such cases a strong immune response does not appear to occur. From our data we conclude that DRw6 may represent an important marker for an immune response gene.

HLA-DRw6 specificity is difficult to define.[6] In the white population of Holland there are two subgroups of HLA-DRw6 related to the cellularly defined specificities HLA-Dw6 and HLA-Dw9, respectively (Schreuder *et al*, paper submitted for publication). In this study we could not distinguish these two DRw6 subgroups serologically. Whether one or both of these subgroups influence the immune response in patients with transplants remains to be studied. Our data suggest that the serological definition of DRw6 as used for this study may be considered as a homogeneous specificity. Possibly DRw6 specificity represents a "blank" on the DR locus, containing only those determinants which are recognised by LB-E12 (MB1, MT1), DR2+DRw6, and MT2 antisera. If so, this would explain why no good monospecific anti-DRw6 reagents have been found. Nevertheless, Dw6-positive and Dw9-positive homozygous typing cells give clear-cut typing responses with cells from DRw6-positive people.

The implications of our findings for clinical renal transplantation are clear. HLA-DRw6-positive patients (about a quarter of the patients on the waiting list of Eurotransplant) should be given only HLA-DR-identical kidney transplants. This policy is less urgent for HLA-DRw6-negative patients. This guideline must remain provisional, however, until we have studied the effect of HLA-A and B matching in these two groups. A larger series of patients will be needed for such an analysis.

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Clinical aspects of delta infection

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Abstract

The clinical features of delta infection were analysed retrospectively in 191 hepatitis B surface antigen (HBsAg) carriers and 592 cases of acute hepatitis B seen over 11 years in the Swedish town of Malmö (population 250 000). With a few exceptions delta infections occurred exclusively in drug addicts.

In the chronic HBsAg-carriers the most common clinical manifestation was an episode of acute hepatitis, which in some individuals became severe with a pronounced rise in serum alanine aminotransferase activity for many months. During the period of delta infection the HBsAg titre was lowered and in three out of 26 cases the patient lost HBsAg altogether and developed hepatitis B surface antibodies (anti-HBs). In one patient the acute hepatitis due to delta infection was fulminant and fatal.

In patients with acute hepatitis B the clinical picture did not distinguish between those with and without simultaneous delta infection. The frequency with which acute hepatitis B was succeeded by a chronic carrier state was the same whether or not the patient was infected simultaneously with the delta agent.

The discovery of the delta agent has improved understanding of the natural history of chronic hepatitis B infection in drug addicts. Thus, instances of acute hepatitis in a chronic carrier, previously termed hepatitis non-A, non-B, may actually be episodes of delta infection.

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Introduction

The delta antigen/antibody system was first described by Rizzetto and coworkers[1] in Italy in 1977 and has later been associated with a transmissible hepatitis agent, the delta agent, which can cause infection only when the hepatitis B virus genome is present. The delta agent is found all over the world.[2] It was first discovered in southern Italy, where it is endemic and associated with serious liver damage,[1] while in Northern Italy, as well as elsewhere in the world, it is prevalent among drug addicts (and haemophiliacs).[2]

Infectivity studies have shown that chimpanzees are susceptible to delta infection.[3] The inocula contained both hepatitis B virus and delta agent. In animals without previous markers of hepatitis B a self-limiting acute hepatitis developed with markers of both hepatitis B virus and delta infection, the

incubation time being 3-4 weeks when highly infectious material was used and 12-13 weeks with less infectious material. In chronic HBsAg-carrying chimpanzees acute self-limiting hepatitis (with a biphasic enzyme pattern in one) was seen. The HBsAg titre was temporarily lowered before delta antibodies developed.

A previous seroepidemiological analysis[4] of about 600 cases of acute type B hepatitis and nearly 200 chronic carriers of HBsAg found in Malmö, Sweden, between 1970 and 1981 showed that delta infection was present almost exclusively in drug addicts. The infection was introduced into this town in 1973. From 1975 onwards about half of all cases of acute type B hepatitis occurring among drug addicts were also infected with the delta agent. Among intravenous drug addicts registered as chronic carriers of HBsAg by 1981, 73% were also carrying markers of delta infection.

Patients and methods

The study population comprised 592 cases of acute hepatitis B seen in Malmö (population 250 000), Sweden, during 1970-81 and 191 chronic carriers of HBsAg registered during the same period. Most patients attended the infectious diseases clinic at the General Hospital, the only one in the town. The remaining cases derived from the town prison, which is a point of referral for sick prisoners in southern Sweden. All files at the hospital and at the prison were re-examined for details of clinical history and laboratory investigations.

Patients were diagnosed as having acute hepatitis B if they had transitory HBsAg during an acute self-limiting disease with a more than five-fold increase in serum alanine aminotransferase or clinical jaundice or both. Four subclinical cases with only transitory HBsAg were included. Forty-six patients were lost to follow-up before HBsAg titres had become negative; in these cases diagnosis was based on enzyme patterns and clinical criteria only. A chronic carrier was defined as a patient who was positive for HBsAg for more than six months. In 22 instances where follow-up was shorter than six months a carrier state was presumed when indicated by the enzyme pattern and the history of the patient. In chronic carriers of HBsAg who had acute delta hepatitis when first seen at the hospital a simultaneous diagnosis of acute hepatitis B was made only if hepatitis B core antibodies (anti-HBc) of the IgM class were present according to certain criteria.[5] Sera from all patients had been stored at −20° and were retrospectively analysed for markers of delta infection.

Tests for markers of delta infection—Solid phase radioimmunoassay for delta antigen in serum and blocking radioimmunoassay for anti-delta were performed as described.[4]

Tests for markers of hepatitis B—HBsAg, anti-HBs, HBeAg, and anti-HBe were measured by commercial radioimmunoassay kits (Abbott laboratories, North Chicago, Illinois, USA). Anti-HBc-IgM was determined by solid phase radioimmunoassay.[5]

Results

CHRONIC CARRIERS OF HBSAG

Among 191 patients delta markers were found in 45, 42 of whom were intravenous drug addicts (table I). In one fatal case delta antigen only was found; in 44 delta antibody was present and in six both antigen and antibody. By serial analysis of frozen sera the time of conversion to anti-delta was established in 26 patients. Evaluation of clinical symptoms at the time of conversion showed that the most common clinical manifestation of delta infection was an episode of acute hepatitis with a rise in serum enzyme activity lasting for up to two months. This was seen in 17 patients. In another eight patients the acute episode was prolonged, lasting up to one year. Finally, in one case the course was fulminant and fatal (table II).

In three patients the enzyme and bilirubin patterns were biphasic. The findings were not otherwise noteworthy. In two patients the acute hepatitis was of the prolonged type; delta antigen was present at the time of the first peak and delta antibodies at the time of the second (fig 1).

Serum alanine aminotransferase values were raised between 10 and 230 times (mean 46 times). Serum bilirubin concentrations were normal in four, raised in 17 (between 1·5 and 30 times, mean 10 times), and not measured in five. In 13 out of 18 instances where sufficient sera were available to permit evaluation in detail HBsAg titres were temporarily lowered at the onset of delta infection. In a

TABLE I—*Presence of delta markers in different groups of chronic HBsAg-carriers*

	Delta markers		Total
	Present	Absent	
Addicts	42	37	79
Homosexuals	0	15	15
Haemophiliacs	1	7	8
Others (with known source of infection)	0	28	28
Unknown source of infection	2	59	61
Total	45*	146	191

*One positive for delta-antigen, 44 for anti-delta.

TABLE II—*Clinical features of delta infection in chronic carriers of HBsAg*

	Addicts	Non-addicts
Acute hepatitis (< 2 months)	17	0
Prolonged hepatitis (> 2 months)	8	0
Fulminant (fatal)	1	0
Subclinical hepatitis		2
Incomplete data	16	1
Total	42	3

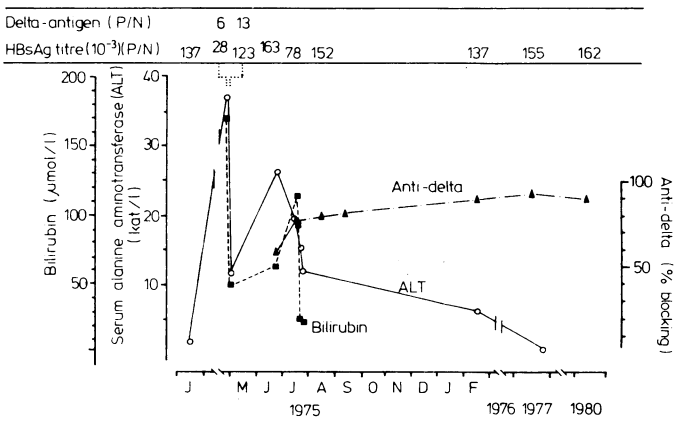

FIG 1—Acute hepatitis caused by the delta agent in a chronic carrier of HBsAg. Serum bilirubin concentrations and aminotransferase activity rose biphasically, delta-antigen was present at the first enzyme peak, and delta-antibody appeared at the time of the second peak.

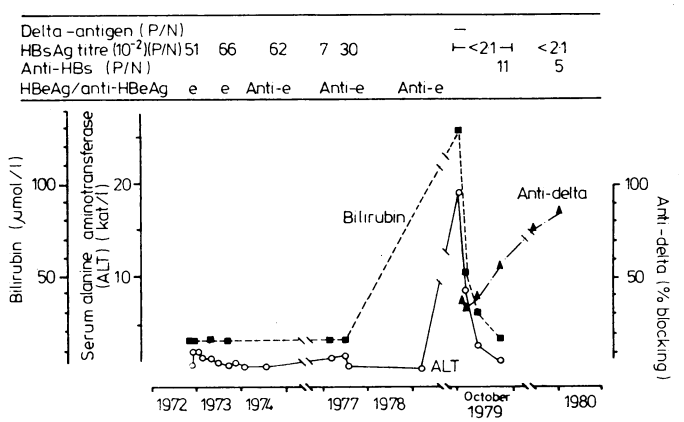

FIG 2—Acute hepatitis caused by the delta agent in a chronic HBsAg carrier after seven years of carriership. After aminotransferase activity and bilirubin concentrations returned to normal values HBsAg disappeared and anti-HBs developed. The anti-delta titre rose gradually during the following months.

further three patients the fall in titre persisted and anti-HBs developed. In two patients only was no fall in HBsAg titre observed. The change in titre occurring simultaneously with the first enzyme and bilirubin peak is shown in fig 1.

To establish whether delta infection is liable to occur at any particular time during the course of a chronic carrier state of HBsAg 25 carriers in whom the onset of both hepatitis B virus infection and delta infection were known were studied. This showed a wide spectrum from simultaneous onset to a time lag of seven years. In 26 patients the HBeAg/anti-HBe status was known at the time of onset of delta infection; eight of these were positive for HBeAg and 18 for anti-HBe. No conversion to anti-HBe during the delta infection was found except in one patient in whom a conversion to anti-HBs occurred simultaneously.

During follow-up of the 191 chronic carriers HBsAg disappeared in 19 after a carrier state lasting from eight months to 10 years (mean 38 months). When clearance occurred among delta-positive carriers the acute delta episode was directly related in time to the disappearance of HBsAg (followed by development of anti-HBs) in three patients. Fig 2 shows the disappearance of HBsAg during an acute episode of hepatitis occurring after seven years of carriership in a young man.

ACUTE HEPATITIS

Of 592 cases of acute hepatitis B, 57 were simultaneously infected with the delta agent, only one of whom was not a drug addict. Of these, 40 had classical acute hepatitis which could not be distinguished from the other cases by either clinical signs or enzyme patterns. In eight a biphasic course was noted (fig 3); there was a double bilirubin and aminotransferase peak, and the delta antibody titre rose during the second peak. One further case had a prolonged course. In eight patients data were inadequate to permit evaluation.

A chronic HBsAg carrier state developed in two of those 57 patients who had simultaneous delta and hepatitis B virus infections, whereas of 535 cases of hepatitis B without delta markers 25 became carriers (table IV). Thus, no difference in development of chronic hepatitis B virus infection could be seen between the two groups.

TABLE III—*No of chronic carriers of HBsAg in whom the antigen cleared during continuous follow-up*

	Antigen clearance in HBsAg carriers:			Total No of carriers
	Total No	Delta-positive	Delta-negative	
Addicts	14	9/42	5/37	79
Homosexual men	0		0/15	15
Haemophiliacs	0	0/1	0/7	8
Others (with known source of infection)	3		3/28	28
Unknown source of infection	2	0/2	2/59	61
Total	19	9/45	10/146	191

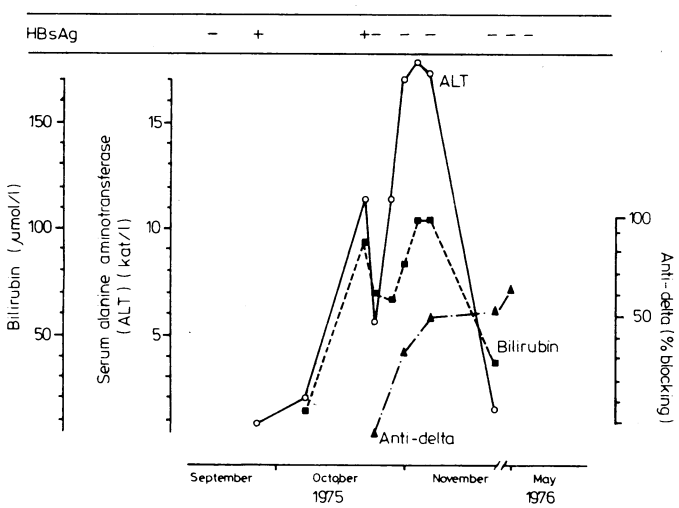

FIG 3—Simultaneous infection with hepatitis B virus and the delta agent causing a biphasic rise in serum bilirubin concentration and aminotransferase activity. Delta-antigen was not detected in this patient; the anti-delta titre rose during the second enzyme peak.

TABLE IV—*Clinical features of simultaneous acute infection with hepatitis B virus and the delta agent compared with type B hepatitis alone*

	No (%) delta-positive	No (%) delta-negative	Statistical significance
Classical acute self-limiting hepatitis	38 (66·6)	489 (91·4)	NS
Classical acute hepatitis progressing to chronic carrier state	2 (3·5)*	25 (4·7)	NS
Biphasic hepatitis	8 (14·1)	15 (2·8)	$p<0·001$
Prolonged acute hepatitis	1 (1·7)	1 (0·2)	NS
Subclinical hepatitis B	0	4 (0·7)	NS
Insufficient data	8 (14·1)	1 (0·2)	NS
	57 (100)	535 (100)	

*Also included among the 45 chronic carriers.

Discussion

Delta infection, as it occurs in our area, is overwhelmingly a phenomenon linked with intravenous drug addiction. Only four non-addicts harboured delta markers: a haemophiliac, a tourist who had received blood transfusions in Spain, and one Turkish and one West German immigrant.[4] The first instances of delta infection occurred in 1973 in two brothers (identical twins) who were both chronic HBsAg carriers and addicts and who were infected five months apart. In the same year the tourist referred to above was infected. From 1975 onwards there was a steady increase in the prevalence of delta markers, 72% of all addict carriers being positive for anti-delta by 1981.

Delta infection presumably spreads by the same routes as the hepatitis B virus, for example, by shared hypodermic needles. The present study also indicated potential infectivity of blood transfusions and coagulation-factor concentrates. Delta infection in a chronic carrier may occur at the beginning of the carrier state or at any time later as long as the carrier state persists.

The HBeAg/anti-HBe system has no bearing on the risk of an individual becoming infected with the delta agent. This accords with the concept that delta infection occurs in the presence of HBsAg whether the hepatitis B virus genome is integrated or not. The lower number of HBeAg-positive cases among delta-infected carriers reflects the natural history of the HBsAg carrier state in addicts, in whom a conversion from HBeAg to anti-HBe usually takes place after a comparatively short time. Our results indicated that the likelihood of a case of acute hepatitis B infection developing into a chronic carrier state was not increased by additional infection with the delta agent. A similar conclusion has been reached by Smedile *et al.*[7]

The one example in our study of delta infection in a chronic carrier leading to fulminant hepatitis and death was unprecedented in our area. It was the only case of fulminant hepatitis B infection among 789 patients with either chronic carriership of HBsAg or acute-type hepatitis B infection registered between 1970 and 1981. An episode of hepatitic activity in a chronic drug addict and carrier should alert the clinician to the possibility of delta infection. In fact, several episodes previously classified by exclusion of hepatitis A as hepatitis non-A, non-B have now been shown by positive criteria to be instances of delta infection. A biphasic bilirubin and enzyme pattern also suggests delta hepatitis.

Delta infection occurring simultaneously with acute hepatitis B is not recognisable by clinical features alone. In a few instances an unusually protracted course may retrospectively suggest delta infection. Of greater relevance is a biphasic pattern with double rises in enzyme activity and bilirubin concentrations. These findings were also noted in animal experiments[3] and in patients in an Italian multicentre study.[6] In our study this pattern occurred with significantly higher frequency in the patients with both delta and hepatitis B virus infections (eight out of

44 cases) than among patients with hepatitis B virus infection alone (15 out of 535 cases) ($p < 0.001$).

An interesting aspect of delta infection in chronic carriers is its connection with a depression in HBsAg titres. In the animal experiments by Rizzetto *et al*[3] a lowering of HBsAg titres was seen in both carrier chimpanzees infected. The titres, however, eventually reverted to preinfection values. In our patients this effect is shown in fig 1, where it was temporary, and in fig 2, where the HBsAg titre stayed permanently below the sensitivity threshold of the assay and anti-HBs developed. This finding has diagnostic and prognostic implications. As the HBsAg titre decreases it may temporarily become difficult to detect the antigen. A correct diagnosis of an HBsAg carrier may then be missed. Previously, when less sensitive assays were used, such diagnostic mistakes were particularly liable to occur.

Delta infection in a chronic carrier is sometimes associated with termination of the carrier state. In our series of 191 HBsAg carriers, 19 lost HBsAg over 12 years' follow-up. Fourteen of these were drug addicts, among whom nine had and five had not demonstrable markers of delta infection. Thus, antigen clearance not infrequently occurs irrespective of delta infection. In three cases, however, clearance coincided with an attack of acute hepatitis and anti-HBs developed in all three cases, which suggests a causal relationship between delta infection and HBsAg clearance, a possibility which should be studied in more detail.

The discovery of the delta agent has improved our understanding of a number of obscure events in the natural history of hepatitis B virus infection in drug addicts. Thus, acute episodes of hepatitis, sometimes called hepatitis non-A, non-B, may actually be instances of delta infection. The clearance of HBsAg not infrequently seen in drug-addict carriers may also be associated with delta infection.

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Raynaud's phenomenon and thermal entrainment: an objective test

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Abstract

A new objective test for diagnosing Raynaud's phenomenon was assessed in practice. The test is based on entrainment of the thermal vasomotor control system and entails non-invasive measurement of blood-flow responses in one hand while alternating thermal stimuli are applied to the contralateral hand. A significant ($p < 0.001$) abnormality of vasomotor control was found in patients with Raynaud's phenomenon compared with normal subjects.

When applied clinically this test is diagnostic and indicates the severity of the disease and the effect of treatment.

Introduction

Assessment and diagnosis of vasospastic disease of the digital vessels are often subjective, depending on the patient's description of the problem and the clinician's skill at interpreting physical signs that are often absent at the time of examination or difficult to elicit. Haemodynamic tests that may aid diagnosis include plethysmography,[1] Doppler studies of the patency of digital arteries,[2] and measurement of digital blood pressure.[3] Unfortunately, the very nature of the intermittent and sometimes elusive vasospasm that occurs in Raynaud's phenomenon renders such tests liable to subjective and objective errors.

We describe a test for Raynaud's phenomenon that does not depend on the production of vasospasm and is free from observer error. The method is an application of thermal entrainment of the vasomotor control system and was developed as a result of investigation of this system in Raynaud's phenomenon.[4]

History

In 1940 Burton and Taylor[5] found that peripheral blood flow is subject to spontaneous flow oscillations that occur at frequencies of less than 0·1 Hz. Kitney[6] subsequently showed that by applying a certain range of periodic thermal stimuli to one hand the spontaneous variations in flow in the contralateral hand could be suppressed and replaced by strong oscillating variations occurring at the same frequency as the stimulus. This phenomenon is called entrainment and occurs when a stimulus is applied to an oscillating system at a frequency approaching its natural resonant frequency.

The ratio of the magnitude of the response of blood flow compared with the magnitude of the temperature stimulus is termed gain and can be precisely measured. It is the gain of the thermal vasomotor control system that is measured in thermal entrainment studies. The

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