AN EXPERIMENTAL MODEL OF HEPATITIS A IN *Macaca mulatta* INFECTED WITH HUMAN HEPATITIS A VIRUS

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The urgency of obtaining an adequate and accessible experimental model of human hepatitis A (HA) is determined by its importance for health and a universal increase in the prevalence of this disease in recent years. At present as a model of HA we use chimpanzees (*Pan troglodites*) [11], marmosets (*Saquinas mystax*) [10], and nocturnal monkeys (*Aotus trivirgatus*) [14]. The more readily available African and Asiatic lower monkeys are considered to be insusceptible to hepatitis A virus (HAV). The view is held that natural infection in these monkeys is accompanied only by seroconversion [8, 12]. Attempts to reproduce the disease experimentally by infecting these animals with human HAV (HHAV) have been unsuccessful [15]. There have been recent reports that in some species of old world lower monkeys (macaques and green monkeys) enzootics of HA are observed, with a combination of characteristic changes [1, 5, 9, 16]. Recently we succeeded in reproducing all the features of the disease (except jaundice) in Java macaques (*Macaca fascicularis*), on infecting them both with HHAV and with strains isolated from rhesus monkeys and African green monkeys developing the disease spontaneously [16].

In the investigation described below an experimental model of HA on rhesus monkeys infected with HHAV was obtained for the first time and characterized from the virologic, biochemical, serologic, and morphologic aspects.

EXPERIMENTAL METHOD

Experiments were carried out on 17 rhesus monkeys (*Macaca mulatta*, 14 males and three females), aged from 3 months to 4 years. The monkeys were imported into the Sukhumi Nursery from Vietnam and used in the experiments at the end of the quarantine period (2 months after importation). Monkeys with neither serologic nor biochemical evidence of infection were selected. Liver biopsy likewise revealed no morphologic changes. Feces from a patient with human HA, collected at the beginning of the icteric period of the disease, and feces from monkeys wiith HA were used for subculture. Material was injected either intravenously or perorally or by a combination of both methods. HAV antigen in samples of feces and in autopsy material and antibodies to them were determined by solid-phase radioimmunoassay (RIA) [2, 3]. Virus particles were detected by immunoelectron microscopy (IEM) [13]. Alanine aminotransferase (AlAT) activity was determined by a micromethod [7] and expressed in micromoles substrate per liter of serum per minute (μmoles/liter/min). Liver biopsy was carried out after injection of ketamine hydrochloride, using a Menghini's needle. Material for histologic investigation was fixed in 10% neutral formalin. Paraffin sections were stained with hematoxylin and eosin. Frozen sections were stained for fat by Goldman's method.

EXPERIMENTAL RESULTS

The culture of HHAV used to infect monkeys (HHAV-1) was obtained from the feces of a patient and was first tested and characterized.

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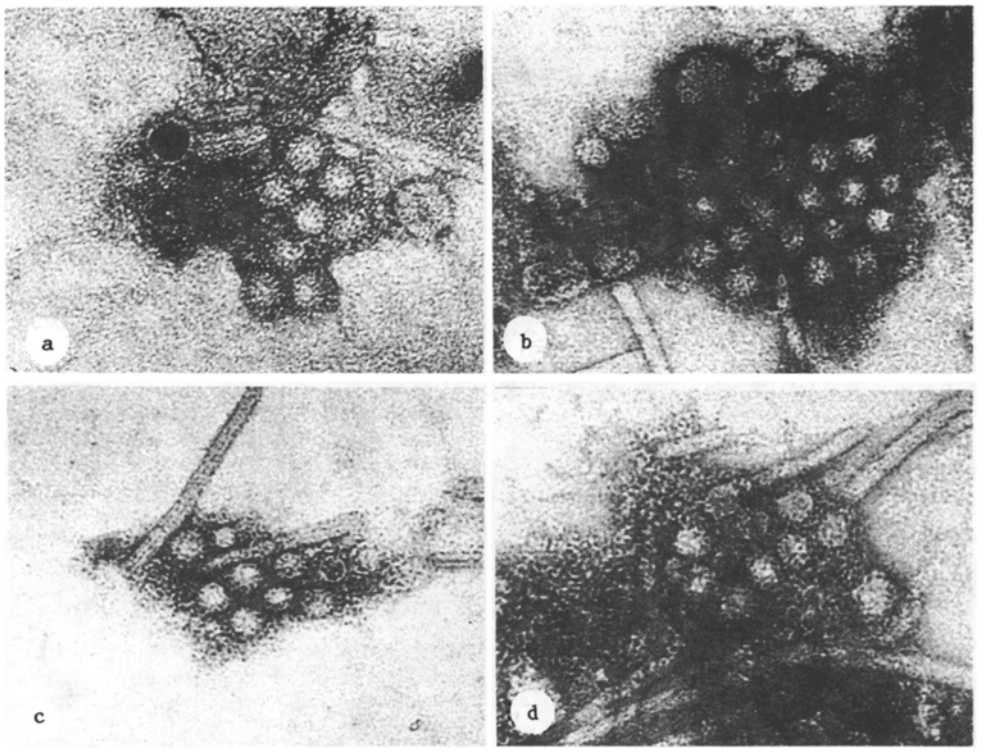

Fig. 1. Immune complexes from hollow and empty virus particles 27-30 nm in diameter. a) Culture of HHAV-1 isolated from feces of a human patient, 170,000×; from monkeys' feces: b) No. 21 595 (second passage), 170,000×; c) No. 21 630 (third passage), 133,000×; d) No. 21 623 (fourth passage), 170,000×.

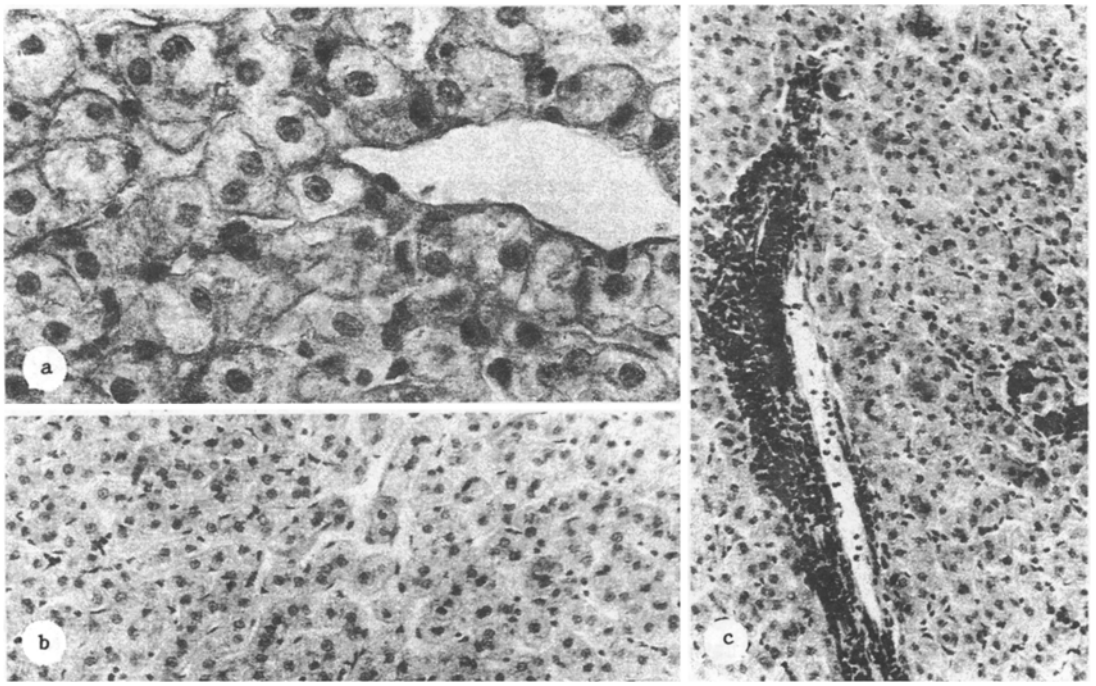

Fig. 2. Liver involvement in rhesus monkey. a) Balloon degeneration of hepatocytes, 800×; b) foci of lysis in hepatic lobule, 200×; c) lymphoid infiltration of portal tracts, foci of intralobular lymphohistiocytic infiltration, 120×. a-c) hematoxylin and eosin.

TABLE 1. Signs of HA in Macaques Infected with HHAV

No. of monkeys	Mode of administration of virus	Signs of infection (days after infection)			
		excretion of HAV with feces	increased AlAT activity	anti-HAV of M class	Changes in liver biopsy specimens
21 520	Intravenously	6—35	22 and 55	13	7—65
21 553	» »	Not found	16—17	21	7—31
21 673	Perorally	6—8	Not found	13	19—26
21 475	» »	11—15	24	21	22—35
21 514	Intravenously + perorally	13—27	13 and 24—27	21	17—51
21 668	» »	10—32	13 and 24—27	21	17—51
21 541	» »	Not found	19 and 34	19	7—34
21 662*	» »	» »	Not found	Not found	7—10*

Legend. *) Monkey killed for investigation on 10th day.

On gradient centrifugation in cesium chloride the main peak of virus activity was observed in the buoyant density zone of 1.34 g/cm^3, characteristic of HAV. According to the results of RIA, it contained a large amount of HAV antigen. After treatment of the material with convalescent serum from a patient with HA, IEM revealed aggregates consisting mainly of hollow virus particles, spherical in shape, and measuring 27-30 nm, covered with a halo of antibodies (Fig. 1a). All the eight macaques infected with this culture developed the disease with its characteristic virologic, biochemical, serologic, and morphologic characteristics. The monkeys were infected both intravenously and perorally with the virus and also by a combination of these methods. Data on the times of appearance of the features of HA and their duration are given in Table 1. The earliest sign of infection was the appearance of the virus in the feces, which was recorded from the 6th-13th day after infection both by RIA and by IEM. The duration of excretion of HAV with the feces varied from 3-4 days to 2-4 weeks. As Table 1 shows, an increase in serum AlAT activity was found not before the 13th-24th day after infection, and it varied in duration from 1 to 5 days. A second rise for 1 day, observed on the 34th and 55th days, occured in two macaques. Changes in AlAT activity could not be found in one monkey. Antibodies to HAV of the M class (titers 1:50,000-1:100,000) were found regularly in all the sick monkeys on the 13th-21st days after infection, and persisted during 70 days of observation. Examination of liver biopsy material showed the development of morphological changes characteristic of acute hepatitis in it, which were observed at different times after infection. In some monkeys they appeared on the 7th day, in others on the 17th-22nd day. Their intensity and duration also varied. In most macaques they lasted 3 weeks, gradually weakening in intensity. In one monkey (No. 21 520) they were found for 2 months, in two others (peroral infection) from 7 to 12 days. The principal morphological changes (balloon degeneration of the hepatocytes, foci of lysis in the hepatic lobule, hypertrophy and hyperplasia of the Kupffer cells, and lymphoid infiltration of the portal tracts, with foci of intralobular lymphohistiocytic infiltration) are illustrated in Fig. 2a-c.

The virus was then subjected to second, third, and fourth passages through nine macaques (three monkeys per passage). Macaques for each passage were infected with virus-containing feces obtained from monkeys developing the disease in the previous passage. All nine animals developed the disease, as shown by the results of their investigation. The laboratory findings for individual macaques in different passages are given in Fig. 3. The earlier appearance of signs of infection in monkeys used for the passages will be noted. Whereas the macaques in the first passage began to excrete the virus with the feces on the 6th-13th days, in all other animals it began to be excreted on the 4th-6th days after passage. The incubation period also was reduced, measured from the time of infection to the time of elevation of the serum transaminase levels. Thus in monkeys of the first passage increased AlAT activity was observed not earlier than 13-24 days after infection, but in most macaques of the second to the fourth passages, it was observed after the 4th-5th day. During passage a longer period of a raised AlAT level, up to 50-70 days, with a wavelike type of curve, was observed (Fig. 3b, d).

Times of appearance of anti-HAV of the M class also were reduced: from 2-3 weeks in the first passage to 1-2 weeks in the 2nd-4th passages. Morphologic changes in the liver biopsy material from monkeys starting with the third passage were discovered earlier — on the 4th-8th day, and they were more severe. Denser portal zones of infiltration and larger foci of death of hepatocytes, with a distinct inflammatory reaction in them, were observed. Intralobular lymphoid infiltration was diffuse in character. As will be clear from Fig. 2, morphological changes in the liver continued to be found until the 70th day after infection (time of observation).

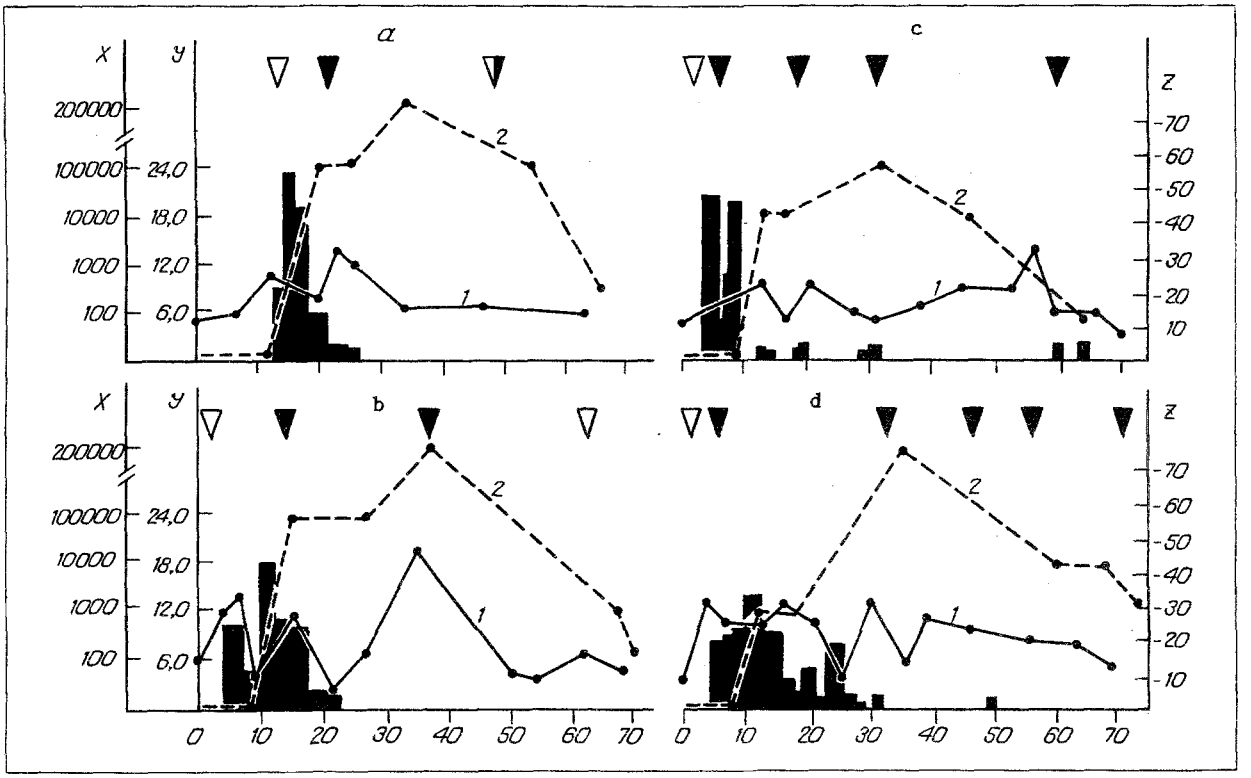

Fig. 3. Laboratory evidence of HA in macaques. a) Macaque No. 21 520 (first passage), b) No. 21 595 (second passage), c) No. 21 630 (third passage), and d) No. 21 623 (fourth passage). Abscissa, days after infection; ordinate: on left, *X*) reciprocal of titer of antibodies to class M HAV. *Y*) AlAT activity (in μmoles/liter/min); on right, *Z*) P/N – ratio of radioactivity of experimental specimen to negative control in RIA. Ratio $\geq$2.1 taken as positive. 1) AlAT level, 2) Antibodies to class M HAV; blank columns – HAV antigen in feces, empty triangles – absence of morphological changes in the liver, filled triangles – distinct morphological changes in the liver, half-filled triangles – mild morphological changes in the liver.

A strain of virus designated HHAV-3, with stable pathogenicity for these animals with a titer of $10^{3.5}$ ID (doses infectious for macaques) was obtained in the passages through macaques. It possessed the antigenic, biophysical, and morphologic properties characteristic of HAV. The virus found in the feces of monkeys in the different passages by IEM had the same morphologic characteristics as the initial HHAV-1. Electron micrographs are shown in Fig. 1.

The results of this investigation indicate that macaques are sensitive to HHAV by both intravenous and peroral infection. Under these circumstances macaques develop a disease with features characteristic of HA: excretion of HAV with the feces, elevation of the AlAT level, the appearance of class M anti-HAV antibodies, and the presence of morphologic changes in the liver. These results show that the HA reproduced in macaques follows a similar type of course to the human disease [4, 6] and that it can serve as an experimental model of this infection. We obtained similar results also when infecting *Macaca fascicularis* [16]. The experimental model of HA in macaques is more readily available and economically advantageous by comparison with the corresponding models on chimpanzees, marmosets, and nocturnal monkeys. It is suggested that it may be used to study the pathogenesis and immunogenesis of the disease and also for testing vaccines against HA and antiviral preparations.

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LIMITATION OF STRESS-INDUCED ACTIVATION OF LIPID PEROXIDATION BY SMALL DOSES OF THYROID HORMONES

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Near-physiological doses of thyroid hormones protect the heart against stress-induced damage to the sarcolemma and mitochondria of the cardiomyocytes and against a fall in the ratio of areas of the mitochondria and myofibrils to the level of the contractile function of the myocardium [1-3]. A leading mechanism of the disturbance of cardiomyocyte structure and function induced by stress is known to be activation of lipid peroxidation (LPO) [7].

Taking account of existing data on the effect of thyroid hormones on enzyme systems connected with the antioxidant system of the cells [8, 12], it can be postulated that one mechanism of the protective effect of thyroid hormones during stress is the limitation by them of LPO processes in the heart.

The aim of this investigation was to study the possibility of limiting stress-induced activation of LPO in the rat myocardium by small doses of thyroid hormones and to study the effect of these hormones on some components of the antioxidant systems of the cells.

EXPERIMENTAL METHOD

Experiments were carried out on 57 noninbred male albino rats weighing 180-220 g. The animals were divided into the following groups: 1st — control (n = 21), end — stress (n = 15), 3rd — thyroid hormone (n = 11), 4th — thyroid + stress (n = 10). Immobilization stress was created by fixing the animals in the supine position for 6 h. Blood samples (taken with a catheter introduced into the carotid artery) and pieces of myocardium were obtained 1 h after the end of immobilization under urethane anesthesia. Thyroid was given by the intragastric route in starch mucilage for 28 days in a daily dose of 1.5-3 mg/100 g, as in [1]. Control rats received starch mucilage only. Activation of LPO both in the myocardium and in the whole animal was judged by determining concentrations of initial and final LPO products respectively in myocardial homogenates (diene conjugates and mal-

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