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TRANSMISSIBLE AGENT IN NON-A, NON-B HEPATITIS

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Summary  Plasma or serum from 4 patients with acute or chronic non-A, non-B post-transfusion hepatitis (P.T.H.) and from a blood-donor implicated in two cases of P.T.H. was inoculated into 5 chimpanzees. Biochemical and histological evidence of hepatitis developed in these 5 chimpanzees but not in a control animal. The mean incubation period in the chimpanzees was 13-4 weeks, compared with 7-7 weeks in the 4 patients with P.T.H. The peak alanine aminotransferase (A.L.T.) levels in the 5 chimpanzees were 265, 212, 219, 70, and 62 I.U/L. Histological changes ranged from mild to conspicuous hepatitis and generally correlated with the degree of A.L.T. elevation. There was no evidence of clinical disease and all animals went on to biochemical and histological recovery. There was no serological evidence of type A or type B hepatitis. Hepatitis was transmitted by serum derived from patients with chronic as well as acute hepatitis, strongly suggesting a chronic carrier state for the agent responsible for non-A, non-B hepatitis. Non-A, non-B hepatitis thus seems to be due to a transmissible agent which can persist and remain infectious for long periods.

Introduction  Infection due to human hepatitis virus A and B can be identified by assays for various serological and enzymatic markers.1-3 At present, 60-90% of post-transfusion hepatitis is serologically unrelated to either of these viral agents.4-6 Neither cytomegalovirus nor the

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<tbody>
<tr>
<td>1</td>
<td>Acute non-A, non-B P.T.H. progressing to C.A.H.</td>
<td>1600/1400</td>
<td>8.4</td>
<td>8</td>
<td>4 (50) 6 (22) 8 (390) 10 (1000) 11 (220)</td>
<td>4.5 ml serum</td>
<td>165</td>
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<tr>
<td>2</td>
<td>Acute non-A, non-B P.T.H. progressing to C.A.H.</td>
<td>978/256</td>
<td>0.2</td>
<td>10</td>
<td>42 (228)</td>
<td>75 ml plasma</td>
<td>186</td>
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<tr>
<td>3</td>
<td>Acute non-A, non-B P.T.H. Recovered</td>
<td>1506/1400</td>
<td>10.8</td>
<td>6</td>
<td>6 (153) 7 (618) 8 (906)</td>
<td>3 ml serum</td>
<td>189</td>
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<tr>
<td>4</td>
<td>Donor with related A.L.T. involved in two cases of acute non-A, non-B P.T.H.</td>
<td>474/714</td>
<td>0.2</td>
<td>N.A.</td>
<td>N.A. (474)</td>
<td>75 ml plasma</td>
<td>191</td>
</tr>
<tr>
<td>5</td>
<td>Acute non-A, non-B P.T.H. progressing to C.A.H.</td>
<td>468/402</td>
<td>0.8</td>
<td>7</td>
<td>35 (180)</td>
<td>75 ml plasma</td>
<td>196</td>
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*When more than one week indicated, 0-3-1 ml samples from each week were pooled and the total volume was given as a single injection.

P.T.H. = post-transfusion hepatitis; C.A.H. = chronic active hepatitis; C.P.H. = chronic persistent hepatitis; N.A. = not applicable;
Epstein-Barr virus has been implicated in such cases, and the existence of at least one additional human hepatitis virus is suspected. No serological marker of this 'non-A, non-B' virus has been defined, nor viral particle has been observed, and no agent has been isolated in tissue culture. We have investigated the blood of patients with acute or chronic non-A, non-B hepatitis for the presence of an agent transmissible to chimpanzees.

**Materials and Methods**

**Human Inocula**

The samples came from subjects identified in a continuing investigation of post-transfusion hepatitis among patients having open heart surgery. Four were patients; the fifth was a blood-donor (see accompanying table). All the patients had received volunteer-donor blood screened for hepatitis-B surface antigen (HBsAg) by solid-phase radioimmunoassay. Blood-samples were obtained weekly or biweekly during the first three months after transfusion and then monthly for a further three months. Additional samples were obtained when liver enzymes were raised; whenever possible, plasmapheresis was performed on patients with suspected acute or chronic non-A, non-B hepatitis.

Post-transfusion hepatitis was diagnosed when, between 2 and 26 weeks after transfusion, alanine aminotransferase (ALT) rose to 2½ times the upper limit of normal (112 U/L) and when a second sample, after at least a week, was over twice the upper limit of normal (90 U/L). Icteric hepatitis was diagnosed when the serum-bilirubin exceeded 2·5 mg/dl. Congenital failure, toxic hepatitis, and other causes of raised hepatic enzymes were excluded as far as possible. Diagnosis of non-A, non-B hepatitis was contingent upon the absence of HBsAg and the absence of antibody seroconversion to HBsAg, hepatitis B core antigen (HBcAg), hepatitis A virus (HAV), cytomegalovirus (CMV), and the Epstein-Barr virus (EBV). Liver biopsy specimens were not obtained in the acute phase of the disease, but were obtained when enzyme abnormalities persisted for more than six months.

Subject no. 4 was a blood-donor. Her blood had been given to two patients in whom non-A, non-B hepatitis developed. In the more recent case she was the only donor who proved, on recall, to have raised alanine and aspartate aminotransferase values. She had no history of previous hepatitis or other medical disease, was not on medication, and denied use of narcotics or heavy intake of alcohol. Her ALT on first recall was 79 U/L and it fluctuated between 56 and 474 during the next six months. She complained of nausea and increased fatigue at this time her hepatic enzymes were at their maximum level, but generally she was asymptomatic. Liver biopsy has not yet been done.

**Study in Chimpanzees**

Selected samples of plasma or serum from the four patients and from the blood-donor were inoculated intravenously into five chimpanzees in volumes ranging from 3 to 75 ml (see table). The chimpanzees were then monitored weekly for ALT, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), and HBsAg. Pre-infection and six-month post-infection samples were also tested for antibody to HBsAg (anti-HBs), antibody to HBcAg (anti-HBc), and antibody to the hepatitis A virus (anti-HAV). When enzyme abnormalities were detected, the animals were plasmapheresed on one or more occasions and serial liver biopsy specimens were obtained.

The chimpanzees were kept at the Laboratory for Experimental Medicine and Surgery in Primates (L.E.M.S.I.P.) in Sterling Forest, New York. Each of the animals had been wild-caught in Sierra Leone, Africa, but were housed at L.E.M.S.I.P. for at least 2 years when this study was started. At the time of the study, the chimpanzees were between 4 and

54 years old. Three of them (nos. 165, 186, and 189) had been experimentally infected with the hepatitis-B virus previously and had anti-HBc and anti-HBs at the beginning of the study. All were negative for HBsAg and all had normal hepatic enzyme levels. Two of the animals (191, 196) had not previously been used in experiments and each was negative for HBsAg, anti-HBc, and anti-HBs. All chimpanzees had anti-HAV at the beginning of the study.

The five chimpanzees were housed in a single room, away from other chimpanzees at L.E.M.S.I.P. A sixth uninoculated control was housed in a separate room. Each chimpanzee was kept in an isolation cage suspended from the wall and equip-
pered from the AD 169 strain of human c.M.v.13 Paired sera were titrated in duplicate for anti-B.V. by immunofluorescence.14 Liver biopsy specimens were obtained percutaneously with ‘TruCut’ disposable needles (Travenol). Biopsy specimens were placed in formalin, embedded in paraffin, sectioned, stained with haematoxylin and eosin, and read under code by H.P.

Results
Chimp 165 had had 18 blood-samples taken in the six months before inoculation with 4.5 ml of acute-phase serum from subject 1. In only one sample did the A.L.T. exceed 25 i.u./l (value of 42) and the A.S.T. was never higher than 27 i.u./l. A mild rise in A.L.T. began three weeks after inoculation and persisted through week 11 (fig. 1). G.G.T.P. followed a similar pattern but did not rise until week five. There were no significant changes in A.S.T. during this early period. A.L.T. began a steep rise during week twelve, peaked at 265 i.u./l during week fourteen and returned to normal by week seventeen. G.G.T.P. followed a similar, but slightly delayed pattern, reaching a peak of 343 i.u./l during week fifteen and returning to normal by week twenty-one. A.S.T. paralleled A.L.T. and G.G.T.P., but the magnitude of change was considerably less. The animal remained HB.Ag negative throughout this time and was positive for anti-HB.A, anti-HB.S, and anti-H.A.V. before the study began. Liver biopsy specimens obtained at the time of peak increases in hepatic enzymes (weeks thirteen and fourteen) showed a moderately severe acute hepatitis (fig. 2). There was variation in the size and staining quality of hepatocyte nuclei and the cytoplasm was irregularly clumped. Some hepatocytes were replaced by inflammatory cells (focal necrosis) and there were moderate numbers of acidophilic bodies. There was a distinct increase in sinusoidal lining cells. The portal tracts were densely infiltrated by mononuclear cells. In general, the border between portal tracts and the surrounding parenchyma was sharp, but in places the limiting plate was eroded and cellular exudate spilled into the parenchyma. Two months later (week twenty-two), when A.L.T., A.S.T., and G.G.T.P. were normal, the liver was histologically normal.

Chimp 168 had had 17 blood-samples taken in the six months before the beginning of this study; the highest A.L.T. was 25 i.u./l and the highest A.S.T. 20 i.u./l. As seen in fig. 1, there was a slight and fluctuant increase of A.L.T. beginning three weeks after the animal received 75 ml of chronic-phase plasma from subject 2. Definite increases of hepatic enzymes did not appear until weeks ten to eleven. The animal remained HB.Ag negative throughout and was positive for anti-HB.A, anti-HB.S, and anti-H.A.V. when the study began. Liver biopsy specimens obtained during weeks thirteen and fourteen showed an acute hepatitis which was histologically similar to that seen in chimpanzee 165, but less conspicuous. Subsequent biopsies (weeks sixteen and eighteen) showed considerable improvement, but a specimen taken during week twenty-two showed increased portal-tract inflammation and focal necrosis, despite improving values for A.L.T. and G.G.T.P.

Chimp 189 had had 9 blood-samples taken in the four months before it received 3 ml of acute-phase serum from subject 3. The highest A.L.T. during this time was 27 and the highest A.S.T. 20. Unlike chimp 165 and

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*Kindly supplied by Dr Lucy Overby of Abbott Laboratories.

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**Fig. 2—Biopsy specimen of liver from chimpanzee 165 at the time alanine aminotransferase was 163 i.u./l (week 14).**

Hematoxylin and eosin, x 250. Note nuclear variation, lobular disarray with "smudgling", increased sinusoidal lining cells, acidophilic bodies, focal necrosis, and portal-tract inflammation with some infiltration of parenchyma.
186, this animal did not show an elevation in A.L.T. and G.G.T.P. three to four weeks after inoculation. There was, however, a definite rise in A.L.T. beginning at week 12 (fig. 1). G.G.T.P. and A.S.T. followed a similar pattern, but the increases were considerably smaller. H.B.Ag was not detected and anti-H.B.a and anti-H.B.c were already present when the study began. Histologically the liver was very similar to that of chimp 186, showing an acute hepatitis with acidophilic bodies, focal necrosis, and a conspicuous portal-tract inflammatory reaction. Subsequent biopsy specimens showed striking improvement.

Chimp 191 had had 13 blood-samples taken in the 6 months before inoculation the highest A.L.T. was 25 i.u./L and the highest A.S.T. 20 i.u./L. The enzyme response to transfusion with 75 ml of chronic-phase plasma from subject 5 was also small, but it was definite and the temporal relation to transfusion was very similar to that in chimps 165, 186, and 189 (fig. 1). A.L.T. reached a maximum at week thirteen and there was a secondary rise during week eighteen. There was no concomitant rise in A.S.T. or G.G.T.P. In this animal H.B.Ag, anti-H.B.a, and anti-H.B.c were never detected. Liver biopsy during week eighteen revealed a mild diffuse hepatitis; although the enzyme abnormalities were much less than in chimp 186, the histological lesions were very similar. Partial resolution had occurred by week twenty-two.

Chimp 183 was an un inoculated control. During the twenty-six week follow-up the highest A.L.T., A.S.T., and G.G.T.P. were 20, 19, and 22 i.u./L. H.B.Ag was not detectable. Anti-H.B.a, anti-H.B.c and anti-H.A.V. were present before the study began. Neither the un inoculated control nor any of the inoculated animals became clinically ill.

Discussion

Indirect evidence that an infectious agent is responsible for non-A, non-B hepatitis has been threefold: (1) a variable, but defined incubation period intermediate between that of type A and type B hepatitis (generally six to twelve weeks); (2) hepatic histology which by haematoxylin and cosin stain is virtually indistinguishable from that of type A or type B viral hepatitis; and (3) a positive correlation between the development of non-A, non-B hepatitis and the source of donor blood—like type B hepatitis, non-A, non-B is about ten times more common after receipt of paid-donor blood than after receipt of volunteer-donor blood.

We chose the chimpanzee as an indicator of a transmissible infectious agent because this animal is susceptible to both hepatitis A and hepatitis B virus infection. Several previous attempts to transmit non-A, non-B hepatitis to chimpanzees have been unsuccessful, but whether the inoculum was non-infectious or the particular chimpanzees were immune could not be ascertained. Lately, Hollinger et al. have recorded modest increases in A.L.T. (maximum 75 i.u./L) in 4 of 3 chimpanzees inoculated with material either from donors implicated in a case of non-A, non-B hepatitis or from recipients with non-A, non-B post-transfusion hepatitis. Minimal histological changes, primarily Kupffer-cell hyperplasia and swelling of hepatocytes, were noted in relation to A.L.T. elevations, but were not absolutely diagnostic of hepatitis.

In the study described here, chimp 165, 196, and 189 showed unequivocal enzymatic and histological evidence of hepatitis. In chimps 191 and 196 the diagnosis of hepatitis was less certain; however, the A.L.T. reached distinctly higher levels after inoculation than in the baseline period, and higher, too, than those in the uninoculated control chimpanzees. In addition, enzyme abnormalities persisted for at least two weeks and the A.L.T. reached a peak at a post-inoculation interval similar to that in the three chimpanzees with unequivocal hepatitis. Lastly, in chimp 186, although enzyme abnormalities were minor, biopsy showed definite hepatitis.

There were several additional observations of interest. First, the incubation period in the chimpanzees, as judged by the first A.L.T. over 50 i.u./L, was somewhat longer than that in the human beings from whom the inocula came (13-4 weeks in the chimpanzees compared with 7-7 weeks in the four patients with post-transfusion hepatitis), though in one patient/chimp pair the incubation periods were almost identical.

Second, the three chimpanzees with the greatest enzyme rises were the ones that had been previously infected with type B hepatitis. We do not feel that this represents recrudescence of the type B infection since each had had repeatedly normal enzymes for at least four months before entry into this study and because each was persistently H.B.c Ag negative. In addition, before the present study was started, each chimpanzee had acquired anti-H.B.a and anti-H.B.c— which generally denote complete recovery from type B virus infection. It is more plausible that the three animals received a more virulent inoculum or that their host response was such as to induce more severe hepatic destruction. Such variability of host response has been clearly demonstrated in type B viral infections of both man and chimpanzees.

Diverse human responses to identical non-A, non-B inocula have also been demonstrated in a retrospective, serological analysis of volunteer studies performed in the early 1950s. Third, non-A, non-B hepatitis was transmitted by serum or plasma obtained either in the acute or in the chronic phase of the disease. This provides good evidence for a chronic carrier state. Only the existence of such a carrier state can account for the large number of non-A, non-B hepatitis cases which derive from seemingly healthy donors. Indeed, this is a key observation in this study.

Fourth, the histological lesion in these chimpanzees was identical to that seen in type B hepatitis, so that whole lobule was involved, and in that streaks of focal necrosis extended to the central zone. In contrast, the changes seen in hepatitis A are primarily peritoneal. These histological differences may account for the tendency of type B and non-A, non-B hepatitis to progress...
to chronic liver disease whereas type A hepatitis seems to be a self-limited acute disease. 32

Fifth, we employed three tests of hepatocellular dysfunction, of which A.L.T. was the most consistent: it was the only enzyme raised in all five chimpanzees, and in four of them it was the one showing greatest abnormality.

G.O.T.P. had a very similar pattern to that of A.L.T. and in one chimpanzee reached a higher and more sustained level. However, in the two chimpanzees with the mildest A.L.T. increases, the G.O.T.P. remained normal. A.S.T. was the last to disappear of the tests.

Lastly, the volume of inoculum did not seem to influence the severity of the ensuing disease; two of the three chimpanzees with severe disease had received less than 4 ml of infectious material, whereas the two with the mildest hepatitis received 75 ml. In future experiments, therefore, smaller inocula can be used. Similarly, the stage of human non-A, non-B hepatitis at which the blood was obtained did not seem to influence the course of the animal which received chronic-phase plasma had a response almost identical to those of the two animals receiving acute-phase serum.

We have shown that blood from human beings with apparent non-A, non-B hepatitis contains an agent capable of causing similar disease in chimpanzees. This animal model may now permit more definitive characterisation of non-A, non-B inocula, including infectivity titres, assessment of cross immunity to determine whether non-A, non-B hepatitis exists in more than one variety; detection of virus material in liver; and the use of such material in development of a serological test for non-A, non-B hepatitis.

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Requests for reprints should be addressed to H. J. A., National Institute of Health Clinical Center, Blood Bank Department, Bethesda, Maryland 20014, U.S.A.

REFERENCES
15. Feinman, S. M., Alter, C. L., Koniol, D. P., Personal communication.
23. Evers, G. J., Provan, P. J., Personal communication.
29. Evers, G. J., Provan, P. J., Personal communication.