STUDIES ON THE TRANSMISSION OF HUMAN VIRAL HEPATITIS TO MARMOSET MONKEYS

I. Transmission of Disease, Serial Passages, and Description of Liver Lesions*

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The lack of a susceptible animal and of an in vitro system for the propagation of the causative agent of viral hepatitis have hindered our full understanding of this disease. The use of human volunteers as the only susceptible experimental subjects has given valuable information but such experiments have been necessarily very limited.

Many nonprimate animal species have been evaluated repeatedly for susceptibility to human viral hepatitis but with negative results. Nonhuman primates were claimed several times to be susceptible, but a review by Evans in 1954 (1) stated that none of these studies had established that the disease could be transmitted to nonhuman primates. Studies on chimpanzees in the Belgian Congo in 1958 (2) again gave only suggestive evidence of transmission of human viral hepatitis. In contrast to the failures of transmission from man to primates, evidence has recently accumulated for the transmission of a disease, indistinguishable from human viral hepatitis, from nonhuman primates to man (3). Such primate-associated human cases may, however, result from several mechanisms. They may represent an original transmission from man to nonhuman primates. Multiplication and excretion of the agent by the primate may then occur with subsequent reinfection of man. Or, man may occasionally become infected with a nonhuman primate hepatitis agent different from that agent which ordinarily causes human viral hepatitis. These observations have stimulated inves-

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tigators to search again for a nonhuman primate susceptible to the elusive agent or agents of human viral hepatitis (4, 5).

For the past 5 yr we have studied small South American nonhuman primates (marmosets) for their possible use as laboratory animals. In attempts to establish their susceptibility to various human infections, we have inoculated these animals with materials from patients suffering from viral hepatitis. The results of these studies are reported here.

Materials and Methods

Marmosets.—Most of the marmosets used in this study were white lipped, hairy faced tamarins (*Saguinus fuscicollis* and *Saguinus nigricollis*) but some crested, barefaced, cotton top tamarins (*Saguinus* (*Oedipus*) *oedipomidas*) were used in initial studies. No differences were observed in the responses of the two types of animals.

TABLE I

Normal Values of Certain Hepatic Tests in Randomly Selected, Wild-Caught Marmosets

Test	Mean	Range	N*
Glutamic oxalacetic transaminase (units)	105	48–204	125
Isocitric dehydrogenase (units)	715	274–1466	122
Bilirubin, direct	0.10	0.0–0.3	128
Bilirubin, indirect	0.10	0.0–0.4	128

* N, number of determinations on which these figures are based.

Handling of Animals.—The general husbandry, experimental procedures, and establishment of biological baseline values for marmosets in our colony have been described previously (6–8). All animals were wild caught and were used in these studies after at least 6 wk in the laboratory, after three consecutive sets of serum enzyme determinations (see below) and after at least one liver biopsy. Only animals with normal enzyme levels and normal liver histology (see section on pathology) were selected for individual experiments. Control and inoculated animals were housed, fed, and handled under identical conditions. Two to four animals of the same experimental group were caged together but the groups were removed as far as possible from each other. Complete isolation was unfortunately impossible in our facilities but the position of cage batteries in different corners of the same room, or whenever possible in different rooms, reduced the possibility of transmission between groups to a minimum. Anesthesia was not necessary for bleeding or biopsy. The animals received no medication except for molybdenized ferrous sulfate suspension added to the drinking water of animals bled more than once a week. In addition all animals received a pediatric multiple vitamin preparation in syrup in their drinking water.

Biochemical Hepatic Tests.—Serum activity of glutamic oxalacetic transaminase (SGOT) was measured by the method of Karmen, Wroblewski, and LaDue (9), that of serum isocitric dehydrogenase (SICD) by the method of Wolfson and Williams-Ashman (10), and serum bilirubin by the method of Evelyn and Malloy (11). Normal values in randomly selected wild-caught adult marmosets of our colony are shown in Table I.

Liver Biopsies.—Liver tissue was obtained with a 40 × 1.40 mm Menghini needle using the two man technique (12). The tissue was fixed in 10% formalin buffered with phosphates

to pH 7.4, sectioned, and stained as routine with hematoxylin and eosin or in periodic acid–Schiff (PAS) reagent before and after diastase digestion of glycogen. Selected sections were subjected to iron stain, connective tissue stains, and silver impregnation.

Cell Cultures.—The preparation and maintenance of primary newborn human kidney, adult marmoset kidney, and diploid human embryonic fibroblast cultures have been described elsewhere (13).

Inoculations.—Inocula were diluted 1:2 in Hanks' balanced salt solution and a 0.5 ml total volume was inoculated per animal. In early experiments material was injected into a tail vein but femoral vein inoculation was used in later groups.

Testing of Inocula.—All materials used for inoculation of marmosets were inoculated before use into standard bacteriological and mycoplasma media and into newborn human and/or adult marmoset kidney cell cultures. The inoculated cell cultures were incubated in a stationary manner for 7 days and three blind passages were performed by transferring frozen and thawed medium and cells from the previous passage. The cultures were observed for the development of cytopathic effect and, in addition, the cultures of the third passage were tested for hemadsorption with chicken, guinea pig, and human type O erythrocytes and for interference by superinfection with vesicular stomatitis virus (14). None of these tests indicated the presence of infectious agents.

Experimental Design

After selection for individual experiments, animals were randomly distributed into experimental and control groups. After inoculation the animals were observed for signs of overt disease and were bled by venipuncture at least once a week. SGOT and SICD activities were measured in each serum sample. If they were elevated, direct-reacting and total serum bilirubin were also determined. SGOT values of 200 units and SICD values of 2000 units were taken as the upper limits of normal. Normal bilirubin values in marmosets, as in other nonhuman primates, are lower than in man, and values above 0.15 mg per 100 ml direct-reacting serum bilirubin and above 0.50 mg per 100 ml total serum bilirubin were considered significantly elevated. Needle biopsies were performed every 2 wk and, in most instances, also on the day the hepatic tests yielded abnormal values for the first time. The biopsies were coded and evaluated under code.

Stool samples were collected from some control and inoculated animals of the experimental groups twice weekly for virus isolation. The specimens were coded, stored at −65°C., and evaluated under code in various tissue cultures.

RESULTS

Six attempts were made to induce hepatitis in marmosets by inoculation of human serums or plasmas. In five instances the material had been obtained from patients with hepatitis, in the sixth the inoculum had been shown *not* to cause hepatitis in human volunteers. Each attempt represents a group of experiments and will be discussed separately.

Group 1.—

D. M., an 18-yr-old student nurse in an institution where infectious hepatitis is endemic (15), developed malaise and anorexia with hepatic tests indicative of hepatitis. She was sick for about 6 wk, with jaundice for 2 wk. Liver function 8 wk after the onset of symptoms was normal.

Serum obtained on the second day of jaundice was inoculated into three marmosets. Only one animal developed enzyme elevations 80 days after inoculation (SGOT 276, SICD 4750) without histological changes in liver biopsy. Serum enzymes were elevated for 2 wk and were again normal for the subsequent 5 wk. Serum from the one questionably positive animal, obtained at the time of the enzyme elevations, was injected into four more marmosets in a subsequent experiment. One of these animals had only slight SGOT elevations 77 and 84 days later (212 and 284, respectively) and the other three remained normal throughout the 4 months of observation. Liver biopsy specimens were again normal in all animals. A third passage in four more marmosets was attempted with serum from the second passage animal, which had had borderline elevation of enzyme activity, but no chemical or histological abnormalities were observed over a period of 12 wk.

Group 2.—

G. B., a 34-year-old surgeon, developed acute hepatitis. He had not knowingly exposed himself to serum hepatitis. He was moderately ill and acute phase serum was obtained on the third day of jaundice during an illness characterized by a maximum total serum bilirubin of 14.4 mg per 100 ml and icterus of 4 wk duration.

The acute phase serum was inoculated into four marmosets, all of which developed abnormal hepatic tests 16–40 days later, liver biopsies also showing definite abnormalities. Serum obtained from one of the animals on the day that elevated serum enzyme activities were first observed was inoculated into a new group of four marmosets. Five serial marmoset-to-marmoset passages were carried out with almost all inoculated animals showing chemical evidence of disease (Text-fig. 1) with peak SGOT activities from 400 to 900 units and SICD activities as high as 12,000 units. Many animals had hyperbilirubinemia, and incubation periods varied but tended to cluster around 20 days, particularly in the later passages.

Group 3.—

B. B., was 36-year-old man who entered the hospital because of anorexia for more than a week, and jaundice allegedly for only 24-hr. He was deeply icteric and had a total serum bilirubin of 35 mg per 100 ml on the day following admission. He died 5 days later in hepatic coma.

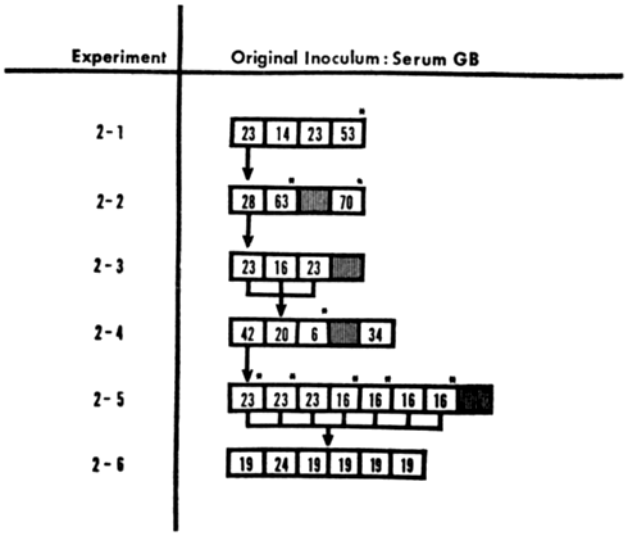
Serum taken on the second hospital day, when the total serum bilirubin had risen to 54 mg per 100 ml, was inoculated into five marmosets and these animals showed no chemical abnormalities during the next 4 months.

Group 4.—

A 9-year-old girl, M. J., entered the hospital with anorexia and nausea for a week and a history of exposure to hepatitis. Her hepatic tests suggested anicteric hepatitis (cephalin flocculation 3+, SGOT 240, serum glutamic pyruvic transaminase 275, bilirubin 0.10 mg

direct-reacting, and 0.75 mg per 100 ml total). She never became jaundiced and hepatic tests were normal 2 wk later. Serum was obtained on the 8th day of her disease.

Serum from M. J. was inoculated into five marmosets and they showed no evidence of hepatitis over the next 4 months.



TEXT-FIG. 1. Serial passages in group 2. Each row of blocks represents a single experiment, each block represents a single animal. Animals which showed no evidence of liver disease throughout the experiment are shown by shaded blocks. Animals with biochemical evidence of hepatitis are shown by open blocks, and the number inside the block is the number of days from inoculation to first abnormal hepatic tests. The vertical arrows originate from the blocks representing animals from which serum for passage was obtained. The asterisks indicate hyperbilirubinemia. (Bilirubin determinations were not done in experiment 2-6 because the available serum was used in other studies.)

Group 5.—

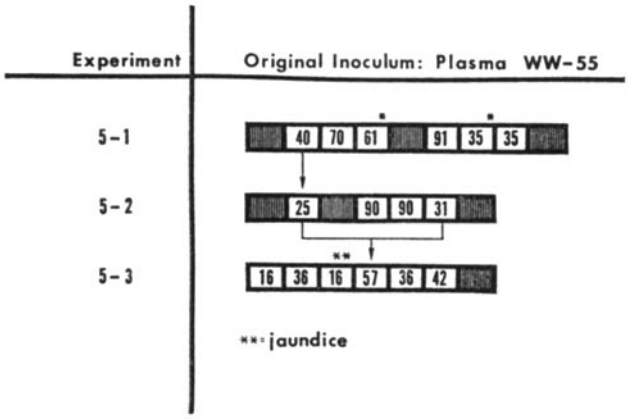
Through the courtesy of Drs. I. W. McLean and Wilton Rightsel of Parke, Davis and Co., Detroit, Mich., aliquots of the original WW-55 plasma and a control plasma were received under a code which was broken only after the completion of the experiment. The WW-55 plasma had induced hepatitis in about 50% of human volunteers with an incubation period of about 35 days (16). The control plasma, on the other hand, had been shown to be nonicterogenic for man.

Both plasmas were inoculated at the same time into two groups of nine marmosets each. The subsequent course of the animals receiving control material is described under group 6. Six of the nine animals which were inoculated with WW-55 plasma developed biochemical evidence of hepatitis in 35–91 days (Text-fig. 2). Needle biopsies showed abnormalities consistent with viral

hepatitis. In two subsequent marmoset-to-marmoset passages, using acute phase marmoset sera as in the earlier attempts, 10 of 14 inoculated animals developed chemical and histological liver disease with incubation periods of 16–90 days.

Group 6.—

This group of experiments consisted of the inoculation and passage of the control plasma referred to in group 5. This plasma had been inoculated into human volunteers without causing any disease (16).



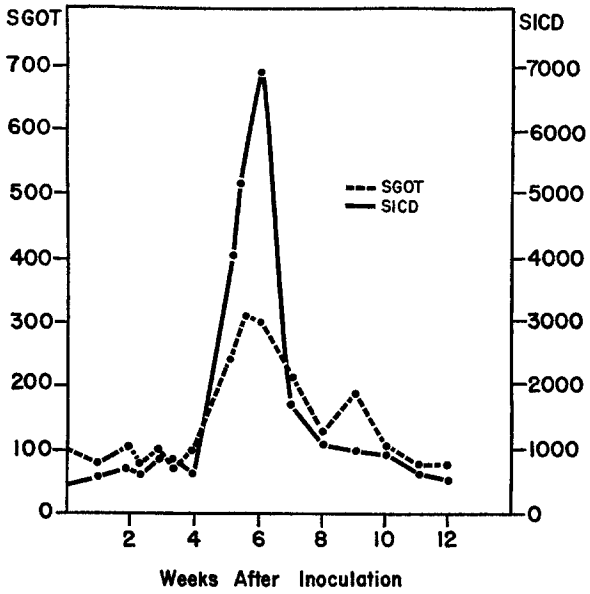
TEXT-FIG. 2. Serial passages in group 5. The explanation of this figure is the same as for Text-fig. 1.

The control plasma was injected into nine marmosets which did not, over the next 5 months, show any evidence of chemical or histological liver disease. Serum harvested from two of these animals 40 days after inoculation, at the same time as the serum for passage was obtained from the animals in group 5, was administered to another group of eight marmosets. These animals did not develop liver disease during the following 5 months. Serums from all animals of this second passage were obtained on the 35th day after inoculation, pooled, and inoculated into a third group of 4 marmosets which remained chemically and histologically normal for the succeeding 4 months.

Other Inoculations.—We also inoculated a preparation of adenovirus type 5 intramuscularly. This agent was isolated by Dr. W. D. Hillis from a chimpanzee involved in an outbreak of chimpanzee-associated hepatitis (17). No abnormalities were observed in these animals during 4 months.

A serum from a patient with typical infectious mononucleosis was inoculated intravenously into five animals. None showed heterophile antibodies, atypical lymphocytosis, serum enzyme rises, or changes of liver biopsy during 4 months.

General and Biochemical Data.—None of the animals with abnormal hepatic tests and/or with histological evidence of liver damage exhibited any signs of overt illness. Serum enzyme activities (SGOT and SICD) rose in animals from three of the five groups inoculated with potentially positive material but the curves of successive serum enzyme values were not the same from animal to animal. Some marmosets had a single "spike" of rather short duration (Text-fig. 3); others had a biphasic curve with two periods of illness (Text-fig. 4) with either the first or the second enzyme elevation higher and longer. Others re-



TEXT-FIG. 3.* Course of biochemical abnormalities in a single marmoset from experiment 2–4. Note the single "spike" of elevated SGOT and SICD activities over a period of 3 wk.

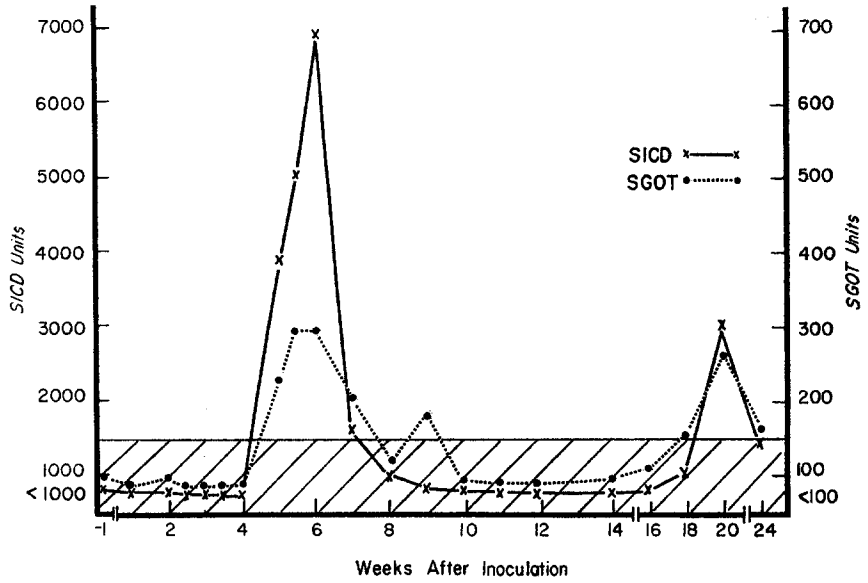
sponded with persistent biochemical abnormalities for many weeks (Text-fig. 5). Serum levels over 0.15 mg per 100 ml direct-reacting bilirubin and 0.50 mg per 100 ml of total bilirubin occurred in 9 of 21 infected animals in group 2 and in 3 of 16 animals in group 5.

Animals of the last passage of group 2 are not included in this count as no bilirubin determinations were performed because all available serum was used for determining various other enzyme levels which will be reported in detail elsewhere.[1]

* Reprinted by permission from: Deinhardt, F., and A. W. Holmes. 1965. Epidemiology and etiology of viral hepatitis. *In* Progress in Liver Diseases. Hans Popper and Fenton Schaffner, editors. Grune and Stratton, New York and London. **2**:380.

[1] Mohr, J., and H. Mattenheimer. Unpublished observations.

The isoenzymes of lactic dehydrogenase were determined by electrophoretic separation in six serums each from control and infected animals during the acute stage of their disease. During acute liver injury (acute stage serums) the fastest moving isoenzyme (which is predominantly present in liver tissue) was increased both in absolute amount and in percentage of total lactic dehydrogenase activity (Table II).[1]

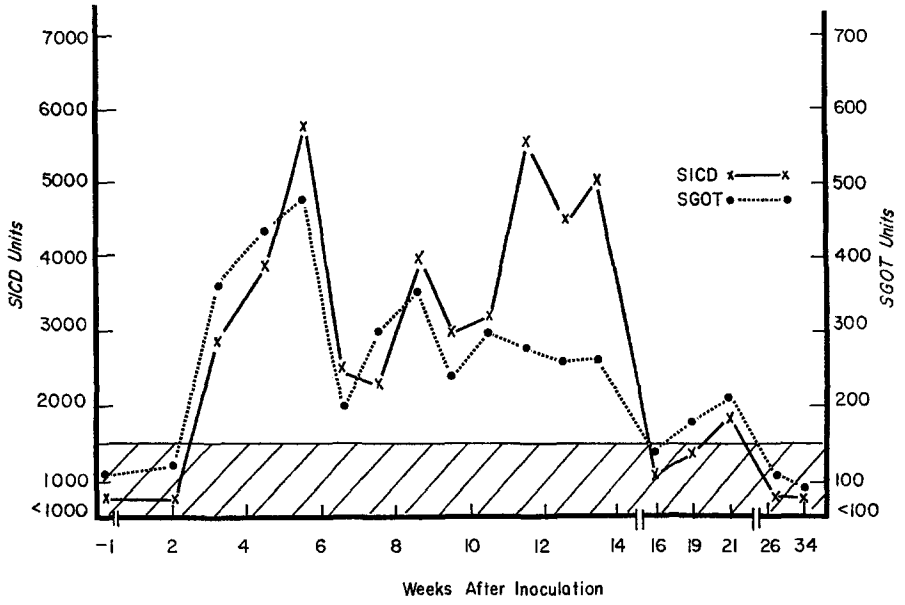


TEXT-FIG. 4. SGOT and SICD levels in a marmoset from experiment 2-4. Note the two episodes of increased enzyme activity occurring about 6 and 20 wk after inoculation.

The evaluation of stool samples from control and inoculated animals is not yet completed but, except for some known enteric viruses recovered from both control and inoculated animals, no agents have been detected which could be linked to the inoculations.

Histopathologic Findings.—The livers of randomly selected untreated control marmosets or marmosets inoculated with control materials revealed, in the studied biopsy specimens, liver cells varying in size and usually containing much glycogen. This increased glycogen content was indicated in hematoxylin eosin sections by fine vacuolated and granulated cytoplasm (Fig. 1 *a*). The nuclei appeared vesicular but often dense and the nucleoli were not always conspicuous. The chromatin was accentuated on the nuclear membrane but was otherwise fairly homogeneous. Binucleated cells were frequent. Brown argentaffin pigment granules without iron reaction, seemingly lipofuscin, were seen around the bile canaliculi and, in effect, outlined them (Fig. 1 *b*). Sinusoidal cells were

moderately prominent, but contained little PAS-positive nonglycogenic material. The walls of the central veins were thin and their lining endothelial cells flat (Fig. 1 *c*). Occasionally, mononuclear inflammatory cells accumulated in sinusoids (Fig. 1 *d*), and very exceptionally they replaced liver cells to produce small focal necroses. The sinusoidal walls were fairly prominent and gave a distinct reaction in aniline blue connective tissue stains as well as with PAS. In silver impregnation, the reticulum framework lined the sinusoids. The portal tracts were, as a rule, poor in cells (Fig. 1 *a*), but in very few instances they



TEXT-FIG. 5. SGOT and SICD levels in a marmoset from experiment 2-1. Note the protracted period during which elevated enzyme activity was found in the serum of this animal.

contained inflammatory cells, either of histiocytic character with PAS-positive cytoplasm or lymphocytes, but only rarely were segmented leukocytes and plasma cells noted (Fig. 1 *e*). Proliferation of bile ductules was not conspicuous. In some specimens, microfilariae were seen in sinusoids.

The specimens from many inoculated animals exhibited changes which were not found in controls, and some alterations found in controls were accentuated.

Alterations in inoculated animals, observed during the week before the first enzyme elevations were noted, included increase in the number and activation of sinusoidal cells (Fig. 2 *b*) with conspicuous focal necroses and portal inflammation with bile duct proliferation (Fig. 2 *a*).

At the height of the enzyme elevations the most conspicuous findings were

alterations of the hepatocytes which differed from those in control specimens in that the cells varied in staining qualities of cytoplasm and of nuclei, even with considerable variation between neighboring cells (Fig. 2 *c*). In hematoxylin and eosin sections, scattered dark cells were seen (Fig. 2 *d*). Moreover, some hepatocytes appeared either denser *in toto* or a portion of the cytoplasm exhibited greater density giving the character of eosinophilic homogenization (Figs. 2 *d*, 3 *a*). In PAS stains without diastase digestion, these dense portions appeared free of glycogen. Moreover, in these stains the variation in neighboring cells was particularly well brought out since they reflected inequalities in glycogen con-

TABLE II

Activity of Lactic Dehydrogenase and Its Isoenzymes in Serum from Normal Marmosets, Marmosets with Histological and Biochemical Evidence of Hepatitis, and in Normal Marmoset Liver Tissue

Test samples		LDH-Isoenzyme					LDH total
		1	2	3	4	5	
Normal marmoset serum	Activity*.....	64.9	24.6	16.8	18.6	21.5	146.4
	% of total activity.....	44.3	16.8	11.5	12.7	14.7	
"Hepatitic" marmoset serum	Activity*.....	262.1	119.4	65.8	60.2	607.9	1115.4
	% of total activity.....	23.4	10.7	5.9	5.4	54.5	
Normal marmoset liver tissue	Activity‡.....	0	0	0	21.6	448.4	470.0
	% of total activity.....	0	0	0	4.6	95.4	

Note the predominance of isoenzyme 5 in whole liver tissue and the absolute and proportional elevation of this isoenzyme in serum from marmosets with acute hepatitis.

* International units/liter.

‡ International units/100 g wet weight.

tent in neighboring cells (Fig. 3 *b*). In addition, some enlarged hepatocytes had a glycogen poor, very light cytoplasm giving a ballooned appearance (Fig. 3 *c*). The nuclei varied in size. Many were more vesicular than were those of controls, and their chromatin appeared irregularly arranged and peculiarly clumped (Fig. 3 *f*). This resulted in a coarse stippling of the nuclei and irregularly outlined unstained zones (Fig. 3 *d*). Occasionally nuclei were ballooned. Focal necroses were numerous and mainly mononuclear cells, either histiocytes or lymphocytes, replaced liver cells (Figs. 3 *a*, 3 *f*). Some foci of necrosis were extensive, resulting in large accumulations of inflammatory cells. Another feature, only found in hepatitis, was inflammation of the central veins. Their walls were thickened and contained inflammatory cells of various characteristics. Moreover, their lining cells were proliferated and bulged into the lumen (Fig. 3 *e*).

The changes found occasionally in controls and markedly accentuated in hepatitis included accumulation of inflammatory cells both in the parenchyma and in the portal tracts. In the parenchyma, the sinusoidal cells appeared far more numerous and a much larger number contained PAS-positive nonglycogenic material. They accumulated sometimes in small clusters in dilated sinusoids (Fig. 4 *a*). The portal tract inflammation was far more severe and more uniform, sparing no portal tract. The same type of inflammatory cells accumulated with occasional admixture of segmented leukocytes. Sometimes bile ductules had proliferated (Fig. 4 *b*). In one marmoset (group 5, third passage) the alterations were more conspicuous than in all others, both in the parenchyma and in the portal tracts (Fig. 4 *c*). The number of focal necroses was considerably greater and the alteration of the hepatocytes involving both nuclei and cytoplasm was much accentuated. Eosinophilic homogenous cells with pyknotic nuclei were seen, although it was not clear whether they were of hepatocytic or Kupffer cell origin (Fig. 4 *d*). Even in small portal tracts, the accumulation of inflammatory cells was impressive and plasma cells were fairly frequent (Fig. 4 *e*). This animal had had elevated enzyme activities since the 23rd day after inoculation (SGOT up to 226 units and SICD up to 8042 units) and died on the 30th day. The SGOT and SICD activities on the day of death were 216 and 4510 units respectively. The marmoset was not clinically ill and death was caused by a tension pneumothorax following liver biopsy.

The morphological alterations persisted in a number of animals for several weeks after the date the serum enzyme activities had returned to normal. These alterations include disarray of the liver cell plates with variation in the appearance of neighboring hepatocytes, few focal necroses, and considerable proliferation of sinusoidal cells (Fig. 5 *a*). Similarly, the portal tracts remained enlarged and densely infiltrated in some instances while the hepatocytes still showed severe alteration; even phlebitis of the hepatic vein tributaries was noted (Fig. 5 *b*). Borderline alterations such as conspicuous focal excess of sinusoidal cells and variations of liver cell size were also noted in some marmosets that had not developed any rises in serum enzyme activities after injection with inocula which, however, had induced chemical liver disease in other animals (see results under groups 2 and 5) (Fig. 5 *c*). In serial biopsies, hepatic changes such as disarray of hepatocytes with focal necrosis and inflammatory reaction preceded the enzyme rise (Fig. 6 *a*), and the full-blown picture of hepatitis was still seen when the enzyme activities were returning to normal levels (Fig. 6 *b*). Even 2 wk later, while the enzyme activities had remained normal, definite hepatic alterations and particularly focal necrosis were still seen (Figs., 6 *c*, 6 *d*).

DISCUSSION

Past experiences have shown that it is necessary to evaluate any apparent transmission of human viral hepatitis to experimental animals with particular caution. A suitably wary approach may be found in a reformulation of Koch's

postulates. Can the causative agent be isolated regularly from patients suffering from hepatitis? Can the agent be passed serially from one animal to another by materials free of other microorganisms? Does the agent produce in inoculated animals a disease identical with or similar to the disease in man? Can a rise in antibodies to the agent be demonstrated both in man and in the experimentally infected animals? Can the morphology, and the chemical, physical, and antigenic characteristics of the agent be determined? And after several animal passages does the agent still induce the same disease if reinoculated into man?

The inoculation of acute serums or plasmas from human cases of viral hepatitis into marmosets induced definite biochemical and morphological hepatitis in two out of five attempts. Of the three failures one gave borderline results and two were totally negative (these two materials were theoretically less likely to contain virus). Inoculations of a control human serum, an acute phase serum from a case of infectious mononucleosis, and an adenovirus type 5 preparation did not induce pathological changes in marmosets. These results are similar to the frequency of virus isolations in other known virus diseases.

Hepatic disease was produced regularly in serial marmoset-to-marmoset passages by transfer of acute marmoset serums. The incubation periods varied considerably in the early passages but became less variable and shorter after two or more animal passages with a simultaneous increase in the severity of the disease. This apparent adaptation of an agent to a new host is observed in many instances when viruses are transferred from one animal species to another. The disease induced in marmosets was probably caused by a microorganism, as a toxic factor would be unlikely to persist through six animal-to-animal passages. Inoculation of bacteriological and mycoplasma media and of various tissue cultures excluded, beyond reasonable doubt, the presence of any known common agents which might be causing hepatitis in marmosets. The use of control animals from the same colony and the blind passage of serums from such animals made it unlikely either that a naturally occurring marmoset agent was transmitted or that a dormant agent was activated by the experimental procedures. However, these possibilities have not been completely eliminated.

The disease demonstrated in marmosets was similar to hepatitis in man. Between 50 and 100% of animals in different experiments developed both significant elevations of SGOT and SICD activities and morphological changes in the liver but none of the animals became overtly sick. The results of other serum enzyme determinations also indicated that liver injury had occurred. Of particular importance, elevation of the lactic dehydrogenase isoenzymes by electrophoresis showed that only the fastest-moving fraction was elevated, and this isoenzyme is predominantly present in liver tissue.[1] Although serum bilirubin of marmosets, as in other nonhuman primates, is normally very low and is not a very sensitive indicator for hepatic disease in nonhuman primates (7), hyper-

bilirubinemia was observed in about 20% of the positive animals. The biochemical disease lasted from 1 to 12 wk and biphasic curves of serum enzymes were not infrequently observed. The morphologic alteration in the liver had many hallmarks of human viral hepatitis, particularly variations in the appearance of neighboring hepatocytes, acidophilic bodies, focal necrosis, portal inflammation with proliferation of bile ductules, and inflammatory reaction of the hepatic vein tributaries (18–20). However, the lesion was not identical with the picture typically seen in human viral hepatitis. In a patas monkey that developed hepatic disease after inoculation with icterogenic material, the histologic appearance was also not identical with that in man with hepatitis (5). The characteristic lesion in marmosets differed from the occasional alterations found in control marmosets which were presumably the result of spontaneous infections, parasitism, or malnutrition. The first changes in liver biopsies were sometimes observed serveral days before significant serum enzyme rises could be measured and they frequently persisted for several weeks after the serum enzyme activities had returned to normal. Morphological alterations were seen also in some inoculated animals which did not develop significant biochemical abnormalities. Some animals used in these studies are being kept for long-term follow-up studies in our laboratories, but it is not known yet whether they will develop recurrent attacks, active chronic hepatitis, or cirrhosis.

No rise in antibodies to the agent has been demonstrated, the physical characteristics of the agent remain unknown, and it is yet to be proved that the agent inducing hepatitis in marmosets is indeed the agent of human viral hepatitis by the reintroduction of the agent after several marmoset passages into man.

Our case for considering that human viral hepatitis has been transmitted to marmosets rests on these results. All of the observations seem compatible with the assumption that the disease in marmosets is caused by "the" or one of the agents of human viral hepatitis and, if this assumption is correct, marmosets may then provide the long sought model system for the study of this disease. But before any assumption based on the findings reported in this publication can be regarded as more than a working hypothesis, the results with marmosets must be both confirmed and extended.

It is unlikely that marmosets are inherently more susceptible than other nonhuman primates, and it is of interest to consider why they should appear so. Nonhuman primates which have been in captivity for some time before use often acquire immunity to human diseases by natural infection through contact with man, as occurred in measles studies with rhesus monkeys (21). Marmosets, by their ferocious character, minimise their contacts with man, and the time spent by marmosets in transit before arrival in the laboratory is usually shorter than for African or Asian primates. Natural infections of marmosets with common human viruses, therefore, should be rare and this was shown in a previous study in which no antibodies to common human viruses could be demonstrated in

marmosets which had been in our colony for 6 wk to 6 months (8). It is likely, therefore, that marmosets have no preexisting immunity to the agents of human viral hepatitis. In addition, it has been postulated that a predamaged liver is a prerequisite for the full expression of hepatitis. Marmoset livers are rarely completely normal, due mainly to heavy parasitic infestations, and this may predispose the animals to a fuller expression of the disease. Future studies with parasite-free colony born animals may answer this question.

SUMMARY

Inoculation of human serums or plasmas obtained during the early acute phase of viral hepatitis induced chemical and morphological hepatic disease in marmosets in two out of five experimental series. The disease was transmissible in series from marmoset to marmoset with an apparent increased virulence of the causative agent in later marmost passages. The chemical evidence for the disease was elevation of the activity of SGOT and SICD and of serum bilirubin. In serial liver biopsy specimens interpreted under code, a hepatitis, exhibiting some of the characteristics of human viral hepatitis, was readily distinguishable from nonspecific changes. The morphological changes preceded the biochemical alterations and persisted after them.

The data reported in these studies indicate that marmosets may be susceptible to human hepatitis. If these observations are confirmed, these animals may provide good experimental models for this disease. Final proof that the hepatitis observed in marmosets is caused by agents of human viral hepatitis is still lacking.

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EXPLANATION OF PLATES

PLATE 81

FIG. 1 *a*–1 *e*. Livers of uninoculated marmosets. Hematoxylin and eosin.

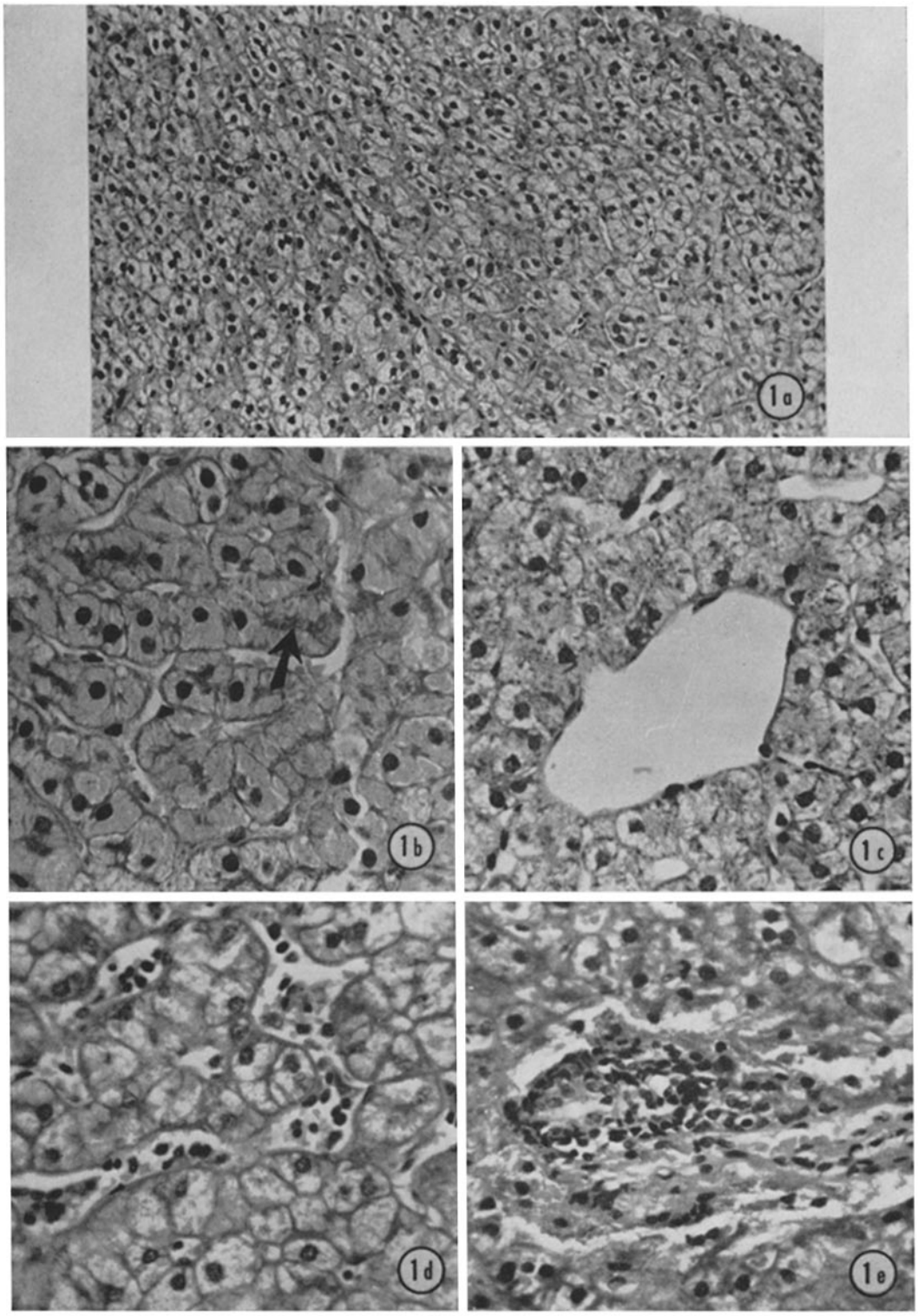
FIG. 1 *a*. The liver cells have equal size and contain finely vacuolated and granulated cytoplasm indicative of high glycogen content. In center is normal portal tract. × 120.

FIG. 1 *b*. Note pigment granules around bile canaliculi and normal sinusoidal cells. × 400.

FIG. 1 *c*. Note thin wall of central vein with flat endothelium. The hepatocytes contain in the centrilobular zone many pigment granules of lipofuscin character. × 400.

FIG. 1 *d*. Focal accumulation of sinusoidal cells, some lymphocytes, no alteration of hepatocytes. × 400.

FIG. 1 *e*. Slight excess of inflammatory cells in portal tract without proliferation of bile ductules. × 120.



(Deinhardt et al.: Hepatitis in marmosets)

PLATE 82

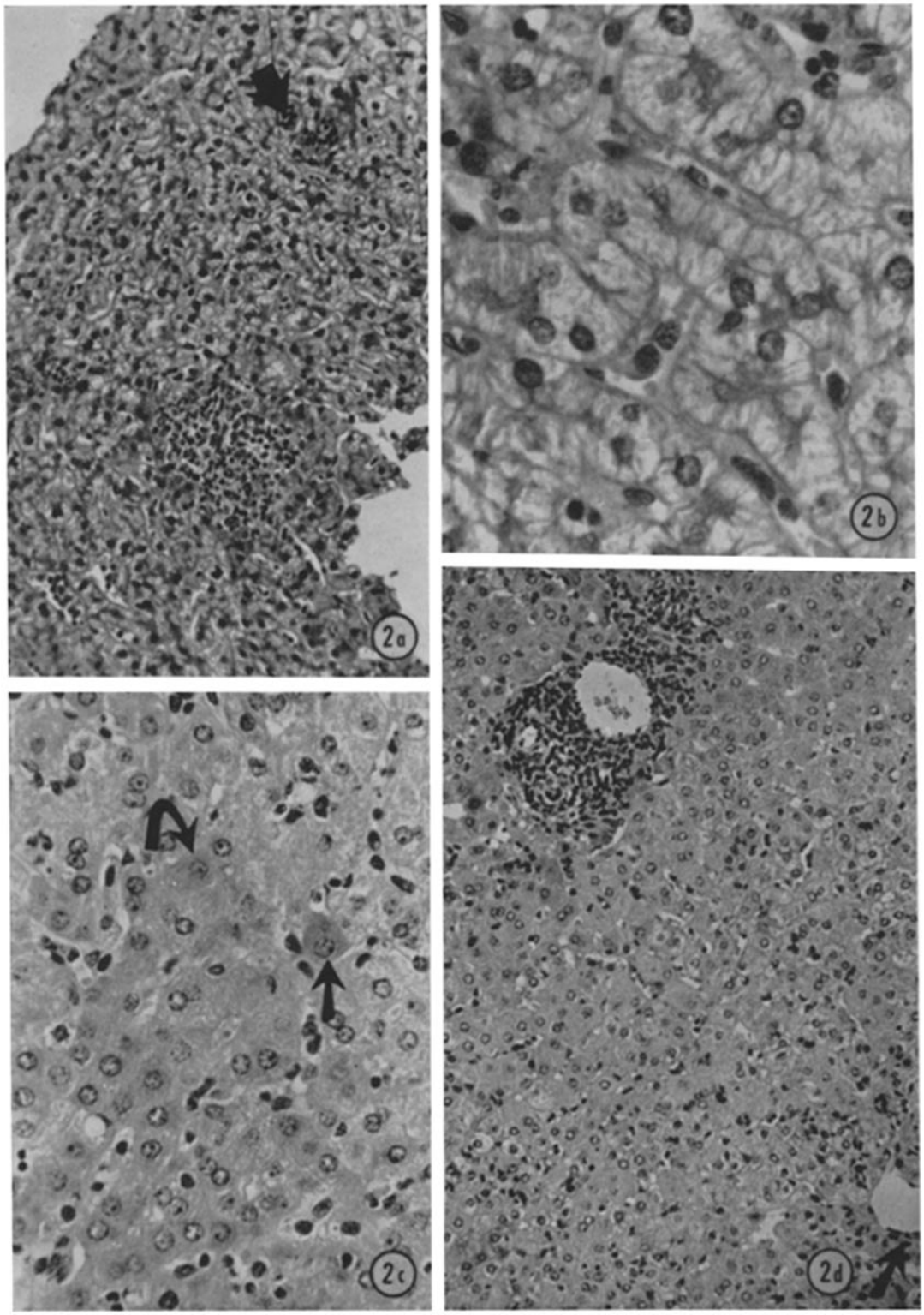
FIG. 2 *a*–2 *d*. Livers of inoculated marmosets. Hematoxylin and eosin.

FIG. 2 *a*. 2 wk after inoculation before rise of enzyme activity (experiment 2-5). Variation in size and staining qualities of hepatocytes. Focal necrosis (arrow). Enlargement of portral tracts with conspicuous inflammatory reaction. × 120.

FIG. 2 *b*. Same animal. Proliferation and activation of sinusoidal cells. × 400.

FIG. 2 *c*. On the peak of rise of enzyme activity (experiment 2-5, different animal). Variation in appearance of neighboring hepatocytes, some are dark (arrow) and others show eosinophilic homogenization (curved arrow). Note clumping of chromatin in nuclei and accumulation of inflammatory cells in sinusoids. × 240.

FIG. 2 *d*. Note severe portal inflammation with slight proliferation of bile ductules. Variation of hepatocytes with accumulation of inflammatory cells within sinusoids and replacing liver cells. Activation of endothelial lining in central vein (arrow). × 120. (Same animal as Fig. 2 *c*.)



(Deinhardt et al.: Hepatitis in marmosets)

PLATE 83

FIG. 3 *a–3 f*. Livers of inoculated marmosets that showed elevated activity of serum enzymes. (All from experiment 2-5.)

FIG. 3 *a*. Variation in appearance of neighboring hepatocytes, both in cytoplasmic staining quality and in nuclei. Note eosinophilic homogenization (fine arrow) and focal necroses (heavy arrow). Hematoxylin and eosin. × 240.

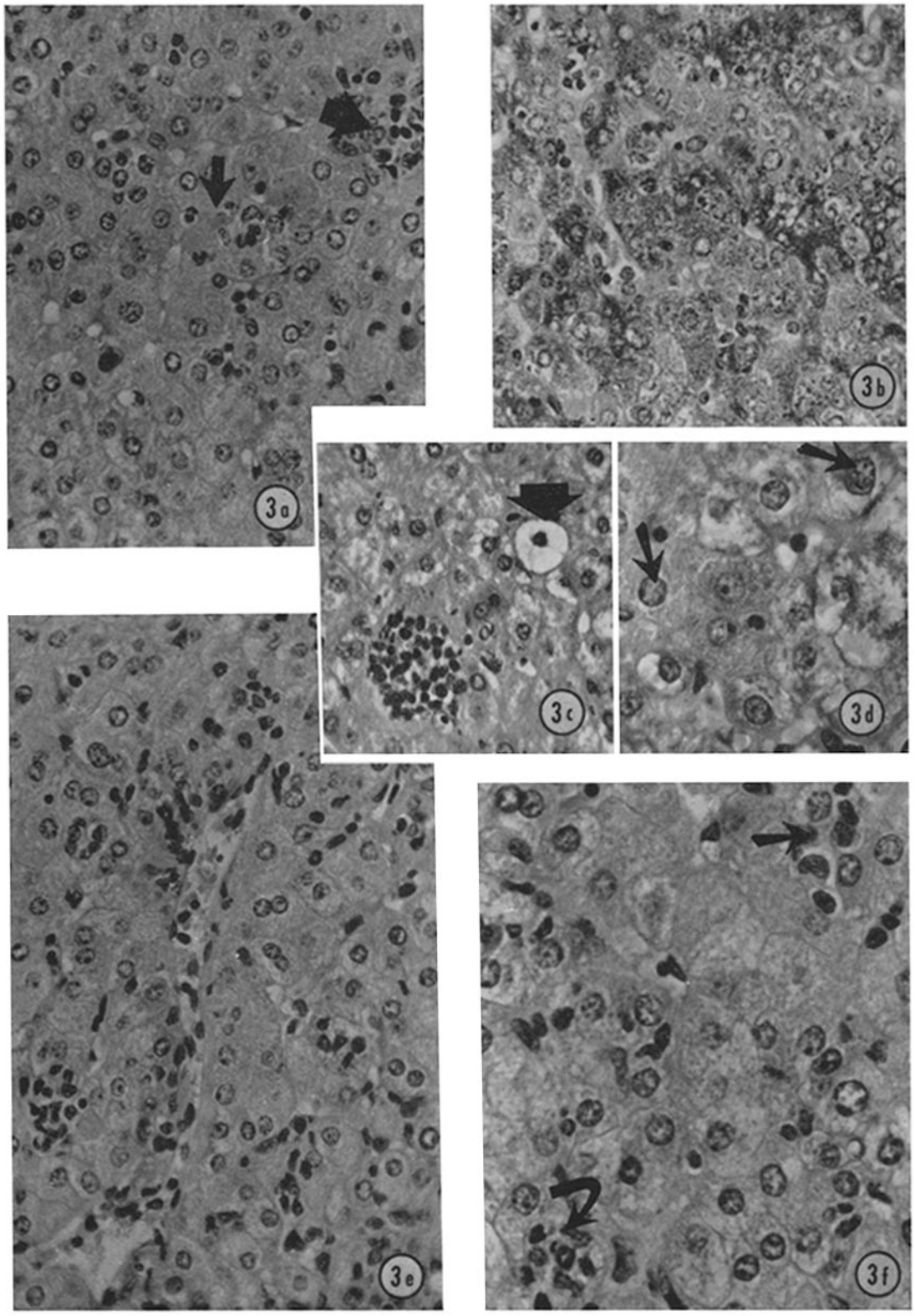
FIG. 3 *b*. Variation in glycogen content of neighboring hepatocytes. PAS stain. × 240.

FIG. 3 *c*. Ballooned hepatocytes with central nucleus and hydropic cytoplasm (arrow) and focal necroses (inflammatory cells replace liver cell which had disappeared). Hematoxylin and eosin. × 240.

FIG. 3 *d*. Irregular arrangement of nuclear chromatin producing stippling as well as central unstained zone (arrows). Hematoxylin and eosin. × 400.

FIG. 3 *e*. Note thickening of central vein with accumulation of inflammatory cells in its wall and mobilization of sinusoidal cells. Hematoxylin and eosin. × 240.

FIG. 3 *f*. Note variation in appearance of neighboring cells involving cytoplasm as well as nuclei. Some nuclei are ballooned. In some hepatocytes the cytoplasm is irregularly clumped. Inflammatory cells accumulate in sinusoids (arrow) or replace liver cells (curved arrow). Hematoxylin and eosin. × 400.



(Deinhardt et al.: Hepatitis in marmosets)

PLATE 84

FIG. 4 *a*–4 *e*. Livers of inoculated marmosets that exhibited rise in enzyme activities. (Experiment 5-3.) Hematoxylin and eosin.

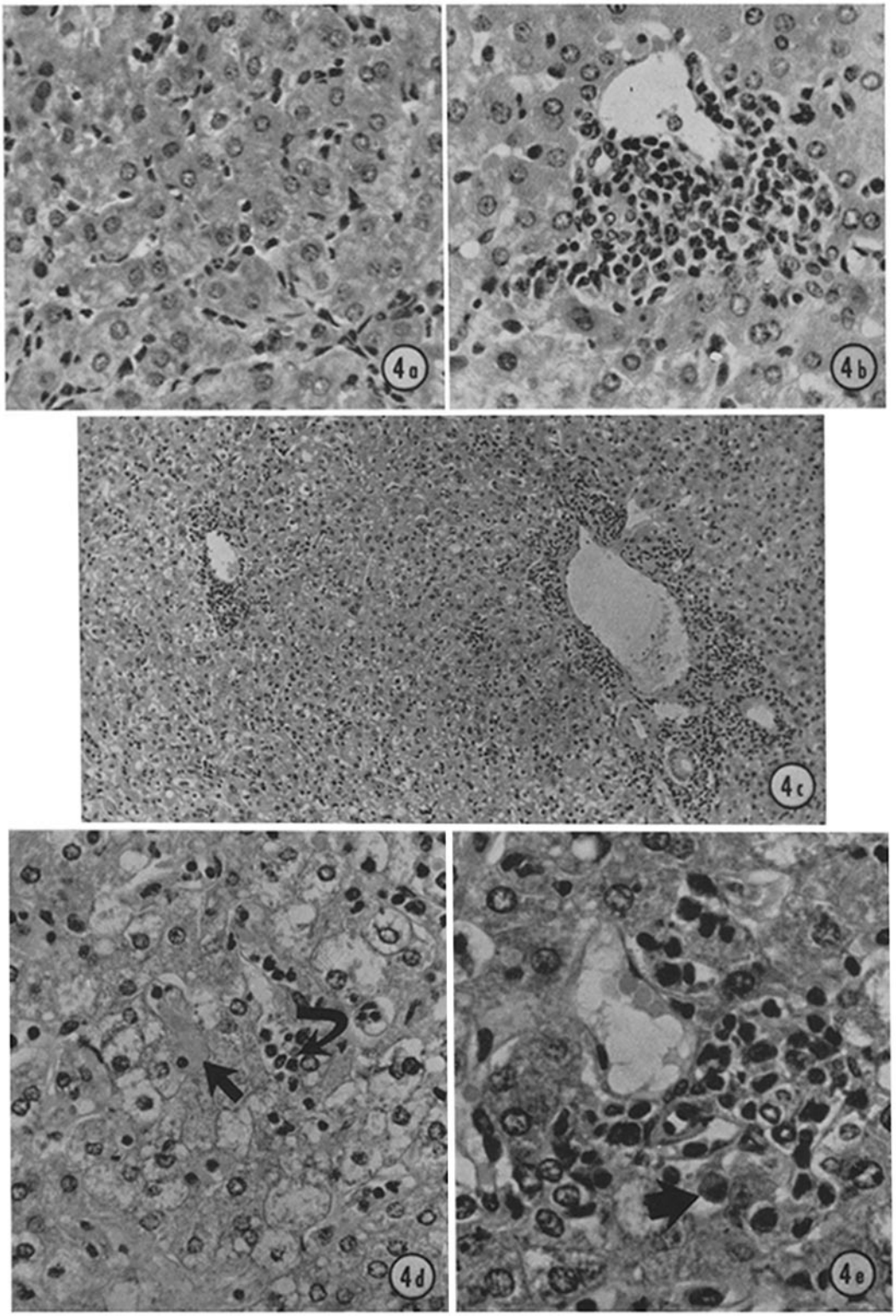
FIG. 4 *a*. Proliferation of sinusoidal cells throughout with focal accumulation of inflammatory cells in dilated sinusoids. × 240.

FIG. 4 *b*. Severe portal inflammatory reaction with accumulation of histiocytes, lymphocytes, and polymorphonuclear leukocytes. Note proliferation of bile ductules. × 240.

FIG. 4 *c*. Conspicuous variation in appearance of liver cells and foci of inflammatory cells in parenchyma. Extensive portal inflammation. × 60.

FIG. 4 *d*. Conspicuous variations of liver cells throughout involving nuclei and cytoplasm. Some cells lining the tissue spaces have a homogeneous eosinophilic cytoplasm resembling acidophilic bodies (arrow). Inflammatory cells accumulate and replace liver cells (curved arrow). × 240.

FIG. 4 *e*. Small portal tract densely infiltrated by a variety of inflammatory cells with conspicuous contribution of plasma cells (arrow). × 400.



(Deinhardt et al.: Hepatitis in marmosets)

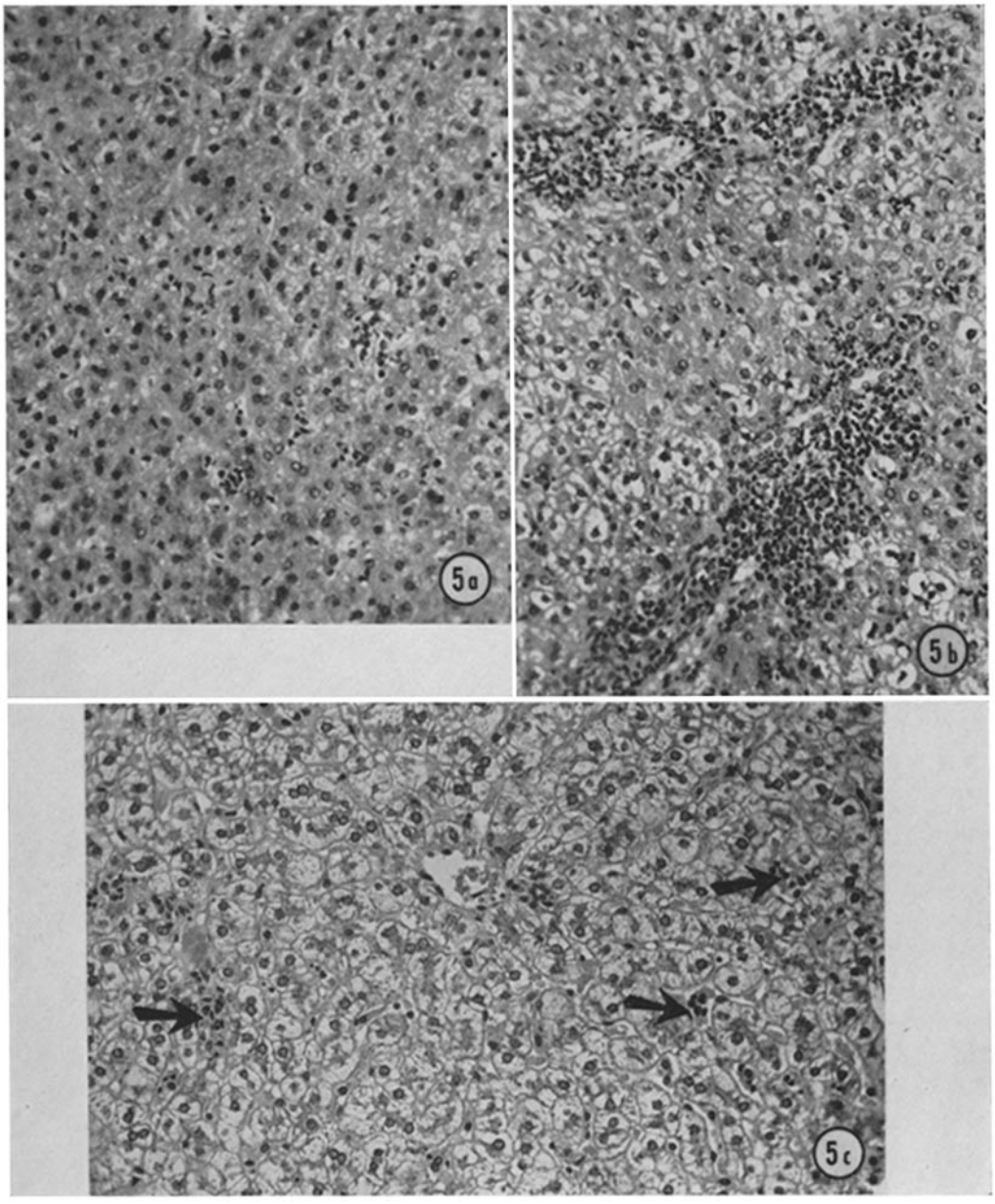
PLATE 85

FIG. 5 *a*–5 *c*. Livers of inoculated marmosets. Hematoxylin and eosin.

FIG. 5 *a*. 6-wk after inoculation and 2 wk after significant enzyme rise with normal enzyme activity at the time of examination. There is considerable variation in arrangement, size and staining qualities of hepatocytes, focal necrosis and accumulation of sinusoidal cells. (Experiment 2-5.) × 120.

FIG. 5 *b*. Same animal 2 wk later with continued normal enzyme activities. Note severe portal inflammatory reaction and parenchymal alteration. × 120.

FIG. 5 *c*. Animal never developed elevated activities of enzymes. (Experiment 2-5.) Note accumulation of inflammatory cells in sinusoids (arrows) and slight disarray of liver cell plates. × 120.



(Deinhardt et al.: Hepatitis in marmosets)

PLATE 86

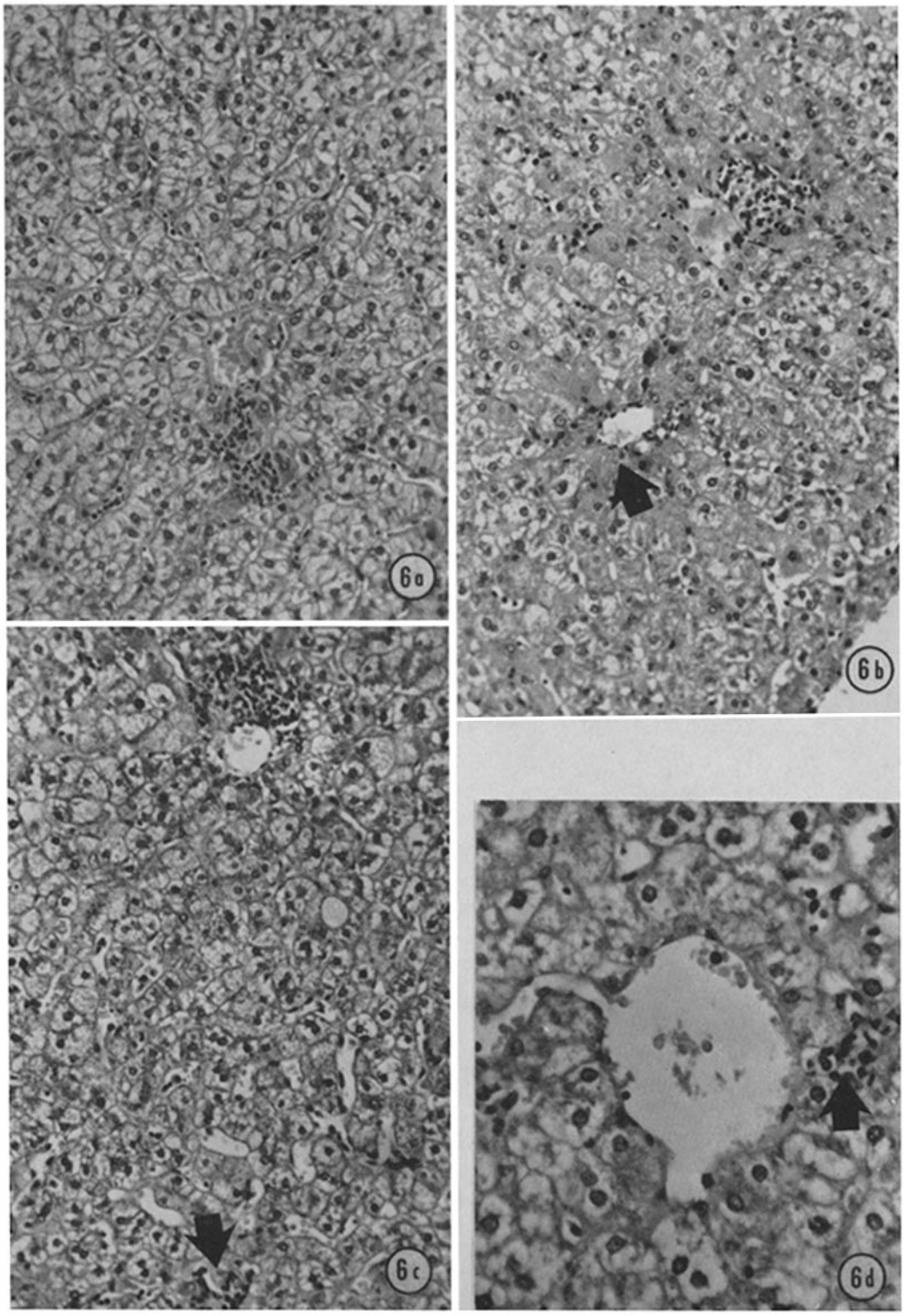
FIG. 6 *a*–6 *d*. Serial liver biopsies from a single marmoset in experiment 2-5. Hematoxylin and eosin.

FIG. 6 *a*. 15 days after inoculation and 1 day before first rise of enzyme activities. Note disarray of liver cell plates, focal necrosis, excess of sinusoidal cells, and portal inflammatory reaction. × 120.

FIG. 6 *b*. 6 wk after inoculation, when the rise of enzyme activities was returning to normal levels. Note variation of neighboring hepatocytes with many dense and ballooned cells, portal infiltration, and inflammatory cells in wall of central vein (arrow). × 120.

FIG. 6 *c*. 8 wk after inoculation. Serum enzyme activity has remained normal. Note slight disarray of liver cells, focal necroses (arrow), and portal inflammatory reaction. × 120.

FIG. 6 *d*. Focal necroses near a central vein (arrow) the wall of which is normal. × 400.



(Deinhardt et al.: Hepatitis in marmosets)