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Ultrastructural Features in Chronic Non-A, Non-B (NANB) Hepatitis: A Controlled Blind Study

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Liver biopsies from 12 patients with chronic Non-A, Non-B (NANB) hepatitis, 7 with hepatitis B surface antigen (HBsAg) positive chronic liver disease, 1 HBsAg positive normal carrier, and 4 patients with non-viral liver disease, were examined by electron microscopy for cytoplasmic and nuclear changes. Aggregates of particles measuring 20–35 nm in diameter were noted in the nuclei of 8 of 12 patients with NANB chronic hepatitis, but not in the other groups. The tubular changes seen in the endoplasmic reticulum (ER) of chimpanzees with NANB hepatitis were not noted in biopsies from any of our patients.

Key words: electron microscopy; Non-A, Non-B hepatitis

INTRODUCTION

Following the development of diagnostic serological tests for hepatitis A and B infection, it became apparent that there were additional unknown viruses causing hepatitis in man and these have been named the Non-A, Non-B (NANB) group [Feinstone et al, 1975; Mosley, 1975]. This group accounts for approximately 90% of post-transfusion hepatitis [Alter et al, 1978; Seeff et al, 1978], and has also been reported in patients receiving blood coagulation factors [Craske et al, 1975; Bamber et al, 1981a], in intravenous drug abusers [Bamber et al, 1981b], in patients and staff in haemodialysis units [Galbraith et al, 1979], and sporadically in the general population [Villajeros et al, 1975; Mosley et al, 1977; Bamber et al, 1981b]. Chronic hepatitis develops in 23–46% of infected patients [Knodell et al, 1977; Berman et al, 1979].

Antigen/antibody systems associated with NANB hepatitis have been reported [Shirachi et al, 1978; Vitvitski et al, 1979; Chircu et al, 1980], but there still remains a controversy concerning their specificity. Virus particles have also been

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seen in the serum of patients with NANB hepatitis [Hantz et al, 1980; Yoshizawa et al, 1980].

Intranuclear particles and specific ultrastructural alterations in the ER have been described in chimpanzees infected with serum from patients with NANB hepatitis [Jackson et al, 1979; Shimizu et al, 1979]. Similar intranuclear particles have also been noted in one patient with acute NANB hepatitis [Gmelin et al, 1980] and in 10 patients with NANB-associated chronic active hepatitis [Grimaud et al, 1980]. Control liver biopsies have not been examined. We have therefore examined, in a blind controlled study, the hepatocytes of 12 patients with NANB-associated chronic liver disease, 7 with HBsAg positive chronic liver disease and 4 with non-viral liver disease, to determine whether the ultrastructural alterations described in chimpanzees are present in man and to establish the specificity of these alterations for NANB hepatitis.

PATIENTS AND METHODS

Three patient groups were studied.

NANB Chronic Liver Disease (Table I)

A diagnosis of acute NANB hepatitis was made if serological markers of acute type A (IgM anti-HAV, Abbott Laboratories) and type B (HBsAg, Ausria Abbott Laboratories) infection were negative in the acute phase of the illness, and in the chronic phase if HBsAg was negative and autoantibodies (ANF, SMA) were in a concentration of less than 1/10. None had consumed more than 80 gm of al-

TABLE I. Clinical, Histological, and Biochemical Data on Patients With Chronic NANB Hepatitis Showing Nuclear Aggregates

Patient	Nuclear changes	Possible source of infection	Incubation period	Histology	Biochemistry at time of liver biopsy		
					AST	ALP	BIL
1	+	Factor VIII	NK	CAH	62	15	8
2	+	Factor VIII	2½ wk	CPH	43	7	10
3	+	Factor VIII	NK	CAH	26	5	27
4	+	Factor VIII	NK	CPH	22	5	10
5	+	Factor VIII	NK	CAH	61	13	15
6	+	Dental surgery	1 mo	CLH	170	20	276
7	+	Dental surgery	1 mo	CPH	86	9	10
8	+	NK	NK	CAH/CIRR	17	8	15
9	–	Factor VIII	3 wk	CAH	150	16	11
10	–	Factor VIII	NK	CPH	23	12	10
11	–	Factor VIII	NK	CPH	64	13	20
12	–	Blood transfusion	4 mo	CAH	111	13	16

AST, aspartate transaminase in IU/liter (upper limit = 15); ALP, alkaline phosphatase in King Armstrong Units (KA)/100 ml (upper limit = 13)—1 KA unit = 17 international units; BIL, bilirubin in mmole/liter (upper limit = 17); CIRR, cirrhosis; CAH, chronic active hepatitis; CPH, chronic persistent hepatitis; CLH, chronic lobular hepatitis; NK, not known.

cohol a day for the previous 2 years or had a history of taking hepatotoxic drugs.

Eight haemophiliac and 4 nonhaemophiliac patients underwent liver biopsies at between 6 and 36 months after either an episode of acute NANB hepatitis (7) or the first detection of abnormal liver function tests (5). Four of the haemophiliac patients demonstrated chronic active hepatitis (CAH) and 4 chronic persistent hepatitis (CPH). Of the 4 nonhaemophiliac patients, 1 had CAH with cirrhosis, 1 CAH without cirrhosis, and 2 had chronic lobular hepatitis. The aspartate transaminase concentration (AST) at the time of the liver biopsies ranged between 17 and 170 IU/liter. The alkaline phosphatase (ALP) ranged between 5 and 20 King Armstrong units/100 ml. It was elevated in 3 patients only. The bilirubin (BIL) ranged between 8 and 276 μmole/liter and again was elevated in 3 patients.

In the haemophilia group 1 patient had received only cryoprecipitate and 7 factor VIII concentrate of mainly commercial source. Four patients with previously normal liver function tests were followed prospectively after an episode of acute hepatitis. The incubation period, defined as the period between the last infusion and the onset of abnormal liver function tests, could be established with certainty in only two patients, and was 2.5 and 3 weeks. Two other patients had received several factor VIII infusions in the 12 weeks prior to the onset of the acute hepatitis, and the incubation period could not be defined. Four further patients were noted to have elevated serum transaminase levels for between 3 to 7 years prior to liver biopsy, but biochemical records were insufficient to define an acute hepatitic episode.

In the nonhaemophilia group, 1 patient had received a blood transfusion 3 months previously, and 2 patients had had dental surgery one month and two months prior to the onset of their acute hepatitis.

HBsAg Positive Chronic Liver Disease

Liver biopsies from 7 patients with HBsAg positive chronic liver disease served as a control group. Five demonstrated CAH and 2 cirrhosis. One patient was an HBsAg positive carrier with normal liver histology.

Non-Viral Liver Disease

Four patients with normal liver histology under the light microscope served as the second group of controls. One patient was found to have Gilbert's syndrome, 1 was being investigated for a carcinoid tumour, 1 demonstrated very mild fatty change, and the fourth patient demonstrated mild liver cell siderosis.

METHODS

The liver biopsy specimens were examined under code. The electron microscopic examination was carried out by two observers who had had no knowledge of the identity of the specimens. The liver biopsy material was fixed in 10% neutral buffered formaldehyde and stained with haematoxylin and eosin for routine histology. A small portion was fixed in 3% glutaraldehyde in Cacodylate buffer, and postfixed in 1% OsO_4. It was then dehydrated in graded alcohols and embedded in LEMIX. Ultrathin sections were stained with Reynolds lead acetate. The sections were examined in an electron microscope (Philips 301).

RESULTS

Ultrathin sections of 8 of the 12 liver biopsies carried out on the patients with Non-A, Non-B hepatitis demonstrated nuclear changes. These consisted of aggregates of nuclear particles (Figs. 1, 2), and less commonly some irregularities in the nuclear membrane with nuclear chromatin margination (Fig. 3). The aggregates were variable in size but usually consisted of approximately 20 particles. The diameter of the particles ranged between 20–35 nm. Within an aggregate the particles demonstrated equal diameters but their outline was not always well defined. It was usually necessary to examine a number of nuclei in order to detect particles. Nuclear particles were not detected in the HBsAg positive and non-viral liver histology group of patients, and therefore were not thought to be normal intranuclear constituents. They were unrelated to perichromatin or interchromatin particles.

We did not find any of the tubular changes of the ER which had previously been described in chimpanzees [Shimizu et al, 1979; Jackson et al, 1979].

DISCUSSION

We have found aggregates of particles, similar to those described by other groups of workers, in the nuclei of hepatocytes of 8 patients with chronic NANB hepatitis, but not in the hepatocytes of patients with chronic HBV infection or those of non-viral liver disease. Margination of nuclear chromatin was found in only two patients with chronic NANB hepatitis. Of the 8 patients with the nuclear particles, 5 patients were haemophiliacs and had received Factor VIII. Three patients demonstrated CAH and 2 CPH. Of the 3 nonhaemophilic patients demonstrating nuclear particles, dental surgery may have been a route of infection in 2. One patient demonstrated CPH, 1 CLH, and 1 CAH with cirrhosis. It is of interest that 2 of the patients demonstrating nuclear aggregates were receiving corticosteroids which may have allowed an increased level of viral replication. We failed to detect nuclear changes or particles in 4 patients with chronic NANB who in all other respects were similar to the patients with nuclear changes.

In 1979 Jackson et al reported a characteristic abnormal derangement of the ER in 4 of 5 chimpanzees which had received serum from 2 patients with NANB chronic hepatitis. This derangement took the form of 'tubules' present throughout the cytoplasm of hepatocytes during the period when the serum-alanine-aminotransferase was raised. The diameter of the 'tubules' formed by the ER alteration ranged from approximately 190 nm for those bounded by a single membrane to 206 nm for those bound by a double one. In similar studies reported by Shimizu et al later in 1979, two different ultrastructural alterations were observed in liver cells of chimpanzees inoculated with plasma derived from one patient with acute NANB hepatitis and one with chronic NANB hepatitis. During the acute phase of the illness in the group of chimpanzees which had received the chronic phase serum, peculiar tubular structures similar to those seen by Jackson et al [1979] were noted. In contrast, these structures were never detected in the liver cells of the second group of chimpanzees that received the acute phase NANB serum. However, nuclear changes, usually associated with aggregates of 20–27 nm particles were found in hepatocytes of the latter animals. These workers therefore

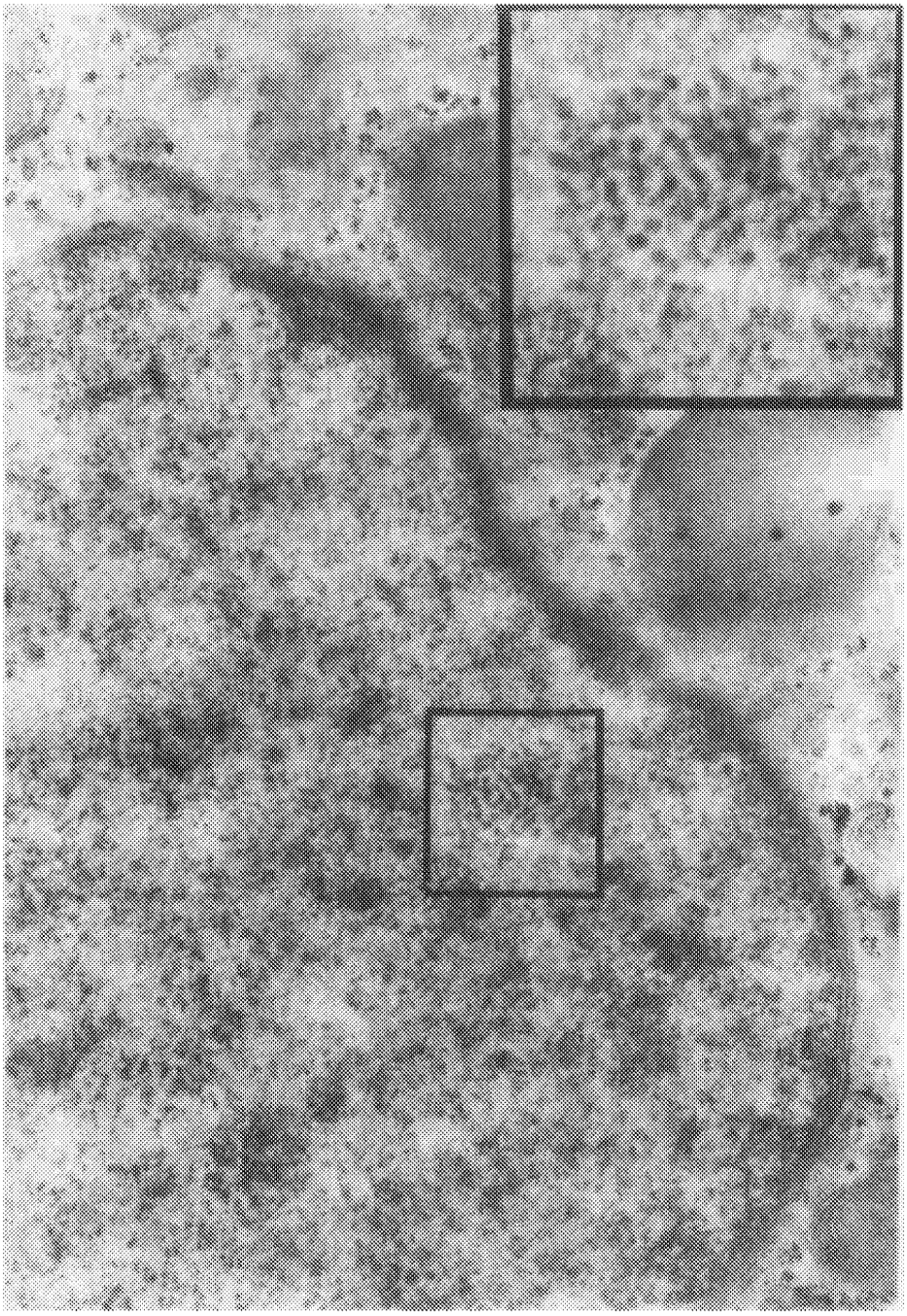

Fig. 1. An hepatocyte from patient 7 when AST was 86 IU/liter. Liver biopsy demonstrated CPH. Note the appearance of aggregates of intranuclear particles. Inset shows a higher magnification of the particles.

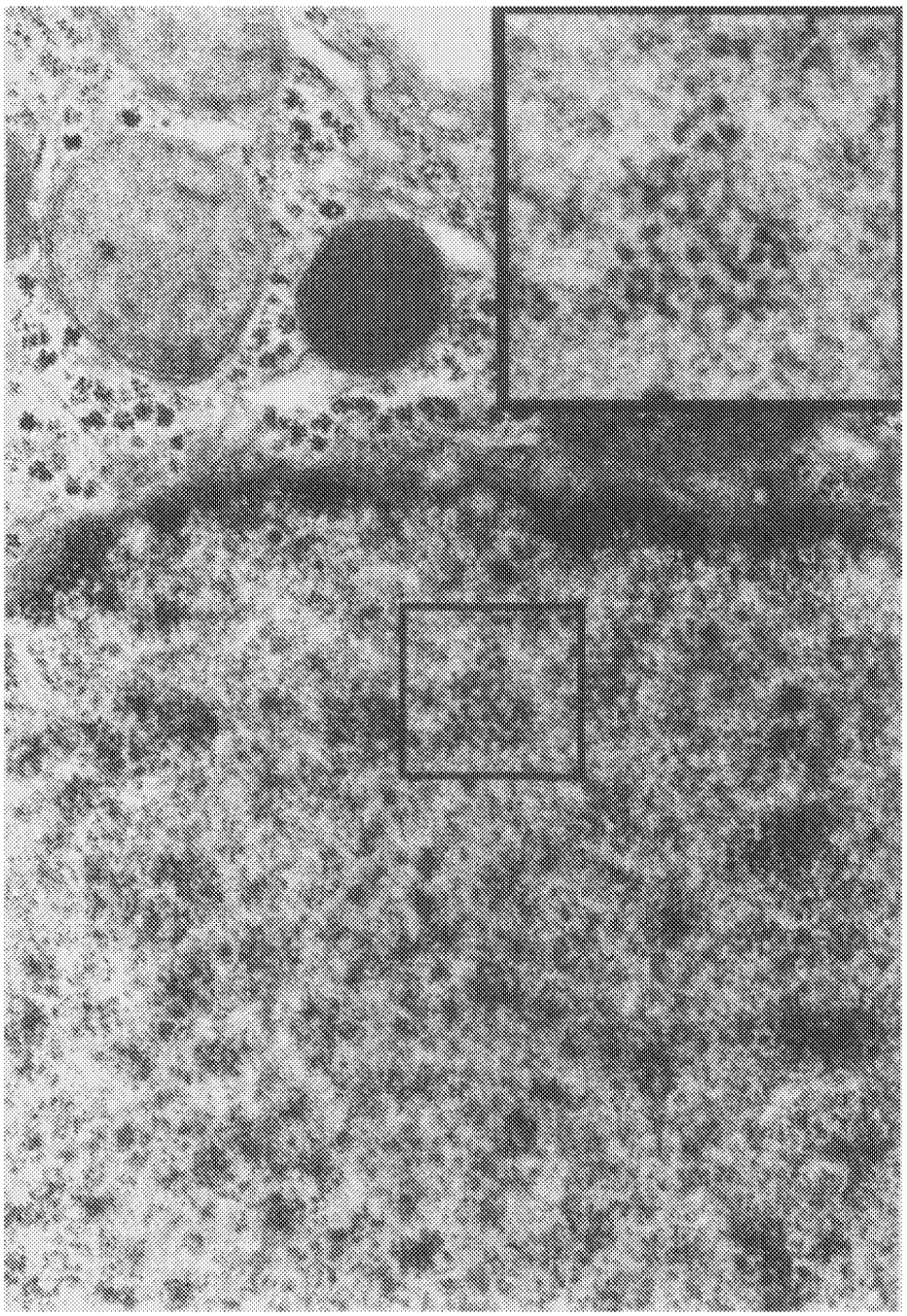

Fig. 2. An hepatocyte from patient 8, when AST was 17 IU/liter. Liver biopsy demonstrated CAH/cirrhosis. Note intranuclear particles. Inset shows a higher magnification of the particles.

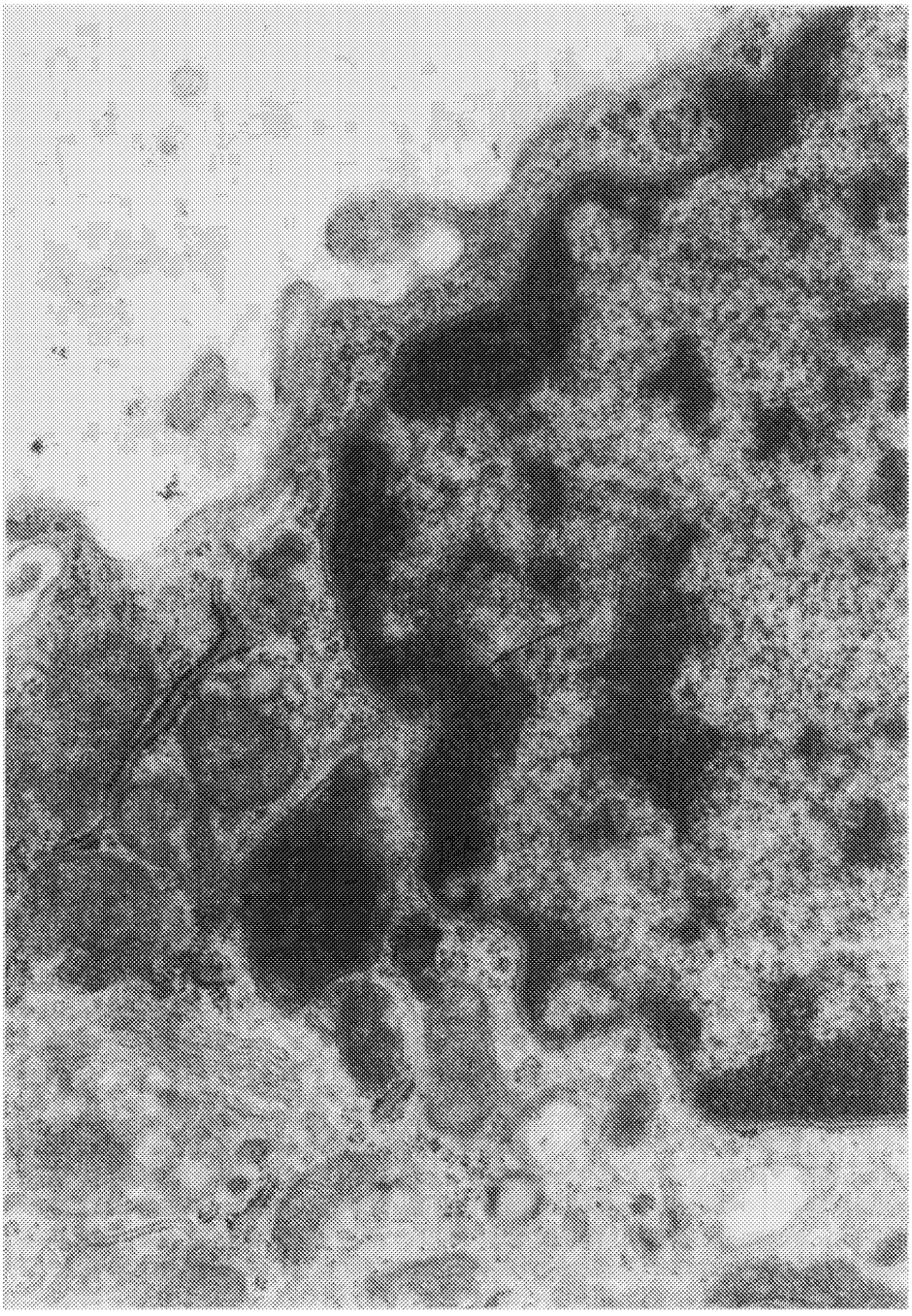

Fig. 3. An hepatocyte from patient 2, demonstrating irregularity in outline and margination of nuclear chromatin (arrow).

suggested that the different ultrastructural alterations produced by different inocula may represent the existence of more than one NANB agent. Electron microscopic studies reported in 1980 by Tsiquaye et al supported this view.

In none of the ultrastructural studies so far carried out on humans have there been the distinctive tubular changes in the ER as described in chimpanzees. It remains possible that these changes are peculiar to NANB hepatitis in chimpanzees or may be detected only in the early phase of an acute hepatitis. It is not clear whether the nuclear particles are virus particles or merely virus-asssociated changes. Studies to determine the specificity of the nuclear particles must await the availability of monospecific anti-viral sera so that immune electron microscopy may be undertaken. At the present time these nuclear particles may be a useful marker allowing positive identification of chronic NANB hepatitis in man.

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