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The Pathology of Viral Hepatitis in Chimpanzees

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Summary. Serial biopsy specimens (up to 21) of 39 chimpanzees who received inocula of defined infectivity containing hepatitis virus A (9 animals), B (7 animals), and non-A-non-B (24 animals) were evaluated under code for light-microscopic alterations. These studies demonstrated the basic pathologic features seen in human viral hepatitis, although to a lesser degree. These included besides hepatocytic degeneration and necrosis lobular and portal reactions of lymphocytic, macrophagic, and sinusoidal cells. Simultaneously determined serum enzyme activities correlated well with histologic parenchymal changes, indicating that diffuse hepatocytic alterations rather than necrosis are the main substrate of functional alterations. Massive necrosis and chronic active hepatitis were never observed. Hepatitis A and B revealed relatively severe changes which in hepatitis A were restricted to the periportal zone. Hepatitis B had a more prolonged course. Hepatitis non-A-non-B appears to represent a lingering disorder with prolonged low-grade activity but may have a transient period of acute hepatocytic degeneration. The histologic changes appeared earliest in hepatitis A, much later in hepatitis B, and intermediate in time in hepatitis non-A-non-B. The histologic features in the three forms of chimpanzee hepatitis may assist in the light-microscopic differentiation of the three forms in man.

Key words: Viral hepatitis – Chimpanzees – Light microscopy.

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Introduction

The chimpanzee is the only animal susceptible to all three types of human hepatitis viruses (A, B, and non-A-non-B). Inoculation with any of these agents has caused reproducible hepatic histologic lesions and functional alterations. The availability to us of material from chimpanzees infected with all three viruses prompted us to undertake a study of the lesions in serial biopsies. In doing so, we attempted (a) to trace the light-microscopic lesions of viral hepatitis in chimpanzees, a model in which serial biopsies may be obtained at short intervals, and to compare them with the established evolution of human viral hepatitis (Altmann, 1971; Ishak, 1976; Popper, 1976), where light-microscopic observation plays a significant role in understanding and management of the disease, (b) to correlate the histologic alterations with functional changes reflected in elevation of enzyme activities conventionally determined in man, and (c) to explore differences in the morphologic evolution of the three types of hepatitis in chimpanzees, to provide guidance in the search for similar differences in man.

Three difficulties were encountered in these histologic studies. One was the far milder degree of hepatitis in chimpanzees compared to man, a fact which may be explained in part by the young age of almost all chimpanzees examined (Hoofnagle et al., 1978). The second was the very small size of some of the biopsy specimens, from which large portal tracts were frequently absent, interfering with our ability to trace the lesion of larger ducts. The third was the occasional finding of minor alterations in control chimpanzees, a feature which interfered with evaluation of subtle changes in infected animals. The last two difficulties were overcome partly by the availability of large numbers of consecutive needle biopsies, which permitted reconstruction of the evolution of the lesions.

Material and Methods

The main study materials were derived from 49 chimpanzees, all between one to six years of age, of both sexes. They were born and raised in primate centers in the United States. Of these, eight received inocula of hepatitis A virus, seven of hepatitis B virus, and 25 of hepatitis non-A-non-B. Five were control animals. One to three biopsies were available from each chimpanzee before inoculation to serve as baseline controls. In addition, single or multiple liver biopsy specimens from infected chimpanzees sent to one of us (H.P.) for consultation were studied as well. Details of animal handling and care were reported previously (Barker et al., 1973; Dienstag et al., 1975; Dienstag et al., 1976; Alter et al., 1978). Non-A-non-B inocula included material obtained from patients with acute hepatitis in which infection with hepatitis A and B viruses, as well as with other agents was excluded; from similarly characterized patients with chronic hepatitis; from presumed non-A-non-B carriers; and from chimpanzees infected with human hepatitis non-A-non-B (Alter et al., 1978). The inoculum consisted of 3 to 75 ml of serum or plasma given intravenously. Chimpanzees which, following the first inoculation, had no elevation of enzyme activities or whose elevated activities had returned to normal and remained so for more than a year after a previous infection, were cross-challenged with another non-A-non-B hepatitis inoculum. Two chimpanzees received inocula of two types, and one animal was inoculated with all three types of human hepatitis virus consecutively and developed lesions characteristic for each of the respective agents.

Liver biopsies were obtained by Menghini needle, initially once weekly and subsequently at longer intervals. In four animals which had received non-A-non-B inocula, a surgical biopsy was

obtained, usually at the time of significant elevations of enzyme activities. Biopsy specimens were fixed in 10% neutral buffered formalin and stained with hematoxylin eosin. Most of the specimens were prepared in other histologic laboratories, but representative blocks of those biopsies showing prominent alterations were obtained and special stains prepared as was done for the material processed completely at the Mount Sinai School of Medicine. These special stains included silver impregnation, chromotrope aniline blue, PAS with and without diastase digestion of glycogen, and iron reaction. During our earlier studies (Dienstag et al., 1975), chimpanzees were subjected to frequent plasmapheresis, and then increasing amounts of iron were found in lobular and portal macrophages, sometimes surrounded by non-iron-containing inflammatory cells; however, these features were disregarded in the histologic evaluation. The number of biopsy specimens varied from 5 to 21 per chimpanzee; all were interpreted initially under code by H.P.

In blood samples obtained at the time of biopsy, and often more frequently, in addition to sero-immunologic studies, the activities of alanine and aspartate aminotransferases were determined in all animals. Also, in the majority, isocitric dehydrogenase and gamma-glutamyl transpeptidase activities were determined as well. Since in this study the emphasis was on light-microscopic features, only the enzyme activities corresponding to liver biopsy findings were considered, particularly since the enzyme activities at other periods revealed no additional information.

Results

Liver specimens obtained in control animals or during the preinoculation period ranging from 3 to 30 weeks revealed minor alterations. These included focal increase and enlargement of sinusoidal lining cells; occasional segmented leukocytes in the sinusoidal lumen; and small areas of focal necrosis not exceeding the space of five hepatocytes in cross section, sometimes associated with few segmented leukocytes. Normal-sized portal tracts occasionally had a slight increase in mononuclear cells but not exceeding ten in cross section.

Features Common to All Three Types of Chimpanzee Hepatitis

The initial lesion in all three forms of hepatitis was degeneration of hepatocytes, associated with accumulation of mononuclear cells (Fig. 1 A). The hepatocellular degeneration was indicated by variations in the staining quality of their cytoplasm and in the size of the nuclei, which varied from dense to large vesicular nuclei with multiple nucleoli. Sinusoidal lining cells were increased in number and enlarged far more than in the occasional lesions in control animals. Within a week the variations in size and staining of hepatocytes became more conspicuous, particularly between neighboring ones, and mitoses were seen. Moreover, multinucleated hepatocytes became prominent; however, the appearance of multinucleated cells was not confined to the height of the lesion but frequently persisted after the disappearance of other lobular alterations. In all three forms, single or small groups of hepatocytes showed distinct eosinophilia, which, presumably, preceeded the formation of acidophilic bodies lying free in the tissue spaces with pyknotic or absent nuclei. Acidophilic bodies were occasionally surrounded by predominantly mononuclear inflammatory cells. These cells replaced hepatocytes which had disappeared, and, in silver stains, small foci of collapse were recognized. Cholestasis was observed only in one episode of hepatitis A and one of hepatitis B, both in the same chimpanzee. It was reflected in dilated bile canaliculi surrounded by multiple hepatocytes in acinar arrange-

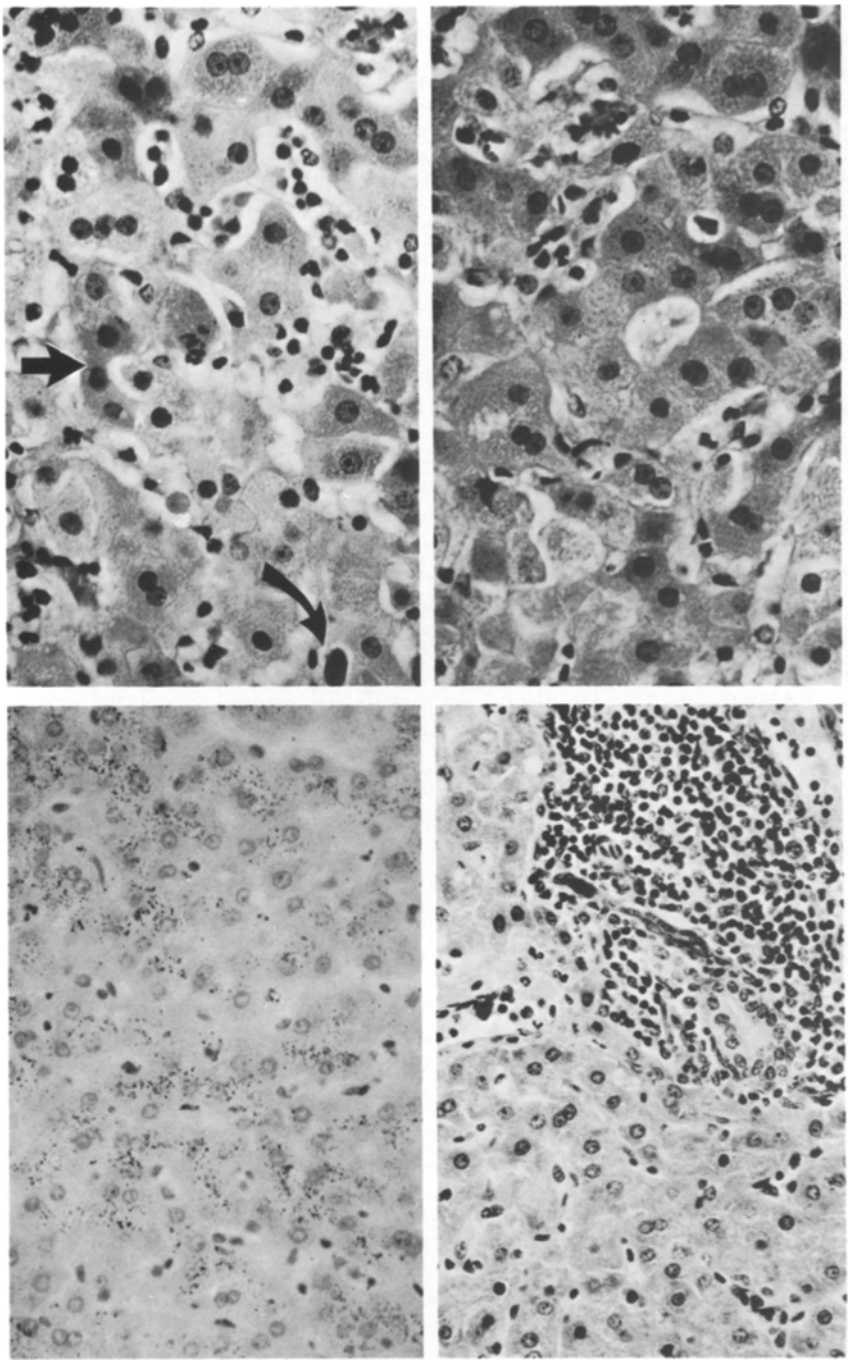

Fig. 1A–D. Characteristic features in chimpanzee viral hepatitis. ×250. **A** Variations in size and staining quality of the hepatocytic cytoplasm. Straight arrow points to acidophilic hepatocytes, curved arrow points to an acidophilic body in the tissue space (without nucleus). Note increased number of sinusoidal cells, lymphocytes and histiocytes. Hematoxylin eosin. **B** Cholestasis reflected in dilated bile canaliculus containing biliary precipitates, surrounded by hepatocytes in acinar arrangement. Otherwise the same changes as in **A** are seen. Hemotoxylin eosin. **C** Iron reaction of fine hepatocytic granules around bile canaliculi and coarse granules in macrophages. **D** Accumulation of lymphoid and histiocytic cells in the portal tract. The bile duct shows minor irregularities of its epithelial lining

ment (Fig. 1 B). During the acute stage, in several animals infected with all three types of hepatitis virus, macrophages showed many coarse iron granules and the hepatocytes contained similar fine granules around bile canaliculi (Fig. 1 C). Granules in both macrophages and hepatocytes were predominantly in the periportal zone. This iron reaction was independent of plasmapheresis and disappeared subsequently. Portal tract inflammation set in simultaneously with or one week after the first lobular alterations (Fig. 1 D). Infiltrating cells were mainly lymphocytes and histiocytes, with rare segmented neutrophilic or eosinophilic leukocytes. In the presence of extensive portal inflammation, lymphocytes aggregated in the center, and histiocytes in the periphery of portal tracts. Sometimes, germinal centers developed in late stages, particularly in hepatitis B and non-A-non-B. The lymphocytes surrounded bile ducts. while both lymphocytes and histiocytes, as well as segmented leukocytes, were around proliferated bile ductules. The bile duct epithelium showed occasional slight variations, including piling up of cells, pyknotic nuclei, and infiltration by lymphocytes.

After acute hepatitis, significant parenchymal lesions subsided and only minimal activation of sinusoidal lining cells remained. Rarely, a transient period of steatosis was noted. Portal infiltration persisted for varying periods, particularly in large portal tracts, and some animals eventually showed slight portal fibrosis. Unfortunately, large portal tracts were not present in all biopsy specimens; therefore, a more definitive evaluation of the evolution of portal fibrosis was not possible.

Correlation Between Biochemical and Histologic Observations

Elevations of alanine aminotransferase (SGPT) activities correlated best with histologic findings, predominantly of degenerating hepatocytes reflected by significant variation in size and staining features (Popper et al., in press). The degree of focal necrosis and inflammatory infiltration did not influence this relation, and no correlation was found between enzyme activities and the portal inflammatory reaction. The first elevation of enzyme activities developed simultaneously with the appearance of hepatocellular alterations. Enzyme elevations preceded histologic changes in only two animals, once by 7 days and once by 19 days. In all other animals hepatocellular alterations appeared 7 to 12 days before elevation of enzyme activities. Resolution of biochemical and histologic changes, however, exhibited less parallelism; portal tract alterations, especially, frequently outlasted elevated enzyme activities by months and exceptionally even by years, depending on the type of hepatitis. Elevations of serum bilirubin were only encountered in the one animal with histologic evidence of cholestasis.

Hepatitis A

In animals inoculated orally or percutaneously, the first histologic manifestations were observed within 7 to 37 days, reflected in focal necrosis. Subsequently,

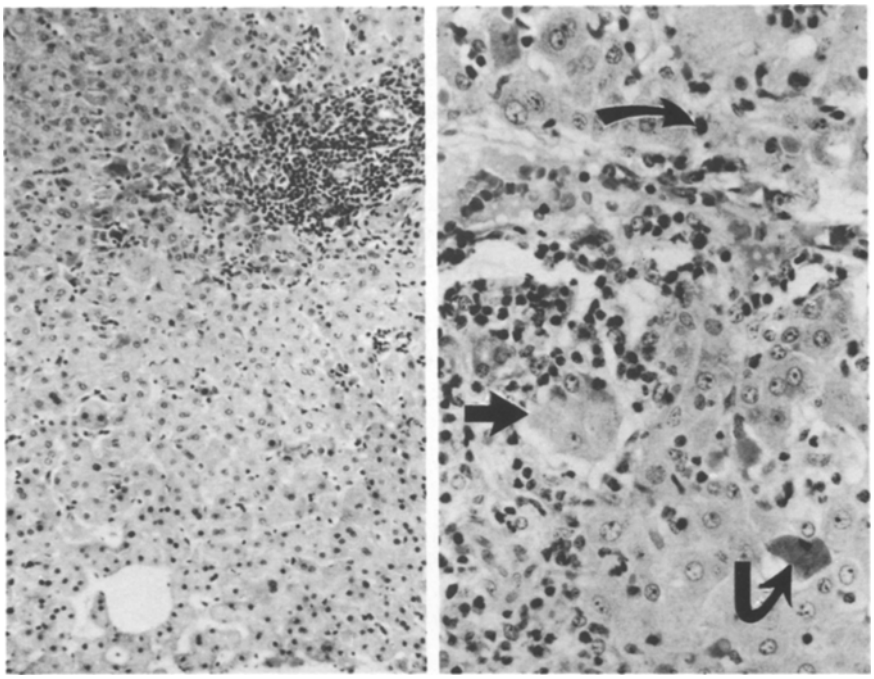

Fig. 2 A and B. Hepatitis A. Hematoxylin eosin. **A** Dense infiltration of the portal tract, mainly by mononuclear cells, which extends into periportal parenchyma, where the hepatocytes show variations in size and staining quality and focal necrosis is found. The changes are less conspicuous in the intermediate zone and almost absent around the hepatic vein tributary which is normal. ×100. **B** Higher magnification view of the periportal zone. Note alterations of the hepatocytes, sometimes in close contact with lymphocytes (*curved arrow*). Hepatocytes in acinar arrangement (*straight arrow*) are also surrounded by inflammatory cells. The inverted arrow points to an acidophilic body with a nuclear remnant. ×240

significant alterations of the hepatocytes in the periportal areas (zone 1 of the acinus of Rappaport) and conspicuous inflammation of portal tracts appeared (Fig. 2A) (Rappaport, 1976). These changes coincided with a sharp rise in SGPT activity (highest observed elevation was 760 I.U.), and in one instance the histologic changes preceded this rise by two weeks. In the periportal zone, lymphocytes and macrophages, most with PAS-positive diastase-resistant granules, accumulated in the tissue spaces or replaced necrotic hepatocytes. Near foci of hepatocellular necrosis, lymphocytes were also in close contact with viable hepatocytes. The dense portal inflammatory exudate, often containing plasma cells, sometimes spilled over into the surrounding parenchyma, resulting in sleeves of periportal necrosis in which hepatocytes persisted in acinar arrangement (Fig. 2B). Bridges of necrosis often connected the portal tracts. The lesions were less conspicuous in the intermediate zone (Fig. 2) and almost entirely absent from the area around the hepatic vein tributaries (zone 3). The tributaries were not involved even at the height of the reaction. The portal and parenchymal lesions regressed despite the persistence of activated, usually iron-containing sinusoidal cells. Within 6 to 8 weeks after inoculation, significant lesions and

elevated enzyme activities had subsided but morphologic alterations outlasted biochemical abnormalities by 14 to 29 days.

Hepatitis B

In the seven chimpanzees studied serially, the first lesion developed within 12 to 16 weeks after exposure, accompanied by a rise of SGOT activities, 1590 I.U. being the highest level observed. The initial lesion consisted of conspicuous activation of sinusoidal lining cells and small collections of focal necrosis throughout the lobule. Within a week, this was accompanied by diffuse, conspicuous variation in staining quality and size of neighboring hepatocytes, which were in close contact with lymphocytes and macrophages, many of them with PAS-positive granules (Fig. 3A). The membranes of the hepatocytes appeared indistinct, and, occasionally, lymphocytes seemed to lie within viable hepatocytes (emperipolesis) (Fig. 3B). Hepatic vein tributaries were surrounded by focal necrosis and their sinusoidal lining cells were activated and included many PAS-positive macrophages. The portal tracts showed dense inflammatory infiltration appearing simultaneously with hepatocytic alterations. The exudate, again containing plasma cells, spilled over into the periportal zone, resulting in destruction of its limiting plate (Fig. 3C). The degree of lobular changes fluctuated in parallel with changes in aminotransferase activities. The duration of significant biochemical and histologic changes varied between 7 and 15 weeks. Toward the end of the acute reaction, the portal-tract alterations were more impressive than the lobular changes. In one instance, residual changes such as sinusoidal cell activation and portal inflammation lasted more than a year longer than aminotransferase elevations.

The degree of the changes varied significantly among chimpanzees. In two, focal necroses lined up to form broad streaks between central and portal canals. The streaks were surrounded by altered hepatocytes and focal necroses (Fig. 3D). In one, an area of confluent necrosis was noted in a periportal zone.

An about 11 year old chimpanzee, imported from abroad, had serum markers, including hepatitis B surface antigen, for at least 3 years, an essentially normal liver with only borderline activation of sinusoidal lining cells, and normal enzyme activities (Fig. 4A). Ground-glass cells, characteristic of the human carrier state, were absent, and Shikata's orcein stain was negative in this as well as in the other seven chimpanzees. In the apparently healthy carrier, however, Dr. M.A. Gerber demonstrated inclusions consisting of surface antigen by immunoperoxidase staining (Fig. 4B), while core antigen could not be stained.

Hepatitis Non-A-Non-B

Four of the 25 chimpanzees inoculated failed to develop significant histologic lesions. In one of these four, SGPT activities were elevated from the 80th to the 90th day after inoculation. The other 21 chimpanzees showed histologic

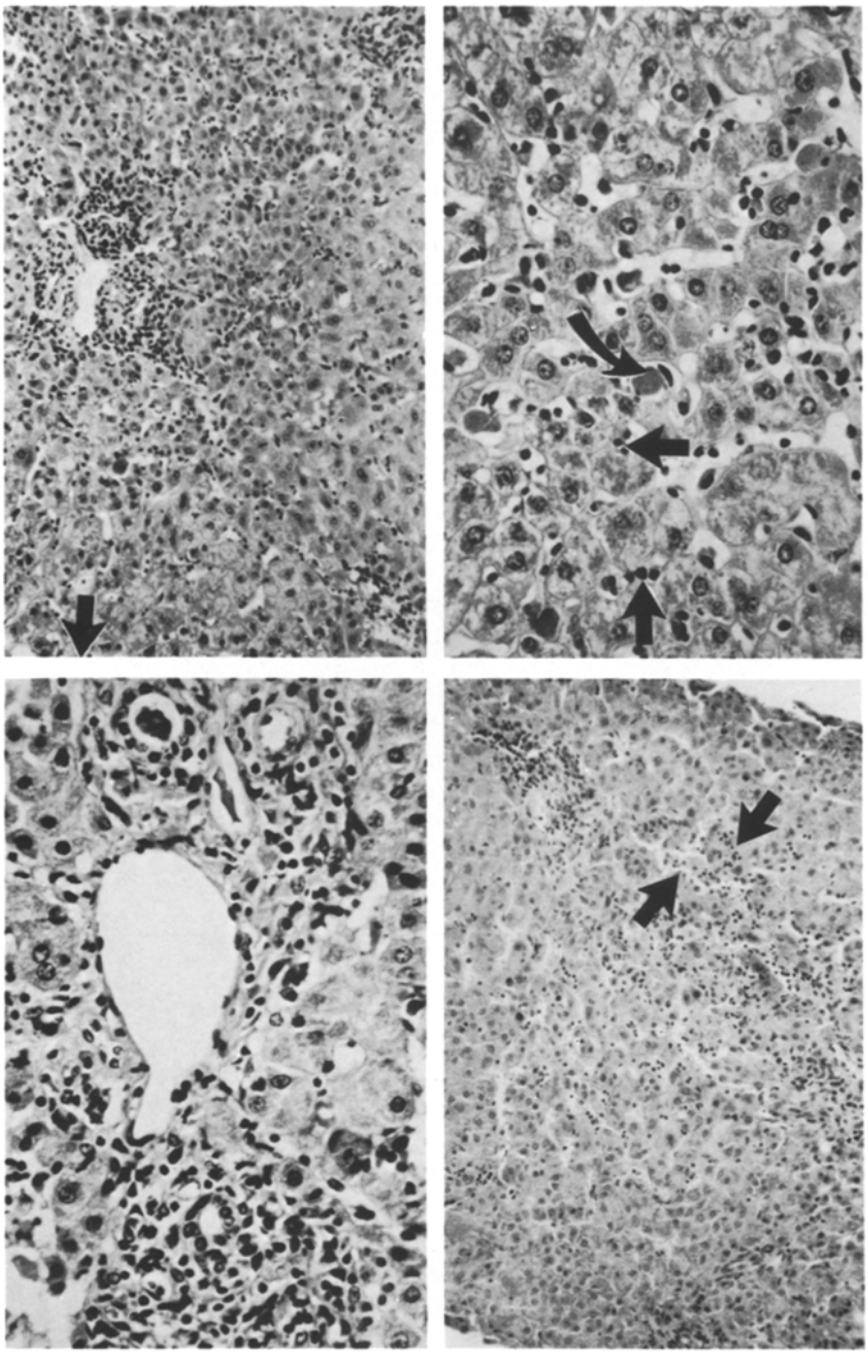

Fig. 3A–D. Hepatitis B. Hematoxylin eosin. **A** Diffuse alterations of the hepatocytes throughout the lobule, extending to the altered central vein (*straight arrow*). Inflammatory infiltration of portal tract. ×100. **B** Diffuse alteration of the hepatocytes reflected in variation of nuclei and cytoplasmic changes. Close contact of lymphocytes with hepatocytes (*straight arrow*). Curved arrow points to acidophilic body. ×240. **C** Inflammatory exudate within portal tracts extending into the surrounding parenchyma, the limiting plate of which is eroded in places. The epithelial lining of ductules is slightly altered. ×240. **D** Streaks of focal necrosis extend from portal tract to central vein. Note alteration of hepatocytes in the parenchyma around the streaks with lymphocytes in close contact with hepatocytes (*arrows*)

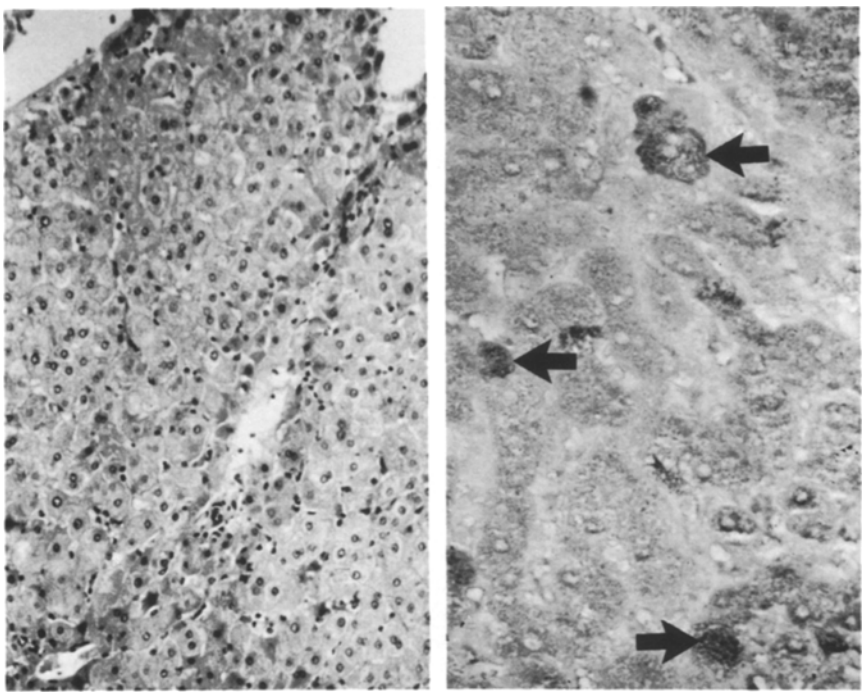

Fig. 4A and B. Thirteen-year-old chronic hepatitis B surface antigen (HBsAg) carrier chimpanzee. **A** The parenchyma shows mainly sinusoidal activation; portal tract in left lower corner is normal. Hematoxylin eosin, ×100. **B** Immunoperoxidase reaction. Arrows point to HBsAg in the cytoplasm of hepatocytes. ×250

alterations within 14 to 100 days, usually accompanied by SGPT elevations below 100. Although they varied in histologic expression, it was possible at least to tentatively reconstruct the evolution of the lesion.

In three chimpanzees, a similarity to the histologic changes in hepatitis B was suggested by the prominence of intralobular lymphocytes (Fig. 5A, B). Also, PAS-positive macrophages appeared frequently within the lobular parenchyma; however, portal inflammation did not extend into the surrounding parenchyma and the limiting plate was not eroded. In one surgical specimen from one of these chimpanzees, focal necroses formed a narrow streak linking central and portal canals and were associated with collapse of the framework. In contrast to findings in hepatitis B, however, focal necrosis was minimal in the vicinity of the streak. In the other 18 chimpanzees, the first lesion was a mild alteration of cytoplasmic staining without nuclear alterations but with conspicuous activation of sinusoidal lining cells, which rarely had PAS-positive granules. Simultaneously, conspicuous inflammation limited to the portal tract appeared (Fig. 5C). These changes were accompanied by minor elevations of SGPT activities to the level of approximately 50 I.U.

In ten of the chimpanzees infected with non-A-non-B hepatitis a transient lesion appeared, lasting from 4 to 7 weeks. It was characterized by conspicuous variation in hepatocytic nuclei, some of them multinucleated, and by severe,

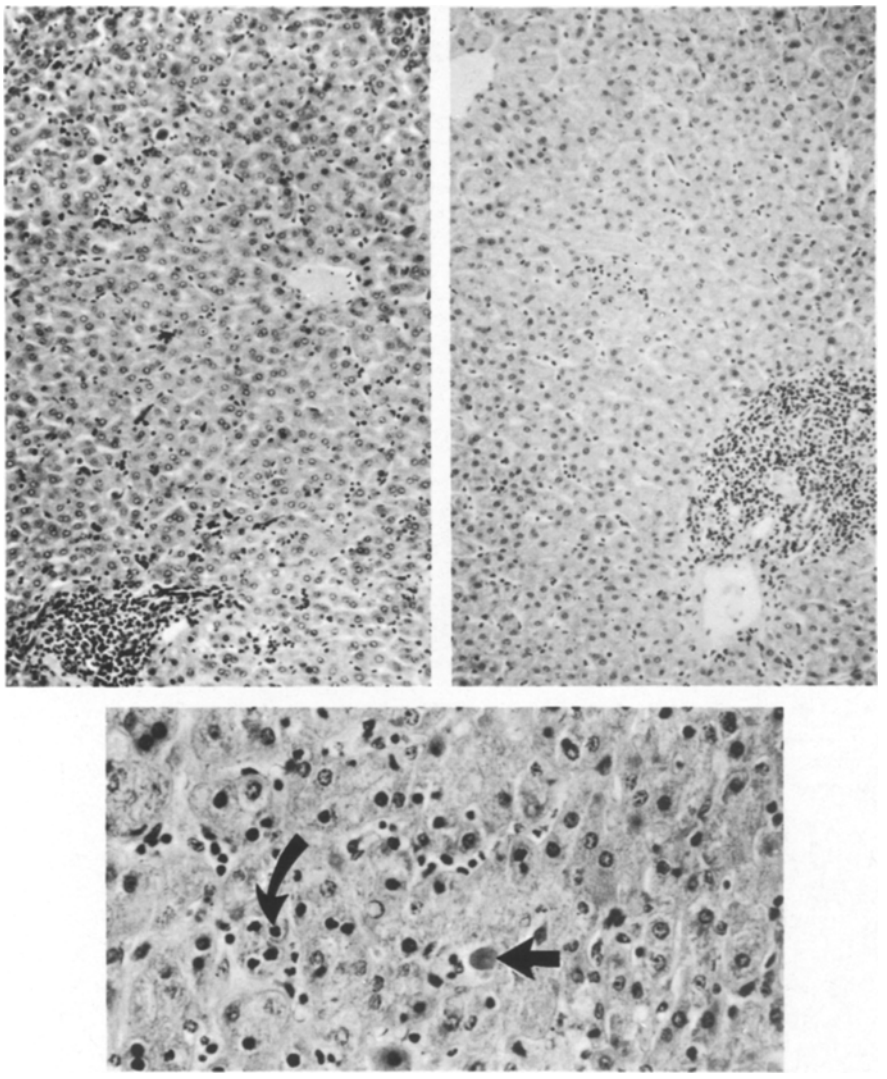

Fig. 5A–C. Hepatitis non-A-non-B. Hematoxylin eosin. **A** Features of hepatitis B. Note hepatocytic alterations and many intralobular lymphocytes. The portal inflammatory infiltration does not extend into the parenchyma. ×100. **B** Higher magnification view of **A**. Note moderate alterations of the hepatocytes, with lymphocytes in contact with hepatocytes. The cell membrane is sometimes indistinct. Areas of focal necrosis (*curved arrow*) and an acidophilic body (*straight arrow*) are noted. **C** Moderate changes of the hepatocyte cytoplasm, but activation of sinusoidal cells and conspicuous infiltration restricted to the portal tract. ×100 (SGPT 89 I.U.)

diffuse alterations of the cytoplasm, which showed irregular clumping and vacuolization, the latter seemingly from fat droplets or from hydropic swelling. The cell membranes appeared intact (Fig. 6A). Few foci of cytolytic necrosis were noted, but acidophilic bodies were conspicuous. The sinusoidal cells, mostly PAS-negative, were activated and relatively few lymphocytes were present. Portal inflammation was prominent but restricted to the tracts in which eosinophiles

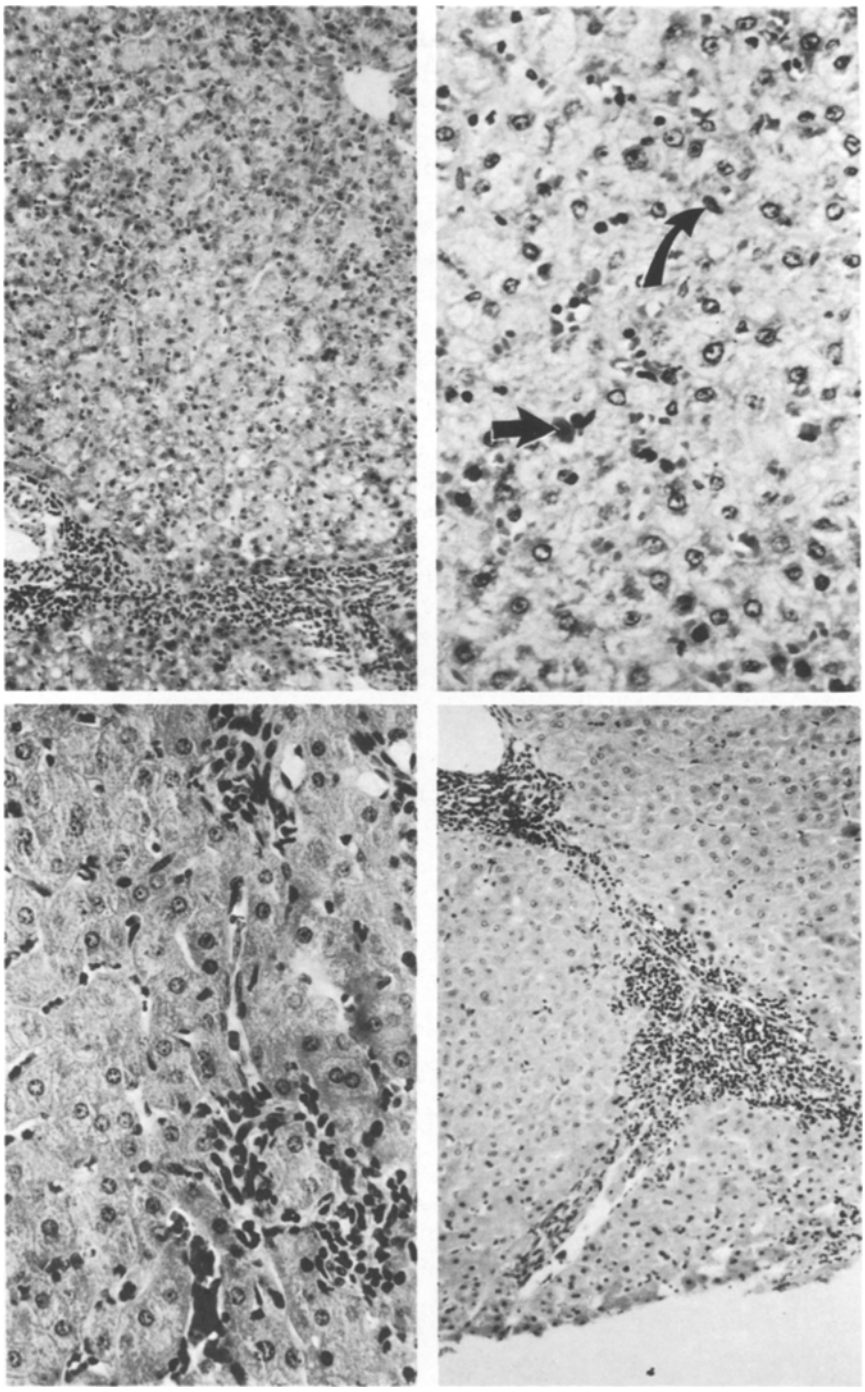

Fig. 6A–D. Hepatitis non-A-non-B. Hematoxylin eosin. **A** Diffuse alterations of hepatocyte cytoplasm, activation of sinusoidal lining cells, and intense portal lymphocytic infiltration not extending into parenchyma. ×100 (SGPT 221 I.U.). **B** Higher magnification view of **A**. Note irregular clumping and vacuolization of hepatocytic cytoplasm, variations in appearance of nuclei, proliferation of sinusoidal lining cells (*curved arrow*) and acidophilic body (*straight arrow*) ×240. **C** Proliferated bile ductules surrounded by inflammatory exudate within lobular parenchyma in late stage. ×240. **D** Dense infiltration of portal tracts by inflammatory cells which extend into the parenchyma in only a few places. Minor lobular changes (late stage). ×100

in some cases were intermixed with lymphoid and histiocytic cells (Fig. 6B). The transient, intralobular lesion was regularly accompanied by a sharp increase in SGPT activity which, in general, did not exceed 300 I.U. The one exception was a chimpanzee with a histologic lesion resembling that of hepatitis B, in which SGPT rose to 422 I.U.

Subsequent to these changes, the livers showed less conspicuous alterations for long periods, in one chimpanzee for several years. These were characterized by activation of sinusoidal lining cells, more prominent in the centrolobular zone, and irregular dilatation of the sinusoids, with only minor alterations of the hepatocytes. Foci of necrosis reappeared at various intervals, frequently in close proximity to proliferated bile ductules which had extended into the peripheral (zone 3) and intermediate (zone 2) regions of the lobule, surrounded by few lymphocytes and histiocytes (Fig. 6C). In silver impregnations, small areas of collapse were noted. Occasionally, however, it was difficult to distinguish these minor alterations from the mild abnormalities in control chimpanzees. Among portal tracts, some were normal, while others showed inflammatory infiltration which was more frequent in the larger than in the smaller tracts. In two chimpanzees, dense, inflammatory infiltration of larger portal tracts was noted (Fig. 6D), and only occasionally did the inflammatory exudate spill over into the surrounding parenchyma. In these instances, intralobular alterations were more pronounced.

Parenchymal and portal lesions were more conspicuous in the chimpanzees which had been challenged previously with another non-A-non-B inoculum. However, in none did acute hepatocellular degeneration develop as observed after the first inoculation, and SGPT activities did not rise above 100 I.U.

Discussion

Chimpanzees proved to be a useful model to study and compare light-microscopic changes following inoculation with human hepatitis A, B, and non-A-non-B agents. These observations confirm a series of individual, less detailed descriptions of hepatitis in chimpanzees, produced by hepatitis A (Dienstag et al., 1975; Dienstag et al., 1976), B (Barker et al., 1973; Dienstag et al., 1976; Schaffner et al., 1977; Hoofnagle et al., 1978), and non-A-non-B (Alter et al., 1978; Tabor et al., 1978; Bradley et al., 1979; Wyke et al., 1979) viruses. In principle, the lesions represent the spectrum of features seen in acute viral hepatitis in man, particularly in the acute stage, namely, hepatocellular degeneration and necrosis associated with an inflammatory intralobular reaction. Portal inflammation appears simultaneously with or slightly later than the lobular reaction, but persists much longer in all forms. These observations suggest the possibility of a different role for the intralobular and portal inflammatory reaction, particularly as it concerns lymphocytes and possibly also PAS-positive macrophages. The close contact lymphocytes with hepatocytes, especially in hepatitis A, and B, may reflect immunologic attack against the hepatocytic plasma membrane which leads to the elimination of hepatocytes, considered the substrate for the histologic picture in viral hepatitis B (Bianchi and Gudat, 1979). The lesser severity of the intralobular lesions in chimpanzees, compared to man, could reflect, in addition to the younger age of the animals, a species

difference in immunologic reactivity. Alterations of bile ducts are never severe. Bile ductules surrounded by inflammatory exudate proliferate in later stages. Transient iron is found in the periportal zone, just as it is frequently seen in man (Altmann, 1971; Popper, 1979a).

The hepatitis induced in chimpanzees does not resemble the lesions in murine (Gledhill et al., 1955) nor canine (Cabasso et al., 1962) viral hepatitis, in which the cytopathogenicity of the virus is incriminated. In contrast to human hepatitis, massive necrosis characterized by sparsity of lymphocytes has not been observed to date in chimpanzees, even chimpanzees that died. The pathogenesis of this particular human reaction remains undefined. Moreover, the features of chronic active hepatitis have not been observed in chimpanzees, even after hepatitis B virus infections when markers of infectivity persist. The fact that severe lesions (spilling-over of portal exudate into the surrounding parenchyma, with destruction of the limiting plate of the parenchyma and with periportal necrosis, as well as bridging necrosis linking central and portal canals) were not followed by features of chronic active or aggressive hepatitis is an important lesson for human pathology and in agreement with recent human experiences (Theodor, 1978; Nisman et al., 1979). Chronic persistent hepatitis, defined as a dense portal inflammatory reaction, generally with preservation of the limiting plate, and often associated with a milder lobular reaction (De Groote et al., 1968), is histologically a less well defined entity. Such lesions have been observed in chimpanzees following inoculation with material from hepatitis non-A-non-B, though mostly in animals which had received a second inoculum.

The histologic changes, initially evaluated blindly, exhibited a surprisingly good correlation with elevation of activities of enzymes used in ascertaining the status of the liver in man. Of the enzymes, alanine aminotransferase was the most informative. The enzyme elevations coincided in general with the appearance of lesions, particularly of lobular changes, and only in the subsiding stage did a dissociation develop in that persisting portal inflammation was not necessarily associated with elevated enzyme activites. The fact that the degree of enzyme elevations correlated so well with the intensity of hepatocellular cytoplasmic changes determined under code, but not necessarily with focal necrosis, confirms the observation in man that in milder degrees of hepatocellular injury the alterations of the viable hepatocytes, reflected in variation of their cytoplasm and nuclei, are the morphologic substrate of hepatocellular dysfunction (Popper, 1979b).

The differences in the three types of chimpanzee hepatitis concern (a) interval between inoculation and appearance of lesions (histologic incubation period), (b) duration of changes, (c) localization of alterations and (d) evolution.

Hepatitis A has, in confirmation of a previous study (Dienstag et al., 1976), the shortest histologic incubation period and the shortest duration; hepatitis B the longest incubation period and sometimes prolonged duration; hepatitis non-A-non-B an intermediate incubation period and the longest duration. In hepatitis A, which may be of considerable severity, the lesions are characteristically prominent in the periportal zone and almost spare the central zone (zone 1) of the parenchyma and the hepatic vein tributaries. Extensive destruction of hepatocytes takes place in the periportal zone. This peripheral predominance does not hold true for marmosets, in which hepatitis A produces a conspicuous

diffuse hepatitis (Holmes et al., 1971). Inoculation with hepatitis A virus did not elicit any histologic lesions in rhesus, African, wooly, and cebus monkeys (Purcell and Dienstag, 1978). It has not been established whether peripheral area predominance characterizes hepatitis A in man. Sparing of centrozonal was observed in biopsy material from experiments in which a mild, transient hepatitis was produced in prisoner volunteers (Joliet, Illinois) who had received the MS-1 of Krugman HAV strain (Boggs et al., 1970). Such peripheral predominance was also conspicuous in 11 needle biopsy specimens from an epidemic of human hepatitis A in Japan, the study of which was made possible by the cooperation of Dr. K. Tanikawa (Tanikawa, 1979).

Hepatitis B, by contrast, exhibits diffuse involvement of the entire lobule. This also includes the hepatic vein tributaries, just as in human viral hepatitis B, and might be explained by the presence of activated Kupffer cells in these tributaries in the norm (Popper, 1977). The severity, judging from both the histologic alterations and the rise in enzyme activities, equals or is greater than that seen in hepatitis A, but the duration and severity vary in different animals, and in some, bridging necrosis was observed which was wide and surrounded by altered hepatocytes in contact with lymphocytes and macrophages. Possibly the diffuse lobular involvement in chimpanzee hepatitis B is related to its potential chronicity in man, in contrast to the periportal localization in hepatitis A, which never becomes chronic (Rakela et al., 1978). Chimpanzee hepatitis B thus is an acute but prolonged disorder of varying intensity with occasionally delayed resolution. A carrier state seems to exist without significant histologic or functional alterations. In contrast with man, in chimpanzees ground-glass cells are absent and Shikata's orcein stains are negative, despite the presence within hepatocytes of hepatitis B surface antigen, as demonstrated immunohistologically.

The existence of non-A-non-B hepatitis has been suggested recently by epidemiologic investigations (Purcell et al., 1976) and established by transmission from man to chimpanzees and from chimpanzees to each other (Alter et al., 1978; Tabor et al., 1978; Bradley et al., 1979; Wyke et al., 1979). The possibility that more than one type of non-A-non-B agent exists has been suggested by differences in incubation periods, by the occurrence of two or more bouts in the same individual or experimental animal, and by differences in ultrastructural changes in experimentally infected chimpanzees (Shimizu et al., 1979). Currently, however, there is insufficient evidence for the existence of more than one histologic pattern on a light-microscopic level. Several chimpanzees showed a resemblance to hepatitis B in that many lymphocytes and PAS-positive macrophages were in close contact with hepatocytes, a feature which was absent throughout illness in the rest of the cases. This suggests a decreased lymphocytic immune attack in the majority of chimpanzees with hepatitis non-A-non-B. Moreover, even in animals with histologic features similar to those of hepatitis B, the portal infiltrate did not spill over into the parenchyma. In the majority of chimpanzees, initial mild hepatocellular alterations were associated with sinusoidal cell activation and portal infiltration and minor elevation of enzyme activities. In most of them, a transient period of conspicuous hepatocellular degeneration developed accompanied by moderate elevation of enzyme activites but not associated with significant lymphocytic infiltration. The pathogenesis of this peculiar

transient hepatocytic lesion requires elucidation. Eventually a chronic stage with less conspicuous alterations sets in. In some chimpanzees it was very prolonged and was characterized by such features as sinusoidal cell activity, transient focal necrosis, and proliferation of bile ductules extending into the lobular parenchyma. The lesion, varying in character, was observed for many months and sometimes years, as were portal inflammation and minor elevations of enzyme activities. While, in general, the appearance of the liver in chimpanzee non-A-non-B hepatitis in this stage corresponded to that of human nonspecific reactive hepatitis or the fading stage of viral hepatitis, it was not always reliably distinguished from the occasional lesions in control animals. In this late stage, however, some chimpanzees had features of human chronic persistent hepatitis.

Thus, chimpanzee hepatitis non-A-non-B is a lingering disorder with a subdued lobular lymphocytic reaction, but with a tendency to transient hepatocytic degeneration, although the role of the second challenge has not been evaluated. These findings in chimpanzees agree with available observations on human hepatitis non-A-non-B reported recently in individuals with chronic hepatitis (Knodel et al., 1977; Berman et al., 1979; Iwarson et al., 1979; Rakela and Redeker, 1979). In man it also seems to be a lingering disease with a higher tendency to chronicity but of lesser severity than hepatitis B. As mentioned above, however, it was not possible by light microscopy to recognize the two types of chimpanzee hepatitis non-A-non-B distinguished by electron microscopy (Shimizu et al., 1979), one with peculiar cytoplasmic inclusions and the other with nuclear alterations.

Although chimpanzee hepatitis non-A-non-B differs from both hepatitis A and B, these observations should still be considered preliminary and should be substantiated by further studies. Nevertheless, tracing the evolution of the three types of viral hepatitis in chimpanzees may provide clues in the search for morphologic differences among the lesions induced in man by different hepatitis viruses.

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