Animal Models for the Study of the Human Hepatitis Viruses and Related Animal Viruses

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Nonhuman primate animal models for infections with the human hepatitis viruses have resulted in rapid advances in our knowledge of these viruses. Nonprimate animals have not been susceptible to these viruses, and the hepatitis B virus and the three non-A, non-B hepatitis viruses (and until recently, hepatitis A virus) have not been transmissible in cell cultures.

Animal models for infections of hepatitis A, hepatitis B, and non-A, non-B hepatitis viruses.

Only the chimpanzee (*Pan troglodytes*) [1], the marmoset (*Saguinus mystax* and *Saguinus labiatus*) [2, 3], and the owl monkey (*Aotus trivirgatus*) [4] have been reproducibly infected with hepatitis A. Only the chimpanzee [5] and gibbon (*Hylobates lar*) [6] have been reproducibly infected with the hepatitis B virus. The chimpanzee [7, 8] has been reproducibly infected with two of the three non-A, non-B hepatitis viruses; two (not the same two) appear to have been transmitted to a small percentage of inoculated marmosets [9, 10].

The phylogenetic relationships between man and the nonhuman primates susceptible to the agents of human hepatitis are shown in Figure 1. Man, a species that originated in the Old World, is susceptible to the viruses of all three types of hepatitis. Nonhuman primates susceptible to the hepatitis A virus (HAV) and the three non-A, non-B hepatitis viruses are found among those originating in both the Old World and the New World. Nonhuman primates known to be susceptible to the hepatitis B virus (HBV) are found only among those originating in the Old World. The usefulness of these animal models for the study of the human hepatitis viruses has depended upon the identification of infectious inocula and the determination of their end-point of infectivity. These inocula have been described in a recent review [11].

Selection of suitable primates

Selection of suitable chimpanzees provides a model for the selection of other known susceptible primates. Of the two species of chimpanzees, *Pan troglodytes* and *Pan paniscus*, only *Pan troglodytes* has been reported to be susceptible to infection by the human hepatitis viruses. There are no published data concerning the relative susceptibility of the three subspecies of *Pan troglodytes*; most studies have utilized the common or masked subspecies. It appears that variant or strain differences among chimpanzees of the masked subsepcies do not affect susceptibility.

Equal susceptibility to HAV and HBV has been observed in male and female chimpanzees. Infant chimpanzees, 18 months of age, and adult chimpanzees believed to be older than 25 years of age, have been shown to be susceptible to infection with the hepatitis A and B viruses if they have not been previously exposed. However, infant chimpanzees have been found to be best for the study of non-A, non-B hepatitis; since there are no universally accepted serologic tests to determine prior exposure to the non-A, non-B hepatitis viruses, these infant chimpanzees are most likely to have had no prior exposure or acquired immunity.

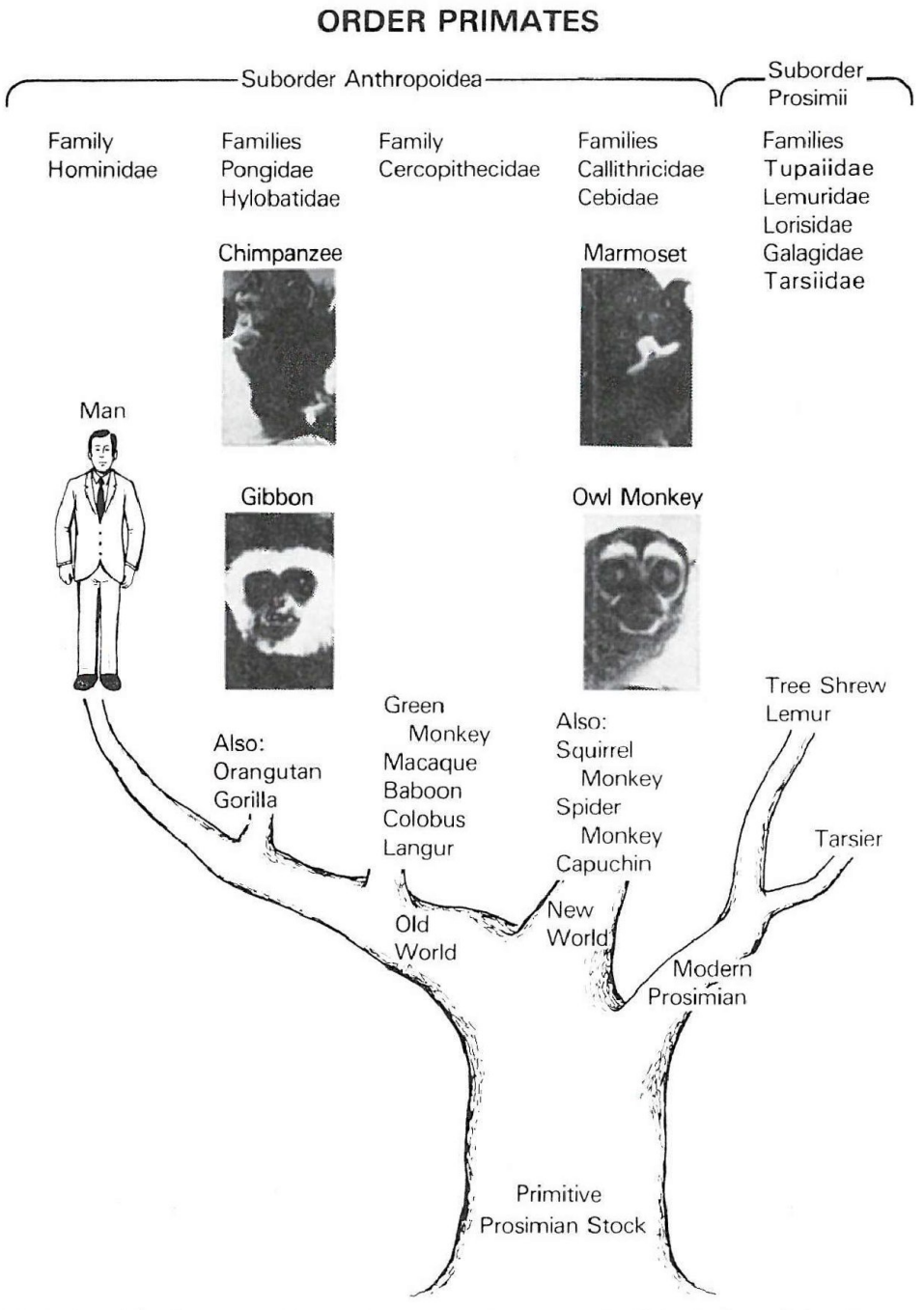

Fig. 1. Relationship between the primate species reproducibly infected by human hepatitis viruses (pictures) and a few other primate species. The photographs are: to left, chimpanzee (*Pan troglodytes*); bottom left, gibbon (*Hylobates lar*); top right, marmoset (*Saguinus labiatus* shown here; also *Saguinus mystax*); bottom right, owl monkey (*Aotus trivirgatus*). Other examples of primates useful for biomedical research are listed by common names. (From reference 11, also by the author).

No difference has been found in the susceptibility to HAV or HBV between seronegative chimpanzees born in African jungles or in breeding colonies in the United States. Even though prior infection with HAV or HBV can be determined by serologic tests, the rare possibility that serologic markers of immunity may have decreased in titer to undetectable levels makes the use of previously unused chimpanzees the best approach when these chimpanzees are available. With regard to non-A, non-B hepatitis, chimpanzees born in breeding colonies have been preferentially used to assure that they are susceptible by minimizing their chance of prior exposure to these viruses.

The value and small numbers of chimpanzees makes it necessary to use all chimpanzees sequentially for the study of more than one hepatitis agent. The possible interference by prior infection with one virus with subsequent infections by another has been evaluated. No viral interference has been shown between HAV and HBV; in fact, simultaneous acute infection with both viruses in a chimpanzee has been shown to result in unusually severe liver disease. Limited viral interference between simultaneous non-A, non-B hepatitis and HBV has been shown in certain situations. In general, it is acceptable to utilize chimpanzees in successive hepatitis studies, provided recovery from prior studies with HAV and HBV is shown serologically, and apparent recovery from non-A, non-B hepatitis has been shown by the return of liver enzymes and liver histology to normal.

Related hepadna viruses of animals as models for human hepatitis

Three viruses of animals, and their close relative, HBV, have been designated "hepadna" viruses. These are the woodchuck hepatitis virus (WHV) which infects *Marmota monax*, Pekin duck hepatitis B virus (DHBV) which infects *Anas domesticus*, and Beechey ground squirrel hepatitis virus (GSHV) which infects *Spermophilus beecheyi*. Although these three viruses are different from each other and from HBV, their similarities to HBV have made them the object of intense study. In particular, the association of WHV with primary hepatocellular carcinoma (PHC) in the woodchuck, analogous to the association between HBV and PHC in man but with a shorter interval infection and the appearance of PHC, has been expected to provide a model in which to study the prevention and treatment of PHC in man. (Paradoxically, PHC has not yet been found in chimpanzees chronically infected with HBV.)

WHV is similar in size and structure to HBV, with a nucleocapsid core surrounded by surface protein; large quantities of excess surface protein are found free in the serum of infected woodchucks [14]. Immunologically, WHV has a surface antigen with substantial cross-reactivity with that of HBV; the core antigens of WHV and HBV, however, have only very limited cross-reactivity [15]. The DNA sequences of the two viruses are about 70 % homologous. The potential susceptibility of chimpanzees to experimental infection with WHV has been suggested by the seroconversion of one inoculated chimpanzee [13]. Among woodchucks captured in the eastern United States, serologic markers of prior or current infection with WHV have been found in 42 % [12], up to two-thirds of which have chronic infection with WHV. PHC was found at necropsy in 60 % of those with chronic WHV infections [12, 13], in few of those with serologic evidence of prior infection and recovery [13], and in none of those which had never been infected [12, 13].

GSHV structurally resembles HBV and WHV, but it is slightly larger and has not been associated with PHC or with severe hepatitis. In one section of California, 85 % of ground squirrels had serologic

markers of prior or current GSHV infection, and 52 % of the total ground squirrel population were chronically infected [16]. Some cross-reactivity is seen between the surface antigens of HBV and GSHV, but cross-reactivity of the core antigens has not been described in detail. The extent of homology between the DNA sequences of HBV and GSHV has not been fully reported. The chimpanzee may perhaps also be susceptible to infection with this virus, since one intravenously inoculated chimpanzee has been reported to have seroconverted [16].

DHBV has been less extensively studied. Structurally it is similar to HBV but is slightly smaller. The extent of antigenic cross-reactivity and DNA homology with the other hepadna viruses has not been reported in detail. Attempts to transmit the virus to chimpanzees have not been reported. Congenital infection with DHBV has been documented as a likely mechanism for its transmission, with viral replication beginning as early as the 12th day of embryonic life [17].

Applications of nonhuman primate models for human hepatitis

The availability of the marmoset model for hepatitis A permitted the development of an inactivated experimental vaccine against hepatitis A even before the virus could be grown in cell culture [18]. The marmoset model contributed to the discovery of a way to propagate HAV *in vitro* in the absence of cytopathic effects. The recognition that HAV had been transmitted *in vitro* [19] was dependent in part on the transmission of hepatitis A to marmosets by inoculation of the supernatant fluid from the infected cell cultures, and in part on the demonstration of HAV in the infected cells using an immunofluorescence test which had been developed using tissues from marmosets and chimpanzees experimentally infected with hepatitis A.

The chimpanzee model for hepatitis B has made possible the development of vaccines to prevent this disease despite the inability to propagate the virus *in vitro*. The safety, immunogenicity, and efficacy of these vaccines were first proved in chimpanzees [20, 21]. Titered HBV inocula were used to challenge vaccinated chimpanzees to document that protection had resulted from the vaccination. The inactivation of specific amounts of HBV infectivity by formalin, pepsin, urea, heating at 60°C for 10 h, and high-titer antibody to HBsAg (anti-HBs) have been shown using titered inocula in chimpanzees [22, 23, 24, 25, 26]. These inactivation studies contributed to the certification of safe hepatitis B vaccines and the development of clotting factor concentrates with a reduced risk of transmitting hepatitis B.

Without the chimpanzee model for human non-A, non-B hepatitis, our knowledge of this disease would be limited to what we have learned from epidemiologic studies. The chimpanzee model has enabled investigators to confirm that the disease "non-A, non-B hepatitis" includes infections of the liver caused by at least three viruses, designated "blood transmitted", "coagulation-factor-transmitted", and "epidemic waterborne" [27]. Only one of the three non-A, non-B hepatitis viruses, the "blood transmitted" virus, when transmitted to chimpanzees, results in characteristic convoluted tubules which can be seen in liver cells by electron microscopy. When chimpanzees which had been infected by this virus and had apparently recovered were subsequently inoculated with the well-documented inocula with the "coagulation-factor-transmitted" virus, second episodes of non-A, non-B hepatitis were observed, in which the characteristic convoluted tubules were absent [28, 29]. The third virus of non-A, non-B hepatitis, the "epidemic waterborne" virus, has not yet been successfully transmitted to chimpanzees, but the number of chimpanzees inoculated has been

too small to draw conclusions. Serum and liver biopsy tissue from infected chimpanzees have provided material to develop experimental serologic tests and to attempt other ways to identify those who are infected with non-A, non-B hepatitis.

The chimpanzee model has been extensively used to evaluate the "blood-transmitted" non-A, non-B hepatitis virus. It has been used to show that this virus can persist in the blood of an asymptomatic carrier for six or more years [30]. The demonstration that an agent of non-A, non-B hepatitis can be inactivated by formalin [31, 32] or by heating at 60°C for 10 h [33] has been made possible by the availability of the chimpanzee model, providing a prospect for the future development of a vaccine against this agent.

Alternative systems: Cell culture

Hepatitis A virus (HAV) was grown in cell culture for the first time in 1978. The list of cell lines in which HAV can be grown now includes fetal rhesus kidney, primary African green monkey kidney, vero (continuous African green monkey kidney), FL (human amnion), human embryo fibroblast (HEF and WI-26), human embryo lung fibroblast (WI-38 and MRC-5), and human embryo kidney. HAV has also been used to infect marmoset liver explants and to superinfect the PLC/PRF/5 human hepatoma cell line. One or more of these cell lines will provide antigenic substrate or virus for a hepatitis A vaccine and a convenient system to evaluate inactivation or attenuation procedures for this vaccine.

Inoculation of many cell lines with hepatitis B virus (HBV) has resulted in no detectable infections, although numerous unconfirmed reports of successes have appeared over the years. However, the recognition that a human hepatoma cell line, PLC/PRF/5, produces HBsAg in the absence of any detectable virus, has provided an experimental system which may eventually improve our understanding of why HBV has not been induced to replicate *in vitro*.

Attempts have been made by several laboratories to grow the three agents of non-A, non-B hepatitis in conventional cell lines; none has resulted in detectable infection. The difficulties in finding alternative *in vivo* and *in vitro* systems in which to study the infectivity of the agents of non-A, non-B hepatitis underline the importance of the chimpanzee animal model.

References

1. *Dienstag, J. L., S. M. Feinstone, H. Purcell, J. H. Hoofnagle, L. F. Barker, W. T. London, H. Popper, J. M. Peterson & A. Z. Kapikian:* Experimental infection of chimpanzees with hepatitis A virus. J. Infect. Dis. *132*: 532—545 (1975).
2. *Holmes, A. W., L. Wolfe, H. Rosenblat & & F. Deinhardt:* Hepatitis in marmosets: Induction of disease with coded specimens from a human volunteer study. Science *165*: 816—817 (1969).
3. *Provost, P. J., V. M. Villarejos & M. R. Hilleman:* Suitability of the rufiventer marmoset as a host animal for human hepatitis A virus. Proc. SEoc. Exp. Biol. Med. *155*: 283—286 (1977).
4. *Le Duc, J. W., S. M. Lemon, C. M. Keenan, R. R. Graham, R. H. Marchwicki & L. N. Binn:* Experimental infection of the New World owl monkey (*Aotus trivirgatus*) with hepatitis A virus. Infect. Immun. *40*: 766—722 (1983).
5. *Barker, L. F., F. V. Chisari, P. P. McGrath, D. W. Dalgard, R. L. Kirchstein, J. D. Almeida, T. S. Edgington, D. G. Sharp & M. R. Peterson:* Transmission of type B viral hepatitis to chimpanzees. J. Infect. Dis. *127*: 648—662 (1973).
6. *Bancroft, W. H., R. Snitbhan, R. M. Scott, M. Tingpalapong, W. T. Watson, P. Tanticharoenyos, J. J. Karwacki & S. Srimarut:* Transmission of hepatitis B virus to gibbons by exposure to human saliva containing hepatitis B surface antigen. J. Infect. Dis. *135*: 79—85 (1977).
7. *Tabor, E., R. J. Gerety, J. A. Drucker L. B. Seeff, J. H. Hoofnagle, D. R. Jackson, M. April, L. F. & G. Pineda-Tamondong:* Transmission of non-A, non-B hepatitis from man to chimpanzee. Lancet. *1*: 463—466 (1978).

8. *Bradley, D. W., L. H. Cook, J. L. Maynard, K. A. McCaustland, J. W. Ebert, G. H. Lolana, R. A. Petzel, R. J. Kantor, A. Heilbrunn, H. A. Fields & B. L. Murphy:* Experimental infection of chimpanzees with antihemophilic (factor VIII) materials: recovery of virus-like particles associated with non-A, non-B hepatitis. J. Med. Virol. *3*: 253—269 (1979).
9. *Feinstone, S. M., H. J. Alter, H. P. Dienes, Y. Shimizu, H. Popper, D. Blackmore, D. Sly, W. T. London & R. H. Purcell:* Non-A, non-B hepatitis in chimpanzees and marmosets. J. Infect. Dis. *144*: 588—598 (1981).
10. *Kane, M. A., D. W. Bradley, S. M. Shrestha,, J. E. Maynard, E. H. Cook, R. P. Misha & D. D. Joshi:* Epidemic non-A, non-B hepatitis in Nepal: Recovery of a possible etiologic agent and transmission studies in marmosets. J. Am. Med. Assoc. *252*: 3140—3145 (1984).
11. *Tabor, E., R. H. Purcell & R. J. Gerety:* Primate animal models and titered inocula for the study of human hepatitis A, hepatitis B, and non-A, non-B hepatitis. J. Med. Primatol. *12*: 305—318 (1983).
12. *Popper, H., J. W. Shin, J. L. Gerin, D. C. Wong, B. H. Hoyer, W. T. London, D. L. Sly & R. H. Purcell:* Woodchuck hepatitis and hepatocellular carcinoma: Correlation of histologic with virologic observations. Hepatology *1:* 91—98 (1981).
13. *Wong, D. C., J. W. Shih, R. H. Purcell, J. L. Gerin & W. T. London:* Natural and experimental infection of woodchucks with woodchuck hepatitis virus, as measured by new, specific assays of woodchuck surface antigen and antibody. J. Clin. Micro. *15*: 484—490 (1982).
14. *Summers, J., J. M. Smolec & R. Snyder:* A virus similar to human hepatitis B virus associated with hepatitis and hepatoma in woodchucks. Proc. Natl. Acad. Sci. USA *75*: 4533—4537 (1978).
15. *Millman, I., T. Halbherr & H. Simmons:* Immunological cross-reactivities of woodchuck and hepatitis B viral antigens. Infect. Immun. *34*: 752—757 (1982).
16. *Marion, P. L., S. S. Knight, F. H. Salazar, H. Popper & W. S. Robinson:* Ground squirrel hepatitis virus infection. Hepatology *3:* 519—527 (1983).
17. *O'Connell, A. P., M. K. Urban & W. T. London:* Naturally occurring infection of Pekin duck embryos by duck hepatitis B virus. Proc. Natl. Acad. Sci. USA *80*: 1703—1706 (1983).
18. *Provost, P. J. & M. R. Hilleman:* An inactivated hepatitis A virus vaccine pre-

pared from infected marmoset liver. Proc. Soc. Exp. Biol. Med. *159*: 201—203 (1978).
19. *Provost, P. J. & M. R. Hilleman:* Propagation of human hepatitis A virus in cell culture *in vitro*. Proc. Soc. Exp. Biol. Med. *160*: 213—221 (1979).
20. *Hilleman, M. D., E. B. Buynak, R. R. Roehm, A. A. Tytell, A. U. Bertland & G. P. Lampson:* Purified and inactivated human hepatitis B vaccine: Progress report. Am. J. Med. *270*: 401—404 (1975).
21. *Purcell, R. H. & J. L. Gerin:* Hepatitis B subunit vaccine: A preliminary report of safety and efficacy tests in chimpanzees. Am. J. Med. Sci. *270:* 395—399 (1975).
22. *Tabor, E., E. Buynak, L. A. Smallwood, P. Snoy, M. Hilleman & R. J. Gerety:* Inactivation of hepatitis B virus by three methods: Treatment with pepsin, urea, or formalin. J. Med. Virol. *11*: 1—9 (1983).
23. *Tabor, E., G. Murano, P. Snoy & R. J. Gerety:* Inactivation of hepatitis B virus by heat in antithrombin III stabilized with citrate. Tromb. Res. *22*: 233—238 (1981).
24. *Heimburger, N., H. Schwinn & R. Mauler:* Factor VIII concentrate, hepatitis-safe: Progress in the treatment of hemophilus A. Gelben Hefte *20*: 165—174 (1980).
25. *Heimburger, N., H. Schwinn, P. Gratz, G. Luben, G. Kumbe & B. Herchenhan:* Factor VIII concentrate, highly purified and heated in solution. Arzneim. Forsch. *31*: 619—622 (1981).
26. *Tabor, E., D. L. Aronson & R. J. Gerety:* Removal of hepatitis-B-virus infectivity from factor-IX complex by hepatitis-B immune-globulin. Lancet *2:* 68—70 (1980).
27. *Tabor, E.:* The three viruses of non-A, non-B hepatitis. Lancet *1*: 743—745 (1985).
28. *Tabor, E., P. Snoy, D. R. Jackson, Z. Schaff, P. M. Blatt & R. J. Gerety:* Additional evidence for more than one agent of human non-A, non-B hepatitis: Transmission and passage studies in chimpanzees. Transfusion *24*: 224—230 (1984).
29. *Hollinger, F. B., I. W. Mosley, W. Szmuness, R. D. Aach, R. L. Peters & C. Stevens:* Transfusion-transmitted viruses study: Experimental evidence for two non-A, non-B hepatitis agents. J. Infect. Dis. *142*: 400—407 (1980).
30. *Tabor, E., L. B. Seeff & R. I. Gerety:* Chronic non-A, non-B hepatitis carrier state: Transmissible agent documented in one patient over a six-year period. N. Engl. J. Med. *303*: 139—143 (1980).
31. *Tabor, E. & R. J. Gerety:* Inactivation of an agent of human non-A, non-B hepatitis by formalin. J. Infect. Dis. *142*: 767—770 (1980).
32. *Yoshizawa, H., Y. Itoh, S. Iwakiri, K. Kitajima, A. Tanaka, T. Tachibana, T. Nakamura, Y. Miyakawa & M. Mayumi:* Non-A, non-B (type 1) hepatitis agent capable of inducing tubular ultrastructures in the hepatocyte cytoplasm of chimpanzees: Inactivation of formalin and heat. Gastroenterology *82*: 502—506 (1982).
33. *Tabor, E. & R. J. Gerety:* The chimpanzee animal model for non-A, non-B hepatitis: New applications. In Szmuness, W., H. J. Alter & J. E. Maynard (eds.): »Viral Hepatitis: 1981 International Symposium«. Philadelphia: Franklin Institute Press, p. 305—317 (1982).

Sammendrag ved S. Alexandersen

Anvendelse af forsøgsdyr i studiet af humane hepatitis virus of relaterede animale virus

Brugen af primater som forsøgsdyr i undersøgelser af humane hepatitis virus har bidraget væsentligt til hvad vi idag ved om disse virus. Andre forsøgsdyr er ikke velegnede til sådanne studier, da de ikke er modtagelige for infektion med humane hepatitis virus, og cellekulturer kan kun bruges i begrænset omfang, idet kun hepatitis A kan dyrkes *in vitro*.

Den oftest anvendte primat til forsøg med hepatitis virus er chimpansen, der er modtagelig for infektion med hepatitis A og B samt to af de tre ikke-A, ikke-B typer. Gibbonaber, marmoset-silkeaber og nataber har også været anvendt.

Betydningen af oplysninger fået fra studiet af tre animale virus tæt beslægtede med hepatitis B omtales. Specielt fremhæves betydningen af studier af skovmurmeldyrets hepatitis virus, idet infektion med dette virus hos skovmurmeldyret, i lighed med hepatitis B virus infektion hos mennesket, er sat i forbindelse med optræden af primært hepatocellulært carcinoma.

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