Serological Relationship of Woodchuck Hepatitis Virus to Human Hepatitis B Virus

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Two antigenic systems of the woodchuck hepatitis virus have been identified. The relationship between viral antigens of the woodchuck hepatitis virus and the human hepatitis B virus was determined by using immunoprecipitation, hemagglutination, and immune electron microscopy techniques. Antigens found on the cores of the two viruses were cross-reactive. Lack of cross-reactivity between the surface antigens of the two viruses in immunodiffusion experiments suggested that the major antigenic determinants of the viral surfaces are different; however, results of passive hemagglutination tests indicated that there are common minor determinants. Nucleic acid homology, as measured by liquid hybridization, was found to be 3 to 5% of the viral genomes. The results of this study provide further evidence that woodchuck hepatitis virus is the second member of a new class of viruses represented by human hepatitis B virus. Since virus-infected woodchucks may acquire chronic hepatitis and hepatocellular carcinoma, these antigens and their respective antibodies will be useful markers for following the course of virus infection in investigations of the oncogenic potential of this class of viruses. The nucleocapsid antigen described may be a class-specific antigen of these viruses and, thus, may be useful in discovering new members of the group.

The properties of human hepatitis B virus (HBV) distinguish it from any known class of DNA-containing viruses. It was recently suggested that a woodchuck hepatitis virus (WHV), found in Marmota monax, is a second member of this novel class of viruses (15). HBV and WHV have the following characteristics in common: (i) infection with either virus results in the accumulation in the blood of large amounts of excess viral coat protein in the form of spherical and tubular particles 20 to 25 nm in diameter (3, 15); (ii) 40- to 50-nm-diameter particles (6, 15) containing a small, partially single-stranded, circular DNA (12, 14, 15) and a DNA polymerase (DNA nucleotidyltransferase) (8, 15) may also be found in the blood and are thought to be the complete virions; and (iii) both viruses are associated with chronic hepatitis and hepatocellular carcinoma (4, 15, 16).

There are two principle antigenic systems of HBV. Determinants called hepatitis B surface antigen (HBsAg) are found both on the surface of the virus particles and also on the 20- to 25-nm particles (3, 6). Antibody to these determinants is designated anti-HBs. Nucleocapsid cores, 27 nm in diameter, can be isolated from viral particles or from infected livers. These cores possess an immunologically distinct antigen, hepatitis B core antigen (HBcAg) (1), and antibody to this determinant is termed anti-HBc.

We have identified the analogous antigenic systems associated with the 20- to 25-nm particles and viral cores of the WHV and describe in this paper antigenic cross-reactivity between the components of the two viruses. In addition, we have found a small region of strong homology between the two viral DNAs. These results further support a close phylogenetic relationship between the two viruses.


MATERIALS AND METHODS

Sera. Sera from HBV-infected individuals were from the collection of B. S. Blumberg (Institute for Cancer Research, Philadelphia, Pa.). Sera from woodchucks were obtained from a colony of M. monax at the Penrose Research Laboratory of the Philadelphia Zoological Society, Philadelphia, Pa. Some of these animals were previously shown to be infected with
WHV as indicated by the presence of particles in the sera similar to those associated with human HBV infections (15).

**Immunoprecipitation methods.** Micro-Ouchterlony double diffusion or immunodiffusion (ID) and counterimmunoelectrophoresis (CIE) were carried out with 0.5% agarose (Aldrich Chemical Co., Inc., Milwaukee, Wis.) in barbital buffer, pH 8.3 (T/2 = 0.04). For CIE experiments involving the woodchuck surface antigen system, it was necessary to use a combination of agar (Noble Special Agar; Difco Laboratories, Detroit, Mich.) (0.2%) and agarose (0.4%) prepared in the same buffer, since no precipitins were visible with standard conditions. After 16 to 48 h of incubation at room temperature, slides were washed with phosphate-buffered saline, pH 7.4 (PBS), for 24 to 48 h, dried, and stained with azocarmine G (Matheson, Coleman & Bell, East Rutherford, N.J.).

**Isolation of IgG.** Immunoglobulin G (IgG) was isolated from human and woodchuck sera with DEAE-Sephadex (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.), depleted in 0.01 M phosphate buffer, pH 6.5, by the batch method of Baumstark et al. (2). IgG preparations were tested (at approximately 5 mg/ml) for purity by immunoelectrophoresis with horse anti-human whole serum.

**Preparation of viral cores from livers.** Viral cores were isolated from a liver obtained at autopsy from a kidney transplant patient who had been treated with immunosuppressive drugs (10) and from the liver of an infected woodchuck. The livers were minced in isotonic saline, homogenized in a Dounce homogenizer or Waring blender, and centrifuged for 5 min at 5,000 rpm in a Sorvall HS-4 rotor. The supernatant containing cores was either used as a crude antigen preparation or subjected to further purification. In the latter case, the crude antigen preparation was layered over a 25-ml 10 to 20% sucrose gradient containing 1 M NaCl and centrifuged at 4°C for 12 h at 27,000 rpm in a Beckman SW27 rotor. The pellet was suspended by sonication in 1 ml of CsCl solution (p = 1.30 g/cm³), overlaid with silicone oil, and centrifuged at 50,000 rpm in a Beckman SW56 rotor for 12 h at 4°C. Fractions were collected and tested for core antigen by CIE. Positive fractions (p = 1.31 to 1.34 g/cm³) were pooled, dialyzed against isotonic saline, and stored at −20°C.

**Purification of HBV and WHV.** HBV and WHV were purified from virus-positive sera by identical procedures. Serum (27 ml) was layered over a 10-ml gradient of 10 to 20% sucrose containing 10 mM Tris-hydrochloride (pH 7.5), 10 mM EDTA, and 1 M NaCl. Viral particles were pelleted by centrifugation for 15 h at 27,000 rpm at 4°C in a Beckman SW27 rotor. The pellets were suspended by sonication in 1 ml of 1 M NaCl, the suspension was layered over a second 10 to 20% sucrose gradient (volume of 4.0 ml), and the virus was pelleted by centrifugation at 50,000 rpm for 3 h at 4°C in a Beckman SW50.1 rotor. The pellets were suspended in a total volume of 1.0 ml of CsCl solution (p = 1.22 g/cm³), overlaid with silicone oil, and centrifuged for 12 to 15 h at 50,000 rpm in a Beckman SW56 rotor. Fractions were collected and assayed for protein and for DNA polymerase activity (15). Fractions positive for DNA polymerase were pooled and stored at 4°C. The major protein peak, banding around p = 1.19 g/cm³, was dialyzed against PBS and used in the preparation of coated sheep erythrocytes (SRBC) for passive hemagglutination assays.

**Preparation of WHsAg- and HBsAg-coated SRBC.** Coated SRBC were prepared by a modification of a method described by Boyden (5). SRBC (Colorado Serum Co., Denver, Colo.) were washed four times with 50 volumes of PBS and resuspended in PBS to form a 10% suspension. The SRBC (0.5 ml) were added to 0.2 ml of a solution of 0.1% tannic acid in PBS and incubated for 15 min at 37°C. The cells were then washed twice with 0.5 ml of PBS, resuspended in 0.1 ml of PBS, and mixed with CsCl gradient-purified woodchuck hepatitis surface antigen (WHsAg) or HBsAg/ayw (120 μg in 0.050 ml of PBS).

After 30 min at 22°C, the cells were collected by centrifugation and suspended in 0.1 ml of PBS containing 0.25% glutaraldehyde. After 5 min at 0°C, 5 ml of PBS containing 0.25% glutaraldehyde was added, and the coated SRBC were allowed to fix overnight at 4°C. The fixed cells were washed once with 5 ml of PBS and resuspended in 0.1 ml of PBS containing crystalline bovine serum albumin per ml and resuspended in a final volume of 5 ml of PBS and 10 mg of bovine serum albumin per ml. Aliquots (0.1 ml) were stored at −70°C and diluted to 1.5 to 2.0 ml before use. Mild sonication in a sonicating water bath was required to disperse the cells completely after thawing. SRBC from the same lot were treated in an identical procedure, except that no antigen was added, and were used as controls for non-viral specific agglutination.

**Assay for hemagglutinating activity.** Fourfold serial dilutions of sera to be assayed for hemagglutinating activity were prepared in TAP buffer (PBS containing 0.005% Tween 80, 5 mg of bovine serum albumin per ml, and 0.0025% polyvinyl pyrrolidone) (17) in a final volume of 30 μl in a V-bottom microtiter plate. Antigen-coated SRBC (20 μl) were added to each well, and the plates were incubated at 22°C. After 1 h, the cells were pelleted by centrifugation at 1,000 × g for 1 min, and agglutination was scored after tilting the plate at an angle of 60° from the horizontal. One hemagglutinating unit was that amount of antiserum required to agglutinate 20 μl of a 0.05% antigen-coated SRBC suspension in the assay described.

**Assay for hemagglutination inhibition.** Samples were assayed for their ability to inhibit agglutination of antigen-coated SRBC by a limiting dilution of antiserum. Two to four hemagglutinating units of antiserum in 25 μl of TAP buffer was mixed with fourfold serial dilutions of antigen (30 μl) in a microtiter plate and incubated at 37°C. After 2 h, 20 μl of antigen-coated SRBC, diluted as described, was added to each well, and the plates were incubated an additional 1 h at 22°C before scoring agglutination. All samples were tested concurrently for their ability to agglutinate uncoated SRBC.

**Preparation of [32P]-cores.** An endogenous DNA polymerase reaction was carried out directly on gradient fractions of CsCl-purified WHV or HBV (0.05 ml) by the addition of 10 mM Tris-hydrochloride (pH 8.0)–20 mM MgCl₂–200 mM each dATP, dGTP, and dCTP–5 μM [32P]dGTP (>100 Ci/mmol)–1 mM dithiothreitol–0.1% Triton X-100 in a final volume of 0.055

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ml. After 2 h at 37°C, the reactions were added to 1.0 ml of CsCl solution (ρ = 1.31 g/cm³), overlaid with silicone oil, and centrifuged at 50,000 rpm for 12 to 15 h at 4°C in a Beckman SW56 rotor. Fractions were collected, and small portions (1 μl) were assayed for acid-precipitable radioactivity (see Fig. 2). The radioactive cores banding at a density of 1.34 g/cm³ were stored at 4°C.

Preparation of WHV or HBV DNA. Purified WHV or HBV in CsCl gradient fractions was precipitated by the addition of 2 volumes of ethanol. The precipitate was washed once with 95% ethanol, dried, and dissolved in 0.1 ml of 10 mM Tris-hydrochloride (pH 7.5)–10 mM EDTA–0.2% sodium dodecyl sulfate–500 μg of pronase per ml. After 2 h at 37°C, the DNA was extracted with 0.1 ml of phenol-chloroform (1:1), adjusted to 0.1 M with NaCl, and precipitated with 2 volumes of ethanol. The invisible precipitate was dissolved in a small volume of water and stored at −20°C. DNA concentrations were determined by the ability of a small portion of the sample (after denaturation) to serve as a template for Escherichia coli DNA polymerase I (14).

Autoradiography. Precipitin lines in ID experiments with purified ³²P-labeled cores (approximately 2,000 cpm per well) mixed with unlabeled carrier cores were visualized by autoradiography. After overnight incubation at room temperature, the ID slides were flooded with distilled water and overlaid with filter paper for drying. They were then placed face down on X-ray film (Cronex 4, Dupont, Wilmington, Delaware), which was exposed overnight before developing.

Electron microscopy. Sera pelleted by centrifugation and fractions of CsCl gradients were examined by negative staining with uranyl acetate on collodion films. Immune electron microscopy was performed by mixing purified viral cores with immune or normal IgG (50 to 300 μg/ml, final concentration). After 10 min at 37°C, the cores were applied to collodion films, negatively stained with 2% uranyl acetate, and examined by electron microscopy.

Electron microscopy of immune precipitin lines. Precipitin lines were formed by ID or CEP between homogenates of infected liver and serum or IgG with antibody activity. The agarose slides were washed with frequent changes of PBS, and, to identify the reacting components, individual precipitin bands were sampled by removing a plug with a Pasteur pipette. The plug was placed in a small glass tube with 10 μl of 3 M sodium thiocyanate, broken up into small pieces with a pipette, and sonicated, and a sample was put on a grid and stained with 2% uranyl acetate for examination by electron microscopy.

RESULTS

WHsAg. Serum from a woodchuck in the colony at the Penrose Research Laboratory was found to react specifically by ID and CEP with sera from WHV-infected animals. The reactivity was found in the IgG fraction eluted with 0.01 M phosphate buffer, pH 6.5, from DEAE-Sephadex (data not presented). Precipitin bands formed with sera from infected woodchucks were extracted and examined by electron microscopy.

Numerous aggregates of 20- to 25-nm spherical particles with occasional tubular forms were seen (Fig. 1). Since these particles resemble in both morphology and density (15) the small spherical particles with HBsAg reactivity, we propose to call the antigen associated with these forms WHsAg. Antibody against the WHsAg would then be designated anti-WHs. As a control for nonspecific trapping of particles, a precipitin band formed between the same virus-positive serum and rabbit antiserum to woodchuck IgG was extracted and examined by electron microscopy. No particles were observed in this immune complex.

Using this woodchuck anti-WHs serum, we investigated the ability of anti-WHs or anti-HBs (from a patient who had received multiple transfusions) to form immune precipitates with either WHsAg or HBsAg by ID and by CEP. Although reactivity by CEP in the homologous systems was readily demonstrable at dilutions of at least 1:4, no cross-reactivity of the two undiluted antisera could be demonstrated (Table 1). Antisera detecting the WHsAg group-specific determinant a and the subtype specific determinants d, y, w, and r did not react by ID or CEP with WHsAg(+) sera. Anti-WHs did not react with HBsAg(+) sera from the subtype reference panel (P1 to P10; Research Resources Branch, National Institute of Allergy and Infectious Dis-

![Fig. 1. Electron micrograph of negatively stained immune aggregate from precipitin band formed between a WHsAg(+) serum and serum with naturally occurring antibody to the virus (anti-WHs (+)). Bar represents 50 nm.](http://jvi.asm.org/)
causes, Bethesda, Md.).

In passive hemagglutination assays, however, a low level of cross-reactivity was detected. Anti-WHs caused specific agglutination of HBsAg-coated SRBC at a dilution 1,000 times lower than that required to agglutinate WHsAg-coated cells. Similarly, human serum with anti-HBs specifically agglutinated WHsAg-coated cells at a dilution approximately 100 times lower than that which agglutinated HBsAg-coated cells (Table 2). Consistent with these findings, unfractonated WHsAg(+) serum did not inhibit the reaction between anti-HBs and HBsAg-coated cells, nor did HBsAg(+) serum inhibit the agglutination of WHsAg-coated cells by anti-WHs. On the other hand, both WHsAg(+) and HBsAg(+) sera were able to inhibit the agglutination of coated cells by the heterologous antiserum.

We investigated the specificity of these observed cross-reactions by using cells coated with either WHsAg or HBsAg in hemagglutination inhibition assays designed to answer the following questions. First, is the cross-reacting determinant on the coated cells present on the surface antigen particles that were used to coat the cells, or was it a contaminant in the purified antigen preparation? Second, are the antibodies which react with cells coated with the heterologous surface antigen directed against determinants present on the homologous surface antigen particles?

### Table 1. Lack of cross-reactivity between HBsAg and WHsAg by ID and CEP

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<tr>
<th>Antiserum</th>
<th>Titer when tested against:</th>
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<tr>
<td></td>
<td>WHsAg(+) serum</td>
</tr>
<tr>
<td></td>
<td>ID</td>
</tr>
<tr>
<td>Anti-WHs</td>
<td>1/4</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>—</td>
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* Serum from a woodchuck in the colony at the Penrose Research Laboratory.

* Serum containing antibodies to the HBsAg group-specific determinant a, from a hemophilia patient who had received multiple transfusions.

* — Not reactive.

### Table 2. Cross-reactivity between HBsAg and WHsAg by hemagglutination

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Titer when tested against:</th>
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<tbody>
<tr>
<td></td>
<td>WHsAg-coated cells</td>
</tr>
<tr>
<td>Anti-WHs</td>
<td>1/1,000,000</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>1/1024</td>
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</tbody>
</table>

* Described in footnotes to Table 1.

Sera containing WHsAg or HBsAg/ayw were centrifuged to equilibrium in a CsCl density gradient, fractions were collected, and the surface antigen peaks were located by hemagglutination inhibition activity (Fig. 2, closed symbols). Each fraction was tested for its ability to inhibit hemagglutination in each of the cross-reacting systems. It was found that only those fractions containing the surface antigen particles had inhibiting activity. Furthermore, these fractions were capable of inhibiting the cross-reactions of cells coated with either surface antigen (Fig. 2, open symbols). These findings are consistent with the cross-reacting determinants being present on the surface antigen particles used to coat the cells. In addition, antibodies participating in the cross-reaction seem to be directed against determinants present on the homologous surface antigen particles. Thus, it appears that a minor fraction (0.1 to 1%) of the antibody activity against WHsAg or HBsAg is directed against determinants shared by both surface antigens.

**WHsAg.** Antigens present on the nucleocapsid cores of the two viruses were compared. Cores were prepared from two sources in each case. [32P]DNA-labeled cores were isolated from purified HBV or WHV after an endogenous

![FIG. 2. Hemagglutination inhibition activity in CsCl gradient fractions of WHsAg(+) and HBsAg(+) sera. A 0.1 ml amount of woodchuck serum (A) or human serum (B) was mixed with 0.9 ml of a solution of CsCl (p = 1.23 g/cm3), overlaid with silicone oil, and centrifuged at 50,000 rpm at 4°C in a Beckman SW56 rotor. After 18 h, fractions were collected and assayed for hemagglutination inhibition activity with the following hemagglutination systems: Anti-WHs versus WHsAg-coated SRBC, (○); Anti-HBs versus WHsAg-coated SRBC, (○); Anti-HBs versus HBsAg-coated SRBC, (△); and Anti-WHs versus HBsAg-coated SRBC, (△). In each system, 2 to 4 hemagglutinating units of antiserum was used to agglutinate the coated SRBC. Densities were determined by refractometry.**
DNA polymerase reaction. The radioactive cores banded at a density of 1.34 g/cm³ in cesium chloride gradients (Fig. 3), whereas a second radioactive peak occasionally appeared at a lower and variable density (see Fig. 3A). The second peak was found in some preparations from both WHV and HBV and was not used in these studies. The peak at 1.34 g/cm³ was examined by electron microscopy and found to consist exclusively of cores. WHV-infected woodchucks were found to carry an antibody which reacted with these cores. Immune electron microscopy with cores purified from WHV particles and IgG isolated from the serum of an infected woodchuck showed specifically aggregated cores decorated with antibody. Three examples of such immune aggregates are shown in Fig. 4 (B, C and D). IgG from the serum of an uninfected woodchuck showed no reaction with WHV cores by immune electron microscopy (Fig. 4). By analogy to HBV, we have called this nucleocapsid antigen woodchuck hepatitis core antigen (WHcAg) and the corresponding antibody anti-WHc.

Cores to be used for immunoprecipitation reactions were prepared from the liver of a WHV-infected woodchuck and from the liver of an immunosuppressed kidney transplant patient infected with HBV. Crude core antigen preparations were found to react with both anti-WHc and anti-HBc (from a chronic carrier of HBV) by CEP and ID (Table 3). The precipitin lines from the CEP reactions were extracted and examined by electron microscopy. The precipitin lines were found to consist of immune complexes and decorated cores (Fig. 5B, C, E, and F), whereas unreacted CsCl-purified cores isolated from liver were free of antibody.

CsCl-purified HBV or WHV cores isolated from infected livers were found to react with IgG fractions prepared from both anti-WHc positive and anti-HBc positive sera by ID. When small amounts of 32P-labeled cores derived from intact virus were mixed with cores isolated from liver, radioactivity was found in the visible precipitin line (Fig. 6). This finding demonstrates not only that HBV and WHV cores have common antigenic determinants but also that these are found on the surfaces of cores isolated from both infected liver and purified virus.

CsCl-purified HBV or WHV cores isolated from infected livers were used to test for antigenic identity by ID as described by Ouchterlony (11) (Fig. 7). Lines of partial identity were formed when IgG with either anti-HBc or anti-WHc reactivity was tested against both core preparations. In each case, the lines in the homologous system were stronger and spurred over the line formed with the heterologous cores.

Nucleic acid homology. Nucleic acid homology between WHV and HBV was measured by liquid hybridization. 32P-labeled probe was prepared by in vitro replication of each denatured viral DNA with *Escherichia coli* DNA polymerase I as previously described (14). The products of the DNA polymerase reactions were subjected to electrophoresis through a 1.5% agarose slab gel, and bands were located by autoradiography. A densitometer tracing of the autoradiograms is shown in Fig. 8. Both radioactive products contained a discrete component repres-
TABLE 3. Cross-reactivity between HBcAg and WHcAg

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<thead>
<tr>
<th>Antiserum</th>
<th>WHcAg</th>
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<th>HBcAg</th>
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<tbody>
<tr>
<td></td>
<td>Liver homogenate</td>
<td>Isolated cores</td>
<td>Liver homogenate</td>
<td>Isolated cores</td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>CEP</td>
<td>ID</td>
<td>CEP</td>
</tr>
<tr>
<td>Anti-WHc(^a)</td>
<td>1/512</td>
<td>1/64</td>
<td>1/256</td>
<td>NT(^b)</td>
</tr>
<tr>
<td>Anti-HBc(^c)</td>
<td>1/64</td>
<td>1/64</td>
<td>1/32</td>
<td>NT</td>
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\(^a\) IgG isolated from the serum of a WHV-infected woodchuck.  
\(^b\) NT, Not tested.  
\(^c\) IgG isolated from the serum of a HBV-infected human.

Fig. 5. Electron micrographs of HBV (A to C) and WHV (D to E) cores. Panels A and D show purified cores in the absence of antibody. Panels B and E are virus cores aggregated by human anti-HBc, and panels C and F are cores clumped by woodchuck anti-WHc. These immune complexes are from samples of precipitin lines formed by CEP between cores from liver homogenates and purified IgG from antibody-positive sera. The linear material above the cores in panel E is strands of agarose gel. Bar represents 50 nm.

senting the in vitro replicated complete strand, as well as a smaller component heterogenous in size derived from the incomplete strand, as previously described for HBV DNA (14). The discrete component, which presumably contained all of the HBV or WHV genome sequences, was used as a probe for investigating homology between the genomes.

The ability of unlabeled purified WHV, HBV, or bovine DNAs to accelerate the annealing of these two probes is shown in Fig. 9. As seen in panel A, WHV DNA showed an ability to accel-
Fig. 6. Immunoprecipitin lines showing reactivity against HBV and WHV cores visualized by staining with azocarmine and by autoradiography. Panels A and B contain an ID pattern with labeled HBV cores from serum and unlabeled HBV cores from liver in the central well and woodchuck serum IgG with anti-WHc reactivity in well I and human serum IgG with anti-HBc reactivity in well 2. (Wells 3 to 6 are empty.) Panels C and D are set up in the same arrangement except that the center well contains both labeled WHV cores from serum and unlabeled WHV cores from liver. In the autoradiographs only the central well is visible.

Fig. 7. Cross-reactivity of WHV and HBV core particles by ID. The center well contains anti-HBc(+) IgG (A) or anti-WHc(+) IgG (B); wells 1 and 3 contain WHV cores, and wells 2 and 4 contain HBV cores. The cores were isolated by CsCl gradient centrifugation of liver homogenates from an infected woodchuck or human.

Extrapolating the final slope attained to a WHV DNA \( C_{\text{S}} = 0 \), one can estimate the increase in annealing due to the WHV DNA to involve approximately 5% of the HBV probe. Similarly, in panel B, annealing of approximately 3% of the HBV probe is accelerated by the presence of unlabeled HBV DNA. Under these conditions, therefore, nucleic acid homology between WHV and HBV is limited to a region consisting of 3 to 5% of the viral genomes, or 100 to 150 base pairs.

**DISCUSSION**

The surface core of the WHV have antigenic determinants which we have called WHSAg and WHcAg, respectively, to denote their relationship to the corresponding antigens of HBV. Anti-HBs is present in the serum of humans with HBV infections; similarly, antibody to the core of WHV (anti-WHc) is found in WHV-infected woodchucks. The pattern of reaction of these antibodies with WHcAg and HBcAg by ID (i.e., spurring and lines of partial identity) indicates that the nucleocapsid components (cores) of the two viruses share major determinants and that, in addition, each has unique antigenic determinants. The presence of similar determinants is also shown by the results of electron microscopic examination of the pre-
Fig. 9. Nucleic acid homology between WHV and HBV DNAs. WHV and HBV DNAs were extracted from virus particles purified through a CsCl isopycnic gradient as previously described (15). Approximately 5 ng of each DNA sample was denatured and radiolabeled in a reaction containing 100 mM Tris-hydrochloride, pH 7.4; 20 mM MgCl₂, 10 μM each dATP, dGTP, and dCTP; 5 μM [³²P]TTP (approximately 350 Ci/mmol); 1 μg of oligonucleotide primers (14); and 0.1 μg of E. coli DNA polymerase (gift of L. Loeb). After 40 min at 15°C, the reaction was terminated with 0.1% sodium dodecyl sulfate and applied to a 1.5% agarose gel to separate the product of the full-length strand from that of the incomplete strand. Approximately 5,000 cpm of [³²P]-labeled probe (full-length strand) was mixed with 10 ng of purified WHV, HBV, or bovine DNA plus 200 μg of bovine DNA, denatured, and annealed at 68°C in 0.2 ml of a solution containing 10 mM Tris-hydrochloride (pH 7.4), 10 mM EDTA, and 1 M NaCl. Aliquots of 0.02 ml were removed at various times and assayed for annealing by resistance to digestion with S1 nuclease (15). The fraction of the probes annealed were normalized to the maximum annealing observed with a vast excess of the homologous DNA, usually around 70%. A: HBV [³²P]DNA probe annealed with unla-beled HBV DNA (△), WHV DNA (○), or bovine DNA (●); B: WHV [³²P]DNA probe annealed with unla-beled HBV DNA (△), WHV DNA (○), or bovine DNA (●).

cipitin lines; cores present in crude homogenates of liver obtained from infected woodchucks or humans are aggregated by virus-positive sera from either woodchucks or humans. As found in HBV infections, cores obtained from intact WHV in the serum share determinants with cores purified from infected liver. The core antigenic specificity shared by HBV and WHV may be thought of as a group-specific antigen for this class of virus. Other animals which are subject to chronic liver disease and hepatocellular carcinoma may be found to have similar viruses, and we would predict that viremic animals would carry an anti-HBC-like (anti-WHc-like) antibody in their blood.

The high degree of cross-reactivity seen between the core antigen systems is not seen between the surface antigen systems. The lack of cross-reactivity in ID and CEP tests, as well as the results of the hemagglutination assays, indicates that it is unlikely that WHV shares the group-specific determinant "a" with HBV; at least, this determinant must not be a major determinant of WHsAg. Similarity between the surface constituents is suggested by the finding that in both HBV and WHV surface antigen systems, the precipitin lines are selectively stained by orcein (B. G. Werner, unpublished data). The cross-reacting determinants are present on particles from WHV- or HBV-infected serum which band at the same density as particles carrying the major surface antigen determinants. The data do not rule out that cross-reacting determinants might be present on a second class of particles banding at the same density.

The CaCl gradient fractions that inhibit the cross-reactions do so at dilutions equal to or greater than those required to inhibit agglutination by a homologous antiserum. This finding suggests that the shared determinants, even though they are less immunogenic, are present in amounts comparable to that of the major viral specific determinants.

A small region (100 to 150 base pairs) of strong nucleic acid homology was detected by liquid hybridization. Since duplexes of less than 100 base pairs are not likely to be readily detected under the hybridization and assay conditions used, it seems likely that this homology represents one or two regions of nearly identical nucleotide sequence. In support of this conclusion, preliminary studies indicate that the homology is located within a restriction fragment representing about 60% of the WHV genome (data not presented). The degree of nucleic acid homology found (3 to 5% of the genomes) is on the order of that detected among papovaviruses. Approximately 5% homology has been reported between simian virus 40 and polyoma virus by heteroduplex analysis (7), and 11 to 12% has been reported between simian virus 40 and BK virus by liquid hybridization (9).

Antigenic cross-reactivity between HBV and WHV is consistent with the finding of nucleic acid homology. At this time, however, it is not clear that the homologous regions in the respective DNAs code for the cross-reacting determinants described in this report. The antigenic and nucleic acid similarities provide further evidence of phylogenetic relatedness between HBV and WHV and support the conclusion that both viruses belong to a novel class of DNA-containing viruses. The antigenic determinants described will be useful markers for following the course of WHV infection and its postulated role in the
development of chronic hepatitis and hepatocellular carcinoma in woodchucks and should increase the potential value of this system as a model for studying the relationship of HBV to human liver cancer.

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LITERATURE CITED