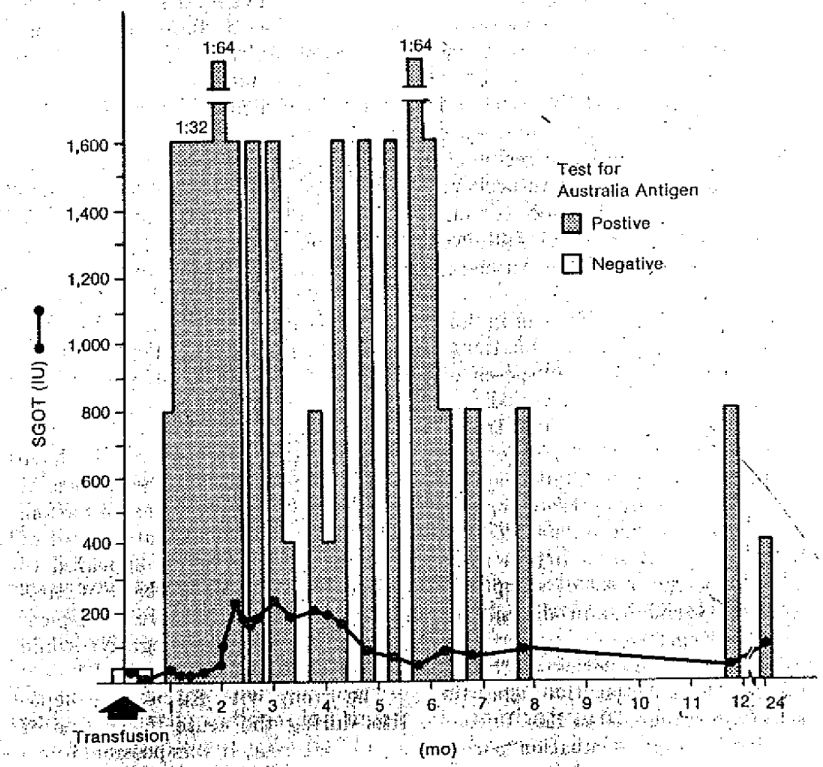


Fig 1.—Schema of commonly observed pattern in patients who develop icteric hepatitis following transfusion of Australia antigen.

Fig 2.—Course of patient who became carrier of Australia antigen following Au-positive blood transfusion. This anicteric pattern was seen in four of six patients who developed carrier state in this study.



trieve all of the donor units in 14 of the 17 Au-negative recipients who developed Au-positive hepatitis, and to retest the specimens with the more sensitive CEP, HA, and CF techniques. It was found that four patients had received Au-positive units which were initially missed. Three of these four cases occurred early in the study when the less sensitive gel diffusion method was in routine use. The donor units of the other ten recipients with Au-positive hepatitis were still Au-negative when retested. Three of the ten had received other blood products, such as fibrinogen and plasma, which may have contained Au but were not tested. The other seven recipients with Au-positive hepatitis could not be accounted for, other than to suggest that they received small amounts of antigen which were undetectable by the best current methods, or that hepatitis had been acquired from a source other than the blood transfusion.

Prophylactic Effect of Normal Human Globulin.—Many of the subjects of this study were given human serum globulin for possible prophylaxis of hepatitis on the option of the attending physician. The preparations used were the common commercially available 16% solutions of pooled normal human globulin prepared by Cohn fractionation. The dose employed varied from 2 to 10 ml given at the time of transfusion and usually repeated one month later. Fourteen (67%) of the 21 Au-positive recipients who developed Au-positive hepatitis received immune serum globulin, and 11 (53%) of the 21 Au-positive recipients who remained well received immune serum globulin. Similarly, in the Au-negative recipient group, ten (59%) of those who developed hepatitis and 25 (34%) of those who remained well had received immune serum globulin. Although the globulin preparations given to these recipients were not tested, other lots of normal human globulin did not contain appreciable anti-Au antibody even by the hemagglutination technique.

Source and Prevalence of Au-Positive Donor Blood.—Table 4 summarizes the prevalence and source of Au-positive units of donor blood during the two-year period of this study. In 1969, a total of 15,932 units were tested, 74% of which were of com-

mercial origin. Fifty-nine units, all from commercial sources, were found to contain Au, for a frequency of 1/83 units. In 1970, more blood was used (17,058 units), but the percentage of commercial blood was reduced (58%). Nevertheless, 92 Au-positive units were still found, including 11 from volunteer sources. Overall, the number of positive units was 13-fold greater in commercial donor blood compared to volunteer blood.

Comment

The high risk of hepatitis associated with the transfusion of blood containing Au has now been confirmed in the substantial number of recipients described here (Table 1). However, it is important to note that recipients of blood which did not contain detectable Au also acquired hepatitis. While the attack rate in such Au-negative recipients was strikingly less (18%) than in recipients of Au, it is significant that some cases of hepatitis did occur. This suggests that the agent associated with Au was not responsible for all cases of posttransfusion hepatitis, and that more than one agent or virus may be involved in causing the syndrome. This idea is supported by the clinical differences in the disease seen in the Au-positive and Au-negative recipient groups. As shown in Table 1, the hepatitis seen in recipients of Au-positive blood tended to be Au-positive during the acute phase while the disease in Au-negative recipients tended to be Au-negative, even when tested for antigen by the more sensitive CF and HAI techniques. In addition, Au-positive hepatitis was more severe than the hepatitis in Au-negative recipients (Table 2).

The incubation periods observed in the two types of hepatitis were quite variable and of no assistance in distinguishing the two groups. There appeared to be a trend toward a longer incubation period in patients with Au-positive hepatitis (mean, 63 days) as compared to patients with Au-negative hepatitis (mean, 45 days). However, there was so much overlap in the range from patient to patient that this difference in average incubation time was not statistically significant. Moreover, the Au-associated form of hepatitis, which presumably represents "homologous serum jaundice" or "long-incubation-type" of hepatitis, was observed to have an incubation period as short as 30 days in one individual. Conversely, the Au-negative hepatitis (short-incubation type?) was observed to have an incubation period as long as 94 days. Thus, the incubation period was unreliable as a clinical feature to differentiate between these two types of hepatitis in the individual patient. It should be noted that the Au titer of the transfused blood had no apparent relation to the severity, incubation period, or antigen positivity of the illness which followed.

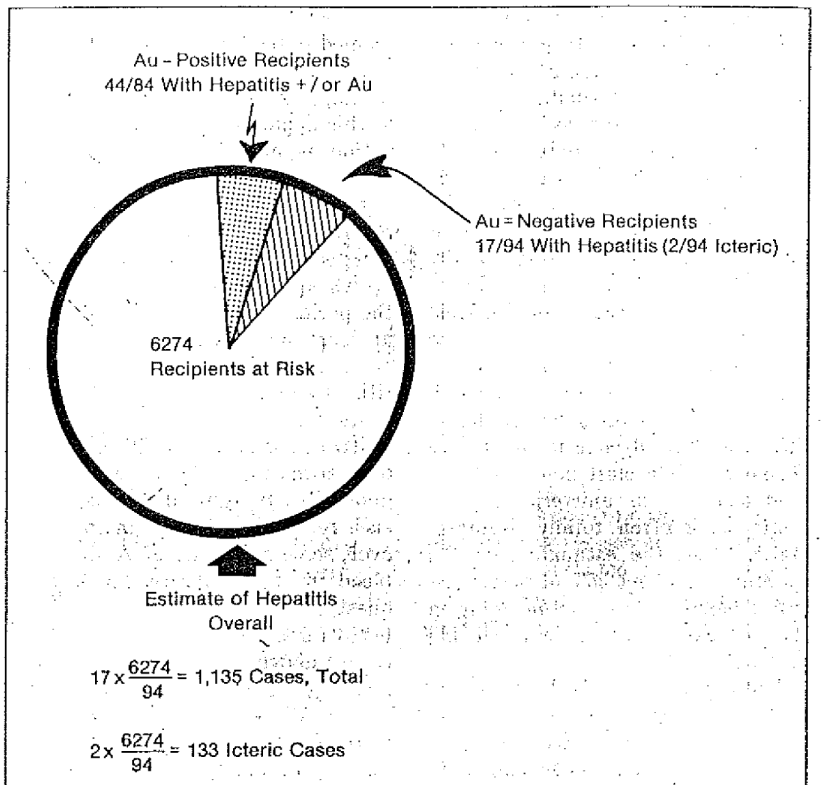


Fig 3.—Estimation of number of cases of hepatitis in total Au-negative recipient population, based on observed cases in prospectively followed Au-negative group. Au-positive and Au-negative recipients followed up in this study represented only small fraction of recipients at risk from transfusion during same period.

Figure 3 provides a perspective on the overall problem of posttransfusion hepatitis and the relative role of Au. Note that the recipients of Au-positive and Au-negative blood who were prospectively followed in this program constituted only a small fraction of the total number of recipients who received transfusions in this hospital during the same period of time. Since all recipients of known Au-positive transfusions were included in the study, the remaining large group of patients not followed up in the protocol were considered to be Au-negative recipients. As illustrated in Fig 3, the frequency of hepatitis in the total Au-negative recipient population at risk can be estimated from the observed cases in the followed group. If this estimate is based on all cases of hepatitis in the Au-negative group—both icteric and anicteric—the number expected in the total population would be quite large (1,135). However, it is probably more relevant to general clinical experience to consider only the icteric cases of hepatitis observed in the Au-negative group. On this basis, it would be calculated that approximately 133 cases of clinically recognizable hepatitis occurred in the total recipient population at risk (and, indeed, such

cases did come to our attention; see Table 2, and below). In comparison, note that 44 patients at most acquired hepatitis attributable to Au (Table 1). Thus, Au-associated hepatitis represented approximately 25% of the total number of cases of icteric hepatitis which might have been expected following blood transfusion in this study. These calculations admittedly favor the Au-associated hepatitis somewhat because all possible cases of Au-associated hepatitis were included whereas only icteric cases of non-Au-hepatitis were considered. However, most Au-associated hepatitis was clinically icteric and severe (Table 2), while most non-Au-associated disease was anicteric and patients were often totally asymptomatic. Thus, the estimate that Au accounts for about 25% of posttransfusion hepatitis has more meaning in the context of the overall clinical problem.

Appropriate care is indicated in extrapolating this experience to hospitals in other areas. Indeed, in populations where Au is less endemic than in New York City, the role of Au in posttransfusion hepatitis may be appreciably less. Nevertheless, the screening of donor blood for Au is still a worthwhile endeavor and the cases prevented would potentially be of a more severe variety. The possible role of other agents in the causation of posttransfusion hepatic damage is of major importance and requires further study. Serologic evidence of transfusion-associated infection by cytomegalovirus and Epstein-Barr virus has been reported, but it is not established that such infections are responsible for hepatic damage.[10,11] Of course, transmission of the agent of infectious hepatitis ("short-incubation" hepatitis) by blood transfusion is an important possibility which cannot be documented at present.

It is noteworthy that six of the 30 patients who manifested Au antigenemia following an Au-positive blood transfusion went on to become carriers of the antigen in excess of six months after recovery from the acute illness. This 20% carrier rate was somewhat surprising and is of significance in terms of the epidemiology of this disease. None of these carriers had recognized immunologic defects, but their response to the infection seemed to be different in that all had a mild acute episode of hepatitis. Again, the pathogenetic significance of this apparently paradoxical observation is not clear at the present time.

Analysis of the cases of alleged posttransfusion hepatitis in Au-negative recipients who were not followed up which were reported to us during the period of the study revealed that at least one third of such alleged cases did not really have viral hepatitis at all (Table 3). This finding suggests that epidemiologic reports of posttransfusion hepatitis may err in overestimating cases as well as the generally recognized tendency of such reports to underestimate. However, some recipients of Au-negative blood in this unobserved group unquestionably developed true viral hepatitis, confirming the finding discussed above of hepatitis in the Au-negative recipient population. Some of the 17 cases of hepatitis which were Au-positive during the acute phase presumably represent units of blood containing Au which escaped detection. This is supported by the reexamination of donor specimens for these recipients. Hepatitis could be accounted for in seven of 14 such patients on the basis of a weakly positive unit which had initially been missed by less-sensitive screening methods or possibly as the result of transfusion of Au-positive blood products which had not been screened. However, the remaining seven recipients with Au-associated hepatitis could not be explained in this way. They may have received very small amounts of antigen which was undetectable even by our most sensitive present techniques. Our current experience with Au detection systems suggests that HAI and CF add little to the efficiency of donor screening by CEP but this problem is under continuing study. The possibility that some patients may have acquired Au-associated hepatitis from a source other than the blood transfusions must also be considered.

Finally, the source and the frequency of donor blood containing Au is of considerable significance in the epidemiology of this disease (Table 4). In this experience, the frequency of Au in commercial or paid donor blood was at least tenfold greater than in volunteer blood. If the estimate above, that Au accounts for only about 25% of posttransfusion hepatitis, is substantially correct, one might anticipate a greater decline in the rate of posttransfusion hepatitis by transfusing only volunteer blood than by screening donors for Au. Furthermore, reduction in the frequency of the non-Au-type of hepatitis might also occur with the exclusive use of volunteer blood. Of course, it is not possible to use only volunteer donors at the present time in large urban hospitals where the requirements for blood greatly exceed the supply of volunteers. It seems clear that resolution of the posttransfusion hepatitis problem will require combined efforts to improve the sensitivity of Au testing and to find methods for detecting other infectious agents in donor blood, as well as to improve blood collection practices.

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Neil B. Kavey, MD, and Harry B. Greenberg, MD, participated in the clinical follow-up of patients and in the development of method. John Gorman, MD, Director, Presbyterian Hospital Blood Bank, permitted us to obtain specimens of donor blood.

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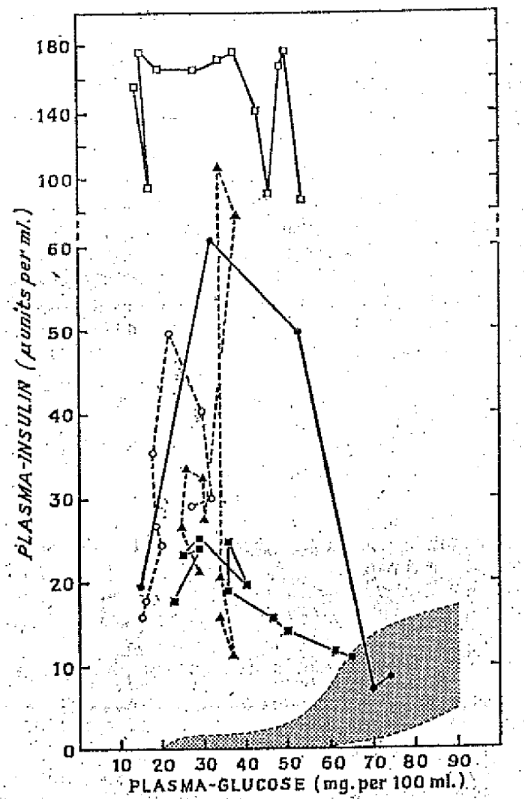

Fig. 4—Five patients with insulinomas in whom there was a temporary rise in plasma-insulin levels in response to fish-insulin-induced hypoglycæmia.

See legend to fig. 2.

which confirmed the diagnosis in the four patients who had normal basal glucose levels after an overnight fast. Although partial suppression of insulin secretion in response to hypoglycæmia was seen in some patients, the plasma-insulin levels remained distinctly abnormal in all the patients studied. In some patients there was a paradoxical increase in insulin secretion during the test. The cause of this is uncertain, but fish insulin contains immunoreactive glucagon, and this may have stimulated insulin secretion.

Hypoglycæmia during a three-day fast has often been used as a diagnostic test for insulinomas, and is an indirect means of demonstrating impaired suppression of insulin secretion. It is not specific unless raised plasma-insulin levels are also demonstrated. Exercise during the fast helps to induce hypoglycæmia, but fish insulin can produce a more certain fall in plasma-glucose over a shorter period. If spontaneous hypoglycæmia has been documented, a positive fish-insulin suppression test is diagnostic of an insulinoma and a fast is unnecessary.

In a patient in whom fasting hypoglycæmia is suspected as a possible cause of a curious attack, the demonstration of normal suppression of insulin secretion during a fish-insulin test probably excludes the diagnosis of an insulinoma. Stimulation tests for insulinomas are not useful in this context, because false-negative results are common.[5,6] A normal fish-insulin test will not exclude other causes of fasting hypoglycæmia, and only a prolonged fast will definitely do this. However, most of the other causes of fasting hypoglycæmia in adults can be easily excluded by other means. Endocrine deficiencies of the pituitary or adrenal, and cirrhosis, are usually severe and clinically apparent before hypoglycæmia occurs. The hypoglycæmia induced by the fish-insulin test provides a stimulation test for cortisol and growth-hormone secretion, with increased plasma levels at the end of the test.[7] Hypoglycæmia induced by ethanol, sulphonylurea, or other drugs, may be suspected from the history, and self-administration of insulin usually induces circulating insulin antibodies. Sarcomas causing hypoglycæmia are usually large, and can be detected by palpating the abdomen, or by a chest X-ray. Thus a normal fish-insulin suppression test combined with clinical assessment and a few simple tests exclude virtually all causes of fasting hypoglycæmia in adults. These tests can be performed on outpatients, and are useful in situations in which fasting hypoglycæmia is a possible, but improbable, cause of curious attacks.

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RISK OF TRANSFUSING BLOOD CONTAINING ANTIBODY TO HEPATITIS-B SURFACE ANTIGEN

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Summary 362 blood-transfusion recipients, whose sera were initially negative for hepatitis-B antigen (HB_sAg), were prospectively followed for clinical and serological evidence of exposure to hepatitis-B virus (H.B.V.) and for the development of hepatitis unrelated to H.B.V. None

of the donor units received by these patients contained detectable HB_sAg. Of the 362 transfusion recipients, 23 (6%) developed 25 episodes of hepatitis; only 4 of these 25 episodes were serologically related to H.B.V. Based on the absence of antibody to HB_sAg (anti-HB_s) prior to transfusion, 278 of the patients were considered susceptible to H.B.V. infection. Of these susceptible patients, 133 received at least one unit of blood containing anti-HB_s; when compared with the 145 who did not receive anti-HB_s, there was no significant difference in biochemical or overt hepatitis B (3/133 vs. 1/145), in serological response to H.B.V. (5/133 vs. 5/145), or in hepatitis unrelated to H.B.V. (11/133 vs. 6/145). It is concluded that blood containing detectable anti-HB_s carries no increased risk of transmitting hepatitis B compared with blood which lacks this antibody.

Introduction

THE risk of developing post-transfusion hepatitis has been markedly reduced by the adoption of universal testing of donor bloods for hepatitis-B antigen (HB_sAg) and by decreased utilisation of commercial blood.[1] However, despite the exclusion of HB_sAg-positive blood donors, some HB_sAg-positive post-transfusion hepatitis continues to occur.[1-4] This could be due to the administration of HB_sAg or other specific antigens associated with hepatitis-B virus (H.B.V.) in quantities below the threshold of current detection methods.[4-8] HB_sAg could also escape detection if that antigen were complexed to and therefore masked by antibody to it (anti-HB_s). In the latter instance, one might detect only circulating anti-HB_s in blood that is potentially infectious.[9] In addition, since the presence of anti-HB_s indicates past exposure to the hepatitis-B virus, the finding of this antibody might be just as valid a reason for donor exclusion as is the currently accepted exclusion based upon a history of clinical hepatitis.[10,11]

Despite these theoretical considerations, blood containing anti-HB_s is still transfused and the few studies performed to date have failed to demonstrate an increased infectivity of such antibody-containing blood; the issue, however, is not totally resolved because the number of individuals followed has been small or because the studies have lacked serological data to assess susceptibility to H.B.V.[12-14] Furthermore, the exclusion of donors with anti-HB_s would severely reduce blood availability, since 5–20% of volunteer donors have anti-HB_s detectable by present, sensitive techniques.[10] We have combined data from three prospective studies of post-transfusion hepatitis in order to determine more clearly if donor blood containing anti-HB_s carries a significantly greater risk of transmitting hepatitis B than blood which lacks this antibody.

Patients and Methods

Design of Studies

Studies were performed in three medical centres: the Washington University Medical Center in St. Louis, the Baylor College of Medicine–Ben Taub General Hospital in Houston, and the National Institutes of Health Clinical Center in Bethesda. The designs of these studies have previously been described.[1-4] Patients were assessed at the Washington University and the Ben Taub General Hospital beginning August, 1971, and at the Clinical Center Blood Bank beginning February, 1970. All patients successfully completed a six-month follow-up by November, 1973.

The Washington University study and the Clinical Center Blood Bank study followed cardiovascular-surgery patients, while the Baylor study followed a randomised sample of general-surgery patients who received blood-transfusions at Ben Taub General Hospital. The three studies are very comparable in design and are summarised in table I. All donor units were tested for antigen and antibody, and recipients were followed at least every two weeks for three months and every month thereafter for three months. Washington University patients were followed every two weeks for six months. Patients were excluded if they received transfusions on more than one occasion or if they received blood derivatives other than plasma, red cells, or whole blood.

Definitions

Hepatitis was diagnosed when, between two and twenty-six weeks following transfusion, alanine aminotransferase (S.G.P.T.) and/or aspartate aminotransferase (S.G.O.T.) rose to at least 2 times the upper limit of normal on 2 successive occasions at least a week apart, and when there was no other obvious explanation for the enzyme elevation. *Icterus* was defined as a bilirubin greater than 2 mg. per 100 ml. *Hepatitis B* was diagnosed when, during an episode of hepatitis, HB_sAg was detected, and/or the patient developed antibody seroconversion. *Seroconversion* was the de-novo appearance, and persistence, of anti-HB_s twenty-one or more days after transfusion in a patient having no pre-existing antibody to the hepatitis-B antigen. *Anamnestic response* was a fourfold or greater rise of anti-HB_s occurring within fourteen days following transfusion in a patient with pre-existing antibody. *Serological response only* was defined as seroconversion, or anamnestic response, or development of HB_sAg in a patient who did not develop enzyme elevations indicative of hepatitis. *Exposure* was measured by development of hepatitis B and/or serological response to H.B.V.

Technique

All donors in this study were tested for HB_sAg by counterelectrophoresis[15,16] prior to transfusion. After transfusion, stored sera from these donors were retested by radioimmunoassay. At Washington University and at Baylor subsequent testing was performed by double-antibody radioimmunoassay (R.I.A.-D.A.)[17-19] and by solid-phase radioimmunoassay (Ausria).[20] At the Clinical Center most specimens, but not all, were retested by Ausria. Patients who were HB_sAg-positive prior to transfusion or who received blood containing HB_sAg were excluded from analysis in this report.

Anti-HB_s was measured by R.I.A.-D.A.[18,21] and by passive hæmagglutination (P.H.A.).[23] These methods have recently been compared.[23] Washington University initially tested for anti-HB_s by R.I.A.-D.A. and confirmed positives by P.H.A.; Baylor screened for antibody by P.H.A. and confirmed positives by R.I.A.-D.A. The Clinical Center Blood Bank tested for antibody by R.I.A.-D.A. initially in the study and by P.H.A. later.

Statistical analysis was performed by Dr Marian Fisher of the Biometrics Research Branch, National Heart and Lung Institute, using Fisher's exact test, two tails. Statistical significance in this study is defined as a P value of 0·05 or less.

Results

362 patients whose pre-transfusion sera were all initially HB_sAg-negative were followed for six months after transfusion. Sera from all of their donors were tested for HB_sAg and anti-HB_s. Table I shows the number of patients and the average number of units received per patient at each participating centre. The proportion of donors with antibody ranged from 9% at the Clinical Center to 15% at Ben Taub, whereas the proportion of recipients with pre-transfusion antibody ranged from 10% to 29%.

Table II provides the clinical and serological response to blood-transfusion in the 362 blood recipients. Among these patients, 25 episodes of hepatitis occurred, 4 of which were ascribed to type-B hepatitis. 2 patients had two distinct episodes of hepatitis—a short-incubation, non-type-B, anicteric illness, and a subsequent long-incubation, HB_sAg-positive, icteric illness. 7 additional patients had a serological response to H.B.V. without biochemical evidence of hepatitis; 1 developed HB_sAg alone, 5 had seroconversion, and 1 had an anamnestic response. All 4 cases of HB_sAg-positive hepatitis were icteric compared with only 5 of 21 non-B hepatitis cases. There were no fatalities attributable to hepatitis in any of the patients followed.

TABLE I—CHARACTERISATION OF DONORS AND PATIENTS IN EACH STUDY CENTRE (ALL DONOR UNITS WERE NEGATIVE FOR HB_sAg* AND WERE TESTED FOR ANTI-HB_s)

Participating centre	No. of recipients followed six months	Average no. of donor units transfused per patient	Commercial blood (%)	Donors anti-HB_s positive (%)	Recipients anti-HB_s positive before transfusion (%)
Washington University Medical Center	105	11·3	0	11	22
Baylor-Ben Taub General Hospital	208	2·8	15†	15	29
Clinical Center Blood Bank	49	18·5	0	9	10

* All donor units were tested by counterelectrophoresis prior to transfusion; subsequently at Washington University and at Baylor all units, and at the Clinical Center most units, were retested by radioimmunoassay.
† 15% of the blood was obtained from a commercial blood-bank service but only one-fourth of these donors were paid.

We first analysed the entire recipient population (362 patients) in order to compare the clinical and serological outcome in those who received antibody-containing blood with those who did not (table II). There was no significant difference in measured H.B.V. exposure (6/172 vs. 5/190) nor in hepatitis unrelated to H.B.V. (13/172 vs. 8/190). Patients were then analysed separately according to whether or not they had anti-HB_s prior to transfusion. 278 recipients did not have pre-existing anti-HB_s; 145 of these recipients received only blood which lacked anti-HB_s while 133 received at least one unit of blood containing anti-HB_s. When these groups were compared there was again no significant difference in measured H.B.V. exposure (5/145 vs. 5/133), in hepatitis B (1/145 vs. 3/133), or in hepatitis unrelated to H.B.V. (6/145 vs. 11/133).

84 patients had antibody to HB_sAg at the time of transfusion. 4 of these patients developed hepatitis, but none was serologically related to H.B.V. An anamnestic serological response to H.B.V. was observed in only 1 of the recipients with pre-existing anti-HB_s; that patient received blood containing anti-HB_s.

Among the 362 recipients in this study there were 21 episodes of hepatitis in which neither HB_sAg nor anti-HB_s could be demonstrated. These non-type-B cases showed no significant association with the presence or absence of pre-existing anti-HB_s in the recipient or with the presence or absence of anti-HB_s in donor blood.

35 additional susceptible patients were followed by the Clinical Center Blood Bank. They were not included in the preceding analysis because greater than 90%, but not all, of their donor sera were tested for anti-HB_s.

All donors were tested for HB_sAg and were found to be negative. Each of these 35 patients received at least one unit of blood containing anti-HB_s. A negative control group for these patients cannot be presented because of the uncertain antibody status of untested donors. None the less, among these 35 additional recipients of blood containing anti-HB_s, none developed type-B hepatitis or serological response to H.B.V.; 2 developed hepatitis unrelated to H.B.V.

Discussion

Seeff et al.,[14] in a preliminary report of a study of over 2000 blood recipients, found that the risk of

TABLE II—CLINICAL AND SEROLOGICAL RESPONSE OF RECIPIENTS TO BLOOD WHICH CONTAINED OR LACKED ANTI-HB_s

Pre-transfusion serological status of recipient	Total no. of recipients	Donor blood contains: HB_sAg	Donor blood contains: Anti-HB_s	No. of recipients	Total cases of hepatitis	Confirmed exposure to H.B.V.: Total hepatitis-B cases*	Serological response only: HB_sAg	Serological response only: Anti-HB_s	Total hepatitis-B exposure	Total non-type-B hepatitis*
No HB_sAg or anti-HB_s	278	—	—	145	7†	1 (1)†	1	3	5	6 (0)†
		—	+	133	14†	3 (3)†	0	2	5	11 (4)†
Pre-existing anti-HB_s only	84	—	—	45	2	0	0	0	0	2 (1)
		—	+	39	2	0	0	1	1	2 (0)
Totals	362			362	25	4 (4)	1	6	11	21 (5)
							7			

* Numbers in parentheses indicate numbers of total cases which were icteric.
† 2 episodes of hepatitis were diagnosed in each of 2 patients; the first episode in each patient was non-type-B and the second was type-B hepatitis.

developing overt or biochemical hepatitis B after receiving at least one unit of blood containing anti-HB_s (1·4%) was not significantly greater than the risk after receiving blood without detectable antigen or antibody (0·6%); both groups had significantly less hepatitis than a third group receiving blood which contained only HB_sAg (13·7%).

Goldfield et al.[24] prospectively studied 29 recipients of anti-HB_s-containing, HB_sAg-negative blood and 103 controls who received blood with neither anti-HB_s nor HB_sAg; recipients of antibody-containing blood did not demonstrate an increased frequency of post-transfusion hepatitis. Gocke and Panick[12] compared 37 recipients of anti-HB_s-containing blood with 136 recipients of anti-HB_s-negative blood, and concluded that antibody-containing blood carried no increased risk of transmitting hepatitis B. In all three studies, the pre-transfusion antibody status of the recipients was not stated and therefore their presumed susceptibility to H.B.V. was unknown. In addition, the post-transfusion serological response of the patients was not given and thus the ability of anti-HB_s-containing blood to elicit an HB_sAg response without hepatitis or to cause seroconversion without disease could not be ascertained.

The present study provides data not only on the development of hepatitis, but also on the development of serological response to H.B.V., and permits analysis of these data in terms of whether or not the recipient was initially susceptible to H.B.V. as judged by the presence of anti-HB_s in the serum before transfusion. When the entire patient population was analysed, neither the risk of hepatitis B nor the frequency of serological exposure to H.B.V. was significantly greater in those transfused with anti-HB_s than in those who did not receive antibody-containing blood. A similar lack of statistical association was observed when only those patients without pre-existing antibody (presumably susceptible patients) or when only patients with pre-existing antibody were analysed.

No cases of biochemical or overt hepatitis B occurred among the 84 recipients with pre-existing anti-HB_s in contrast to the 4 cases which developed among the 278 presumably susceptible recipients. Although these differences are not statistically significant, they are consistent with previous studies[25,26] which indicate that anicteric or icteric hepatitis B in patients with pre-existing anti-HB_s is extremely unusual.

The total hepatitis risk for patients in this study was 6·4%. Only 1·1% of recipients developed hepatitis B; hence only 16% of the total hepatitis was related to H.B.V. As expected, there was no relationship between the frequency of non-type-B hepatitis and the presence of anti-HB_s in either the donor or the recipient prior to transfusion.

We conclude from our data and other studies that the risk of exposure to hepatitis-B virus or of development of HB_sAg-positive hepatitis following transfusion of anti-HB_s-containing blood is not significantly greater than that observed following the transfusion of blood which lacks detectable anti-HB_s. The data do not support exclusion of donor blood containing anti-HB_s.

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SEASONAL OCCURRENCE OF COMPLEX VENTRICULAR SEPTAL DEFECT

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Summary The seasonal occurrence of births of children with ventricular septal defects (V.S.D.) was examined for a series of 302 cases from New England. The overall series showed a moderate peak in the summer, which was entirely attributable to a strong tendency for complex V.S.D. to occur in summer. Complex V.S.D. occurred 4·4 times more frequently in urban counties than rural counties, and the seasonal trend was strongest in urban areas. The seasonal peak was not associated with birth-weight,