AETIOLOGY AND NATURAL HISTORY
OF NON-A, NON-B VIRAL HEPATITIS

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CHAPTER 1

INTRODUCTION

Viral hepatitis is a disease which has been recognised since the days of Hippocrates, and reports closely simulating what is today understood under this term, can be traced over the centuries.

Pope Zacharias in the 8th century suggested that some forms of jaundice (morbus regius) might be transmissible (103) but a whole spectrum of different diseases was included in the category of jaundice, and knowledge of the diverse aetiology of it was limited until perhaps the 19th century. Then smaller or bigger outbreaks started being reported with increasing frequency, and malaria, yellow fever, other haemorrhagic fevers, bacterial infections and leptospirosis are thought today to have been the causes of some of them, besides viral hepatitis, which was known by the term "epidemic catarrhal jaundice" - as opposed to "infectious jaundice" (or Weil's disease).

One of the first classical descriptions of viral hepatitis comes from Bremen, where Lurman in 1885 reported 191 cases of hepatitis among 1289 shipyard workers, all of whom had received inoculations with a smallpox vaccine containing
glycerated human lymph several months before the onset of symptoms (104). With this report the concept of oral or airborne modes of transmission thought to be responsible for the spread of "catarrhal jaundice" came to an end. Later Cockayne, 1912 (105) put forward the hypothesis that "catarrhal jaundice" as a specific disease (could be) due to an unknown organism endemic over a wide area and appearing not uncommonly in restricted and very rarely in widespread epidemics" and he proposed that "infective hepatitis would more accurately express the condition than catarrhal jaundice".

During the first half of this century viral hepatitis was separated into two different entities: of epidemic and blood-borne hepatitis (106, 107) and epidemics of the latter were described in patients attending venereal disease, (108,109) diabetes, (110) and rheumatoid arthritis clinics, (111), in recipients of blood and blood products (112,113), and in military personnel who were inoculated with yellow fever vaccine (114,115). Epidemiologic investigations incriminated contaminated needles and syringes, blood and blood products, and the human serum component of the vaccines as the sources of infection.

Human transmission experiments were initiated (116,117) to study the morbidity and patterns of disease better, and extensive investigations in infectious materials derived from patients were undertaken (especially during World War II).
A major breakthrough towards our modern understanding of viral hepatitis came in 1965, when Blumberg et al. (118) reported the discovery of an isoprecipitin (antibody), present in the sera of many patients with haemophilia who had been transfused, which reacted with a protein (the "Australian antigen"), found in sera of 10% of patients with leukaemia and Australian aborigines, but absent from the sera of many United Stated populations tested.

At about the same time Krugman and his colleagues were investigating patterns of hepatitis in an institutionalised population (at Willowbrook State School) for the mentally retarded and came out with extensive evidence for the existence of two types of viral hepatitis with distinct epidemiological, clinical and immunological features: viral hepatitis A (or infectious hepatitis due to MS-1 -virus and viral hepatitis B (or serum hepatitis due to Ms - 2 virus)(119 - 124). Recommendations for the use of immune serum gamma globulin in the prevention of infection soon followed (122, 123). The relation of hepatitis B with the Australia antigen was demonstrated (120). The hepatitis B virus was isolated by immunological techniques (125,126,127) and visualised by electron microscopy (126). Three distinct antigen-antibody systems were defined with which assessment of viral properties and host reactions was facilitated and the modes of transmission were further investigated (127, 128, 129). Transmission experiments in both chimpanzees and man permitted more detailed descriptions of clinical and histologic manifestations (129, 130, 131).
As soon as serologic tests for detection of hepatitis B virus (HBV) became available several studies indicated that a significant proportion of parenterally transmitted hepatitis could not be related to this virus (102, 132), and the explanation was given that either the tests were not sensitive enough, or that parenterally transmitted type A hepatitis virus (HAV) was responsible for them. However certain features, like incubation periods, were not in accordance with such hypotheses (133). Also when, measures were taken to eliminate carriers of hepatitis B surface antigen from the blood donor pool the observation was made that the total number of cases of post-transfusion hepatitis was not significantly altered. And reports kept streaming in about patients who had recovered from multiple episodes of acute hepatitis, for each one of which a different virus was implicated (26,44). People had already started speaking about Non-A, Non-B viral agents involved in the pathogenesis of viral hepatitis. The development of sensitive radiommunoassays for the detection of both HAV and HBV followed and investigators again failed to implicate these viruses in a significant proportion of cases with acute hepatitis (52,34, 136).

The entity of non-A, non-B (NANB) hepatitis evolved thus, by serologic exclusion of viruses known to cause acute hepatitis in man. Today still, diagnosis of NANB acute hepatitis depends on the exclusion of all other possible causes of it, including bacterial, rickettsial, fungal, parasitic, viral and toxic agents. In particular drug-
and toxic-hepatitis is excluded by a detailed history (and in ambiguous cases by arbitrarily taking as cut-off-point of incubation period a minimum of 2 weeks since virtually all toxic-hepatitis are expected to be manifested in less than that); hepatitis B is excluded by serologic tests for HB surface antigen and IgM-specific anti-HB core antibody; hepatitis A by serologic tests for IgM-specific anti-HAV antibody; Epstein-Barr virus (EBV) infection by testing for IgM-anti EBV antibody; Cytomegalovirus (CMV) infection by testing for IgM-anti CMV antibody; and other bacterial etc. causes by detailed clinical examination, history and appropriate serologic tests.
CHAPTER II

EPIEDEMILOGIC EVIDENCE FOR MORE THAN ONE AGENT IN NON-A, NON B HEPATITIS. (NANB-HEPATITIS)

Non-A, Non-B hepatitis has a worldwide distribution. Although most of our current knowledge about it came from studies performed in the economically and technologically developed countries, reports of similar studies done in the developing and underdeveloped countries keep streaming in, in the modern medical literature.

From several reports we know today that NANB - hepatitis may account for more than 90% of the cases of posttransfusion hepatitis (see also table I) (1,2,3,4,5). The importance of this figure can only be fully appreciated if we take into account the fact that in different centres (mostly developed countries) the overall incidence of hepatitis in recipients of only volunteer donor blood can range from 7 - 17% (1,2,3,4,5) despite all measures to protect the recipients from infective blood, and increases proportionately with the amount of blood transfused (6).

Patients in cardio-vascular surgical units (3,7), multiply transfused patients with haemophilia (8) or thalassemia (9,10) (see Table II) are at an especially high risk of developing NANB-hepatitis. Commerical preparations of factor VIII and IX concentrates (11,12,13,14,15), of fibrinogen (16), and cryoprecipitate (15) have been implicated in the transmission of disease in both humans and in animals (see chapter IV).
Risk factors, associated with increased incidence of transmission of NANB - hepatitis after a transfusion seem to be the number of units transfused (17), the abnormal alanine aminotransferase (ALT) activity in the donor specimen (18) and the antibody to the hepatitis B surface antigen in the donor blood (17). Of course transfusion of blood from commercial blood banks or paid donors increases the average attack rate of clinical and subclinical NANB hepatitis from 7% (when only blood from community service agencies is transfused) to up to 54% (when one or more units from paid donors is transfused) (19).

The age distribution of patients with NANB - post - transfusion hepatitis differs in the various series according to the particular group studied (haemophiliacs, thalassemics, people with cardiovascular disease). Overall, though, one can say that all age groups are susceptible, but perhaps the very young show less evidence of overt disease (7), as is true with many other infections.

Overall, again, the male sex seems to be in slightly greater risk for developing NANB - posttransfusion hepatitis (table III) but in other studies this difference is hardly evident.

NANB - hepatitis has also been reported from haemodialysis units (20,21,22) and renal transplantation units, where the incidence of it is great for both the patients (attack rates annually up to 16%, in one study) and the staff (annual
attack rates up to 2.3%) (24). It seems, however, that for some unknown reason the NANB agents are far less often responsible for outbreaks of hepatitis in these units compared to Hepatitis B Virus (25).

Drug addicts are also a well-recognised group with high incidence of hepatitis in general and it seems that NANB agents are major causes of morbidity in this particular group. (26,85). Mosley et al, 1977 (26) reports that as many as 53% of the hepatitis episodes among this population group can be classified (by exclusion) as Non-A, non-B, whereas others (27) record lower percentages (25%).

Needlestick exposure of health-care workers and investigators in their every-day practice and in laboratories (28,29), outbreaks in plasmapheresis units (30), and oncology units (31), transmission to volunteers by inoculation of icterogenic serum (32), infection by inadvertent inoculation during evaluation of anti-malarial chemotherapy programs (33) have also been reported enhancing the belief that the parenteral route is of major importance in the transmission of the NANB agent (s).

The term sporadic hepatitis has been applied to those patients who are either (a) spontaneously seeking medical care in time when no epidemic is occurring in a place, or (b) are identified by investigators (with clinical, biochemical, serological etc., examinations) during extensive general population surveys. The prevalence of NANB -hepatitis among sporadic cases greatly varies from place to place,
and from time to time. (see table IV). Villarejos et al, 1975 (34) from Costa-Rica reports that 11% of cases of sporadic hepatitis are due to NANB agent(s). Papaevangelou et al, from Greece, observes a shift in the prevalence from 9% to 18% over the last decade (35,36) explained possibly because of the elimination of the paid blood donors system and the subsequent decrease in the prevalence of hepatitis B in the country. Studies in an urban population (in West London) by Farrow et al., 1981 (48) estimate that 13% of hepatitis sporadically occurring is of NANB aetiology, a number confirmed by Bamber et al., 1983 (28) while studying a hospital population (in North London). Alter et al, 1983 (38) gives higher figures from the United States. As many as 42% of cases there are NANB hepatitis, in contrast with previous figures (37) that estimated it to 25%. Alter's figures though come from studies in the community whereas Dienstag's (37) from hospitalised patients. The highest figures come from Japan (49) where, in different studies NANB - hepatitis accounts for 45 - 47% of total cases of sporadic hepatitis. It must be pointed out, though, that we are dealing with a non-homogeneous group of patients; that if detailed history is taken, the disease can be associated with a blood transfusion, use of parenteral drugs, employment as health workers in patient or laboratory work, ingestion of contaminated food (esp. raw shellfish), personal contact with others who had hepatitis, travel overseas (esp. to India) (28,38,85,48) (see also table V) and perhaps with surgical and dental procedures, homosexual contact and imprisonment (48,38).
The patients with sporadic NANB - hepatitis tend to be young adults (usually under the age of 30) (28, 38) and there is a predominance of males among them, which is similar but rather smaller than that for hepatitis B (27,28,38,48) cases. Black people, people of lower educational and socio-economic status are generally in greater risk of acquiring this disease (38). And people with NANB-hepatitis tend to have a history of past clinical hepatitis, probably due to other viruses (HBV, HAV etc.) (38).

Truely epidemic NANB-hepatitis, has only been reported from India (39). In Kashmir, in late 1978 - early 1979 an epidemic hepatitis occurred, with mode of spread similar to that of hepatitis A, high incidence of anicteric cases and mean incubation period of 15 days, but with high incidence of cholestatic cases and, most important of all, without any serologic evidence of acute-phase immune reaction to HBV or HAV. This taken together with the facts that hepatitis A in India occurs in a very young age, affecting virtually the whole population which thereafter becomes immune, whereas the age distribution of the patients in this epidemic was between 11 and 40 years old, suggests that probably a non-A, non-B agent was involved.

The possibility of a toxic aetiology in this epidemic was dismissed by Khuroo et al, 1980 (39). Although epidemic toxic hepatitis have been described in the area (138,139), they tended to have their own distinctive histologic findings in the liver specimens. Moreover there was no history
or other evidence of exposure to any known hepatotoxin.
On the other hand the gross faecal contamination of a particular water supply and the statistically significant clustering of cases around it add further weight towards a viral aetiology. It is of further interest that the Delhi epidemic of viral hepatitis in 1955 - 56 (137) had many epidemiological, clinical and histological similarities with that of Kashmir.

Intrafamilial spread of NANB-hepatitis (34) and spread within an institution for handicapped children (40) have also been described.

Vertical transmission, i.e. from mother to foetus in utero, of NANB-agent(s) has been recorded in 6 out of 9 infants born to women who had acute NANB-hepatitis during the third trimester of pregnancy (41).

At the time, there seems to be some ambiguity as to whether sexual transmission occurs. Bamber et al, 1983 (28) found no evidence of such a mode of transmission among the sporadic NANB-hepatitis cases seen in the Royal Free Hospital in London. Alter et al, 1982 (38), though, doing a community survey, found that "personal contact" (including in at least one case intimate sexual contact) with hepatitis patients was associated with cases with NANB-disease significantly more often than with controls, whereas the association for "homosexuality" missed statistical significance. And Duermayer et al, 1983 (57), who claim that they have produced an ELISA specifically detecting a NANB-hepatitis antigen and its antibody (see chapter V) find that 43% of a sample
of 54 prostitutes had evidence of either antigen or antibody in their sera.

It becomes clear, therefore, from the account of the already presented data, that two major patterns of NANB-hepatitis tend to emerge (42): The one primarily reported from western countries, which is associated with parenteral transmission and follows more or less the epidemiologic patterns of hepatitis B virus (HBV) infection. The second seems to have a non-parenteral mode of transmission, occurs in epidemics (more often reported from India) and more closely resembles epidemiologic patterns of hepatitis A virus (HAV) infection. Unfortunately, the lack of specific serologic tests still hinders the complete study of these diseases.

However, there is further evidence that things are even more complex than that. At the time following the introduction of serologic tests for HBV infection, people started reporting two non-HBV acute episodes of hepatitis in the same patient, and especially in drug addicts (43,44). Karvountzis et al., 1975(44), for example, studying 28 patients with two episodes of acute viral hepatitis and evidence for parenteral transmission, found that in all patients at least one bout was not related with HBV infection and in 2 of them both bouts seemed incompatible with it. Then Mosley et al, 1979(26) described the occurrence of 30 episodes of acute hepatitis in 13 drug addicts. Since serologic tests to identify HAV infection were also available then, he was able to demonstrate clearly that 3 of his patients had
at least 2 bouts of acute NANB-hepatitis, probably due to two unrelated immunologically viruses. Norkrans et al., 1980(27) also described multiple acute episodes in drug addicts (71 episodes in 33 patients) 25% of which were due to NANB agents; at least one of those patients experienced two sequential episodes of acute NANB hepatitis. Also Galbraith et al., 1979(20) described outbreaks of NANB hepatitis in 29 haemodialysis patients, 7 of who had two discrete episodes of acute NANB disease.

Similar multiple attacks had been reported in haemophiliacs treated with commercial factor VIII and factor IX concentrates (11, 12, 45). In fact it was this epidemiological evidence that paved the way to the transmission experiments, that will be discussed later, which brought additional evidence for the existence of more than one parenterally-transmitted NANB-agents.

Finally, one additional point in the argument for at least two (and possibly three) agents-viruses involved in the aetiology of NANB-hepatitis stems from the studies on the sporadic NANB-cases. As mentioned already, if a detailed questionnaire centering on the particular risk factors is filled in, the revelation of the same two broad patterns of acquiring the disease will be made: a parenteral and a non-parenteral, and even then a significant proportion of patients will remain, ranging from 14-29% in different studies (28, 85), for who no known or identifiable way of exposure will be revealed.
CHAPTER III
ACUTE HEPATITIS

CLINICAL EVIDENCE FOR THE EXISTENCE OF MULTIPLE NANB AGENTS

The clinical course of a typical acute hepatitis can be distinguished in: (a) the incubation period, (b) the acute disease phase and (c) the convalescent phase. Further the acute disease phase consists of a period of prodromal symptoms and signs and the icteric phase. There are often asymptomatic cases and/or anicteric cases, and there is of course a continuous flow from one "phase" into the other and the limits are often not so distinct. In general terms, one can say that the symptomatology of the acute NANB-hepatitis and the laboratory findings are the same as for acute A or B hepatitis, and for details one is referred to any of the standard textbooks of general medicine or hepatology (ref. 100). There are, though, differences in the frequency and the severity of them, and what I will try to do here is analyse those differences and peculiarities in the course of acute NANB-hepatitis, which are thought to be more or less characteristic of this disease and point out to its multiple aetiology.

Acute NANB hepatitis has been shown to occur sporadically, epidemically and endemically especially in populations (well studied in long prospective studies) receiving parenteral inoculations of various agents either for beneficial purposes (transfusion of blood, plasma, blood factors) or accidentally ("needlestick" exposure) or as a habit (drug-addicts).
The incubation period of NANB-hepatitis can vary over a wide range (from 7 days to more than 180 days) (14, 39, 46, 101,102). In fact it seems inappropriately wide, even to the non-expert's eyes, to support the existence of only one causative agent for the disease.

A short-incubation period (7-28 days) parenterally transmitted NANB hepatitis has been reported by Bamber et al, 1981(46) in patients with haemophilia and Von Willenbrandt's disease receiving National Health Service or commercial factor VIII concentrates or cryoprecipitates. Other interesting features were: in 50% of patients, it was asymptomatic and anicteric, but in 50% it had a severe symptomatology and jaundice; none of their patients recovered within 6 months, but proceeded to the development of chronic liver disease (see chapter VI); intense lobular sinusoidal mononuclear cell infiltration (noted also in chimpanzees with this infection) (47), fatty changes and damaged bile ducts in the liver histology were some of the features, not commonly seen in viral hepatitis. Other investigators (11,12) have also described NANB-hepatitis with uniformly short-incubation periods in haemophiliacs (again) receiving commercial preparations of factor VIII, but in those studies progress to chronicity was less often and the disease was considered rather mild.

The possibility of a toxic aetiology, especially for the short-incubation NANB-hepatitis has always puzzled the
investigators (8, 11, 12, 39, 46) and, despite making sure to have a full account of drug history and exposure to known or potential hepatotoxins, nobody can exclude their causative role in some such cases. It must be stressed here, though, that toxic hepatitis usually manifests itself in less than 1 week (or at most 10 days) after the exposure, and indeed this period of time (or by others two weeks) is sometimes taken arbitrarily by investigators as a cut off point before proclaiming a bout of acute hepatitis as Non-A, non-B.

Several groups reported the development of acute NANB-hepatitis with a long-incubation period (of more than 6 weeks) (1, 11, 14, 101, 102). In the study by Wyke et al, 1979 (14) the incubation period varied from 42 to 103 days (mean 65 days) and of 17 patients receiving the concentrates of coagulation factor IX (both from commercial and non-commercial sources) on account of chronic liver disease, 4 developed hepatitis (24%) and in 3 cases the illness proved fatal. A chimpanzee receiving concentrate from the same batch developed hepatitis, too (see next chapter). 2 other patients with heart-disease, undergoing operation, received of the same batch of concentrate and produced NANB-hepatitis after 55 and 70 days. All of the patients showed considerable elevations of their bilirubins and transaminases. On the whole, though, Wyke thinks that this kind of NANB-hepatitis is a rather mild, usually subclinical and anicteric illness often overlooked because of underlying diseases or because of acquired immunity of the people receiving the infectious blood-products.
Prince et al, 1974(102) similarly reported the development of a long incubation (mean 8 weeks) post-transfusion NANB acute hepatitis in patients who had undergone cardiovascular surgery in New York. The acute disease was again mild, 61% of cases were anicteric, mean peak of SGPT elevation was 259 units (by the kinetic-spectrophotometric method) and median duration of it was 10.5 weeks.

Feinstone et al, 1975 (101) studied 22 patients who had a total of 23 episodes of transfusion-associated NANB-hepatitis after corrective cardiac operations in Bethesda, Maryland. The incubation periods varied from 2 to 15 weeks (mean 7.3 weeks) but the bouts of NANB-hepatitis tended to form two groups really - one with an incubation period around 4.5 weeks and the second with an incubation period around 9 weeks.

The epidemic NANB-hepatitis in Kashmir, India, reported by Khuroo et al, 1980 (ref 39) had a short-incubation period (mean 15 days); there was a high incidence of fulminant hepatitis (5%) cases, of cholestatic cases (20%) and of secondary cases among contacts of patients; the biochemical changes were mild and returned to normal within 2 to 6 weeks; further there was evidence of faecal-oral transmission of the disease through the contamination of a particular water supply.

Lately, in a fascinating report by Ohori et al, 1983(ref 7) two types of NANB-hepatitis occuring in a cardiovascular
surgical unit have been reported. The one produced two waves of an epidemic during two consecutive years and subsided, whereas the other seemed to go in a non-epidemic way affecting patients in this unit at a steady rate (both before and after the two epidemic waves). These had incubation periods of 24 and 17 days respectively (means), which were not significantly different statistically, but showed other differences. A larger prevalence of immune complexes was found in the epidemic (80%) than in the non-epidemic (8%) hepatitis. The former had significantly more pronounced symptomatology (jaundice in 83%) and biochemical abnormalities than the latter (jaundice in 23%). About 33% of the patients with the epidemic-type hepatitis progressed to chronic liver disease.

Sporadic acute NANB-hepatitis seems to be a rather mild disease, according to Bamber et al, 1983 (ref 28). Their 22 patients had rather mild elevations of bilirubin (less than 260 umol/l) and transaminases (less than 800 I.U.); some experienced prodromal symptoms like fever (27% of patients), arthralgias (18%) and rash (5%); about 10% progressed to chronicity as evidenced by continued abnormal biochemical results. And Farrow et al, 1981(48) doing a similar survey in an urban community (West London) reported similar results: compared with patients with hepatitis B, those suffering from NANB disease had a milder disease, with a significantly shorter duration of jaundice (in the icteric cases), with less common occurrence of arthralgia (26%) or skin rash (15%), and with significantly milder ALT elevations.
From all the above data, therefore, one can say that:

(a) Two ranges of incubation periods seem to attract the interest of investigators: a short-one (i.e. from 2 to 4 weeks approximately) and a long one (i.e from 6 to more than 20). Post-transfusion NANB hepatitis, well documented in prospectively followed up transfused patients, has either a short or a long incubation period. The same applies to NANB hepatitis following infusion of certain blood factors. The epidemic type of NANB hepatitis being reported from India is of a short incubation period. And it will be shown later (see chapter IV) that the agents causing the short or the long incubation period parentally-transmitted NANB hepatitis have been associated with different ultrastructural changes in the liver of chimpanzees inoculated with them.

(b) The severity of the acute disease, assessed by the presence or absence of symptoms, signs and jaundice and the level of the biochemical abnormalities, has also been shown to range considerably, and there is no doubt that it is associated to both the virulence of the causative agent(s) and the intensity of the immune response of the host. The proportion of asymptomatic and/or anicteric cases differs from study to study (see table VI), but it is a common belief that NANB-hepatitis as a whole is a rather mild disease as compared to hepatitis type B, for example. In most of the studies the peak transaminase elevations were lower than those usually observed in hepatitis B, as were
the peak bilirubin levels (85, 28). In association with the different epidemiological backgrounds, though, no distinct differences in the severity of the acute clinical presentation can yet be recognised.

Transmission studies in chimpanzees have demonstrated a biphasic pattern of aminotransferase abnormality which seems to be characteristic of acute NANB disease (77).

This has also been confirmed in man, but, as Tateda et al, 1979 (4) showed, there can be three patterns of aminotransferase elevations: the monophasic, the biphasic and the plateau type (Figure 1). Those patients exhibiting the monophasic type seemed to have a shorter incubation period and the clinical manifestations were relatively severe, whereas those with the biphasic type had the most severe clinical manifestations. Other investigators have confirmed the finding of great and unpredictable fluctuations of the aminotransferases during the course of acute NANB-hepatitis (28, 46, 79).

The duration of the symptoms, the jaundice and the biochemical abnormalities varies widely from a few days to many weeks. More often the symptoms will disappear within a week of the onset of jaundice (fatigue can go on for many months), the jaundice will clear within 4 weeks of onset, and the aminotransferases will return to normal within a 3 month period (28, 39, 48, 85, 97, 102), although they can go
on fluctuating up and down for many months before they subside (81, 78).

Fulminant hepatitis occurs infrequently, in acute NANB-hepatitis, as evidenced by an eightfold reduction in fatalities associated with post-transfusion hepatitis after the introduction of donor screening programs for the elimination of hepatitis B virus (82). Nevertheless, Khuroo et al, 1980(39) reports a rather high incidence of fulminant hepatitis (5% of 275 icteric cases, which in conjunction with the low incidence of anicteric-asymptomatic patients among contacts, which was 27%, justifies, we think, the term "high incidence of fulminant cases") in the epidemic in Kashmir, India (83% of those patients with the fulminant disease died). Also in the report by Ohori et al, 1983(7) which we have already mentioned, fulminant hepatitis occurred more often among the patients with the epidemic type (who were more often icteric and symptomatic and had more often circulating immune complexes in their acute phase sera), than those with the non-epidemic one.

According to a report by the Acute Hepatic Failure Study Group (83) as many as 34% of cases of fulminant hepatitis may be attributed to NANB-agent(s) (second most common aetiology after HBV) and the mortality rate may be as high as 87% (second most grave prognosis after drug-associated fulminant disease). Especially grave is the prognosis for the young adult group (ages between 15-44 years), whereas
survival rates among males and females were comparable in both this study and that of Khuroo et al, 1980(39).

The serum-sickness-like syndrome, often found in HBV-related hepatitis, is rather unusual in NANB-hepatitis. Nevertheless urticaria, purpura, rash, arthralgias and aplastic anaemia have been reported by Perillo et al, 1981(84) and circulating immune complexes may be a very common feature of the disease (see also chapter V). Dienstag et al, 1979(56) reports circulating immune complexes in as many as 70% of patients immediately before or coincident with acute disease and during the early convalescent period, while Ohori et al, 1983(7) finds them in as many as 80% of patients with the epidemic and only 8% of the non-epidemic type of NANB-hepatitis studied in a surgical unit.

Viraemia has been shown to occur throughout the period of clinical illness. Chimpanzee studies by Tabor et al, 1979(77) indicate that viraemia occurs during the acute disease near the time of the first aminotransferase elevation (and possibly starts a week earlier); it also persists at least until a week after the peak aminotransferase elevation.

Historically the NANB-acute hepatitis is not strikingly different from other viral hepatitises: centrilobular liver cell swelling and usually limited amount of necrosis (focal or zonal), portal inflammation, acidophilic bodies, a few liver cell mitoses (binucleated hepatocytes), cells with prominent nucleoli. Features like fatty changes of
hepatocytes, both intracanalicular and intracellular cholestasis, prominent Kupffer cell hyperplasia and mononuclear cell infiltration are viewed by most as more characteristic and are certainly reported in greater frequency with acute NANB disease (39, 43, 46).

Spontaneous resolution is one of the possible outcomes of the disease, and usually occurs within 12 weeks after the onset (85). However, one of the most important consequences of acute NANB-hepatitis is its tendency for progression to chronic liver disease, which will be the subject of chapter VI. The frequency with which it occurs, though, is certainly associated with the different epidemiological backgrounds and the diverse aetiology (as is true for hepatitis A and B). The puzzling observation with NANB-disease is that aminotransferase levels can fluctuate markedly during acute disease (78, 81), and that complete resolutions have been reported after 1, 2 and even 3 years (81). Investigators often use the histologic description of "resolving acute hepatitis" to describe the atypical findings. So, is the usually ascribed 6-month-period cut-off point enough to designate the transition of acute to chronic NANB-hepatitis?
As is frequent when dealing with infectious diseases, in order to prove the infective nature of a disease and isolate and study the causative agents, an animal model is required in which to transmit the disease (i.e. produce a clinicopathological entity into the animal that resembles that of the human being using infective material from man) and from which to recover the agent.

In 1978, Alter et al (47) reported the induction of hepatitis in five chimpanzees, biochemically and histologically confirmed, using plasma and serum from 4 patients with acute or chronic NANB-hepatitis after transfusion and from a blood donor who was implicated in 2 other cases of post-transfusion hepatitis.

Similarly, Tabor et al, 1978 (29) transmitted NANB hepatitis to chimpanzees using serum from a patient with chronic hepatitis whose blood caused acute infection in a nurse following a needlestick accidental exposure, and with serum from 2 blood donors, who transmitted clinical hepatitis to recipients.
Hollinger et al, 1978 (59) of the Transfusion-Transmitted-Viruses-Study (T.T.V.S) group in the United States used serum from 3 blood donors, who were implicated in two cases of NANB-hepatitis, as well as sera from recipients of blood collected at least 12 days before clinical illness to induce the infection in chimpanzees.

Wyke et al, 1979 (14) provided evidence for a transmissible NANB-hepatitis agent in human blood clotting factor concentrates by inducing NANB infection experimentally in chimpanzees. He used material derived from four different batches of factor IX concentrates made from both commercial and non-commercial sources. Some of these batches were used to treat patients with chronic liver disease or in cardiac surgery units and were implicated in the induction of NANB disease in a proportion of them (as mentioned earlier). Three male chimpanzees held at the Primate Unit of the London School of Hygiene and Tropical Medicine were inoculated (66). Chimpanzee Jeremy received material from the same batch of factor IX given to those patients. Chimpanzee George received a similar product, which was not used in treatment of human patients. A positive control inoculum provided by Dr H J Alter and Dr R H Purcell (47) was administered intravenously to a third chimpanzee, Victor. All three chimpanzees developed acute hepatitis, biochemically and histologically, after an incubation period of about 10 weeks, whereas before the inoculation they were normal. Chimpanzee George had previously suffered from hepatitis A and B, and the other two were given hepatitis A antibody
before the inoculation. It was interesting that chimpanzee George developed hepatitis from a batch of factor IX not used at all in patient treatment (i.e. of unknown infectivity and presumed safe) and this alarmed physicians and the WHO (60) in the potential risk of inducing a serious, potentially lethal infection in patients treated for other conditions.

Bradley et al, 1979 (61) managed to transmit NANB-hepatitis to four chimpanzees by the infusion of material from three batches of factor VIII concentrates commercially prepared, which were implicated in cases of hepatitis in 2 patients in the United States. The incubation periods in the patients were short (20 and 38 days). The incubation periods in the chimpanzees ranged from 18 to 53 days, and tended to become even shorter after second passage in other chimpanzees or after inoculation of concentrated chimpanzee liver material.

K. Tsiquaye and A.J. Zuckerman, 1979, 1980 (62,66) reported the induction of a fourth episode of acute hepatitis in chimpanzee George (see above) using the same batch of factor VIII as Drs D. Bradley and J.E. Maynard above, and at a time when he had completely recovered from the NANB-hepatitis induced by the factor IX concentrate. Not only that, but chimpanzee George developed the ultrastructural changes characteristic of short-incubation hepatitis (see below) (66), in spite of the fact that he had also developed those of the long-incubation NANB hepatitis during the earlier bout of the disease.
Reciprocally another chimpanzee, Bern, convalescent from NANB-hepatitis previously induced by infection with the same agent present in the factor VIII concentrate mentioned, suffered a second attack of acute NANB-disease when subsequently inoculated with material from that factor IX concentrate also used in chimpanzee George (63).

These brilliantly organized and executed cross-challenge studies established what was already suggested by epidemiological and clinical findings, namely the existence of at least two immunologically distinct NANB-hepatitis agents (viruses). Yoshizawa et al, 1980, 1981 (16, 139) confirmed this in a similar series of experiments. The observation of electron microscopy (E/M) of certain ultrastructural changes happening in hepatocytes of the infected chimpanzees added further weight to this concept.

Shimizu et al, 1979 (64) inoculated four chimpanzees intravenously with chimpanzee plasma containing an agent (strain F), originally derived from a patient with chronic NANB-post-transfusion hepatitis and passaged twice in chimpanzees. All animals developed hepatitis with a mean incubation period (to peak ALT level) of 11 weeks (long). In liver biopsies regularly obtained from the animals, cytoplasmic abnormalities were observed in the hepatocytes of all four chimpanzees. These occurred in the cisternae of the dilated rough endoplasmic reticulum (E.R.), were circular in cross-section and composed of 2 parallel walls when cut longitudinally
So they appeared to be tubular (see figures 2, 3, 4). The walls of the tubules were constructed of double-unit membranes with electron-opaque material inbetween (trilaminar). The total thickness of the wall was 20-25 nm. The E.R. was contiguous with both the outer and inner membranes. The diameter of the tubules ranged between 150 and 300nm, and the longest tubule observed was 2.2um. Characteristically, the nuclei of the hepatocytes from these chimpanzees appeared to be normal.

During the same experimental work, 5 other chimpanzees were inoculated with material (strain H) from another patient with acute NANB-hepatitis. Two of these animals developed ALT elevations with incubation periods of 6 and 7 weeks (short). In liver biopsies taken regularly from the animals, the hepatocytes of all five animals, contained distinct nuclear changes. The nuclei of the hepatocytes appeared to be heterogeneous in density, condensed and irregular in shape. Intranuclear aggregates of particles, measuring 20-27 nm in diameter were found in hepatocytes of four of the five chimpanzees. In the cytoplasm, mitochondrial cristae were dilated and the E.R. was distorted.

Both the cytoplasmic abnormalities and the nuclear changes described appeared at the time of ALT elevation (wherever such elevation happened at all). Cytoplasmic tubular structures and nuclear abnormalities were never observed in specimens from the same animal or in the same cell in this study. Cytoplasmic changes were universally encountered in almost all hepatocytes; nuclear changes were seen only in a portion of them.
Since then several reports (65,66,67,68) have confirmed the findings by Shimizu et al., and thus the cytoplasmic tubular structures, were universally associated with acute NANB-hepatitis of long-incubation period, and the nuclear changes and the intranuclear particles with acute NANB-hepatitis of short-incubation period (in chimpanzees only); the existence of two agents (viruses) both transmitted by the parental inoculation of infective material was once more strongly suggested.

However, in a similar study by Burk K·H et al, 1981 (69) the observation was made that the same inoculum (inoc. A) could be implicated in the development of either intranuclear particles (in one chimpanzee) or cytoplasmic tubular structures (in a second one); and a second inoculum (inoc. B) was implicated in the development of both changes in the hepatocytes of a third chimpanzee. But the authors themselves could not dismiss the possibility that their inocula were not "pure" and in essence contained two NANB agents resulting in two distinct ultrastructural changes.

Yoshizawa et al, 1981 (139) who also demonstrated the existence of two immunologically distinct NANB-viruses, observed that what they called NANB-1 virus produces cytoplasmic tubular structures in the hepatocytes of chimpanzees similar to those described by Shimizu et al and others (64,65,66,67,68). However, in other chimpanzees infected with their NANB-2 virus, they could not find nuclear changes, or particles, nor cytoplasmic abnormalities (the chimpanzees had hepatitis both biochemically and histologically, though).
Lately, Sidhu et al, 1983 (141) while investigating 21 cases of the Acquired Immunodeficiency Syndrome (AIDS), reported the visualisation of two types of ultrastructural entities (in humans). One of them, what they called "test tube and ring shaped forms" (TRF) was present in lymphocytes and rarely in other cell types of 39% of those patients with the syndrome (and in 76% of those patients from the same series, for whom specimens from many body sources were available). These TRFs looked very much the same as the cytoplasmic tubular forms that have been described from the hepatocytes of chimpanzees with NANB-hepatitis (but never from human cases, see next chapter). This striking resemblance has been pointed out by several other investigators (142,143,144). Schaff et al, 1983 (142) have suggested that these structures in AIDS patients could be explained by the presence of chronic NANB-hepatitis in the background. Similar structures have also been reported from single cases of multiple sclerosis (145) and human T-cell leukemia (146). It is speculated, therefore, that the cytoplasmic tubular structures represent a nonspecific cellular reaction common to several agents, at least some of which could be viral (since viruses are implicated in the aetiology of AIDS, human T-cell leukemia and NANB-hepatitis). Even more recently, however, attempts to produce the same ultrastructural changes in chimpanzees after inoculation with material from AIDS patients failed, either because of the long incubation period of AIDS or because they are not characteristic of this disease (K.N. Tsiquaye, personal communication).
Various virus-like particles have been reported by different investigators in liver cells of humans and chimpanzees as well as in plasma and urine from humans, with NANB-hepatitis (see Chapter V). Bradley et al., 1980 (67) reported the existence of crystalline structures, containing 25 to 30nm particles, in the cytoplasm of endothelial of Kupffer cells in acute phase liver biopsies obtained from three chimpanzees inoculated with either factor VIII materials or "H-strain" plasma.

Particles identical to those described by Bradley et al. were examined carefully by McCaul et al., 1982 (70) using the Markham rotation technique (71) and they concluded that these 25 -30nm particles are rather non-viral, but just a reflection of pathological response of the host cell in NANB-hepatitis, especially since they have also been visualised in liver cells of chimpanzees infected with hepatitis A (67).

Burk et al., 1981 (69), on the other hand, observed intracytoplasmic particles with a diameter of 37 ± 2 nm in hepatocytes of a chimpanzee (in addition to and distinct from both the nuclear or cytoplasmic changes described already), present again in highly-ordered crystalline arrays. They were morphologically homogeneous and consisted of an apparent inner core and outer shell, and had a virus-like appearance. Similar structures have been found in human cases of NANB-hepatitis and will be described in the next chapter. They seemed similar to, but smaller than the characteristic
Dane-particles described years ago in association with HBV infection. However, since electron microscopy of rat hepatocytes and hepatomas, has demonstrated that the crystalloid entities contained in microbodies have morphological similarities to the $37 + 2\text{nm}$ particles within the liver hepatocytes of this chimpanzee (140), one must still be careful in characterising them as viruses.
CHAPTER V

SEROLOGY

The development of a sufficiently sensitive serologic test for detecting the causative agent(s) of NANB-hepatitis specifically has been a major objective of many laboratories and investigators all over the world for the last 6 or 7 years. Its significance in our understanding and further exploring of the nature of these agents is colossal and can not be overemphasised. Several reports announcing the development of such specific tests have been published, and a short account of some of the major ones not only reveals some of the great difficulties towards the objective, but also adds further evidence for the multiplicity of these agents.

Shirachi et al, 1978 (50) was among the first to announce the detection of a certain "specific" agent of NANB-hepatitis by immunodiffusion in 178 out of 268 serum specimens sequentially obtained throughout the incubation period and during the clinical course from 13 patients (and repeatedly for each one of them) with long-incubation post-transfusion hepatitis, to which no other known causative agent could be assigned. However, the peak period of antigen detection could not be found; antibodies against the antigen (called then the "HC antigen") were detected in only 4 of 13 patients despite long follow-up in the recovery phase; "HC-antigen" was
also found in 4 out of 10 patients with short-incubation post-transfusion NANB hepatitis; "HC-antigen" was detected in some patients before blood transfusion and in patients not transfused at all; and finally their control sera did not cover a wide enough spectrum of diseases and "healthy" people.

Vitviski et al, 1979 (53) produced yet another assay by immunodiffusion using again sera from repeatedly transfused and convalescent patients against sera from 14 patients with early acute NANB-hepatitis. To assess the specificity of the test, sera from 17 patients with A-, B-, and drug hepatitis were also processed. The 14 patients in the study suffered NANB-hepatitis of long incubation period (more than 6 weeks), after having been transfused. "NANB-antigen" was detected in 12 (86%) of the 14 patients, but in none of the other groups. The level of "antigenaemia" correlated well with the level of aminotransferases in the serum, and the "NANB antigen" was also demonstrated by immunodiffusion in liver extracts from patients with chronic NANB hepatitis with "antigenaemia". Further on by means of fluorescein-labelled immunoglobulins, with "NANB-antibody" activity, they showed fluorescence in foci of hepatocytes on liver sections of these patients, but not on those of 6 control human livers.

At about the same time Prince et al, 1978 (51) using radio-immunoassay (RIA) and Tabor et al, 1979 (52) using counter-electrophoresis reported the discovery of similar antigen-antibody systems specifically associated with NANB infection.
In June 1980, just prior to the international workshop on hepatitis in Vienna, Austria, a panel of coded sera, was distributed to 15 laboratories actively engaged in the development of NANB tests. Analysis of the results showed that no laboratory managed to break the code successfully, and there were tremendous problems with both specificity and reproducibility. However, there were also problems with the constitution of the panel itself, particularly in the small number of biologically tested negative controls. So a second panel of paired sera, including 7 negative controls (from 2 liver-disease patients, and 5 highly pedigreed donors with normal ALT and repeatedly safe recent blood donations), 8 proven infectious and 3 probably infectious were distributed to seven of those laboratories. The results were equally disappointing. All of the tests lacked in specificity (the differences were statistically insignificant), while for each one of the laboratories the results on the paired sera had from 50 - 100% concordance (54). Thus it was concluded that agar gel diffusion, counterelectrophoretic and immunofluorescent methods were all, beyond doubt, detecting not a non-A, non-B antigen, but probably some factor unrelated to infectivity.

Then came the study by Suh et al, 1981 (55) to shed some light in the confusion. An immunodiffusion system detecting an "antigen" was devised using again acute-phase and recovery sera from patients with transmission-proven NANB acute hepatitis. In an elaborate and meticulously planned and
executed series of experiments, they showed that the "antigen" was also present in a high proportion of patients with other liver diseases, as well as in conditions with high levels of circulating immune complexes in the serum. Manipulating the sera by various techniques (fractionation of "antigen"-containing sera by column chromatography, treatment with polyethylene glycol, and reduction and alkylation) they were able to show that the so called antigenic activity was probably due to the immune complexes rather than to specific viral components.

Indeed other investigators have drawn attention to the high levels of circulating immune complexes in non-A, non-B hepatitis (7,56). So Suh et al concluded that despite their failure to confirm detection of an antigen/antibody system in serum, circulating virus must be present there, as demonstrated by the infectivity of blood from NANB hepatitis carriers and the transmission experiments (see Chapter IV). Antigen, though, may only be produced in small quantities and immediately bound with antibody in the formation of immune complexes, and thus effectively hidden from detection so far.

Taking into account the advices by Suh et al., to investigate further the specificity of the NANB- "antigen" and "antibody" assays, Duermayer et al, 1983 (57) came up lately with yet another assay, an ELISA, and claims that this can detect an antigen (termed DS-antigen) related to NANB-hepatitis.
A relatively high prevalence of this was found, as expected, in patients with haemophilia (9%), who are regularly treated with blood-clotting factor-concentrates. Antibodies to DS-antigen were found in 48% of these patients. The DS-antigen was also found in 0.6% of volunteer blood donors in West Germany (out of 1400), and antibody in 3% of such sera. Interestingly a high prevalence of antibodies to DS-antigen (41%) was found in prostitutes, suggesting a possible sexual route of transmission.

We have already described in the previous chapter the virus-like particles observed by investigators, in liver sections and other infective materials derived from chimpanzees. Similar particles, however, have also been described from human materials lately.

Coursaget et al, 1979 (58) found virus-like particles in the acute-phase serum from one haemodialysis patient with NANB hepatitis, and in the acute-phase urine of two other icteric NANB cases. The particles had an electron-dense core and a closely bound envelope. Each one was 60nm in diameter, with a core of 40nm, resembling the structure of a toga virus.

Similarly, Prince et al, 1979 (72) observed particles measuring 37nm in diameter in human plasma derived from a patient with NANB-hepatitis.
Hantz et al, 1980 (73) also reported the visualisation of small spheres and filaments 15-25nm in diameter, along with 35-40nm particles, very similar to Dane-particles in sera of patients with NANB-hepatitis. These could not be detected in neither patients with drug associated, or type A hepatitis, nor cases with obstructive jaundice also examined. These particles did not express Hepatitis B surface antigen activity, but were associated in three patients with significant endogenous DNA-polymerase activity, which further suggests that they might have been viral in nature.

Finally, Cabral et al, 1981 (68) doing electron-microscopy of biopsies of human cases of NANB-hepatitis reported characteristic **nuclear changes** in hepatocytes similar to those already described in some chimpanzee liver sections (64,66) (see Chapter IV) consisting of: increased lobulization of the nucleus, condensation and margination of chromatin, thickening of the nuclear membrane; and the presence of 3 categories of intranuclear particles. The first consisted of particles measuring approximately 25+ 2nm diameter within the nucleus of hepatocytes (identical to those described by Shimizu et al, 64, in nuclei of chimpanzee hepatocytes). The second consisted of particles measuring approximately 4-14nm in diameter, smaller than any virus capsid yet identified within nuclei. The third consisted of particles (in clusters) measuring approximately 35nm in diameter and situated at the inner periphery of the nucleus. It is worthwhile noting
that these last particles closely resembled both in appearance and location microspheres of perichromatin granules. Such microspheres have been reported in nuclei of human lung epithelial and W138 cells infected with CMV (74,75) and in glial cells infected with herpes simplex virus (76) and are presumed to represent viral nucleoids, or morphologic substrates of viral premessenger RNA (76).
As it has been shown in many studies of hepatitis B, persistence or recurrence of biochemical abnormalities and/or clinical symptoms and signs for more than 6 months is strongly associated with continuing liver damage histologically shown. Continuing histological abnormalities predispose and eventually evolve in clinically and/or biochemically evident liver disease. Thus it has been conventionally agreed that persistence of the biochemical and histological abnormalities of the liver is the hallmark of progression to "chronic liver disease", a term used to include many different clinico-pathological entities associated with considerable morbidity and mortality.

At this point we think it is necessary to mention that, what is referred to as "biochemical abnormalities" by most of the investigators is mainly increased (at least 2½ times above normal) levels of alanine aminotransferase (ALT, SGPT) and/or aspartate aminotransferase (AST, SGOT) (or transaminases according to the older nomenclature). As "histologic abnormalities" compatible with chronic liver disease are mainly understood the following:

(a) **Chronic persistent hepatitis (CPH)** is characterised by expansion of the portal zone by mononuclear cells and some fibrosis. The limiting plate of liver cells between portal zones and liver cell columns is intact. Piecemeal necrosis of liver cells is absent.
(b) **Chronic lobular hepatitis (CLH)** is sometimes termed prolonged or unresolved acute hepatitis. The picture is predominantly that of intralobular inflammation and necrosis. Piecemeal necrosis and bridging necrosis are not seen.

(c) **Chronic active hepatitis (CAH)** is marked by the presence of inflammatory infiltrate, primarily of lymphocytes and plasma cells, which greatly expands the portal areas, and also extends into the liver lobule, causing erosion of the limiting plate and piecemeal necrosis. In its most severe form, confluent necrosis extends from portal zones to centrizonal areas, the reticulum framework collapses and connective tissue septa start invading into the liver cell columns (bridging necrosis), with isolation of groups of liver cells in the form of rosettes.

(d) **Liver cirrhosis (LC)** is defined as widespread diffuse fibrosis with nodule formation (regenerating liver cells with abnormal architecture)

(e) **Hepatocellular carcinoma (HCC)** The tumor cells are smaller than normal cells with granular cytoplasm and large hyperchromatic nuclei; mitoses are conspicuous; giant cells may be seen; the stroma is scanty and the tumour cells have blood spaces between them. (100)

The frequency with which biochemical and histologic abnormalities persist for at least 6 months after the onset of the acute episode of NANB-hepatitis greatly varies from series to
series (see table VII). Thus we see that for post-transfusion hepatitis, persisting biochemical abnormalities are encountered in 23 - 60% of patients with acute disease, after close follow-up. For the sporadic NANB cases prolonged transaminasaemia is seen in 7 - 42.5% of patients with the acute disease.

Interestingly, as shown in the study by Rakela et al, 1979 (80), if we try to analyse further the group of sporadic cases, chronic biochemical abnormalities are found more often in patients with a history of past transfusion (54%) and among the drug-addicts (58% of chronicity) and less often in the group of people with no obvious risk factor in their history (20%). Now, although this difference can be due to other factors (severity of underlying disease, degree of immunosuppression, etc.) most investigators tend to attribute it to the multiplicity of causative agents (28, 46, 7, 80). In support of this latter view may come (a) the reports by Khuroo et al, 1980 (39, 91) who did not observe any progression to chronicity in the vast majority of patients in the epidemic in Kashmir, India (1%); (b) the report by Bamber et al, 1981 (46) who observed that all of their patients (100%) treated with a batch of factor VIII concentrate developed biochemically and histologically proven chronic NANB-liver disease, following the acute episode; (c) the report by Hruby and Schauf, 1978 (12) who did not observe any such progression in a similar group of patients (based on clinical and biochemical criteria only).
It has been pointed out, however, that although persistently elevated aminotransferase levels are associated with continuing liver damage, the normalization of these does not exclude chronic disease (79, 86). So besides regularly checking the aminotransferase levels, doing liver biopsies repeatedly, and examining them histologically is considered a necessity after the 6 month period, if one is to evaluate adequately the hepatitis status of patients who had had serum biochemical resolution and in patients with continuing abnormalities, also. So much so, since Soloway et al, 1971, (87) has clearly shown that the percutaneous biopsy technique, picking only a liver sample, is subject to both observer error and sample error, and accurate estimation of the occurrence of chronic liver disease by examination of just one sample is impossible. Further, transmission studies to chimpanzees (despite the financial, practical and other problems they carry) are an alternative, especially for the identification of the "healthy" carrier state, who may otherwise be difficult to trace at the moment, since no specific serologic markers have yet been widely recognized.

Indeed, the presence of such an infectious chronic carrier state in NANB-disease has been unequivocally suggested by the transmission studies (see earlier chapter). Hoofnagle et al, 1977 (32) reports that sera, collected from blood donors up to 385 days after being implicated in causing NANB-hepatitis to recipients, could cause again disease in volunteers inoculated with them. Alter et al, 1978 (47) successfully transmitted NANB-hepatitis to a chimpanzee
using serum from a blood donor with fluctuating ALT (56-474 U.I./L) who was also implicated in 2 cases of NANB-hepatitis in humans. Tabor et al, 1978 (29) also transmitted the disease to two chimpanzees using two different inocula. One derived from a donor implicated in a case of NANB-disease in a recipient 5 years earlier, and another derived from a second donor whose blood caused disease to the recipient 1½ years earlier. Again, by chimpanzee-transmission studies, Tabor et al, 1980 (88) documented the persistence of an agent of NANB origin in the blood of an asymptomatic patient for six years (the longest reported), even at a time when aminotransferase levels had temporarily returned to normal.

Hollinger et al, 1978 (59) managed to transmit the disease to chimpanzees using sera from 3 blood donors with consistently normal ALT levels, who, nevertheless, were suspected of having transmitted the disease to 2 recipients earlier.

Duermayer et al, 1983 (57), during their effort to produce the ELISA system described in a previous chapter, successfully transmitted NANB-hepatitis in a chimpanzee using some from the DS-antigen containing serum derived from a haemophiliac with persistently (more than 2 years) elevated ALT (100-300 U.I./L).

From the Transfusion-Transmitted-Viruses-Study by Aach et al, 1981 (18) we are informed that the incidence of NANB-Post-Transfusion Hepatitis among recipients of single blood or plasma units can vary from 4% (when donor ALT is less than 14 U.I./L) to 50% (when donor ALT is more
than 60 U.I./l). Moreover, 35% of donors with ALT levels of more than 60 U.I./l and 47% with more than 60 U.I./l are associated with NANB-hepatitis in recipients, as compared with 13% only with ALT levels of less than 30 U.I./l.

These data emphasize, on one hand, that completely asymptomatic and with normal liver function tests people can, nevertheless, be carriers of the NANB agents; and on the other hand, that the higher the ALT is, the more likely they are to transmit the disease. Further, the hepatitis risk in recipients increased dramatically if more than one unit of blood with an elevated (more than 45 U.I./l) ALT was administered: 10 out of 11 patients receiving two such units developed NANB-hepatitis. They also showed that by eliminating blood from donors with ALT levels of more than 45 U.I./l, one could expect to reduce the incidence of post-transfusion hepatitis (PTH) by 30-40%. On the other hand, up to 70% of PTH will not be prevented by screening donors for ALT simply because of the existence of the asymptomatic "healthy" carriers. All the above results were confirmed in a similar study by Alter et al, 1981 (89), and the proposal has been made to eliminate blood derived from donors with high ALT levels. However, this does not prove very practical in view of the high demands in blood and blood products in various hospital units on one hand, and the high prevalence of elevated ALT among blood donors in different areas all over the world (90). Besides, elevated ALT can also be due to racial differences, increased alcohol consumption, higher prevalence of other diseases, consumption of hepatotoxic drugs (like acetaminophen), and even sexual differences.
(male donors showing significantly more often elevated ALT than females) (90).

One interesting question which arises therefore is if there is a more reliable way to identify NANB-carriers and exclude them from the blood donor population, and whether liver biopsy of healthy individuals could be a justifiable and efficient method of doing it, in view of our lack of a specific serologic test for NANB-agents. The histologic status of chronic carriers of NANB-agents is as yet unknown, though.

As has been mentioned earlier, there are many studies today that have evaluated the development of chronic forms of liver disease following an acute attack of NANB-hepatitis. Histologic examination of liver specimens from many of these studies suggests that all forms of chronic hepatitis, as well as cirrhosis of the liver do occur, but in variable frequencies (see table VII).

In the study by Knodell et al, 1977 (92) out of 10 chronic NANB-post-transfusion hepatitis patients, 8 showed CAH in liver biopsy specimens. It was also observed that multiply transfused patients were more likely to progress to chronicity, and that the administration of immune serum globulin before the transfusion was associated with a lower frequency of chronic infections.
Rakela et al, 1979 (80) prospectively studied transfused patients and of those developing chronic NANB-hepatitis, 7 were biopsied. Three had evidently CPH, 3 CAH and one cirrhosis. This last patient eventually died of secondary hepatic failure 42 months after his acute hepatitis, giving evidence that chronic liver disease is a potentially lethal complication of acute NANB-hepatitis.

Berman et al, 1979 (78) in a similar prospective study in previously transfused people, observed that 2 of 8 biopsied chronic patients had CPH, 6 had CAH and that one of those with CAH also showed evidence of early cirrhosis. The investigators, however, after prolonged follow-up, pointed out that most of their patients with CPH and CAH tended to improve both clinically and biochemically as time passed, which together with the absence of bridging necrosis or active cirrhosis histologically, allowed them to expect a better prognosis than that usually attributed to CAH.

Koretz et al, 1976, 1980 (93, 81) in similar prospective studies found a higher proportion of patients with CPH (10 out of 18 biopsied) apart from the patients with CAH (7) and cirrhosis (1). Again none of those with CAH had bridging necrosis histologically, and when 5 of them were put on immunosuppressive treatment, 2 demonstrated CPH, while another progressed to become the second cirrhotic in the series. Two untreated originally CAH patients eventually regressed to CPH spontaneously after 2.5 and 3 years, suggesting
again that, although progression to more serious liver disease does occur, improvement seems to be the rule. Alter and Hoofnagle, 1982 (54) seem to share the same opinion. Although most of their 20 patients had CAH, it was a clinically and histologically mild form of it, and the patients tended to improve with time. Nevertheless, 2 of their CAH patients progressed to cirrhosis, while a further two had cirrhosis on their initial biopsy, 11 and 16 years respectively after their acute episode of NANB post-transfusion hepatitis.

Realdi et al, 1982 (79), however, is not so optimistic. After prolonged follow-up of their 13 patients, they were able to detect progression to cirrhosis in 5 of them (39%), one dying eventually of gastrointestinal bleeding. Interestingly, the progression was asymptomatic in most patients, the clinical and biochemical features were not indicative of it, and liver histology was proven essential in accurately identifying the severity of the chronic liver disease.

Bamber et al, 1981 (46) studied the progression to chronic liver disease of 10 patients with haemophilia and Von Willenbrandt's disease who received infusions of factor VIII concentrates and developed short incubation acute NANB-hepatitis. All 10 progressed to chronicity and of 5 chronic cases biopsied, 3 had evidence of CAH and 2 of CPH. This contrasts with the report by Hruby & Shauf, 1978 (12) who did a similar study in 6 haemophiliac children with acute short-incubation NANB-hepatitis developing after single infusion of commercial factor VIII concentrate. In their
only one patient with prolonged course of the disease, they only found "minimal inflammatory changes" in the liver biopsy. And of 6 haemophiliacs with short-incubation symptomatic acute NANB-hepatitis reported by Kim et al, 1980 (8), 3 had prolonged (of more than 6 months duration) ALT elevation, but histologic characterisation of their liver injury was inhibited by reluctance to do liver biopsy, due to their underlying disease.

The group of sporadic acute NANB-hepatitis patients presents many difficulties for the investigator who wants to study them prospectively. It is a non-homogeneous group, comprised of patients with diverse mode of exposure to the NANB-agents, diverse socio-economic and educational status, some of who have little or no hygienic education (like the drug-addicts) and present many problems to management and follow-up. It would be expected though that different subgroups would show different patterns in the frequency of progression to chronicity and the histological picture of their (chronic) disease. Those with a history of transfusion for example would be expected to progress to chronic liver disease more often, as Bamber et al, 1981 (46) suggests, and histologically to fall within the patterns already described.

In drug-addicts chronic liver disease following acute NANB-hepatitis is frequent as mentioned earlier. Rakela and Redeker, 1981 (94) claim that CPH is the predominant histologic feature in these patients, but they have described the
development of CAH with cirrhosis in at least 2 drug-addicts in one series, one of who died with hepatic failure. Norkrans et al, 1979 (85) also documented CPH as the predominant form of chronic disease in sporadic cases, in who drug-addiction was the predominant epidemiological background. Three out of 4 of the chronic cases biopsied in this study had CPH (and 2 were drug-addicts) and the fourth had cirrhosis following post-transfusion acute NANB-hepatitis.

Summarizing, we observe that there seems to be no doubt about the propensity of post-transfusion (and parenterally-transmitted in general) acute NANB-hepatitis to progress to chronic liver disease, of which CAH is the most frequently seen entity (3, 78, 80, 79, 54). It seems, though, that there is still quite an amount of controversy regarding the severity and the prognosis of it (78, 80, 81, 93, 79). In the sporadic cases chronicity does occur, CPH being the more likely outcome, especially among the drug-addicts subset and some of those with unknown exposure (46, 94, 85). With regard to the usually epidemic non-parenterally transmitted hepatitis, more often reported from India, but which is also likely to account for at least some of the cases with sporadic NANB-hepatitis without obvious exposure in the developed countries, chronicity seems to be virtually unknown (46, 91, 39).

To try to give one reason for this diversity would be rather difficult. Certainly many factors are responsible —the
variable amount of viral bodies administered, the immune status of the infected individual are just two of them. More likely, there is the possibility of multiple NANB viral agents one of which may be associated with non-parenteral transmission and no progress to chronicity; and probably 2 viral agents both associated with transfusion of blood or blood products or with parenteral exposure in general, and of which one is further associated with an unusually high tendency for the development of CAH and cirrhosis (94, 46, 79, 92).

There has been quite an amount of debate whether certain risk factors are associated with the development of chronic NANB-hepatitis and cirrhosis.

Immunosuppressed patients, in general, show a great tendency to chronic liver disease. Of people undergoing renal transplantation (23) and contracting acute hepatitis as many as 80% might go on to chronicity. Galbraith et al, 1975, 1979 (96, 20) reported an outbreak of NANB-hepatitis in a haemodialysis unit. 28% of 29 cases showed elevated ALT for more than 6 months. Liver biopsies performed in 5 of them showed that 3 had CAH (2 with superimposed cirrhosis, as well) and 2 had CPH. One patient died 6 years after the acute episode from hepatic failure.

It is still unclear whether chronicity results in babies with congenital acute NANB-hepatitis (reported by Tong et al, 1978, 41).
According to Knodell et al, 1977 (92) and Kiyosawa et al, 1982 (97) sex seems not to influence the propensity to chronicity, in contrast with HBV infection which seems to cause chronic liver disease considerably more often in males. Berman et al, 1979 (78), however, reports that 75% of those who developed chronic hepatitis in their series were men (male to female ratio = 3:1), whereas men constituted only 50% of the acute NANB-hepatitis cases who were prospectively followed up; and Rakela et al, 1979 (80) reports similar figures (see table III).

The severity of symptoms, the severity of peak amino-transferase elevation and the presence or absence of jaundice during the acute stage probably do not indicate a higher risk for progression to chronicity (Berman et al, 1979; Koretz et al, 1980; Realdi et al, 1982; Kiyosawa et al, 1982; 78, 81, 79, 97). Nevertheless other investigators have emphasized that symptomatic acute disease was significantly more frequent in the group of patients who developed chronic liver disease than in the group whose acute hepatitis resolved (Knodell et al, 1977; 92); that chronicity followed acute anicteric hepatitis significantly more often than icteric hepatitis (Rakela et al, 1979; 80); or that among anicteric patients there was a tendency to chronicity if SGPT (ALT) levels during acute disease exceeded 300 U.I./l (Berman et al, 1979; 78).

Today there is overwhelming evidence for an aetiological association between prolonged HBV infection and hepatocellular
carcinoma (HCC). Such evidence, though, is still lacking for NANB-agents and long prospective studies, as well as a specific serologic test for the detection of NANB viral antigens are required to prove it. Nevertheless, recently data start accumulating pointing to a possible association.

Anderson et al, 1982 (98), reported the case of a 51 year old woman with persistently elevated aminotransferases, with a history of cholecystectomy and carbimazole treatment for thyrotoxicosis, who was serologically negative for markers of HBV, HAV, CMV and EBV infections. On liver biopsy she showed peculiar ultrastructural changes which among other included:

(a) intranuclear cytoplasmic pseudo-inclusions which have been reported in malignant apudoma tissue;

(b) alterations in the arrangement of mitochondrial cristae and intramitochondrial rod-like inclusions also observed in many carcinomas (lung, breast); and

(c) intranuclear glycogen deposits and hypertrophy and dilatation of Golgi complexes which together with the intranuclear cytoplasmic infoldings (a) are regarded as features of human hepatocellular carcinoma. Cells with these features were few in the liver biopsy, but it is well known that HCC is initiated as a clonal focal growth.

Out of 108 patients with HCC reviewed in a retrospective study by Kiyosawa et al, 1982 (97) in Japan, 53 were thought to have no evidence of HBV chronic infection when sera
and liver tissues were tested for markers of HBV infection, and were named NANB-HCC. Of these 53 patients, 7 (13%) had a definite history of blood transfusion, and of these 7, 4 (57%) had a history of post-transfusion hepatitis.

The mean interval from the date of blood transfusion to the diagnosis of NANB-HCC was 23.5 years and differed significantly from the mean interval to the diagnosis of NANB chronic hepatitis (13.5 years). Taking also into account that NANB-acute hepatitis accounts for more than 90% of post-transfusion hepatitis (1, 2, 3, 4, 5) it is highly probable that these 4 patients (at least) had developed their hepatocellular carcinoma as a consequence of a chronic infection with some NANB agent(s).

However, the frequency of development of HCC in patients with NANB-chronic liver disease is unknown. It can be speculated that at least part of the HCC without history or serological markers of HBV infection is due to the NANB virus(es), but until virus-specific antigens are identified and specific serologic tests for NANB-hepatitis become available, the investigation will be hampered. So much so, since the hepatocellular carcinoma is the cumulative result of several cofactors which include genetic, immunological, nutritional and hormonal factors, mycotoxins, pyrrozolidone alkaloids, chemical carcinogens and other environmental influences; and hepatitis-B virus and perhaps the non-A, non-B virus(es) act either as a carcinogen or a cocarcinogen in the persistently infected hepatocytes (99).
ABSTRACT

Multiple bacteria, rickettsiae, fungi, parasites, viruses and toxic agents are capable of producing the clinical syndrome that we understand today under the term "hepatitis", which was also well recognised over the centuries as a serious disease often associated with considerable mortality.

In recent years the application of sensitive immunoassays has clearly demonstrated that a variable portion of cases of acute hepatitis and about 90% of post-transfusion hepatitis cannot be classified by aetiologic agent and are designated therefore as non-A, non-B hepatitis.

Epidemiological and clinical studies have indicated two major patterns of transmission of non-A, non-B hepatitis - one by the parental route and a second by the non-parental route. Clinical observations suggest further the possible existence of at least two non-A, non-B agents transmitted parenterally that are immunologically distinct. Transmission studies in chimpanzees not only confirm this, but also reveal strain-specific ultrastructural changes in the hepatocytes of infected animals.
Acute non-A, non-B hepatitis runs a rather mild course, but spontaneous resolution of it fails to occur in up to 60% of cases. A chronic asymptomatic, yet infectious carrier state has clearly been demonstrated, and all kinds of chronic hepatitis have been repeatedly recognised. Although chronic non-A, non-B hepatitis is usually mild, further progression to cirrhosis can occur in variable frequency. The development of hepatocellular carcinoma on a background of a prolonged NANB-infection has not yet been unequivocally established, but the indications are that it can occur.

Multiple serologic systems claiming to be detecting NANB-viral agents have been developed, but their specificity has been proven poor, and thus our better understanding of this challenging disease is still hampered.
Table 1
Incidence of acute NANB post-transfusion (P.T.) hepatitis in recipients of only volunteers blood

<table>
<thead>
<tr>
<th></th>
<th>Number of total hepatitis patients</th>
<th>Overall P.T. hepatitis incidence in the particular centre</th>
<th>NANB-hepatitis (% of total hepatitis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knodell et al (1977)</td>
<td>279</td>
<td>17%</td>
<td>94%</td>
</tr>
<tr>
<td>Aach et al (1978)</td>
<td>389</td>
<td>7.5%</td>
<td>90%</td>
</tr>
<tr>
<td>Sieff et al (1977)</td>
<td>539</td>
<td>7%</td>
<td>95%</td>
</tr>
<tr>
<td>Tateda et al (1979)</td>
<td>1082</td>
<td>11.5%</td>
<td>93%</td>
</tr>
<tr>
<td>Alter et al (1978)</td>
<td>728</td>
<td>8%</td>
<td>93%</td>
</tr>
<tr>
<td>Berman et al (1979)</td>
<td>30</td>
<td>7.7%</td>
<td>87%</td>
</tr>
</tbody>
</table>

N.B. Overall hepatitis ranges from 7 - 17% between centres (from developed countries) but the proportion of NANB-hepatitis is relatively constant with a range of 90-95% in the big studies.
Table II

Relative frequency of all types of post-transfusion hepatitis among 250 polytransfused homozygous B-thalassemic children in Greece

(Papaevangelou et al, 1980) (10)

<table>
<thead>
<tr>
<th>Type of hepatitis</th>
<th>Number of cases</th>
<th>Percentage of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hep. A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hep. B</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Hep. non-A, non-B</td>
<td>14</td>
<td>78</td>
</tr>
<tr>
<td>Cytomegalovirus hep.</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

NB. In Greece the non-A, non-B viruses appears to be the most important agents of post-transfusion hepatitis among thalasssemics.
Table III
Distribution of patients with acute and chronic NANB-hepatitis in different studies in relation with sex

<table>
<thead>
<tr>
<th>Epidemiological background</th>
<th>Number of cases</th>
<th>Male</th>
<th>Female</th>
<th>M/F Ratio</th>
<th>Number of cases</th>
<th>Male</th>
<th>Female</th>
<th>M/F Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berman et al (1979)</td>
<td>post-transfusion</td>
<td>26</td>
<td>50%</td>
<td>50%</td>
<td>1:1</td>
<td>12</td>
<td>75%</td>
<td>25%</td>
</tr>
<tr>
<td>Rakela &amp; Redeker (1979)</td>
<td>sporadic</td>
<td>45</td>
<td>NA*</td>
<td>NA</td>
<td>-</td>
<td>18</td>
<td>77%</td>
<td>23%</td>
</tr>
<tr>
<td></td>
<td>post-transfusion</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>86%</td>
<td>14%</td>
</tr>
<tr>
<td></td>
<td>drug-addicts</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>non-obvious</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>25%</td>
<td>75%</td>
</tr>
<tr>
<td>Knodell et al (1977)</td>
<td>post-transfusion</td>
<td>44</td>
<td>64%</td>
<td>36%</td>
<td>1.7:1</td>
<td>10</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>Farrow et al (1981)</td>
<td>sporadic</td>
<td>48</td>
<td>58%</td>
<td>42%</td>
<td>1.4:1</td>
<td>NA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bamber et al (1983)</td>
<td>sporadic</td>
<td>22</td>
<td>77%</td>
<td>23%</td>
<td>3.3:1</td>
<td>NA</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*NA = data not available
Table IV
Prevalence of NANB hepatitis among sporadic cases

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Farrow et al (1981) (U.K.)</td>
<td>368</td>
<td>215(58)</td>
<td>98(27)</td>
<td>5(1.5)</td>
<td>2(0.5)</td>
<td>-</td>
<td>48(13)</td>
</tr>
<tr>
<td>Bamber et al (1983) (U.K.)</td>
<td>172</td>
<td>88(51)</td>
<td>58(34)</td>
<td>-</td>
<td>4(2)</td>
<td>-</td>
<td>22(13)</td>
</tr>
<tr>
<td>Norkrans et al (1979) (Sweden)</td>
<td>480</td>
<td>107(22)</td>
<td>297(62)</td>
<td>-</td>
<td>-</td>
<td>1(0.2)</td>
<td>63(13)</td>
</tr>
<tr>
<td>Alter et al (1982) (USA)</td>
<td>295</td>
<td>30(10)</td>
<td>141(48)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>124(42)</td>
</tr>
<tr>
<td>Papäevangelou et al (1979) (Greece)</td>
<td>222</td>
<td>24(11)</td>
<td>178(80)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20(9)</td>
</tr>
<tr>
<td>Papäevangelou et al (1979) (Greece)</td>
<td>(13)</td>
<td>(69)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(18)</td>
</tr>
<tr>
<td>Villarejos et al (1981)* (Costa Rica)</td>
<td>1284</td>
<td>937(73)</td>
<td>325(25)</td>
<td></td>
<td></td>
<td></td>
<td>22(1.7)</td>
</tr>
<tr>
<td>National Hospitals** (1980) (Japan)</td>
<td>167</td>
<td>20(12)</td>
<td>71(425)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>76(45.5)</td>
</tr>
<tr>
<td>University of Tokyo** (1980) (Japan)</td>
<td>90</td>
<td>17(19)</td>
<td>30(33)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>43(48)</td>
</tr>
<tr>
<td>Shimshu Univ. Hosp.** (1980) (Japan)</td>
<td>108</td>
<td>21(21)</td>
<td>33(32)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>48(47)</td>
</tr>
</tbody>
</table>

* from R J Gerety's Non-A, non-B hepatitis, 1981 (pp 158-188)
** from R J Gerety's Non-A, non-B hepatitis, 1981 (pp 158)
### Table V

**Risk factors associated with sporadic NANB-disease**

#### Major
- Blood transfusions \((38, 28, 48, 85)^*\)
- Parental drug use \((38, 28, 85)\)
- Employment in hospital patient care or laboratory work \("needlestick exposure\) \((38, 28, 48, 85)\)
- Personal contact with other persons with hepatitis \((38, 48)\)
- Raw shellfish ingestion \((38, 28)\)

#### Probable
- Visit to endemic areas \((38, 28, 85)\)
- Various invasive procedures \((i.e.\) surgical, dental, ear-piercing, vaccinations) \((38, 28, 48)\)
- Homosexual contact \((38)\)
- Heterosexual contact \((57)\)
- Imprisonment/institutional dwelling \((38, 28, 48)\)
- Tattooing \((85)\)

* Numbers in parentheses refer to studies from which these data come. However, it must be emphasised that this table is mostly based on the study by Alter et al, 1982 \((38)\) which is comparing patients with NANB-hepatitis on one hand with matched control subjects on the other.
Table VI
Clinical aspects of patients with acute NANB hepatitis.
Relative frequency of asymptomatic and anicteric cases*

<table>
<thead>
<tr>
<th>Epidemiological background</th>
<th>Number of cases</th>
<th>Asymptomatic</th>
<th>Anicteric</th>
<th>Peak ALT elevation (times of normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koretz et al** (1980)</td>
<td>31</td>
<td>23%</td>
<td>74%</td>
<td>6-66</td>
</tr>
<tr>
<td>Prince et al (1974)</td>
<td>35</td>
<td>69%</td>
<td>83%</td>
<td>2-82</td>
</tr>
<tr>
<td>Realdi et al (1982)</td>
<td>NA</td>
<td>NA</td>
<td>61%</td>
<td>more than 2.5</td>
</tr>
<tr>
<td>Berman et al (1979)</td>
<td>15</td>
<td>24%</td>
<td>54%</td>
<td>more than 3</td>
</tr>
<tr>
<td>Bamber et al (1981)</td>
<td>26</td>
<td>NA</td>
<td>70%</td>
<td>more than 2.5</td>
</tr>
<tr>
<td>Ohori et al (1983)</td>
<td>10</td>
<td>50%</td>
<td>60%</td>
<td>more than 2.5</td>
</tr>
<tr>
<td>(non-epidemic)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(epidemic)</td>
<td>13</td>
<td>NA</td>
<td>77%</td>
<td>more than 3</td>
</tr>
<tr>
<td>Khuroo et al*** (1980)</td>
<td>24</td>
<td>NA</td>
<td>17%</td>
<td>more than 3</td>
</tr>
<tr>
<td>epidemic</td>
<td>128</td>
<td>NA</td>
<td>27%</td>
<td>more than 2</td>
</tr>
</tbody>
</table>

* The transfused patients who subsequently develop acute NANB-hepatitis constitute a unique group which can be prospectively followed-up and for which the exact incidence of symptomatic-asymptomatic, icteric-anicteric, and sub-clinical hepatitis can be accurately assessed.

** Two separate studies

*** Khuroo's results are included for comparison. The 128 patients were relatives of patients with NANB-hepatitis during the epidemic in Kashmir, India, who were followed up.

Note The similarity in the frequency of anicteric cases between reports by Ohori (epidemic, post-transfusion) and Khuroo et al (epidemic) and the difference with the rest.
<table>
<thead>
<tr>
<th>Study</th>
<th>Number of cases</th>
<th>Epidemiologic background</th>
<th>Aminotransferase elevations no(%)</th>
<th>Liver biopsy results*</th>
<th>Cirrhosis (+CAH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>More than 6 months</td>
<td>More than 1 year</td>
<td>Number of cases</td>
</tr>
<tr>
<td>Knodell et al (1977)</td>
<td>44</td>
<td>post-transfusion</td>
<td>16(36)</td>
<td>10(23)</td>
<td>10</td>
</tr>
<tr>
<td>Seef et al (1978)</td>
<td>119</td>
<td>&quot;</td>
<td>31(36)</td>
<td>12(10)</td>
<td>NA</td>
</tr>
<tr>
<td>Aach et al (1978)</td>
<td>65</td>
<td>&quot;</td>
<td>36(55)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Berman et al (1979)</td>
<td>26</td>
<td>&quot;</td>
<td>12(46)</td>
<td>12(46)</td>
<td>8</td>
</tr>
<tr>
<td>Koretz et al (1980)</td>
<td>66</td>
<td>&quot;</td>
<td>46(70)</td>
<td>40(60)</td>
<td>18</td>
</tr>
<tr>
<td>Realdi et al (1982)**</td>
<td>15</td>
<td>&quot;</td>
<td>8(54)</td>
<td>NA</td>
<td>7</td>
</tr>
<tr>
<td>Kiyosawa et al (1982)</td>
<td>70</td>
<td>&quot;</td>
<td>46(66)</td>
<td>32(46)</td>
<td>14</td>
</tr>
<tr>
<td>Rakela &amp; Redeker (1979)</td>
<td>13</td>
<td>post-transfusion</td>
<td>7(54)</td>
<td>7(54)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>drug-addicts</td>
<td>NA</td>
<td>7(58)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>non-obvious</td>
<td>NA</td>
<td>4(20)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>total (sporadic)***</td>
<td>NA</td>
<td>18(40)</td>
<td>-</td>
</tr>
<tr>
<td>Norkrans et al (1979)</td>
<td>63</td>
<td>sporadic</td>
<td>4(7)</td>
<td>NA</td>
<td>4</td>
</tr>
<tr>
<td>Bamber et al (1983)</td>
<td>22</td>
<td>&quot;</td>
<td>2(10)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Alter et al (1982)</td>
<td>80</td>
<td>&quot;</td>
<td>34(42.5)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Khuroo et al (1980)</td>
<td>275</td>
<td>epidemic</td>
<td>4(1.3)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bamber et al (1981)</td>
<td>10</td>
<td>haemophiliacs</td>
<td>10(100)</td>
<td>5(50)</td>
<td>7</td>
</tr>
<tr>
<td>Kim et al (1980)</td>
<td>6</td>
<td>&quot;</td>
<td>3(50)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

* CPH = chronic persistent hepatitis, CAH = chronic active hepatitis, CLH = chronic lobular hepatitis (or resolving hepatitis)
NA = data not available

** in this study 15 acute NANB-hepatitis patients were prospectively followed up, and 6 additional chronic hepatitis patients were evaluated

*** For Rakela & Redeker's study only
Aminotransferase elevations in acute NANB-hepatitis has been shown to follow three patterns (Tateda et al, 1979; 4) of which the biphasic type seems to be the most characteristic.
Fig. 2. Electron microscopic picture from a hepatocyte of a chimpanzee infected with a NANB-virus. Cytoplasmic abnormalities associated with the endoplasmic reticulum and looking like (concentric) tubules in cross section are clearly seen (x 30,000).
Fig. 3. Electron microscopic picture from a hepatocyte of a chimpanzee infected with the same NANB-virus. Parallel lengths of electron dense "tubules" are seen to be associated definitely with the endoplasmic reticulum (x 68,000).
Fig. 4. Again the parallel lengths of electron dense "tubules" associated with the endoplasmic reticulum. Five concentric tubules in longitudinal section can be seen here (x 68,000).

Figures 2, 3, 4 have been kindly supplied by Dr K N Tsiquaye (Microbiology Department, L.S.H.T.M).


36. Papaevangelou G, Roumeliotou A, Contoyannis P: Serological diagnosis of the various types of acute viral hepatitis and changes of the relative frequency of them during the last three years in Greece. Latrike, 39: 43-48, 1981.


45. Bradley DW, Cook EH, Maynard JE, McCaustland KA, Ebert JW, Dolana GH, Petzel RA, Kantor RJ, Heilbrunn A,


113. Bason PB: Jaundice occurring one to four months after transfusion of blood or plasma: report of seven cases. JAMA 121: 1332-1334, 1943.


