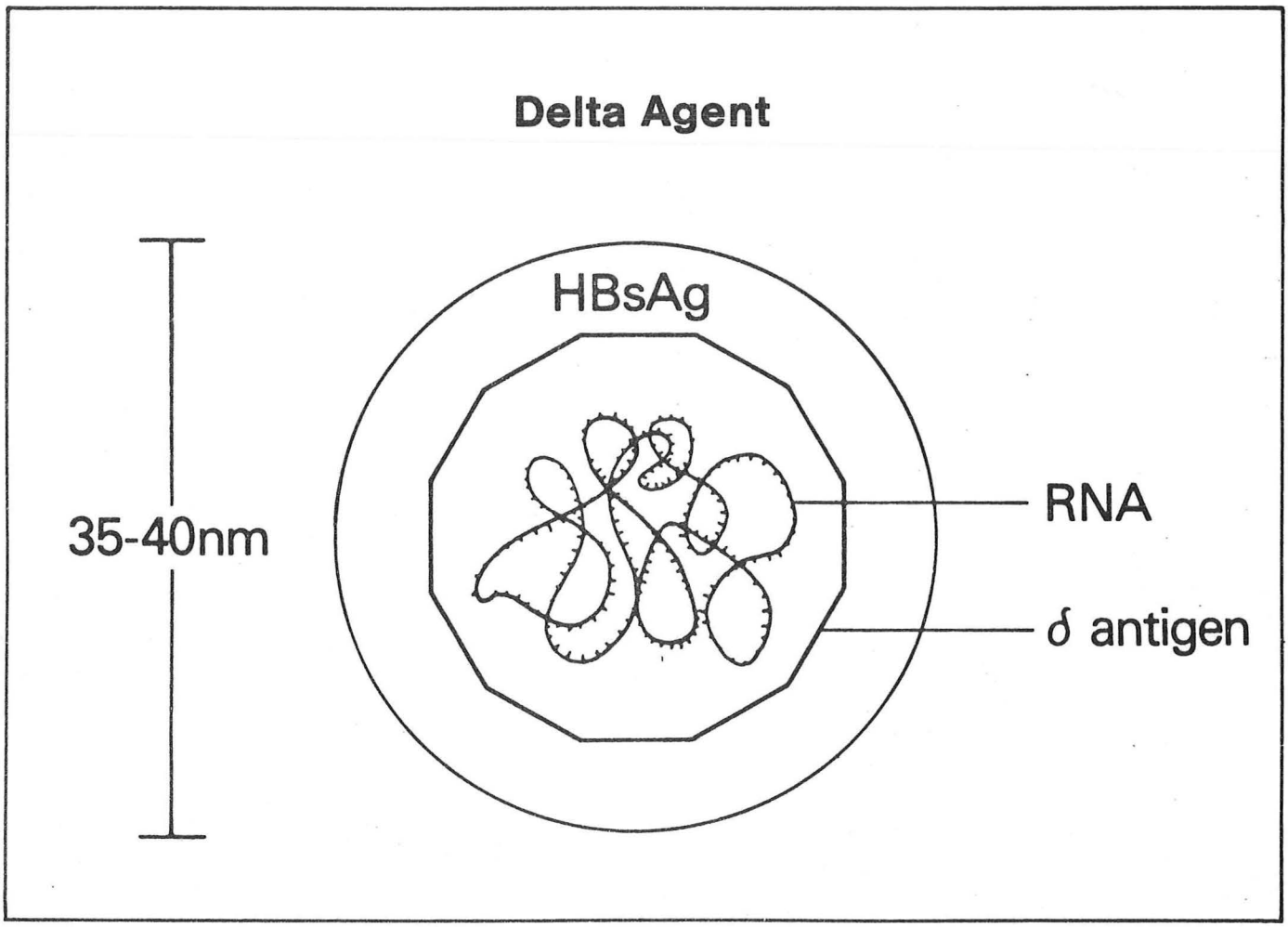
Liver
Hepatitis

DELTA AGENT: HEPATITIS D?



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INTRODUCTION

In 1977, Rizzetto and coworkers first reported the existence of a new antigen/antibody system associated with hepatitis B (74). They termed the new specificities delta/anti-delta to distinguish them from the previously described hepatitis B surface antigen (HBsAg)/antibody to HBsAg (anti-HBs) hepatitis B core antigen (HBcAg)/antibody to HBcAg (anti-HBc) and hepatitis Be antigen (HBeAg)/antibody to HBeAg (anti-HBe) systems. In the initial report, delta antigen was detected in the hepatocyte nuclei of Italian patients with HBsAg positive liver disease by direct immunofluorescence. The antiserum used contained antibodies to HBcAg but also reacted with additional biopsies which did not contain core particles at electron microscopy, thereby indicating the antiserum included antibodies to two nuclear antigens, HBcAg and the newly discovered delta antigen. The association of delta antigen with chronic liver disease was suggested by the failure to find delta antigen in liver biopsies of HBsAg positive patients without chronic hepatitis (74).

Since 1977, extensive investigations have established that delta agent, immunologically identified by the delta antigen/anti-delta antibody system, is a unique hepatitis agent. Delta agent is distinct from HBV, although dependent on HBV for its expression and replication. Recently it has been proposed that a new nomenclature be used for delta hepatitis and delta agent, to reflect the distinct clinical, epidemiological and histopathological features of acute and chronic delta infection.

TABLE I

Nomenclature

Current	Recommended
Delta hepatitis	Type D Hepatitis
Delta Agent (δ)	Hepatitis D Virus (HDV)
Delta Antigen (δAg)	Hepatitis D Antigen (HDAg)
Antibody to delta Ag (anti-delta)	Antibody to HDAg (anti-HD)

From: Rizzetto et al, Prog. Clin. Bio. Res. 143:xx-xxi, 1984.
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HEPATITIS VIRUSES:

Viruses are small infectious agents (20-300nm diameter) containing one kind of nucleic acid (RNA or DNA) as their genome. Replication of viruses occurs in living cells, using host-derived sources of energy, synthetic systems and precursors of viral proteins and nucleic acids. In Figure 1 the processes normally used by the cell are indicated as continuous lines, while those apparently only used in viral infections are broken.

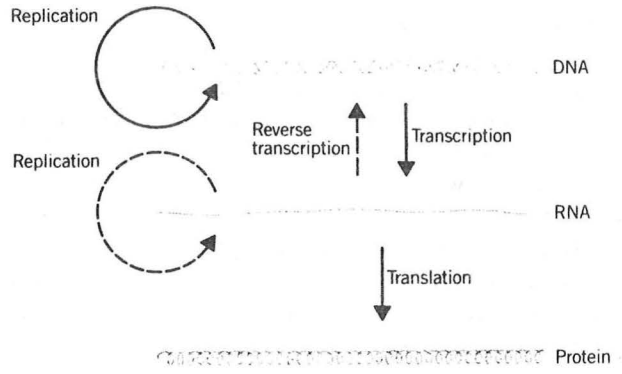

Figure 1
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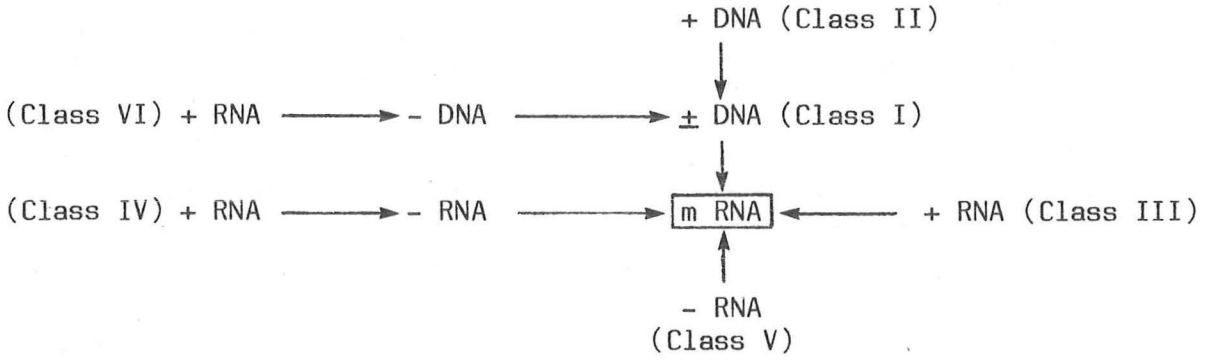
Viruses can be separated into families on the basis of their nucleic acid genome and the mode of replication of the virus particle. Baltimore described a classification system based on the specific mechanism for mRNA synthesis used by each class of virus (4).

VIRUS CLASSIFICATION

Pathway of Nucleic Acid Transcription



From: Baltimore, Bact. Rev. 35:235-241, 1971.
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DNA viruses (Table II, Figure 2) are found in classes I and II. Class I viruses have a genome of double-stranded DNA and class II viruses are single-stranded DNA. The hepadnaviruses are a unique group of DNA viruses causing hepatitis in man and a variety of animals.

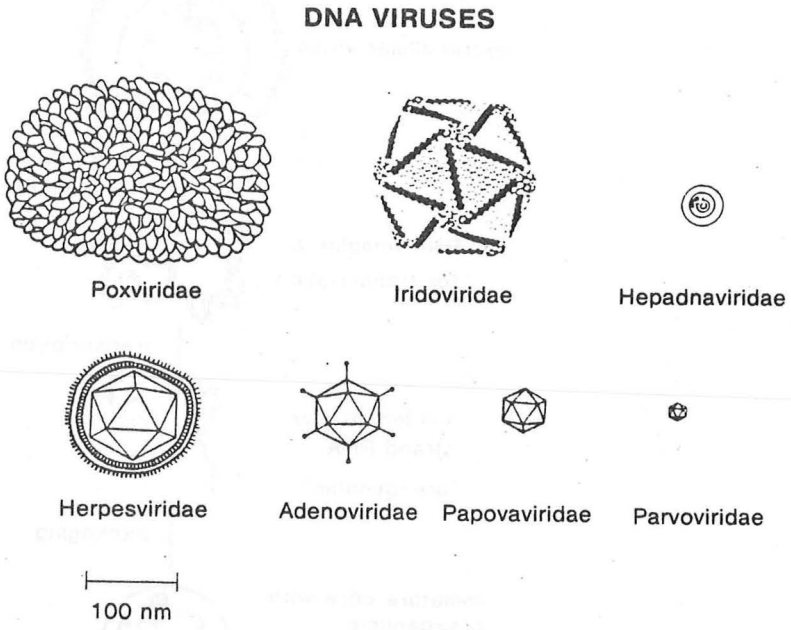


Figure 2 - modified from Fenner F, White DO: Medical Virology 2nd ed. 1976 ©1976 Academic Press, Inc.

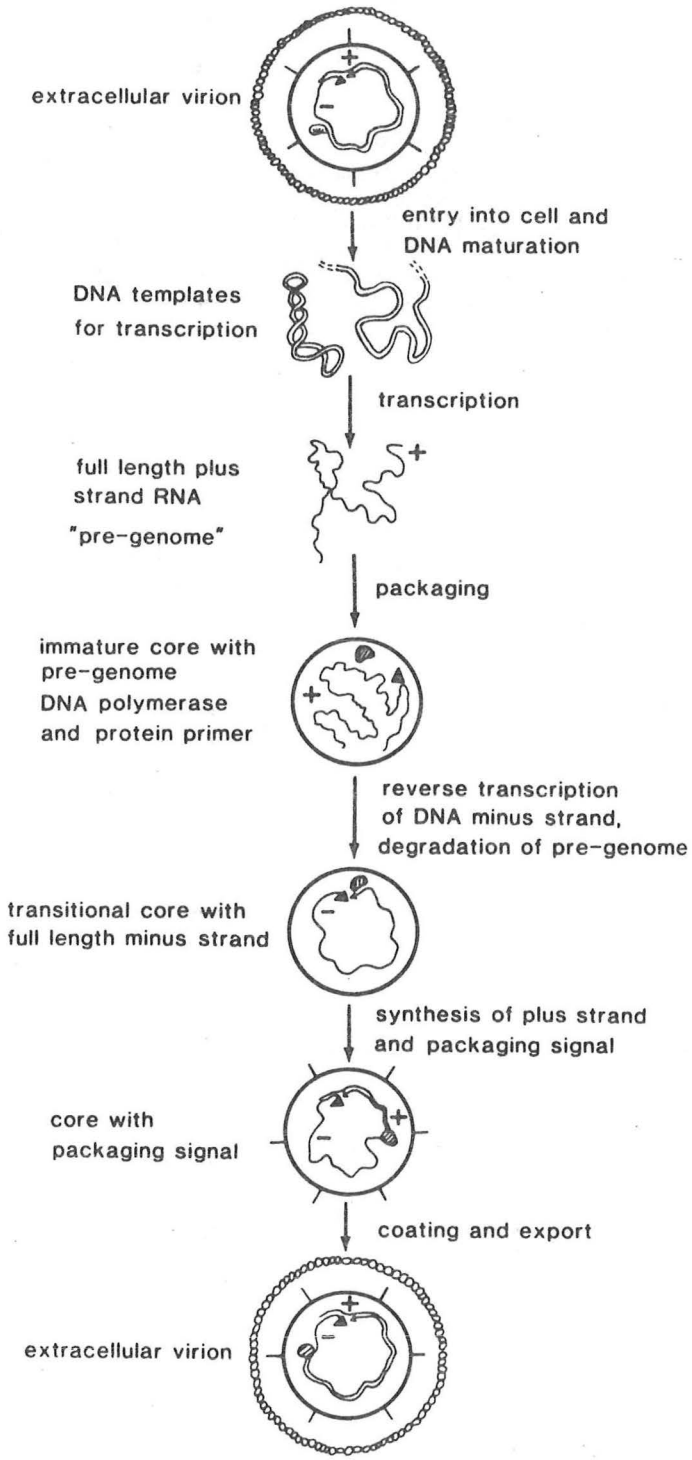
TABLE II

DNA Viruses

Class	Virus Particle (nm)	Nucleic Acid Core		Virus Family
		Type	Molecular Weight x 10^6	
I	70-230x400	DS	20-240	Adenoviridae Herpesviridae Poxviridae
	45-55	DS-circular	3-5	Papovaviridae
	42	DS-circular	1.6	Hepadnaviridae
II	18-26	SS	1.5-2.2	Parvoviridae

DS = double standard; SS = single standard

The hepadnaviridae family includes hepatitis B virus (HBV), woodchuck hepatitis virus (WHV), Beechey ground squirrel hepatitis virus (GSHV) and Pekin duck hepatitis B virus (DHBV) (101). A fifth hepatitis virus, found in tree squirrels (tree squirrel hepatitis virus, TSHV) has recently been described (24). The hepatitis viruses are distinct from other DNA viruses since they undergo reverse transcription during the process of replication, as shown in Figure 3 (102).



A general outline for the replication of the genome of HBV-like viruses.

Figure 3 - from: Summers J and Mason WS, Hepatology 2:61S-66S, 1982. ©1982 AASLD

RNA viruses (Table III, Figure 3) are found in classes III-VI. Class III viruses have a double-stranded RNA genome, class IV viruses have a single-stranded RNA virion identical in base sequence to its mRNA and class V viruses have a messenger RNA complementary to the base sequence of the single-stranded RNA genome. The retroviruses (class VI) include all RNA tumor viruses. Reverse transcription of retroviruses produces double-stranded DNA which may become integrated into host DNA. Some retroviruses are replication-defective and require a helper retrovirus. Hepatitis A virus is a picornavirus (class IV). Delta agent (hepatitis D virus) is a unique RNA virus that requires a helper DNA virus (HBV usually) for replication.

TABLE III

RNA Viruses

Class	Virus Particle (nm)	Nucleic Acid Core		Virus Family
		Type	Molecular Weight $\times 10^6$	
III	60-80	DS-segmented	12-19	Reoviridae
IV	20-30	SS	2-4	Picornaviridae (Hepatitis A) Togaviridae Coronaviridae
	50-300	SS-segmented	3-15	Arenaviridae Bunyaviridae
V	150-300	SS	3-8	Rhabdoviridae Paramoxyviridae
	80-120	SS-segmented	5	Orthomyxoviridae
VI	100	SS	7-10	Retroviridae

DS = double stranded; SS = single stranded

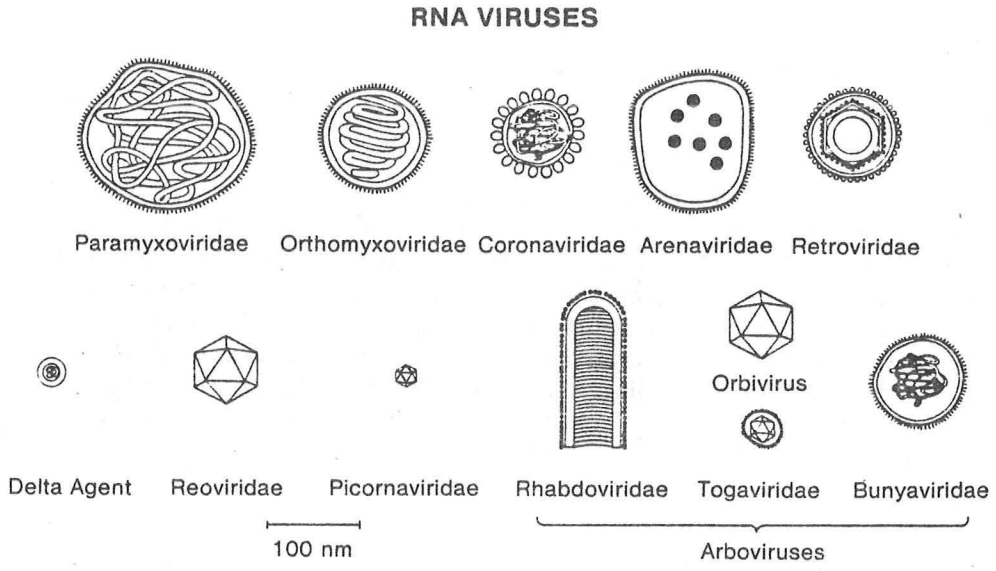


Figure 4 - modified from Fenner F, White DO: Medical Virology 2nd ed. 1976 ©1976 Academic Press

DELTA AGENT (Hepatitis D Virus):

The delta agent is a 35-37nm particle found in hepatocyte nuclei and less often in the serum during delta infection (5,78). The outer coat of the delta particle is HBsAg, coded for by the hepatitis B virus (78). The inner portion includes a nucleic acid (RNA) and a protein (delta antigen).

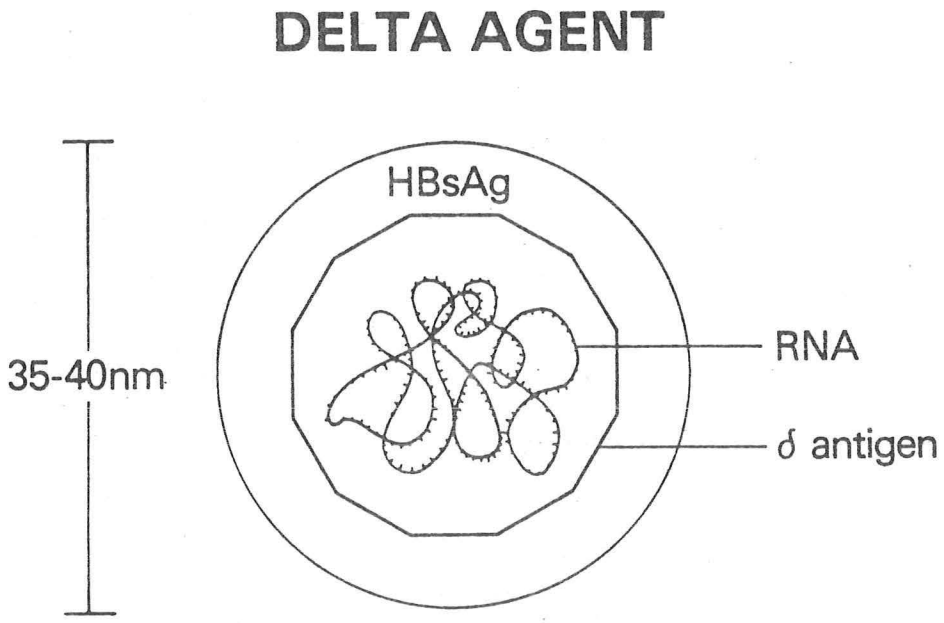
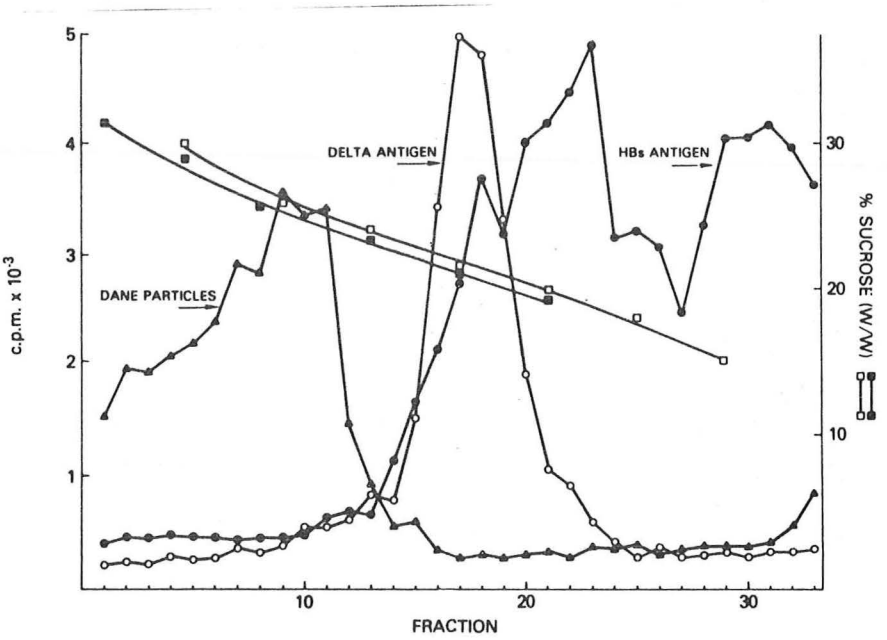


Figure 5
From: Hoofnagle J, 1983 AASLD Postgraduate Course (with permission)

Delta agent (hepatitis D virus, HDV) particles were analyzed following separation from serum of chimpanzees infected with both hepatitis B and delta agent (75). Delta antigen immunoreactivity was isolated with a discrete subpopulation of HBsAg particles. The delta agent particles banded in cesium chloride at 1.24-1.25g/cm^3 and sedimented with a mobility between that of the hepatitis B virion (Dane particles 42nm) and that of the 22nm form of HBsAg (Figure 6). Electron microscopic examination revealed particles of heterogeneous appearance, the predominant form being 35-37nm in diameter. The particles contained delta antigen activity and were precipitated by antibodies to HBsAg.



Rate zonal sedimentation, in 10–30% sucrose, of δ, HBsAg, and Dane particle-associated HBcAg. Fractions 22–27 from the CsCl gradient in Figure 5 were pelleted. This and a pellet from a Dane particle preparation from human blood were each resuspended in 400 μl PBS and layered in separate tubes onto 11 ml of a linear 10–30% sucrose (w/w) gradient with a 0.5 ml cushion of 65% sucrose (w/w) at the bottom. The samples were centrifuged at 40,000 rpm for 110 minutes at 4°C in a SW 41 rotor, and the gradients were collected from the bottom (fraction 1) in 33 fractions of 350 μl each. Fractions were assayed for δ at a 1/5 dilution, for HBsAg at a 1/500 dilution, and for HBcAg at a 1/5 dilution.

Figure 6
From: Rizzetto et al, Proc. Natl. Acad. Sci. (USA) 77:6124-6128, 1980.
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Delta antigen (hepatitis D antigen, HDAg) was initially extracted from human liver tissue obtained at autopsy (81). The delta antigen was extracted and solubilized by sonication and treatment with 6M guanidine HCl. A microtiter solid-phase radioimmunoassay was developed and used for partial characterization of delta. As shown in Table IV, equilibrium centrifugation:gel filtration and chemical and enzymatic treatments revealed delta antigen to be a protein of approximately 68,000 molecular

weight, with a buoyant density of 1.28g/cm^3 in cesium chloride, stable to heat, acid, nucleases and glycosidases but inactivated by alkali and proteases (81). A blocking solid-phase radioimmunoassay for measuring anti-delta (antibody to HDAg, anti-HD) was developed using standard preparations of delta antigen. A standard anti-delta antiserum was obtained from a HBsAg carrier whose serum contained a high titer (1:1000) of anti-delta by blocking immunofluorescence.

TABLE IV
Effect of chemical and enzymatic treatment on the activity of delta antigen

Treatment	% Loss of Activity
Nonidet P-40, 0.5%	0
Tween 80, 0.5%	0
Ether	0
Guanidine-HCl 6M	62
Trichloracetic acid, 1%	92
DNase I, 1%	0
RNase A, 1%	0
Glycosidase, 0.5%	0
Chymotrypsin, 0.1%	69
Pronase, 0.1%	96

From: Rizzetto et al, J. Immunol. 125:318-324, 1980
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More recently, Fields and coworkers have purified and partially characterized delta antigen from chimpanzee liver material (26). A major peak obtained after preparative isoelectric focusing (pI 3.77) contained polypeptides corresponding to molecular weights of 97,000, 79,000 and 66,000. Further characterization awaits immunologic detection of delta specific polypeptides.

In addition to delta antigen activity, a low molecular weight RNA was found to be temporally associated with delta antigen in the serum of infected chimpanzees and co-purified with the delta-antigen associated particles (78). The nucleic acid was identified as a discrete band of ethidium bromide fluorescence in samples that contained delta antigen activity. The nucleic acid was digested by RNase A but not by DNase I and the delta-antigen-associated RNA was estimated to have a molecular weight of approximately 0.5 x 10^6. Similar delta agent particles have been isolated from human serum (5). These latter particles were also a unique subpopulation of HBsAg particles containing delta antigen and were associated with a small RNA (0.55 x 10^6 molecular weight).

The RNA (1.75 kilobases) associated with delta agent is smaller than the smallest RNA viruses, the picornaviruses (molecular wt 2-4 x 10^6) but larger than that of viroids. The RNA has the coding capacity for a protein of about 62,000 daltons and therefore may code for the protein represented

by delta antigen. The delta-associated RNA does not have a 3' poly (A) tail (41). This indicates that the delta agent either has the properties of a negative strand virus (where the complementary plus strand serves as messenger RNA) or that it resembles histone or plant RNAs, which serve as messengers but lack a poly (A) tail. The RNA has been partially cloned and sequenced and a cDNA probe obtained by reverse transcription (41).

The cDNA probe reacts with regions unique to delta-associated RNA and not with HBV-DNA. In addition cDNA probes of HBV do not hybridize with delta-associated RNA (41). Furthermore similarly prepared delta-associated RNA and 28S and 18S ribosomal RNA do not comigrate in comparative electrophoresis, thereby ruling out the hypothesis that delta-associated RNA is host ribosomal RNA (41). Therefore delta agent-associated RNA is a unique, non-host RNA that has no homology with its helper virus HBV. The relationship of delta agent and its associated RNA to other viruses and virus-like particles has not been completely defined. Similar viruses and virus-like particles are compared with delta agent in Table V.

Table V

Virus-like Particles

Particle	Nucleic Acid		Replication	Capsid
	Type	Molecular Weight $\times 10^6$		
Viroid	RNA	0.11 - 0.13	self-replicating	none
Defective Interfering Virus	RNA	<std	helper-std virus	standard
	DNA	<std	helper-std virus	standard
Virus Satellite RNA	RNA	0.1 - 0.5	helper RNA virus	satellite or helper
Delta Agent	RNA	0.5	helper DNA virus (HBV)	viral/helper (delta antigen + HBsAg)

std = standard virus, source of defective interfering virus.

Since delta agent requires a helper virus for replication it is unlike viroids, which are self-replicating (9). In addition, viroids differ in that they do not have a capsid or protein coat (21). The initial description and subsequent characterization of delta agent have demonstrated that delta agent is defective, requiring HBV or a similar virus for replication. In addition delta agent may interfere with HBV replication. HBcAg is found less often in liver biopsies containing delta antigen (6,65,74,100) and longitudinal studies have demonstrated a decrease in titers of HB-related antigens during delta infection (6,51,75).

Defective interfering viruses which interact with either DNA or RNA viruses (including retroviruses) have been described (42). Defective interfering viruses differ from delta agent. They arise spontaneously from the standard DNA or RNA virus and require the presence of the standard virus for replication. The defective viruses have an incomplete viral genome and are therefore viral deletion mutants. They interfere specifically with intracellular growth of the homologous standard virus. The molecular basis of the interference may be different in each system, for example by inhibiting virion RNA replication or by competing for a limiting viral protein such as a procapsid (polivirus).

Defective Interfering Viruses

1. Contain only part of viral genome
   smaller and lighter than standard virus

2. Contain normal viral structural proteins
   (may be coded for only by standard virus)

3. Replicate only in presence of helper standard virus

4. Interfere specifically with intracellular growth of
   homologous standard virus

Delta agent (hepatitis D virus) is not a defective interfering virus as classically defined. The properties of delta agent that are similar to those of defective interfering viruses include its requirement for a helper virus in order to replicate and its interference in growth of the helper virus. However, the presumed delta agent genome is RNA and does not arise from the genome of its helper virus, HBV (41). In addition woodchuck hepatitis virus (WHV) can substitute for HBV (64), but has only some homology with HBV (101).

Delta agent may most closely resemble viral satellite RNAs. These are small, replicating, nonessential encapsidated RNAs only found in plants to date (3,43). The RNAs are defective, requiring a helper virus in order to replicate. In addition some of the RNAs do not seem to represent a part of the viral genome RNA of the helper virus. Some are like delta agent in that they can be encapsidated in the coat protein of the helper virus. Since the molecular weights of some viral satellite RNAs may be of the order of 0.5×10^6 daltons, they may have coding potential for proteins such as satellite-associated proteins similar to delta antigen. Some satellite RNAs interfere with the helper virus. One potential method of interference is by competing for coat proteins, made only by the helper virus.

Satellite Viral RNAs

1. Contain either part of viral genome or different viral genome
2. Contain viral structural proteins coded by satellite or helper RNA
3. Replicate only in presence of helper virus (may be standard virus)
4. May interfere with growth of helper virus

Thus delta agent is like a viral satellite RNA. It contains structural proteins which may be coded for by delta-associated RNA (delta antigen) as well as the structural protein (HBsAg) coded for by the helper virus HBV. In addition delta agent replicates only in the presence of its helper virus (usually HBV) and may interfere with HBV replication. However, viral satellite RNAs have only been described in plants and in all cases both the satellite and helper virus have RNA genomes. Delta agent is therefore unique, being an RNA particle requiring a DNA virus for replication. McDonald and colleagues have suggested that delta infection may be a model for A.I.D.S. (50). They propose that the A.I.D.S. agent may also be a defective particle, activated in the presence of another virus such as cytomegalovirus or human T cell leukemia virus.

Delta Agent - Components

1. RNA - single-stranded, ?linear
   molecular weight 5×10^5 daltons
2. delta antigen - protein
   molecular weight 68,000 daltons
   ?coded by RNA
3. HBsAg - coat protein
   coded by HBV
4. ?other proteins

DETECTION OF THE DELTA SYSTEM:

Delta Antigen

Initially, delta antigen was detected in liver biopsies of HBsAg-positive patients by direct immunofluorescence (1,74). It was found predominantly in the nuclei but occasional cells displayed cytoplasmic fluorescense (14,74,100). Although other markers for hepatitis B infection such as HBcAg and HBsAg were sometimes detected by specific immunofluorescence the majority of delta-positive liver samples were negative for these markers (6,74). Additional studies demonstrated that host-derived

antibodies to delta antigen (anti-delta) were also present in frozen tissue sections (74). They could be detected using fluorescein labelled antibody to human IgG and the delta antigen-anti-delta immune complexes fixed complement. Similiar findings have been reported for HBcAg in that HBcAg/anti-HBc complement-fixing immune complexes can be detected in frozen tissue. Intrahepatic immune complexes of HBcAg and delta antigen have also been demonstrated in chimpanzees infected with HBV and delta agent (76).

The presence of such host-derived anti-delta in tissue sections precluded detection of anti-delta antibodies in test serum, except by blocking of direct immunofluorescence. In 1981, Stocklin and coworkers reported that delta antigen could also be detected by immunofluorescence of conventional formalin-fixed paraffin sections following pronase treatment (100). This observation allowed detection of delta antigen in retrospective studies. For example, in the U.K. delta antigen was detected in liver biopsies from 1971 (107). In addition, host derived anti-delta was not present in paraffin sections following pronase treatment. Therefore, indirect immunofluorescence for detection of anti-delta was possible. Other investigators have shown that immunoperoxidase techniques are also suitable (34,71) and that araldite sections can be used (34).

Following successful extraction of delta antigen from human liver tissue obtained at autopsy of a delta-positive subject, Rizzetto and colleagues developed a sensitive solid-phase radioimmunoassay for both delta antigen and anti-delta (81,82).

Solid Phase RIA for Delta Antigen

1. Standard anti-delta serum coated microtiter plates
2. Delta antigen test samples
3. ^{125}I-anti-delta IgG standard antiserum

Delta antigen extracted from human liver was detected by the solid-phase RIA. Detection of delta antigen in serum required detergent treatment of serum samples to expose the antigen because it is an internal component of a subpopulation of HBsAg particles (78). In experimental delta infection of chimpanzees, delta antigen was detected transiently in serum when delta antigen levels in liver were at their highest (75,78). Resolution of delta hepatitis was followed by seroconversion from delta antigen positivity to to anti-delta (75). Delta antigen was first detected in human serum of a hepatitis B surface antigen carrier who was acutely infected with delta agent (6). As in the animal studies, delta antigen was found in 35-37 nm HBsAg particles and was detected after detergent treatment. Since then delta antigen has been detected in human serum by both solid-phase radio- and enzyme-linked immunoassays (81,86,89). These assays, although sensitive, have been unable to detect delta antigen in sera containing high titers of anti-delta, even though such sera are demonstrably infectious (75). Recently, cDNA probes obtained by reverse transcription of delta-associated RNA have been used to detect delta agent components in serum by blot hybridization (97). Approximately 80% of

patients with anti-delta and chronic liver disease had detectable levels of serum delta-associated RNA. All were positive for intrahepatic delta antigen by immunofluorescence but negative for serum delta antigen by RIA. Similar technology should be useful in the future for determining the presence of delta antigen-containing particles in serum.

Delta Antibody

Antibody to delta antigen (anti-delta) was first detected in serum by direct immunofluorescence (74). Rizzetto and colleagues in Turin detected delta antigen by chance when they observed that fluorescein-conjugated antiserum to HBcAg also gave a nuclear staining reaction with HBsAg positive liver biopsy specimens in which no core particles could be seen after extensive ultrastructural search (74). The detection of delta antigen in liver was therefore by use of naturally occurring anti-delta antibody. Initially other sera were tested for anti-delta antibody by their ability to block direct immunofluorescence of the standard fluorescein-conjugated antiserum (1,17,74). The presence in frozen tissue sections of immune complexes of host-derived antibody and delta antigen prevented the use of indirect immunofluorescence (74,76). After the discovery that pronase-treated paraffin sections were suitable for detection of delta antigen, an indirect immunofluorescence assay was possible (100). More sensitive radioimmunoassays have since been developed (81,82).

Solid Phase RIA for Delta Antibody

1. Standard anti-delta serum coated microtiter plates
2. Standard delta antigen reagent from human liver
3. Test serum
4. Standard ^{125}I-anti-delta IgG

The solid phase RIA for anti-delta is a blocking assay, depending on the presence of antibody in the test serum to block binding by an ^{125}I-labelled anti-delta standard. An enzyme-linked immunoassay has also been developed for measurement of anti-delta (19). Measurement of specific IgM anti-delta has been achieved by using rabbit anti-human IgM specific for μ chains to coat microtiter plates (95).

RIA for IgM-specific Delta Antibody

1. Rabbit anti-u serum coated microtiter plates
2. Test serum
3. Standard delta antigen
4. Standard ^{125}I anti-delta IgG

The specificities of the assays for both delta antigen and antibody have been extensively tested. There is no crossreactivity with HBsAg/anti-HBs, HBcAg/anti-HBc, HBeAg/anti-HBe or any component in serum of patients without HBV markers. The assays require human or chimpanzee samples of delta antigen and antibody, thereby limiting the availability of testing.

PREVALENCE OF DELTA INFECTION:

Delta infection is endemic in Southern Italy but has been detected world-wide (82). The prevalence of delta markers in any given population is dependent on the presence of liver disease within that population and on the prevalence of hepatitis B infection. Delta infection has not been reported in the absence of hepatitis B infection, therefore all individuals positive for delta antigen or anti-delta have either HBsAg or anti-HBs, in addition to their delta markers. The general significance of the various combinations of HB and delta markers is outlined below (modified from references 23,79,88,94,95). Individual cases may vary.

Significance of Delta Markers

HBV Marker	Delta Marker	Comment
HBsAg IgM anti-HBc		acute or active HBV
	delta Ag	acute or active delta
	IgM anti-delta	acute or chronic delta
	IgG anti-delta	chronic delta
HBsAg IgG anti-HBc		chronic HBV
Anti-HBs		previous HBV
	delta Ag (1 case)	acute delta
	IgM anti-delta	resolving delta (?active delta)
	IgG anti-delta	late resolving delta (?active delta)

In surveying large populations, most studies have concentrated on the prevalence of anti-delta antibodies in HBsAg-positive individuals. This population may transmit delta infection since delta associated RNA has been found in their serum (97). Anti-HBs positive individuals with anti-delta appear to have resolving simultaneous infection with both HBV and delta, and therefore are an unlikely future source of infection. However, follow-up studies in this group are necessary for confirmation. Delta antigen, if present in serum, has always been associated with active delta infection in either chronic carriers or in cases of simultaneous acute infection with B and delta. The reporting of acute hepatitis with delta antigenemia in one case with pre-existing anti-HBs (77) suggests that delta infection may also occur in circumstances other than acute and chronic HBV infection. Confirmatory studies are needed.

Delta Infection - Population at Risk

1. Asymptomatic HBsAg carriers

   Parenteral drug users
   Polytransfused individuals
   Homosexuals

2. Acute hepatitis B

3. Chronic HB liver disease

Within the HBV positive population there are 3 main categories: individuals who are asymptomatic carriers, those who have acute hepatitis and those with chronic liver disease. Details of delta infection in the latter two groups are contained in pages 28-45. Among asymptomatic carriers, various subgroups have been identified with respect to the prevalence of delta markers. The prevalence of delta markers in HBsAg carriers, without evidence of liver disease and other than IV drug users and polytransfused individuals, is shown in Table VI. In most countries delta markers were absent or rare. These data indicate that potential reservoirs of delta infection exist not only in Italy but also in the U.S. among volunteer blood donors. High prevalence of delta infection has also been reported in Africa, South America and the Middle East (66).

TABLE VI

Prevalence of Delta Markers in HBsAg Carriers

Source	Delta Markers*	%Positive	Reference
HBsAg positive blood donors			
U.K.	0/250	0	104
Germany	1/266	0.4	27,85
U.S. I	67/1316	4.1	52
U.S. II	74/1916	3.9	53
Symptomless HBsAg carriers			
Italy	6/83	7.2	82
Sweden	2/94	2.0	38
Greece	1/73	1.4	37
France	0/70	0	105
Japan	0/33	0	82
China	0/163	0	44
Australia	0/43	0	110

*Anti-delta except for U.S. I and II
U.S. I: Volunteer donors in Florida, Hawaii, Kansas and Texas; 6 delta antigen and 61 anti-delta positive.
U.S. II: American Red Cross; 2 delta antigen and 72 anti-delta positive.

Higher rates of positivity for delta markers are found in IV drug users (Table VII) and polytransfused individuals such as hemophiliacs and thalassemics (Table VIII). However, the homosexual HBsAg carrier population does not seem to have an increased risk of delta infection (Table IX). This may be due to separation of subcultures or may merely reflect earlier introduction of delta infection to the parenteral drug using group.

The data in Table VII indicate that HBsAg carriers in the IV drug using population have a very high prevalence of delta markers. The finding of anti-delta together with anti-HBs is likely to indicate recovery from acute hepatitis B and acute delta infection acquired simultaneously. The anti-delta antibody under these circumstances is usually IgM type and transient (23,95). However, the large number of HBsAg positive IV drug users with evidence of delta infection suggests that this group is at risk for continued exposure to delta hepatitis.

TABLE VII

Prevalence of Delta Markers in Drug Addicts

Origin	Reference	Delta Markers (% positive)			
		HBsAg positive		anti-HBs positive	
Europe*	73	9/33	(27%)	13/156	(8.3%)
U.K.**	61,104,108	47/159	(30%)	4/55	(7.3%)
France*	105	12/20	(60%)	-	
Germany	85	4/8	(50%)	1/40	(2.5%)
Sweden	39	62/116	(53%)	-	
Norway	39	21/64	(33%)	-	
U.S.-TX	52	15/29	(52%)	-	
Dallas	91	16/32	(50%)	-	
Australia	109,110	3/8	(38%)	3/34	(8.8%)
Total		189/469	(40%)	21/285	(7.4%)

*symptomless
**includes acute hepatitis and/or chronic liver disease

In most of these studies, anti-delta but not delta antigen was measured. In the studies reporting results of delta antigen testing (52,61,105,109) only one (61) had more than 2 cases positive and in this study one-third of drug users had acute hepatitis. Thus, asymptomatic drug users have a high prevalence of anti-delta antibody. Further characterization of the titer and immunoglobulin class of the antibody may identify HBsAg carriers who are not infectious for delta hepatitis, however, the uniformly high rate of positivity in geographically distant areas suggests the infectivity potential is very high. In the future, use of technology such as cDNA probes will allow identification of individuals having detectable delta agent in addition to anti-delta antibody (97).

Retrospective studies in Sweden have indicated that delta infection was introduced there in 1973. In the analysis of infection of drug users in Malmoe, none of 26 samples was positive for anti-delta before 1973 (38). From 1973-1975, 12 of 37 samples were positive (32%); 1976-1978 saw a further increase (27/54, 50%) and in the final 3 year period, 72% of HBsAg positive samples from IV drug users also contained anti-delta (41/57). These results suggest that delta infection spreads rapidly once introduced to a community of HBsAg carriers subject to repeated exposure to infective material.

TABLE VIII

Prevalence of Delta Markers in Polytransfused Individuals

Origin	Reference	Delta Markers (% positive)			
		HBsAg positive		anti-Hbs positive	
Thalassemia					
Italy	80	2/28	(7%)	0/283	
Hemophilia					
Italy	20,80	11/22	(50%)	2/142	(1.5%)
Germany	20,80	3/8	(38%)	1/36	(2.7%)
Sweden	38	1/7	(14%)	-	
U.K.	20	2/11	(18%)	-	
U.S. - MD	80	10/20	(50%)	0/14	
NJ, PA	79	8/18	(44%)	2/27	(7.4%)
Australia	109	0/10		0/46	

The incidence of delta markers in the serum of polytransfused individuals (Table VIII) is variable, but generally similar to the pattern observed in HBsAg carriers without liver disease, excluding IV drug users (Table VI). The lack of evidence of delta infection in Australia contrasts with the findings in the U.S. The Australian hemophiliac population has a high incidence of HBV exposure and 63% have anti-HBs which is probably protective. In the presence of anti-HBs, delta exposure is unlikely to result in hepatitis or development of delta markers since chimpanzees with anti-HBs were not susceptible to delta infection (75). However, delta infection in an IV drug user with anti-HBs has been reported (88).

TABLE IX

Delta Markers in Homosexual HBsAg Carriers

Origin	Reference	Delta Markers
Denmark	133	1/17
Sweden	133	1*/31
U.K.	13	1/50
U.S.-IL	124	0/500

*Also drug addict

In marked contrast to the drug user population, delta markers are rarely present in homosexual HBsAg carriers in the studies thus far reported. Of 40 HBsAg carriers in California, 13 were positive for anti-delta, of whom 2 were homosexuals (80). Additional surveys (Table IX) have shown a relatively low rate of delta markers. Introduction of the infectious agent to this subpopulation may result in rapid spread, since familial and horizontal spread have been described (12,84,96). In Dallas, two homosexual HBsAg carriers have been found to have anti-delta antibody (92) and spread of delta infection within the homosexual population may occur.

TRANSMISSION OF DELTA HEPATITIS:

Delta infection is similar to hepatitis B in the apparent modes of transmission. Thus, infection may be acquired parenterally or nonparenterally by percutaneous or permucosal exposure.

Modes of Transmission of Delta Infection

1. Parenteral - blood and blood products
2. Nonparenteral - percutaneous and permucosal horizontal
3. Vertical - maternal-infant

Parenteral Transmission

Blood and blood products screened by third generation assays for HBsAg appear to have negligible risks of transmitting delta infection. However, delta antibody has been detected in commercial gamma globulin preparations (62,63). A retrospective study of anti-delta antibody occurrence in immune serum globulin (ISG) preparations made from pooled plasma failed to detect anti-delta antibody in ISG manufactured since 1973 (Table X). Of interest, anti-delta antibody was detected in all other time periods, including 1944 (62). The decrease in prevalence of anti-delta coincided with the introduction of routine screening for HBsAg in donor units. A decrease in the titer of anti-HBc was also observed with the elimination of HBsAg. Anti-delta was detectable in 78% of ISG preparations containing detectable HBsAg. Experimental studies in chimpanzees have demonstrated that the procedures used to inactivate hepatitis B virus in HB vaccines also inactivate delta agent. This finding is supported by the observation of Dienstag and coworkers in their study of chronic HBsAg carriers given HB vaccine (22). No evidence of delta infection was found in vaccine recipients.

TABLE X

Antibody to Delta Agent in Immune Serum Globulins

Production	Anti-delta antibody (% positive)	
1944	1/1	
1962-1965	6/8	(75%)
1967-1970	16/21	(76%)
1971-1972	5/11	(45%)
1973-1977	0/9	

From: Ponzetto et al, Int. Symposium Viral Hepatitis, 1984.

Commercial concentrates used in replacement therapy, such as cryoprecipitate, may be infective even though routine HBsAg measurements are performed before pooling. Single donors having undetectable HBsAg may still carry delta agent in sufficient concentration to result in infection. For example, recent studies have demonstrated that serum from chimpanzees may be infectious for HBV at a rate of 10^6 infectious doses/ml whereas the same serum is infectious for delta (in HBsAg carriers) at a rate of 10^{11} infectious doses/ml (67). The use of concentrates prepared from large numbers of donors increases the risk of including delta agent and infecting HBsAg carriers. The presence of infectious concentrations of delta agent without infectious concentrations of HBV has been demonstrated (67). Thus, anti-hemophiliac factor containing delta agent was not infectious unless there was simultaneous infection with HBV. The risk of transmitting HBV and delta simultaneously with blood or blood products is less than the risk of infectivity for delta alone although the continued occurrence of hepatitis B following transfusion demonstrates that the potential is present. Additional evidence of parenteral transmission of delta infection has been reported recently (48). Delta infection spread among 5 HBsAg carriers using the same artificial kidney for dialysis following the admission of a drug addict with anti-delta. These studies indicate that individuals at risk for parenteral transmission of HBV are also at risk for delta infection.

Nonparenteral Transmission

Endemic areas of delta infection exist in Italy without apparent parenteral routes of infection (96). Furthermore, high prevalence of delta infection has been found in isolated areas of Colombia and Venezuela in South America, in Africa and the Middle East (66). In these populations, delta markers are not associated with drug addicts and polytransfused individuals as they are in the U.S. and Northern Europe. Nonparenteral transmission of delta infection is likely to be similar to nonparenteral transmission of HBV with infection following percutaneous or permucosal exposure. Intimate contact and poor sanitary conditions are likely to contribute.

TABLE XI

Nonparenteral Transmission of Delta Infection:
Family Studies

Index Case	Hepatitis Markers in Family	
	HBsAg	HBsAg + anti-delta
HBsAg only (n=151)	94/565 (16.6%)	2/565 (0.35%)
HBsAg + anti-delta (n=82)	83/376 (22.1%)	45/376 (12%)

From: Rocca et al, Prog. Clin. Bio. Res. 143:133-137, 1983.

In Italy, familial clustering of delta infection has been demonstrated in a number of studies (12,84,96) and is illustrated in Tables XI and XII. Intrafamily spread was apparently spouse-to-spouse or sibling-to-sibling in 75% of instances (34/45) and between parent and child in 25% (11/45) in one study (84, Table XI). Nonparenteral transmission of HBV in anti-delta negative families was also analyzed. It was spouse-to-spouse or sibling-to-sibling in 32% (20/63) but between parent and child in 68% (43/63). These results suggest that horizontal transmission within families is more important than vertical transmission in delta infection when compared with HBV. The increased association of delta infection with anti-HBe may be responsible, since infectivity of HBV correlates with HBeAg (see also pp. 42-44).

TABLE XII

Nonparenteral Transmission of Delta Infection:
Family Studies

Hepatitis Markers in Family at Initial Screen	Delta Infection	
	Initial	Follow-up
HBsAg only (n=162)	0/162	2/162
HBsAg + Delta (n=64)	51/64	55/64

From: Caporaso et al, Prog. Clin. Bio. Res. 143:139-143, 1983

An additional Italian study prospectively examined the transmission of delta infection within families (12, Table XII). At the initial screening, 162 members of 83 families were HBsAg positive and no family members were positive for anti-delta. During follow-up 2 members developed anti-delta (1.2%). In contrast, 64 members of 35 families were HBsAg positive and at least one family member had anti-delta. In 27 families, all HBsAg positive members had anti-delta. Of the remaining 22 members of 8 families, 9 were anti-delta positive initially and 4 of the remaining 13 acquired anti-delta during follow-up (30.8%). This study indicates that new delta infection occurs significantly more often in families with evidence of pre-existing delta infection.

Nonparenteral transmission is also thought to be responsible for the recent epidemic of delta virus infection and severe hepatitis among the Yucpa Indians of Venezuela (36). The epidemic appeared to result from delta virus superinfection of hepatitis B carriers. The mode of transmission was thought to be through close personal contact via open skin lesions. The three villages with severe outbreaks were also characterized by poor economic status and crowding. Thus delta infection appears to be similar to hepatitis B infection with transmission by percutaneous and permucosal exposure.

TABLE XIII

Vertical Transmission of HBV

Maternal Serology	HBsAg in Infants	
	Initial	Follow-up
HBeAg	5/5 (100%)	3/4 (75%)
anti-HBe	6/64 (9%)	0/5
neither	1/4 (25%)	-

From: Zanetti et al, J. Med. Virol. 9:139-148, 1982

Vertical Transmission

HBV infection may be transmitted from mother to child during the perinatal period. Studies have demonstrated that there is an increased incidence of vertical transmission with HBeAg. A prospective study in Italy investigated the risk of vertical transmission of HBV by HBsAg positive mothers. The effect of the presence of HBeAg and of delta infection was examined (111). Whereas all infants of HBeAg mothers became HBsAg positive at 2-3 months of age, only 6 of 64 babies of anti-HBe mothers became HBsAg positive (Table XIII).

Table XIV

Vertical Transmission of Delta Infection

Maternal Serology*	Infant Serology
	HBsAg + anti-delta
HBeAg + anti-delta	1/1
anti-HBe + anti-delta	0/6

*From anti-delta positive in 5 with HBeAg and 64 with anti-HBe, Table XIII.
From: Zanetti et al, J. Med. Virol. 9:139-148, 1982

The prevalence of delta markers was low in this study (7/73, 9.6%). All infants born of mothers with anti-delta had detectable anti-delta at birth from transfer of maternal antibody. Only the baby born to the HBeAg positive mother developed HBsAg and anti-delta (Table XIII).

Vertical Transmission of Delta Infection

1. HBsAg positivity - 481
   455/12,965 screened (3.5%)
   26 in vaccine study

2. HBeAg/anti-HBe status
   HBeAg 22/455 (4.8%)
   26/26 (vaccine study)
   anti-HBe 419/455 (92.1%)
   neither 14/455 (3.1%)

3. delta markers

HBeAg positive	3/48	(6.7%)
anti-HBe	33/419	(7.9%)
neither	0/14	

Recently, Zanetti and coworkers have reported the results of a large study of vertical transmission of delta infection (112). Only one of 36 babies born to mothers with anti-delta and anti-HBe developed infection with HBV and delta (Table XIV). Of the three babies born to HBeAg and anti-delta positive mothers, two received gamma globulin followed by vaccine and did not develop HBV or delta infection. The third baby was not

given gamma globulin nor vaccinated and developed HBsAg, HBeAg and anti-delta as evidence of infection, and this case had been previously described (94,111).

TABLE XV

Vertical Transmission of Delta Infection

Maternal Serology*	Infant Serology
	HBsAg + anti-delta
HBeAg + anti-delta (n=3)	1/3 (2 vaccinated)
anti-HBe + anti-delta (n=33)	1/33

*From 48 with HBeAg and 419 with anti-HBe
From: Zanetti et al, Prog. Clin. Bio. Res. 143:127-132, 1983

These data suggest that prevention of vertical transmission of HBV infection with vaccination also prevents vertical transmission of delta infection. Since most studies indicate that delta infection requires HBV infection for replication, this finding is not surprising. Although the numbers are small, the low rate of infectivity in the maternal combination of anti-delta and anti-HBe suggests that delta infection may be protective. This conclusion is supported by the lower incidence of apparent parent to child transmission of infection in family studies (84). In addition, the usual association of anti-delta with anti-HBe rather than HBeAg (see pages 42-44) also decreases the likelihood of maternal-infant transmission of delta infection.

In summary, transmission studies of delta infection support the hypothesis that delta infection is spread by similar modes to HBV. However, vertical transmission appears to be less important and horizontal transmission within families and closed communities may be more important in delta infection than in HBV infection.

DELTA HEPATITIS (Hepatitis D): Animal Studies

The delta antigen/anti-delta system of markers was first described by Rizzetto and coworkers in 1977 (74). The demonstration of the infectivity and pathogenicity of the delta agent was achieved by transmission studies in the chimpanzee, an animal model of HBV infection (74,78).

The initial animal studies of delta agent were carried out using chimpanzees (74). A series of experiments examined the infectivity of serum obtained from patients with HBV and delta infection. Chimpanzees susceptible to both HBV and delta first developed acute hepatitis B (Figure 7) and then evidence of delta infection appeared in most, generally two to three weeks later. Initially, delta antigen was present in the liver, detected by immunofluorescence, and in some animals delta antigen transiently appeared in the blood. Following recovery, HBV antigens and delta antigen disappeared. Anti-delta antibody was found in some but not all animals (Figure 7). Occasionally delta infection and HBV infection occurred simultaneously rather than sequentially and sometimes delta infection preceded HBV (67).

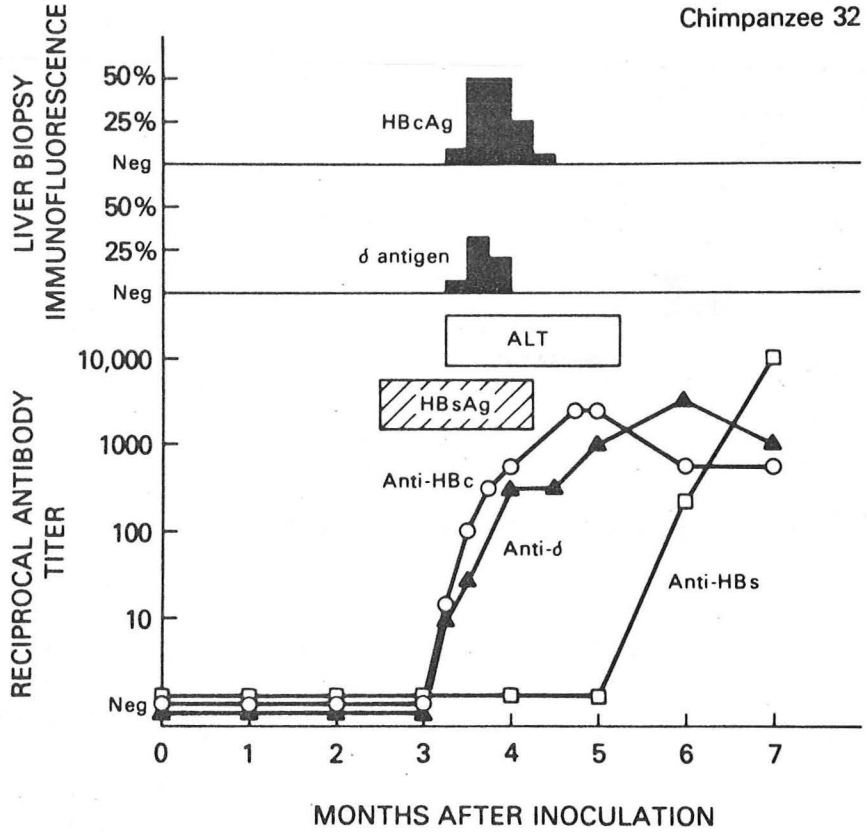

Figure 7
From: Hoofnagle J, 1983 AASLD Postgraduate Course (with permission)

Chimpanzees with chronic HBV infection were also innoculated with serum containing markers of both HBV and delta infection (anti-delta antibody). After a shorter incubation, delta antigen was found in the liver and transient delta antigenemia occurred at the peak of delta antigen positivity in the liver (Figure 8). Coincident with delta infection, HBsAg in serum and liver decreased. Following seroconversion to anti-delta, markers of HBV infection returned to normal.

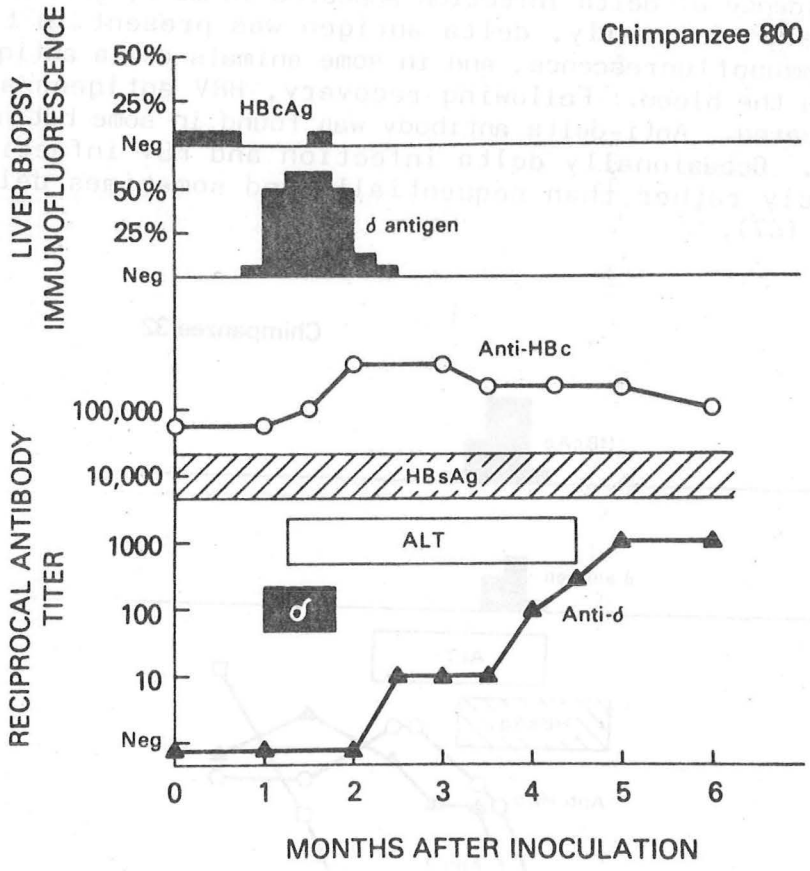

Figure 8
From: Hoofnagle J, 1983 AASLD Postgraduate Course (with permission)

Delta infection was thus able to be transmitted to chimpanzees and the characteristics of the infection were similar to those in humans (vide infra). Simultaneous infection of HBV and delta in susceptible animals resulted in a biphasic response with HBsAg and HBV liver disease appearing first followed by delta infection. In HBsAg carrier animals, the incubation period between inoculation and infection was shorter. The delta antigenemia occurring in some animals enabled investigators to establish that delta agent was coexisting with HBsAg from the carrier animal (subtype adw)[74] and not with HBsAg from the inoculum (subtype ayw). Additional studies have demonstrated that chimpanzees immune to HBV cannot be infected with delta agent (74). Serial passage of delta agent in chimpanzees resulted in a progressive shortening of the incubation period and a progressive increase in the severity of the associated hepatitis (67).

TABLE XVI

Chimpanzee Studies of Delta Infection

HBV Status	Delta Infection
Susceptible	13/15
HBsAg	10/10
Immune	0/5

From: Purcell et al, Prog. Clin. Bio. Res. 143:79-89, 1983.

Chimpanzee studies have also demonstrated lack of crossimmunity between non-A non-B hepatitis and delta infection (67,77). However, limited experiments have suggested that delta agent and non-A non-B hepatitis virus may interfere with the synthesis of each other (67). Additional studies have measured the relative infectivity of HBV and delta agent in a "standard" inoculum (67). The infectivity titer of HBV (measured in HBV susceptible animals) was 10^6 infectious units/ml while that of delta agent(measured in HBsAg carriers) was $>10^{11}$ infectious units/ml. The greater infectivity of delta agent allowed demonstration of the inability of delta agent to replicate without HBV infection. Preliminary reports of chronic delta infection in chimpanzees have recently appeared (25,31). Previous studies had demonstrated only acute short-lived delta infection. Follow-up studies of chronically infected chimpanzees will increase knowledge of the epidemiology and molecular biology of delta agent. In addition, the larger supply of delta antigen and antibody potentially made available from chronically infected animals may allow wider experimentation.

Studies with other animals have demonstrated that delta infection can be transmitted to woodchucks chronically infected with woodchuck hepatitis virus (64). The infection was similar to that observed in humans and chimpanzees. Delta infection resulted in a decrease in the synthesis of woodchuck hepatitis virus (WHV) and delta containing particles with a surface coat of woodchuck hepatitis surface antigen were found. These experiments illustrated the similarities of WHV and HBV infection. In addition they demonstrated that a property of delta agent is the ability to transcapsidate, using surface antigen from the hepatitis virus already infecting the host.

DELTA HEPATITIS (Hepatitis D): Clinical Aspects

Delta agent infection in man as in animals is closely linked to infection with hepatitis B. Both delta and HBV may be acquired simultaneously or delta superinfection may occur in a chronic (or acute) hepatitis B carrier. Delta hepatitis results in acute self-limited disease or progression to chronic delta infection.

Delta Hepatitis

Delta agent may be introduced simultaneously with HBV or may be successfully transmitted during acute or chronic HBV infection. Differentiation of these possible clinical settings can often be achieved by using serologic tests of both hepatitis B and delta infection. Simultaneous acute infection with HBV and delta is likely if the individual was previously negative for HB markers, using sensitive third generation assays for measurement. A presumptive diagnosis of simultaneous infection has also been made when the acute hepatitis was self-limited and resolution of both hepatitis B and delta hepatitis occurred (51,55,86,89,96). However, resolution of chronic hepatitis B has been documented following superinfection with delta agent (51).

Diagnosis of Simultaneous HBV and Delta Infection

1. HBsAg negative → Acute hepatitis B and delta hepatitis
2. Acute hepatitis B and delta hepatitis → Resolution
3. ?Acute hepatitis B and delta hepatitis with IgM anti-HBc

An alternative method of diagnosing simultaneously acquired hepatitis B and delta hepatitis has been used in a number of studies (51,57). They measured IgM anti-HBc as an indicator of recent infection in HBsAg positive individuals (29,46,90). Chronic hepatitis B was presumed when anti-HBc was not IgM. Recently, Sjogren and Hoofnagle have reported that IgM anti-HBc can be persistent at high titers in patients with clinically active disease (93). The presence of HBeAg positivity in longitudinal follow-up for 1-9 years correlated with persistent IgM anti-HBc. However, high titers of IgM anti-HBc were also demonstrated in 5 of 18 patients who seroconverted from HBeAg to anti-HBe.

TABLE XVII

IgM Antibody to HBcAg:
Correlation with Active Hepatitis B

Group	Markers of Hepatitis B			
	HBeAg	anti-HBe	IgM-anti HBc* (mean ratio)	ALT** (mean U/L)
Initial (n=34)	+	-	9.0	251
Follow-up				
I (n=16)	+	-	8.2	elevated
II (n=13)	-	+	1.9	35
III (n=5)	-	+	7.9	elevated

*Negative <2.1; **Normal <40
From: Sjogren and Hoofnagle, Int. Symp. Viral Hepatitis, 1984

These data illustrate the complexity of diagnosing simultaneous infection with hepatitis B and delta agent, indicating that each of the methods used has potential problems. Therefore, the incidence of delta superinfection in chronic HBsAg carriers may be higher than previously estimated. The course of the acute infection and its outcome are different if the individual exposed to delta agent is already chronically infected with HBV, hence the attempts to differentiate the two modes.

Simultaneous Acute Hepatitis (B and delta)

Two large population studies have examined the prevalence of simultaneous infection with hepatitis B and delta agent. In Italy, the overall incidence of simultaneous infection was 14% of acute hepatitis B (96). There was marked geographic variability, with no delta infection in one area of southern Italy (Potenza 0/27) and 91% coinfection in part of Naples (32/35) in southern Italy. In northern Italy, drug addicts accounted for 8/13 delta positive infections in Turin and 18/25 in Milan. Drug addicts were not identified among cases in Southern Italy.

TABLE XVIII

Simultaneous Acute Hepatitis B and Delta Infection*

Origin (Ref)	Delta Markers (% positive)		Comments
	Delta antigen	anti-delta	
Italy (96)	14/687 (2%)	85/687 (12%)	geographic variability
Sweden (51)	-	57/592 (10%)	drug addicts** 56/57
Sweden (55)	-	4/89 (4%)	drug addicts 2/4
Ireland (86)	53/212 (25%)	19/212 (9%)	all 212 drug addicts
Scotland (89)	4/14 (29%)	1/14 (7%)	13 drug addicts
US-Illinois (52)	1/97 (1%)	6/97 (6%)	no clinical details
Italy (23)	-	39/147 (27%)	geographic variability

*Defined by acute self-limited disease - except US study (no definition)
**4 cases were individuals with sexual or non-sexual contact with drug addicts

In Sweden, a retrospective study of simultaneous hepatitis B and delta infection has been carried out (38,51). Of 592 cases of acute hepatitis B occurring betwen 1970 and 1981, 57 were simultaneously infected with delta agent. One individual developed hepatitis B and delta infection in 1973 following a transfusion in Spain and 4 cases were individuals with sexual or non-sexual contact with drug addicts (2 each). The remaining 52 cases of simultaneous acute infection occurred in drug addicts. Delta infection associated with drug addiction was not detected before 1975. Since 1975 the yearly prevalence of simultaneous delta infection varied from 22% to 54% in drug addicts and their associates.

TABLE XIX

Simultaneous Hepatitis B and Delta Infection in Sweden

Time Period	Drug Addicts and Associates	Others
1970-1974	0/163	1/162
1975-1980	54/132	0/142

From: Hansson et al, J. Inf. Dis. 146:472-478, 1982.

A retrospective analysis of the course of hepatitis in these patients was carried out. There was no difference in the clinical features of simultaneous acute infection with hepatitis B virus and delta agent when compared with hepatitis B alone, except for the occurrence of biphasic hepatitis (Table XX). In concurrent delta infection, biphasic hepatitis was observed in 8/57 (14%) whereas it was seen in 15 of 535 cases of hepatitis B (3%). Overall, even patients with biphasic hepatitis were more likely to have only hepatitis B (15/23, 65%).

TABLE XX

Clinical Features of Acute Hepatitis B
With and Without Delta Infection

	HB + Delta Infection n=57		Hepatitis B n=535	
Classical acute hepatitis	38	(67%)	489	(91%)
Biphasic hepatitis	8	(14%)*	15	(3%)
Prolonged acute hepatitis	1	(2%)	1	
Subclinical hepatitis B	0		4	(1%)
Acute hepatitis → chronic carrier	2**	(13%)	25	(5%)
Insufficient data	8	(14%)	1	

*Significantly different
**Acute hepatitis B defined by IgM anti-HBc initially
From: Moestrup et al, Br. Med. J. 286:87-90, 1983
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This important study suggests that delta infection, when acquired simultaneously with hepatitis B, does not significantly alter the outcome of acute hepatitis. The occurrence of biphasic hepatitis, the only differing clinical feature, was also observed in some chimpanzees simultaneously infected (67). Other authors have suggested that delta infection may increase the likelihood of fulminant hepatitis (13,30,72). No case of fulminant hepatitis occurred in the Swedish study in patients having simultaneous infection with hepatitis B and delta agent, nor was fulminant hepatitis observed with acute hepatitis B alone (51). The only case of fulminant hepatitis was in a chronic HBsAg carrier superinfected with delta agent. The lack of correlation of delta agent with severe acute disease may reflect genetic or viral differences. The lack of fulminant hepatitis B alone suggests that such differences may exist.

Two individuals classified as simultaneous acute hepatitis B and delta infection became chronic carriers of HBsAg. In these cases, the diagnosis of acute hepatitis B initially was based upon the finding of IgM anti-HBc. More recent evidence suggests that these two individuals may have had ongoing hepatitis B at the time of presentation (29,46,90,93). If so, it is interesting to speculate that delta infection may in fact decrease the incidence of chronic hepatitis B when both are acquired simultaneously. None of 55 acute simultaneous cases became chronic (adjusted for 2 chronic carriers included previously), compared with 25/535 acute hepatitis B developing chronic hepatitis B.

TABLE XXI

Epidemic Hepatitis B in Irish Drug Addicts

	Acute Hepatitis Serology	
	HBsAg	anti-HBs
1971 - 1973	5	0
1974 - 1976	16	6
1977 - 1979	33	3
1980 - Aug. 1981	140	18

From: Shattock et al, Irish J. Med. Sci. 151:334-338, 1981.

Additional support for the hypothesis that simultaneous acquisition of hepatitis B and delta infection is not clinically different from hepatitis B alone comes from a study of an epidemic of hepatitis B among Irish drug addicts (86-89). Of 212 drug addicts with acute hepatitis B, 140 were considered to have acute hepatitis B alone and 72 had simultaneous delta infection.

TABLE XXII

Simultaneous Hepatitis B and Delta Infection
in Irish Drug Addicts

	Initial Serology			
Acute Hepatitis	HBV	Delta antigen*	Anti-delta	Follow-up
I Acute hepatitis B	140/212	0	0	2 carriers 1 death (fulminant)
II Simultaneous B + delta	72/212	53	19	4 carriers 1 death (14 months)

*Present initial 10 days only, 85% seroconversion to anti-delta (often transient)
From: Shattock and Fielding, Br. Med. J. 286:1279-1280, 1983

There were no significant differences in clinical findings between those with or without delta antigenemia. In all cases, the antigen was present only during the early acute phase and was cleared with 10 days, with seroconversion to anti-delta in 85%. In some cases the anti-delta antibody was transient, as noted previously (94,95). Only one patient (delta negative) died with fulminant hepatitis. Long-term follow-up has demonstrated progression to chronic hepatitis B in 2/140 with acute hepatitis B alone and in 4/72 with simultaneous hepatitis B and delta infection. Restrospective analysis of stored sera may suggest that some of the latter cases represented acute delta infection complicating inapparent HBsAg carrier state.

Finally, an Italian study of simultaneous hepatitis B and delta infection has also concluded that there is no increased severity of disease with the combination (94). The diagnosis of sporadic simultaneous B + delta hepatitis was based on the onset of HBsAg-positive acute hepatitis in individuals without a previous history of liver disease and on development of rising titers of anti-delta. All 42 patients in this study resolved both hepatitis B and delta infection. One patient had a low titer of IgG anti-delta 18 months after resolution of hepatitis. The remaining 41 patients had transient anti-delta, which disappeared after 3 weeks (39 patients) to 5 months (2 patients).

Thus, the literature available to date indicates that delta infection, when acquired simultaneously with hepatitis B, does not increase the severity of hepatitis B. Two studies detailed below concluded differently, in that delta infection was found with high frequency in fulminant hepa-

titis (30,99). Inclusion of chronic carriers with delta superinfection may account for the disparate findings. The lack of effect of delta agent in these cases may reflect the obligatory role of continuing HBV replication for delta agent replication. Natural resolution of hepatitis B would therefore result in resolution of delta infection, in the absence of other helper viruses.

Acute Delta Hepatitis in Chronic HBsAg Carriers

The diagnosis of acute delta hepatitis in chronic HBsAg carriers is also complex. The diagnosis is presumed when known HBsAg carriers seroconvert for anti-delta antibody or develop delta antigenemia (38). In addition, the transient presence of IgM anti-delta indicates acute delta infection (95). Delta antigenemia has been demonstrated in one patient with pre-existing anti-HBs (88). Concurrent with the delta antigenemia there was acute hepatitis. This apparent contradiction of delta infection without evidence of replication of HBV may result from the coexistence of HBsAg (undetectable) with anti-HBs which has been documented to occur in a number of different situations (106). Delta antigen has also been detected in the liver after conversion of HBsAg to anti-HBs (49). This may represent a similar occurrence of undetectable but present HBsAg.

TABLE XXIII

IgM Antibodies to Delta Antigen:
Clinical Significance

Category	Serology		IgM anti-delta
	HBsAg	anti-delta*	
Acute delta hepatitis	+	low titer (transient)	20/20
Resolving hepatitis B and delta infection	anti-HBs	low titer	0/16
Chronic delta hepatitis	+	high titer	17/17
Carrier (normal liver)	+	high titer	0/13

*Blocking anti-delta, includes IgG and IgM
From: Smedile et al, J. Med. Virol. 9:131-138, 1982
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As shown in Table XXI, acute delta infection in chronic HBsAg carriers with resolution of the delta hepatitis is characterized by transient IgM anti-delta. In individuals with resolving simultaneous infection with hepatitis B and delta agent, IgM anti-delta was not found and blocking (IgG) anti-delta was of low titer. Measurement of IgM anti-delta at

earlier time points or with a more sensitive assay may result in demonstration of transient low levels (88,95). In contrast, cases of chronic delta infection in HBsAg positive individuals were characterized by high titers of IgM anti-delta antibody. In addition, some asymptomatic HBsAg carriers also had chronic delta infection with high titered IgM anti-delta. This latter finding suggests individuals may be asymptomatic carriers of both HBV and delta agent. Other authors have used the presence of IgM anti-delta or rising titers of IgG anti-delta or both to diagnose acute hepatitis.

Diagnosis of Acute Delta Hepatitis in HBsAg Carriers

1. Delta antigen newly present
2. Seroconversion to anti-delta antibody
3. Transient low titer IgM anti-delta
4. IgM anti-delta followed by IgG anti-delta
5. Rising titers of IgG anti-delta

In the retrospective study of Farci and coworkers, some patients initially classified as acute hepatitis B were finally diagnosed as having acute delta infection complicating inapparent HBsAg carrier state (23). They studied patients with acute self-limited or progressive hepatitis B (i.e. initially acute presentation but never resolved). Delta infection was observed in both groups but the characteristics were different.

TABLE XXIV

Delta Infection and Acute Hepatitis B

Diagnosis		IgM anti-HBc	Delta Serology
Initial	Final		
Acute hepatitis B	Same→Resolved	+	0/106
Acute hepatitis B + delta infection	Same→Resolved	+	39/39*
Acute hepatitis B	Chronic hepatitis B	+	0/24
Acute hepatitis B + delta infection	Chronic delta + HBsAg carrier	2/34	34/34**

*transient IgM anti-delta
**IgM then IgG anti-delta
From: Farci et al, Gastroenterology 85:669-673, 1983

Acute delta infection complicating inapparent HBsAg carrier state was characterized by low incidence of IgM anti-HBc and by sequential development of IgM and IgG anti-delta antibody. Simultaneous acute hepatitis B and delta infection was characterized by IgM anti-HBc, transient IgM anti-delta and resolution of both hepatitis B and delta infection. These data also suggest that all simultaneous HBV and delta infection resolves but delta infection in HBsAg carriers results in chronic and progressive delta infection. However, there may be bias in this retrospective study with respect to patient selection.

There are a number of studies reporting the outcome of superinfection with delta agent in chronic carriers of HBsAg. In Sweden, a retrospective longitudinal study of delta infection in HBsAg carriers has been carried out (38,51). The majority of HBsAg carriers positive for delta markers were drug addicts.

TABLE XXV

Delta Infection in HBsAg Carriers in Sweden

Source	Delta Markers (% positive)	
	Delta Ag	anti-delta
Drug addicts	1/79 (1.3%)	41/79 (52%)
Others	0/122 -	3/112 (2.7%)
Total	1/191 (0.5%)	44/191 (23%)

From: Hansson et al, J. Infect. Dis. 146:472-478, 1982.

Of the three non-addicts with evidence of delta infection, two were immigrants to Sweden and one was a hemophiliac. All three had both anti-delta and HBsAg present in the first available serum sample. Retrospective analysis of the drug addict population having delta markers was incomplete in 16 (10 with anti-delta present in the first serum sample, 6 seroconverted but complete clinical details were not available). In the remaining 26 individuals, 17 developed acute hepatitis with clinical manifestations for <2 months. Another eight patients had acute hepatitis with a prolonged course (up to 1 year). Two of these patients had a biphasic hepatitis with delta antigen present in the serum during the first peak and delta antibody at the time of the second.

TABLE XXVI

Acute Delta Hepatitis in HBsAg Carriers

Serologic Group	No.	Comments
Delta antigen only	1/43	Fulminant hepatitis
Seroconversion (delta Ag in 6)	31/45	Acute hepatitis 17 (1 biphasic) Prolonged hepatitis 8 (2 biphasic) Incomplete data 6
Anti-delta initially	13/45	Includes 3 non-addicts

From: Hansson et al, J. Infect. Dis. 146:472-478, 1982
Moestrup et al, Br. Med. J. 286:87-90, 1983

In 13 of 18 instances where sufficient sera were available to permit evaluation, HBsAg titers were temporarily lowered at the onset of delta infection. In an additional three patients, the fall in HBsAg titer was followed by disappearance of HBsAg and anti-HBs developed. In only 2 of 18 patients was there no decrease in HBsAg titer. Clearance of HBsAg occurred in 19 chronic carriers of HBsAg, 14 drug addicts and 5 others. The presence of delta infection did not prevent HBsAg clearance and was temporally associated with clearance in 3 cases as outlined above. Long-term inhibition (>3 years) of HBsAg synthesis and transient anti-HBs positivity has also been observed with delta infection (28).

TABLE XXVII

Clearance of HBsAg in Chronic Carriers

	HBsAg Clearance*	
	Delta Markers	no Delta
Drug addicts	9/42**	5/37
Others	0/3	5/109

*Chronic carriers for 8 months - 10 years
**HBsAg clearance with acute delta infection 3/9
From: Moestrup et al, Br. Med. J. 286:87-90, 1983

An Italian study has also documented the effects of delta superinfection in asymptomatic chronic HBsAg carriers (94). They found inapparent delta infection in 2 individuals and acute hepatitis coincident with appearance of IgM anti-delta in 5 cases. Hepatitis persisted in 3 of these. Before the onset of delta infection, only one of the 7 asymptomatic HBsAg carriers had evidence of ongoing liver disease, 3 developed chronic hepatitis following delta superinfection.

TABLE XXVIII

Epidemic Delta Infection in Venezuelan Indians
September 1979 - June 1981

Acute Hepatitis n=81	No.	(%)
Hepatitis A	5	(6%)
Hepatitis B	10	(12%)
Simultaneous B + delta	6	(7%)
Delta hepatitis (HBsAg carrier)	43	(53%)
Delta + B ?type	6	(7%)
Unknown	11	(14%)

From: Hadler et al, Ann. Int. Med. 100:339-344, 1984

Recently, Hadler and colleagues have studied an epidemic of acute hepatitis amongst Yupca Indians in Venezuela (36). Serologic testing showed that HBV infection was highly endemic and that most patients with a history of acute hepatitis had evidence of delta agent infection. They concluded that most of the episodes of acute hepatitis were due to delta superinfection of chronic HBsAg carriers. In the 149 cases of hepatitis, there were 34 deaths and chronic infection was detected in an additional 22. Delta markers in liver or serum were found in 11 of 13 patients who died.

TABLE XXIX

Severe Hepatitis in Venezuelan Indians*

Outcome	No.	Delta Markers
Fulminant hepatitis	25 (17%)	3/4
Subacute hepatitis (died)	9 (6%)	8/9
Chronic hepatitis	22 (15%)	

*n = 149
From: Hadler et al, Ann. Int. Med. 100:339-344, 1984

A study of the severity of chronic liver disease in HBsAg carriers was also carried out. Fifty-six patients with chronic HBsAg positivity were identified and 52 of these were followed. Delta markers were more common with increasing severity of liver disease. Delta infection was present in 4/23 without apparent liver disease, 6/8 with mild disease and in all 21 patients with moderate or severe disease. Therefore, patients with delta infection in addition to hepatitis B were more likely to have progressive chronic hepatitis. Thus, delta superinfection in this group of chronic carriers was associated with a high rate of both fulminant hepatitis (17%) and progressive liver disease. It is not known whether other factors such as socioeconomic status and availability of medical care contributed to these findings.

Fulminant Hepatitis

The role of delta infection in apparent fulminant hepatitis B has not been completely defined. The complexity of both hepatitis B and delta serologies often precludes definitive diagnoses. Clearly, delta infection can cause acute fulminant hepatitis in HBsAg carriers (38,51,68). Similarly, hepatitis A and non-A non-B hepatitis complicating chronic hepatitis B can be fulminant (56,58,70) although other cases are not (15). In well-documented episodes of simultaneous acquisition of hepatitis B and delta infection, fulminant hepatitis has been rare (38,86-89). The problems arise when simultaneous infection is presumed or the diagnosis is based upon the finding of IgM anti-HBc (98,99).

TABLE XXX

Fulminant Hepatitis B:
Role of Delta Infection

Clinical Group	IgM-HBc	Delta Markers
Acute hepatitis B*	+	25/82 (30%)
Chronic hepatitis B + acute hepatitis	-	18/29 (62%)
Total		43/111 (39%)

*May also include patients with active (not acute) hepatitis B
From: Smedile et al, Lancet ii:945-947, 1982.

In the study by Smedile and coworkers, delta infection was detected in 43 of 111 patients with fulminant hepatitis in Europe (39%) with little geographic variability. Of the 43 patients, 7 had delta antigen, 20 had IgM anti-delta alone and 16 had IgM anti-delta with rising titers of IgG anti-delta. Thus, all 43 patients had serology consistent with acute delta infection. However, only 25 of the 43 had IgM anti-HBc, therefore the remaining 18 represented chronic carriers and some of the 25 with IgM anti-HBc may also have represented chronic carriers. Of interest, 11 of the 68 delta negative patients also lacked IgM anti-HBc. The possibility that these patients also had superinfection, but not with delta agent,

remains. In Greece, 10 of 65 cases of fulminant hepatitis were caused by non-A, non-B infection superimposed on HBsAg carrier status (56). In a retrospective study of U.S. patients with fulminant hepatitis, Tabor and colleagues found evidence of delta infection in 1 of 6 patients with fulminant hepatitis B and none of 6 additional patients with non-B fulminant hepatitis (103). They concluded that delta infection did not play a role in fulminant hepatitis in the U.S. A recent study from Los Angeles found differently (30).

TABLE XXXI

Fulminant Hepatitis B:
Role of Delta Infection - Los Angeles 1969-1983

Hepatitis B	Delta Markers	Mortality
Non-fulminant hepatitis B	3/91 (3%)	0
Fulminant hepatitis B	24/71 (34%)	46/70 (66%)
Acute hepatitis B	0/46	33/46 (72%)
Acute B + acute delta	19/19	9/19 (47%)
Chronic B + acute delta	5/5	4/5 (80%)

From: Govindarajan et al, Prog. Clin. Bio. Res. 143:235-236, 1983

The role of delta infection in fulminant hepatitis B in the Los Angeles area has been recently examined (30). The authors found that delta markers were present in 24 of 71 patients with fulminant hepatitis. In contrast, only 3 of 91 patients with non-fulminant viral hepatitis B had delta markers. Further evaluation allowed the distinction between simultaneous acute B and delta from delta infection of chronic B carriers, but details of the methodology used were not given. This study suggests that delta infection occurring simultaneously with HBV or in the HBsAg carriers does have an increased risk of fulminant disease. The mortality rate was less in patients with simultaneous hepatitis B and delta infection than with acute hepatitis B alone or chronic B with acute delta, but the differences were not statistically significant.

In conclusion, the evidence that simultaneously acquired hepatitis B and delta infection results in increased incidence of fulminant hepatitis is not conclusive. Genetic or other factors may influence the severity of simultaneous infection. The superinfection of HBsAg carriers with delta infection does appear to result in increasingly severe disease.

Chronic Hepatitis

Chronic hepatitis B with chronic delta infection is found in many geographic areas. Initially described in Italy, it has now been reported throughout the world. The prevalence of delta infection varies in different areas but in almost all areas chronic hepatitis has a higher prevalence of concurrent delta infection than acute hepatitis.

TABLE XXXII

Delta Infection in Hepatitis B

Origin	Reference	Delta Markers (% positive)	
		Acute Hepatitis	Chronic Hepatitis
Italy - northern	80	6/109 (5%)	48/180 (27%)
- southern		32/35 (91%)	29/49 (59%)
Italy (drug addicts)	73	63/98 (64%)	41/63 (65%)
Total		101/242 (42%)	118/292 (40%)
Sweden	55	4/89 (4%)	11/46 (24%)
Scandinavia (addicts)	55,73	5/43 (12%)	16/27 (59%)
Kuwait	54	4/30 (13%)	25/38 (66%)
Australia	109	0/13	4/40 (10%)
U.S. New Jersey	82	1/26 (4%)	12/41 (29%)
U.S. Los Angeles	30,33	3/91 (3%)	18/57 (32%)
Total		14/201 (7%)	68/192 (35%)

Carriers with chronic delta liver disease usually have active hepatitis or cirrhosis (14,32,59,74,100), a minority have non-progressive chronic hepatitis (chronic persistent hepatitis, chronic lobular hepatitis). In longitudinal studies, progression from non-cirrhotic chronic active hepatitis to active cirrhosis has been observed commonly with delta infection (14).

TABLE XXXIII

Delta Infection in Chronic Hepatitis:
Correlation with Histology

Origin	Reference	Delta Markers (% positive)	
		CPH	CAH
Italy	14,59,74	11/64 (17%)	41/152 (27%)
U.S.	32	1/23 (4%)	18/57 (32%)
Switzerland	100	1/137 (0.7%)	9/175 (5%)

CPH - chronic persistent hepatitis
CAH - chronic active hepatitis

Liver biopsies obtained from patients with chronic delta infection are similar in histopathology to those from patients with chronic hepatitis B of like severity. Characteristic differences are found with immunofluorescence and electron microscopy. Delta antigen is often detected by immunofluorescence in the nuclei of varying numbers of hepatocytes and occasionally cytoplasmic delta antigen is observed. Immunofluorescent detection of HBcAg in nuclei is decreased in delta infection as is the detection of membrane-bound HBsAg (6). Ultrastructurally, intranuclear particles of irregular shape and 20-30 nm in size have been observed in delta infection (14/23, 61%) but they were also found in non-A, non-B hepatitis (10/11, 91%) and occasionally in hepatitis B (2/7, 29%), thereby demonstrating non-specificity (11). Similar intracellular particles have been observed in chimpanzees with delta infection and non-A, non-B hepatitis. Cytoplasmic tubular structures have also been identified in the livers of chimpanzees infected with delta agent (45). Similar structures have also been seen in chimpanzees with non-A, non-B hepatitis but they were not observed in human studies (10). Therefore no characteristic findings have been attributed to delta infection except in immunologic studies. Recently Rizzetto and coworkers described the finding of autoantibodies to microsomes of liver and kidney in patients with chronic delta infection (18). It was present in 13% of 81 such patients. The significance of this observation is unknown.

TABLE XXXIV

Progressive Liver Disease with Delta Infection

Histology	Total Group	Longitudinal Study	
		Initial	Follow-up
Cirrhosis	32/137	-	31/75
CAH	93/137	64/75	32/75
CPH/CLH	12/137	11/75	12/75

CAH chronic active hepatitis; CPH chronic persistent hepatitis
CLH chronic lobular hepatitis;
From: Rizzetto et al, Ann. Int. Med. 98:437-441, 1983

Progressive liver disease associated with delta infection has been observed in a number of studies. In 11 hemophiliacs with chronic liver disease investigated by Rizzetto and colleagues, progressive disease was only observed in the one patient with delta infection (47). In a large study reported by Rizzetto and coworkers, 101 patients with intrahepatic delta antigen were followed for 2-6 years (83). Initially 26 patients had cirrhosis and an additional 31 patients developed cirrhosis in follow-up. Treatment with immunosuppressive therapy (prednisone or azathioprine) did not alter progression. During follow-up 13 patients died, 11 with liver failure and 2 with hematemesis. Unfortunately, a delta-negative group of

matched chronic hepatitis B individuals was not compared. Therefore predictors of poor outcome due to delta infection could not be separated from those related only to hepatitis B.

Bonino and coworkers followed 10 patients with chronic persistent hepatitis and 23 patients with chronic active hepatitis (7). Delta infection was associated with progression of liver disease to cirrhosis in 3 of 4 patients with CPH (unchanged or improved biopsy in 6 patients without delta infection). In CAH, 5 of 13 patients with delta infection demonstrated progression to cirrhosis compared with only 1 of 9 patients without delta infection. Bonino and colleagues also have reported that levamisole, like immunosuppressive therapy, is ineffective in delta infection (2). Furthermore, agents that may alter HBV replication such as DNA inhibitors will also be ineffective in delta infection unless they have a secondary action via HBV. A larger longitudinal study by Colombo and colleagues in Milan also demonstrated progressive liver disease associated with chronic hepatitis B (14). In contrast to the findings of Bonino and colleagues in Turin, progression occurred both with and without intrahepatic delta antigen as evidence of delta infection (Table XXXV). Very few patients (4/100) were HBeAg positive which may suggest a different patient population from those of Rizzetto and Bonino in Turin. Some of the patients without hepatic delta antigen by immunofluorescence may have had serologic evidence of delta infection. However, this study illustrates the need for controlled evaluation before concluding that all progression of hepatitis is due to concurrent delta infection.

TABLE XXXV

Progressive Liver Disease in Hepatitis B

Histology	Intrahepatic Delta Antigen*	
	Present	Absent
Improved	3/35 (9%)	8/66 (12%)
Unchanged	27/35 (77%)	41/66 (62%)
Deteriorated	5/35 (14%)	17/66 (26%)

*No statistically significant differences
From: Colombo et al, Gastroenterology 85:235-239, 1983

The determinants of a fulminant or chronic progressive course, following delta infection in hepatitis B carriers, have not been identified. Rizzetto has suggested that the type of pre-existing HBV infection may be important (72). He concluded that an active HBV infection with HBeAg present was more likely to be associated with severe acute illness. In contrast, delta infection occurring with inactive HBV infection and anti-HBe would be more likely to develop chronicity. In Sweden, the HBeAg/anti-HBe status of chronic HBsAg carriers had no effect on the risk

of becoming infected with delta agent (51). Furthermore, delta infection did not alter the HBeAg/anti-HBe status except in one patient who seroconverted for both HBsAg and HBeAg. This finding suggests that the HBeAg/anti-HBe system is stable during delta infection. Therefore associations between delta infection and the e system may also be stable throughout the disease.

TABLE XXXVI

HBeAg/Anti-HBe System and Delta Infection

Histology	Delta Markers (% positive)	
	HBeAg positive	anti-HBe positive
Acute hepatitis	31/113 (27%)	2/9 (22%)
Fulminant hepatitis	11/26 (42%)	14/25 (56%)
Chronic hepatitis	25/166 (16%)	124/306 (42%)

From references 23,60,96,99

In studies examining the interaction of delta infection and the e system, the prevalence of delta markers was similar in HBeAg positive and anti-HBe positive acute hepatitis (23) and fulminant hepatitis (99). An association between HBeAg positive disease and fulminant hepatitis was not established in these studies since fulminant hepatitis with delta markers occurred equally in HBeAg and anti-HBe individuals. However, the individuals with anti-HBe may represent chronic carriers with delta superinfection. This is suggested by the finding that 8 of the 14 individuals with delta and anti-HBe lacked IgM anti-HBc. In chronic hepatitis, delta infection was found statistically more often in anti-HBe disease than HBeAg disease. Therefore these results support the possibility that delta infection in anti-HBe individuals is more likely to progress to chronicity.

Table XXXVII

Delta Infection and HBeAg/Anti-HBe Status

	Hepatitis B		
	Acute	Fulminant	Chronic
Delta Positive			
HBeAg positive	31/33 (94%)	11/25 (44%)	25/149 (17%)
anti-HBe positive	2/33 (6%)	14/25 (56%)	124/149 (83%)
Delta Negative			
HBeAg positive	113/122 (93%)	15/26 (58%)	141/323 (44%)
anti-HBe positive	9/122 (7%)	11/26 (42%)	182/323 (56%)

From references 23,60,96,99

In these studies, >90% of acute hepatitis was associated with HBeAg whereas only about 50% of fulminant hepatitis had HBeAg positivity. The increased incidence of anti-HBe in fulminant hepatitis was not entirely due to fulminant delta hepatitis in chronic HBsAg carriers since anti-HBe was observed in 42% of delta negative fulminant hepatitis. The possibility remains that superinfection with non-A, non-B viruses may also be contributing. In the study of fulminant hepatitis, 56 patients had no e system markers (14 delta-positive, 42 delta-negative) and therefore it is difficult to draw firm conclusions regarding the interactions. There was a statistically significant increased incidence of anti-HBe in delta positive chronic hepatitis ($p<0.001$ by the chi square analysis) when compared with delta negative hepatitis. This association suggests that concurrent delta infection increases the risk of chronic hepatitis in anti-HBe positive HBsAg carriers. In a pediatric population of 50 untreated patients with chronic hepatitis B, a longitudinal study demonstrated that persistent HBeAg positivity seen in 38 patients was associated with continued disease activity (8). Spontaneous seroconversion from HBeAg to anti-HBe occurred in 13 patients and was followed by improvement and normalization of liver function tests. In the 9 patients initially anti-HBe positive, 7 normalized liver function tests. The two patients with anti-HBe initially and progressive disease had intrahepatic delta antigen. Delta antigen was not found in liver biopsies of any HBeAg positive patients. These results also suggest that delta infection worsens the outcome of anti-HBe positive patients.

The possibility that measurement of HBV-DNA in serum of patients may allow definition of determinants of delta infection has also been addressed (6). HBV-DNA in the serum indicates active hepatitis B virus replication in the liver. Previous workers have demonstrated that other markers of HBV replication are decreased with intercurrent delta infection (38,51). Bonino and coworkers found that HBV-DNA was present in the serum of 9/12 patients with delta infection and was detectable in 5/8 patients with no other markers of active replication (HBeAg negative, HBcAg negative and DNA polymerase negative). In a follow-up study (7) levels of HBV-DNA were found to be persistent over a period of time (mean 4 years). Progression of liver disease histologically could not be predicted on the basis of active HBV replication and in many patients the presence of delta infection was associated with progressive disease.

TABLE XXXVIII
HBV Replication and Chronic Delta Infection

	HBcAg HBeAg DNA-Polymerase	HBV-DNA	Histology* I	Histology* II
Delta Positive	+	+	1/4	3/4
	-	+	2/5	3/5
	-	-	0	3/3
Delta Negative	+	+	2/7	5/7
	-	+	5/5	0
	-	-	5/6	1/6

I = normal liver or chronic persistent hepatitis; II = chronic active hepatitis + cirrhosis; From: Bonino et al, Hepatology 1:386 -391, 1981

When histology is compared with markers of replication, it can be seen that active replication as shown by HBcAg, HBeAg, DNA polymerase and HBV-DNA in the serum is associated with an increased incidence of chronic active hepatitis and cirrhosis. The addition of delta infection to low or undetectable levels of HBV also resulted in more active histology. These data suggest that delta infection may result in progressive disease when HBV replication is low, as shown in the follow-up studies (7). In addition, the presence of HBV-DNA in sera of HBsAg carriers with intrahepatic delta antigen is consistent with infectivity for both HBV and delta despite undetectable HBeAg, HBcAg and DNA polymerase.

In summary, the data indicate that chronic delta infection occurs in chronic hepatitis B carriers throughout the world. It is associated with more severe liver disease on biopsy and development of cirrhosis with subsequent hepatic failure. Immunosuppressive therapy does not alter this course. The respective contributions of delta infection and hepatitis B infection have not been completely elucidated. Chronic delta infection does not result in specific histopathologic changes, rather it increases the severity and activity of the liver disesase. Determinants of the course of delta infection have not been defined, however, chronic hepatitis is more common in anti-HBe individuals with delta infection. Additional studies utilizing measurement of delta agent associated RNA may give greater insight into delta infection.

Hepatoma

One of the common complications of chronic hepatitis B is the development of hepatoma. A number of studies have examined the prevalence of delta infection in hepatoma (14,16,109). As yet no relationship has been found. In Italy 4 of 19 patients with hepatoma had delta antigen in the liver, compared with 17/33 with active cirrhosis, 26/82 with chronic active hepatitis and 3/22 in chronic persistent hepatitis (14). Thus, delta infection was not more frequent in hepatoma than in other forms of progressive liver disease. In Los Angeles, delta infection was found in one of 39 patients (2.5%) with hepatoma but 18 of 57 patients (31.5%) with chronic hepatitis B (33). In Australia, delta markers were found in none of 6 HBsAg positive hepatoma patients (109). Thus, no specific association has been observed for delta infection and hepatitis B related hepatoma.

CONCLUSIONS:

Delta agent is an incomplete RNA virus which requires hepatitis B or a similar helper virus for replication. Replication of delta is associated with decreased replication of the helper virus. As such, delta agent is unique being an incomplete RNA virus requiring a helper DNA virus. Delta agent is endemic in Italy and in areas of South America, Africa and the Middle East. Throughout the world, HBsAg carriers with continued parenteral exposure such as drug addicts and hemophiliacs have high rates of delta infection. Delta agent is transmitted by parenteral and nonparenteral routes like hepatitis B but vertical transmission is less important. Delta infection can occur simultaneously with hepatitis B. Under these circumstances the outcome of the infection appears to be dependent on the host response to hepatitis B. When chronic HBsAg carriers are infected with

delta agent, there may be acute self-limited hepatitis, or development of chronic delta infection. The acute hepatitis in both chronic HBsAg carriers and in those simultaneously infected can be fulminant. Chronic delta infection is associated with progressive liver disease that does not respond to immunosuppressive therapy. Additional studies of this agent will increase knowledge not only of basic molecular virology but also of clinical liver disease.

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