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AN OUTBREAK OF TYPE B HEPATITIS ASSOCIATED WITH TRANSFUSION OF PLASMA PROTEIN FRACTION

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Pattison, C. P., C. A. Klein, R. T. Leger, J. E. Maynard (Phoenix Laboratories Div., Bureau of Epidemiology, CDC, 4402 North Seventh St., Phoenix, AZ 85014), K. R. Berquist, J. H. Hoofnagle, L. F. Barker, J. A. Bryan and D. E. Smith. An outbreak of type B hepatitis associated with transfusion of plasma protein fraction. *Am J Epidemiol* 103: 399–407, 1976.

An outbreak of type B hepatitis followed transfusion with a single lot of plasma protein fraction (PPF) at a 1200-bed hospital in June and July 1973. Of 51 recipients of the product, 31 were available as a study population and 19 (61%) had an illness compatible with hepatitis. Epidemiologic and serologic investigations provided firm evidence that this material was the vehicle for transmission of disease to its recipients. Recipients of four other PPF lots from the same manufacturer were studied. Two of these lots were also associated with extremely high clinical hepatitis attack rates (45% and 55%). The other two lots, which had been prepared from donor plasma contributing to the composition of the initially-studied PPF lot, failed to produce clinical illness, although one of these lots was associated with a high prevalence of hepatitis B seropositivity in recipients. Thus, a broad spectrum of clinical and serologic responses was evident in PPF produced from similar donor plasma and pasteurized in the same bulk container. This study is the first to incriminate heat-treated PPF in transmission of type B hepatitis and suggests the need for further studies of the effect of pasteurization cycles on inactivation of hepatitis B virus.

blood transfusion; disease outbreaks; hepatitis, post-transfusion; hepatitis, type B; plasma protein fractions; serology

INTRODUCTION

Plasma protein fraction (PPF) (a biological solution of water and human plasma derived protein of which at least 83 per cent of the total protein must be albumin and containing no more than 17 per cent globulins with no more than 1 per cent being gamma globulin (1)) and normal serum albumin (NSA) have gained wide clinical acceptance as plasma volume ex-

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Abbreviations: anti-HB_c, antibody to hepatitis B core antigen; anti-HB_s, antibody to hepatitis B surface antigen; CVS, cardiovascular surgery service; HB_sAg, hepatitis B surface antigen; NSA, normal serum albumin; PPF, plasma protein fraction; PTH, post-transfusion hepatitis.

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panders for use in surgery and for treatment of hypovolemic shock (2–5). Presumably because of heat treatment (60 C for 10 hours), which is a required manufacturing step for both PPF and NSA, neither product has been associated with transmission of viral hepatitis in over 25 years of clinical experience (6–8). The safety of these "low risk" plasma products has undoubtedly contributed to their popularity.

An unusual increase in reported cases of post-transfusion hepatitis (PTH) occurred following transfusions in June 1973 at a 1200-bed general hospital in a large urban center in Indiana. Most cases were diagnosed as type B hepatitis by the presence of hepatitis B surface antigen (HB_sAg, Australia antigen) in acute illness phase serum. Preliminary investigation revealed that most of the patients involved had received PPF from a single lot. This report describes the investigation of the outbreak and its probable association with inadequately-heated PPF.

Materials and methods

Case-finding and definition. Review of records related to the occurrence of hepatitis both at the hospital in question and in the surrounding community for the epidemic period and the previous year was undertaken and included: (a) review of Indiana State Health Department records of reported viral hepatitis during 1972 and 1973; (b) examination of PTH reports to the community blood bank of the city in which the hospital was located; (c) review of viral hepatitis cases reported to the infection control committee of the hospital in question; (d) tabulation of monthly hospital discharge records of viral hepatitis; (e) examination of employee health service records for suspect or documented hepatitis among the hospital personnel during 1973; (f) review of hospital laboratory records related to the diagnosis of type B hepatitis for patients admitted to the hospital during 1973; and (g) analysis of PTH reports to a special surveillance system kept by the hospital blood bank for 1972 and 1973. In addition, investigators undertook a comprehensive chart review for each patient involved in the outbreak.

For additional case finding based on interviews with patients, a case of clinical viral hepatitis was defined by the presence of jaundice or dark urine or by the occurrence of elevated serum transaminase (at least two times normal) when accompanied by at least two symptoms (malaise, anorexia, nausea, vomiting, abdominal pain, or arthralgias).

Diagnosis of a clinical case of hepatitis as type B was based on positive serologic findings appropriate to the time of sampling: HB_sAg in acute illness phase serum or the presence of only antibody to hepatitis B core antigen (anti-HB_c) in convalescent serum. The presence of anti-HB_c and antibody to HB_sAg (anti-HB_s) or anti-HB_s alone in convalescent serum was considered evidence of previous infection with hepatitis B virus.

Analysis of transfusion records. The hospital blood bank provided records on lot size, lot number, and date of receipt for all shipments of blood derivatives; all ward transfusion requests for human biologicals; and distribution of all dispensed blood bank products, including data on unit or lot number and the name of each patient recipient. These blood bank records were supplemented by a surveillance system maintained by the blood bank to record the occurrence of PTH in patients transfused at the hospital.

Investigation of the implicated PPF. The manufacturer of the PPF incriminated in the outbreak supplied information with regard to manufacturing procedure, sources of donor plasma, and commercial distribution of the product.

Laboratory investigations. During the investigation serum samples were collected from patients who developed hepatitis, other recipients of incriminated or suspect

lots of PPF, and patients chosen to serve as controls. In addition, certain lots of PPF were made available for serologic testing. Serum samples were tested for HB_sAg by solid phase radioimmunoassay (Ausria™: Abbott Laboratories) (9), anti-HB_s by passive hemagglutination (Virgo Reagents: Electro-Nucleonics) (10), and anti-HB_c by complement fixation (11). For passive hemagglutination or complement fixation a titer of $\geq 1{:}8$ was considered positive.

Results of the Investigation

Surveillance methods failed to identify any unusual incidence of hepatitis in the community or at the hospital in question, except for that related to transfusions administered in the hospital. Cases of post-transfusion hepatitis reported to the hospital blood bank surveillance system during 1972, 1973, and the first six months of 1974 are shown in figure 1 by month of transfusion. In 1973 notable increases in cases of PTH occurred for transfusions between April and October, with a peak of cases from transfusions during June. For 13,313 units of blood transfused at the hospital during 1972, 18 cases of PTH were reported to the blood bank; from 13,741 units transfused during 1973, 36 such cases were reported. The difference in PTH rates between 1972 (1.35/1000 units) and 1973 (2.62/1000 units) was statistically significant ($p < .05$). During the two years there was no major change in blood donor source: 93 per cent of blood transfused at the hospital derived from volunteer donors with an HB_sAg detection rate of 2/1000 donated units by radioimmunoassay and 7 per cent from commercial donors with an HB_sAg rate of 4/1000 units. In 1972, all blood was screened and found to be nonreactive for HB_sAg by counterelectrophoresis; in 1973, the more sensitive and reliable radioimmunoassay method was used.

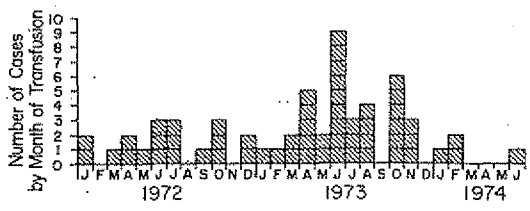

FIGURE 1. Cases of post-transfusion hepatitis reported to hospital blood bank January 1972–July 1974.

Initial investigation was directed toward the nine cases of PTH reported from transfusions in June 1973. Eight of the cases received PPF from a single lot (lot B) of one manufacturer; five of the eight (63 per cent) were positive for HB_sAg at the time of their illness. A line listing of all recipients of PPF lot B was then obtained for follow-up.

All units of PPF lot B used at this hospital had been administered to 50 patients during June and July and one patient in September; 15 patients died (one with hepatitis) within three months of receipt of the PPF, and two additional patients died of chronic gastrointestinal illness with persistent antecedent jaundice and transaminase elevation, which precluded a diagnosis of possible superimposed viral hepatitis. Of the 34 surviving recipients, 33 were contacted for follow-up interview and serologic sampling between eight and 10 months following transfusion; 19 of these individuals experienced post-transfusion hepatitis. The 20 patients (19 living, one dead) who contracted hepatitis had been hospitalized on six different hospital services: 12 were cardiovascular surgery patients, three orthopedic surgery, two general surgery, and one on each of three other services, general medicine, acute renal medicine, and urology. No additional common factor could be identified among the 20 patients which might serve as a vehicle for transmission of hepatitis except for one who had received two units of factor IX concentrate, a plasma derivative with a high risk for PTH (12–14). Three PTH patients had received no blood or blood derivatives other than PPF lot B.

The remaining 14 interviewed recipients

of lot B did not develop signs or symptoms of PTH. Two of these individuals were chronic hemodialysis patients. These two recipients, because of the multiplicity of hepatitis B exposures common to dialysis patients (15, 16), together with the patient who was the recipient of factor IX concentrate, were excluded from further analysis aimed at incrimination of the PPF lot. Accordingly, 31 recipients (19 ill, 12 not ill) comprised the study population which was then examined for the occurrence of PTH according to date of first exposure to lot B. As shown in figure 2, clinical attack rates among recipients of PPF lot B by week of first exposure to this lot were strikingly uniform, implying a close association between transfusion with this product and development of hepatitis.

To further examine the association between lot B and post-transfusion hepatitis, it was decided to examine the experience of patients who had transfusions on the cardiovascular surgery service (CVS) in June and July of 1973. This group included 19 of the 31 members of the study population, as well as a control group consisting of all CVS patients who underwent transfusion-requiring procedures during the same time period but who did not receive lot B. Detailed case-control investigation of the CVS service was considered appropriate because 58 per cent (11/19) of the hepatitis cases in the lot B study group occurred on this service, the service utilized a substantial amount of lot B, and limitation of the investigation to one surgical service would act to control for other variables which might potentially affect routes of disease transmission.

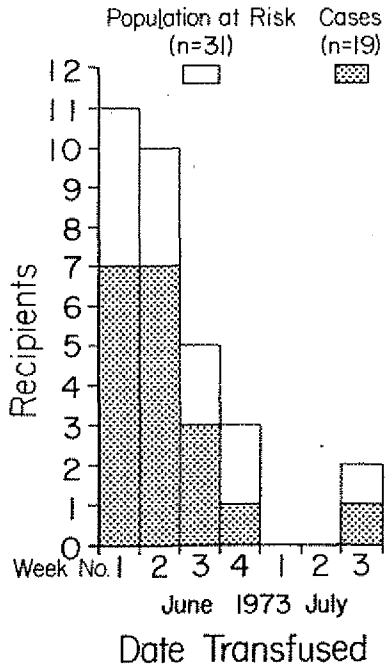

FIGURE 2. Cases of post-transfusion hepatitis among recipients of PPF lot B by week of initial exposure to this lot.

The final CVS control group included 18 of 21 patients undergoing surgery during the time period designated who did not receive PPF lot B. Of the three patients excluded, two received factor IX concentrate and one refused to participate in the study.

The control group matched very closely the CVS lot B study group with respect to average age (55.2 years vs. 53.6 years), average amount of blood (6.9 vs. 7.7 units), NSA (5.1 vs. 4.6 units), and other PPF (0.4 vs. 0.6 units) transfused, and severity and type of operation (61 per cent vs. 63 per cent had open heart surgery). Furthermore, it did not differ significantly from the study group with respect to other possible factors which might influence hepatitis acquisition; e.g., exposure to other individuals with hepatitis, shellfish consumption, foreign travel, dental manipulations, medical or dental injections, tattooing, ear-piercing, acupuncture, or previous hepatitis, hemodialysis, or transfusion. None of the patients in the control group developed clinical hepatitis compared with 11 patients (58 per cent) in the CVS study group that received lot B ($p < .001$).

The strong association between transfusion with PPF lot B and the development of clinical hepatitis was further supported by analysis of serologic data from blood

samples collected from 29 of the 31 members of the lot B study group during the follow-up interviews. As shown in table 1, 90 per cent of these individuals had serologic evidence of prior infection with hepatitis B virus. The finding of anti-HB_c in such a high proportion of samples also provided evidence for the recency of these infections. Table 2 compares serologic results from testing of the 19 recipients of lot B who were given transfusions on the CVS service with 17 of the 18 individuals in the CVS control group who agreed to submit a sample. Whereas 89 per cent of CVS recipients of lot B had evidence of prior infection with hepatitis B virus, only 12 per cent of the controls showed such evidence. The absence of anti-HB_c in the serum of the control individuals indicated that the anti-HB_s found in two might not reflect infection of recent origin.

Analysis of cases of post-transfusion hepatitis reported to the blood bank surveillance system for patients receiving transfusions in April and October 1973 revealed that four of the five PTH patients given transfusions in April had received PPF lot A from the same manufacturer, and that four of the six transfused in October, and one of three transfused in November, had received PPF lot C from the same supplier.

Analysis of blood bank records showed that 25 individuals had received units from lot A; 11 had died within three months of transfusion (one with hepatitis). Of the 14 survivors, two could not be contacted, two were chronic hemodialysis patients, and two had received factor IX concentrate. Four of the remaining eight recipients gave a history compatible with hepatitis; thus, the clinical attack rate in the nine patients (eight living, one dead) in the lot A study group was 55 per cent.

Analysis of transfusion date in regard to lot C revealed 62 recipients of this material. Fifteen had died prior to follow-up with no pre-mortem evidence of viral hepatitis, five could not be contacted, seven were chronic hemodialysis patients, and two received factor IX concentrate. Of the remaining 33 individuals, 15 (45 per cent) developed clinical hepatitis.

Follow-up serologic studies on the non-excluded recipients of lots A and C who consented to give a blood specimen are

TABLE 1

Follow-up serologic studies of PPF lot B recipients

Clinical status	No. tested	No. (%) with				
		HB_sAg and anti-HB_c	Anti-HB_c alone	Anti-HB_s alone	Anti-HB_s and anti-HB_c	Total seropositive
Hepatitis	17	2 (12)	6 (35)	2 (12)	5 (29)	15 (88)
No hepatitis	12	4 (33)	1 (8)	4 (33)	2 (17)	11 (92)
Total	29	6 (21)	7 (24)	6 (21)	7 (24)	26 (90)

TABLE 2

Follow-up serologic studies of patients transfused on cardiovascular surgery service (June–July 1973)

Transfusion status	No. tested	No. (%) with				
		HB_sAg and anti-HB_c	Anti-HB_c alone	Anti-HB_s alone	Anti-HB_s and anti-HB_c	Total seropositive
Recipient of lot B	19	3 (16)	4 (21)	5 (26)	5 (26)	17 (89)
Did not receive lot B	17	0	0	2 (12)	0	2 (12)

shown in table 3. Specimens were obtained between five and 12 months subsequent to transfusion with these lots; thus, a large number of lot C recipients were HB_eAg positive at the time of sampling. At least 75 per cent of recipients of both lots had evidence of prior infection with hepatitis B virus and, as in the case of lot B, the high prevalence of anti-HB_c provided serologic demonstration of the relative recency of infection.

Review of manufacturing records did not demonstrate any procedural abnormality unique to the production of implicated lots which could account for the presumed infectivity of lots A, B, and C. Review by the manufacturer of production equipment and details of pasteurization, however, indicated the possibility of incomplete heating of part of each lot. Following cold ethanol fractionation (17) of the plasma pools and reconstitution of the resulting protein precipitates, each PPF lot was pasteurized (60 C for 10 hours) in bulk, prior to bottling into 250 or 500 cc vials. The structure of the bulk-pasteurizing tank allowed for the possibility that a 50 cc sequestered segment from each lot might not be adequately mixed with the remaining bulk and thus not be subjected to the complete heating cycle.

Because of the association of this manufacturer's PPF with PTH and the inference of inadequate pasteurization of all bulk-heated pooled plasma products, an immediate recall of all PPF and of 5 per cent NSA (also pasteurized in bulk) was instituted by the manufacturer; 25 per cent NSA, heated in final containers, was not included in the recall.

Attempts to contact other recipients of PPF lots A, B, and C at other hospitals were unsuccessful. Unlike the hospital that provided the basis for this investigation, most hospitals were found to dispense plasma products through the pharmacy or central supply with no maintenance of distribution records. For example, 831 vials of lot B were distributed to 19 hospitals. Only five of these hospitals (including the one in this study) dispensed the PPF by lot number. Three of the other four hospitals received less than 10 units. The fourth recieved 20 units, but these were transfused into only four patients, three of whom died within three months of transfusion (without history of hepatitis), and the fourth patient could not be contacted.

Subsequent to the initial investigation, recipients of two additional PPF lots (D and E) from this pharmaceutical company also were studied. These lots were derived from two 3000-liter plasma pools from which the originally implicated lot B was prepared. Lot D was derived from one of the pools (pool 1) and lot E was derived from the other plasma pool (pool 2). Lot B had been derived from a mixture of pools 1 and 2. Of the 27 available members of the lot D study group, only one (4 per cent) developed PTH, and this patient also received lot A. Of 17 lot E recipients, two (12 per cent) developed clinical hepatitis; one of these PTH patients also received lot B and the other received four additional lots of PPF but was not seropositive at the time

TABLE 3

Follow-up serologic studies on recipients of PPF lots A and C

PPF status	No. tested	No. (%) with				
		HB_eAg and anti-HB_c	Anti-HB_c alone	Anti-HB_e alone	Anti-HB_e and anti-HB_c	Total seropositive
Recipient of lot A	8	0	5 (63)	0	1 (13)	6 (75)
Recipient of lot C	27	11 (41)	3 (11)	6 (22)	1 (4)	21 (78)

the blood specimen was drawn, suggesting that his illness was not hepatitis B. Serologic testing of 18 of 24 individuals who had received only lot D revealed one (6 per cent) with HB_sAg, two (11 per cent) with anti-HB_s alone, four (22 per cent) with anti-HB_c alone, and four (22 per cent) with anti-HB_s and anti-HB_c (overall seropositivity in 11 of 18, 61 per cent). Of five individuals who were serologically tested and had received only lot E, none were seropositive for HB_sAg, anti-HB_s, or anti-HB_c. These data suggest that lot D was infective but not pathogenic, that lot E was not infective and that the infectivity of lots B and D were derived from the single plasma pool common to the preparation of both lots.

Samples of the five PPF lots (A–E) were available for testing. All were strongly reactive for HB_sAg by radioimmunoassay and reversed passive hemagglutination but negative by the less sensitive counterelectrophoresis technique. The five lots did not differ significantly in HB_sAg titer.

Discussion

The data from this study provide firm epidemologic evidence that PPF lot B was the vehicle of transmission of viral hepatitis, type B, to recipients of that product and substantial inference of pathogenicity for two lots and infectivity for one other produced by the same manufacturer.

Fractionation of pooled plasma by the Cohn, cold ethanol procedure yields several therapeutically useful fractions which can be classified as having either "low risk" or "high risk" of transmitting viral hepatitis. Fibrinogen (Cohn fraction I), anti-hemophilic factor, and factor II, VII, IX, X complex are clearly "high risk" plasma products (18–21); in the decision to use them, weight must be given to the high probability of thereby transmitting viral hepatitis. On the other hand, immune serum globulin (prepared from Cohn fraction II), PPF (fraction IV-4 + V), and NSA (fraction V) are "low risk" products, not heretofore etiologically associated with PTH (21).

Recent studies (22, 23) have demonstrated that when human plasma containing HB_sAg is fractionated by the cold ethanol technique, HB_sAg is distributed predominantly with the alpha globulins; most HB_sAg is found in fractions III and IV, while only small amounts are demonstrable in fractions I and V, and little or none is detectable in fraction II. The lack of infectivity of PPF and NSA, despite their possible contamination with HB_sAg, is thought to result from the fact that these materials are pasteurized. While heating at 60 C for 10 hours appears to destroy infectivity in these plasma derivatives, it does not destroy HB_sAg reactivity. Viral antigens frequently will survive inactivation processes which destroy infectivity, and the presence of HB_sAg in pasteurized products does not per se imply the simultaneous presence of infectious virus. Testing of commercial lots of plasma derivatives from several manufacturers has revealed that prior to 1972, 86 per cent of PPF lots were positive for HB_sAg by radioimmunoassay (21). Since the institution of routine screening of plasma donors for HB_sAg in 1972, the prevalence of HB_sAg-positive lots of PPF has fallen. At the present time, approximately 24 per cent of commercially prepared lots of PPF are HB_sAg positive when tested by radioimmunoassay (30).

Data from this study indicate that five lots of PPF, all subject to terminal bulk pasteurization in a tank with a particular configuration which might allow for incomplete basic heating of the contents, were not equally pathogenic or infective. It is reasonable to consider that the pathogenicity associated with lots A, B and C in contrast to evidence of infectivity only, associated with lot D, might have been related to a larger quantity of surviving infectious hepatitis B virus in the former lots. Therefore, the existence of other un-

known factors related to the pasteurization process and allowing for differences in "pasteurization breakthrough" must be considered. In this regard, it is pertinent to review the data upon which efficacy of pasteurization is based.

The bulk of direct supporting data derives from two human volunteer studies conducted over 20 years ago. In one (24), 10 cc of an unheated mixture of icterogenic plasma and NSA was inoculated into each of five volunteers, of whom three developed subclinical hepatitis. None of 10 individuals inoculated with a similar amount of heat-treated material (60 C for 10 hours) experienced disease. In a larger study (31) using NSA and stable plasma protein solution (similar to the present day PPF) prepared from icterogenic plasma, no hepatitis was detected in 20 recipients of heated NSA or in 10 patients who received the heated plasma protein solution, even though both products were infectious prior to heating and both have subsequently been found to be HB_sAg positive by radioimmunoassay. However, another study (25) showed that four of 10 individuals receiving one cc each of icterogenic plasma heated 60 C for two hours and five of 10 individuals receiving the same material heated for four hours developed hepatitis. From these studies it would be difficult to interpolate a true viral heat inactivation curve with any precision. Furthermore, a recent report (26) has demonstrated development of serum transaminase elevation in an individual following parenteral inoculation of a small (2 cc) quantity of pasteurized high-titered HB_sAg-positive serum. Anti-HB_s responses have been documented following parenterally-administered pooled human protein solutions having HB_sAg reactivity following pasteurization, presumably resulting from immunogenicity, but not infectivity, of these materials (27, 28).

The absence of identifiable cases of post-transfusion hepatitis traceable to PPF has heretofore lent support to the concept that pasteurization is effective in destroying infectivity associated with the presence of HB_sAg in this material. However, as shown in this study, the lack of adequate distribution records for these fractions and the fact that their use is often associated with the transfusion of blood would tend to obscure any etiologic role that might otherwise be attributable to them. Without such records the source of infection in this outbreak would have escaped detection.

In view of the foregoing, it is our opinion that further studies of the effect of pasteurization cycles on inactivation of hepatitis B virus should be undertaken. In the meantime the imminent federal requirement (29) to test all plasma for HB_sAg by the most sensitive available methods (radioimmunoassay or reversed passive hemagglutination) should help to insure the safety of these plasma derivatives.

Results of this study suggest the need for reevaluation of prospective PTH studies, in which the possible contribution of "low risk" plasma products is usually ignored. Our results also show that routine blood bank surveillance for PTH is a potentially powerful epidemiologic tool. By this rather informal means, nearly 40 per cent of cases of PTH were detected and a major cause of morbidity from transfusion was elucidated.

Finally, this study demonstrates the potential usefulness of testing for anti-HB_c in epidemiologic studies of type B hepatitis. Testing for only HB_sAg and anti-HB_s will frequently yield negative results during the early stages of convalescence from type B hepatitis, a period (three to six months after exposure) which is frequently chosen to sample for the incidence of serologic evidence of type B hepatitis. In our study 23 per cent of PTH patients were positive only for anti-HB_c and would have been undetected by HB_sAg and anti-HB_s testing alone.

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