

Hepatitis in the Marmoset, *Saguinus mystax* (35049)

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(Introduced by H. M. Meyer)

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Saguinus nigricollis and *S. oedipus*, two species of marmosets, have been shown to develop signs of hepatitis when inoculated with serum or plasma from patients with acute viral hepatitis (1-3). Hillis (4) mentioned experiments in which material received from Deinhardt induced changes in *S. mystax* compatible with those reported by Deinhardt *et al.* (1). It has also been found recently that the serum from some hepatitis patients contains Australia, SH or hepatitis-associated antigen (HAA) which is located on 20-m μ , virus-like particles (5-7). We have investigated the effect of inoculating *S. mystax* with the transmissible agent described by Deinhardt *et al.* (1), with HAA positive acute phase serum from a hepatitis patient, and with HAA negative acute phase sera from patients with infectious (short incubation) hepatitis.

Materials and Methods. Marmosets. *Saguinus mystax* (the white mustached marmoset) were used exclusively. They had been maintained in our facilities for more than 1 year and were caged individually during the experiments. Filaria, trypanosomes, or both were found in some of the animals. Uninoculated, control animals were maintained in a separate room from the inoculated animals. They were maintained by one man who cared for the control animals and then the experimental animals and did not return to the room housing the control animals the same day.

The experiments described here were performed using marmosets procured in October, 1967. Since that time, *S. mystax* have been unavailable and thus, these experiments have been limited.

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Inocula. Three types of inocula were used:

1. A pool of marmoset sera (H118) was supplied by Dr. F. Deinhardt, Presbyterian-St. Luke's Hospital, Chicago, Illinois. This material had been passed five times exclusively in marmosets which were originally inoculated with serum from a patient (G. B.) with acute hepatitis as described by Deinhardt *et al.* (1). The H118 pool was diluted 1:10 in 0.85% NaCl immediately before inoculation.

2. Serum M-2 was obtained from a patient with acute viral hepatitis and had a HAA complement-fixing titer of 1:320. Cesium chloride density gradient fractions, 1.18 to 1.25 g/cm³, from this serum were pooled and dialyzed against 0.85% NaCl as described previously (8).

3. A pool, M-3, was prepared from six sera obtained during acute illness from three volunteers infected orally with human serum containing the MS-1 strain of infectious or short incubation hepatitis agent (9). The six sera were provided by Col. Marcel E. Conrad, Walter Reed Army Institute of Research, Washington, D.C. They possess no HAA. The M-3 pool was diluted 1:5 in 0.85% NaCl.

Experimental designs. Expt. 1. Eight marmosets were inoculated intramuscularly with 1 ml each of the H118 pool and eight were used as uninoculated controls. Liver biopsies were obtained from all animals prior to inoculation. Blood samples were obtained at least once a week for enzyme determinations. At 21 and 28 days postinoculation, one control and one inoculated animal were sacrificed; also, one inoculated animal was sacrificed on day 37 and one on day 42.

Expt. 2. Six marmosets were inoculated intramuscularly with 1 ml each of serum M-2

(HAA positive) and six were similarly inoculated with HAA containing fraction of serum M-2. Blood samples were collected weekly for enzyme determinations and assays for the presence of HAA. Liver biopsies were not performed unless transaminase levels were elevated.

Expt. 3. Eight marmosets were inoculated intramuscularly with 1 ml each of pool M-3, the pooled acute phase sera from three patients with short incubation hepatitis (HAA negative). Two of these marmosets had been inoculated previously with pool H118, two with whole serum M-2 containing HAA, two with the partially purified HAA from serum M-2, and two had not been previously inoculated. Biopsies were obtained from all animals before inoculation. Blood samples were collected weekly for enzyme determinations and biopsies were performed when elevated enzyme levels were noted.

Determinations of HAA. HAA was measured by a microtiter, complement-fixation method described previously (10). Also, sera from selected animals were examined by electron microscope for the presence of 20-m μ virus-like particles as previously described (8).

Transaminase determinations. Blood was drawn from femoral vessels into syringes containing 150 units of sodium heparin, USP in 0.1 ml of 0.85% NaCl. Heparin was used to decrease the hemolysis in the samples which was noted after clotting occurred. Plasma glutamin-oxalacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT) were determined by the Sigma-Frankel method (11).

Liver biopsies and histologic evaluation. Liver biopsy specimens were obtained from all animals at least 2 weeks before the animals were inoculated. The biopsy specimens were obtained by Menghini needles, 1.2 to 1.4 mm diam (12). Specimens were fixed either in Bouin's fixative for 2 hr or mercuric sublimate-formalin (B-5) fixative for 24 hr (13). During Expt. 1, six marmosets were exsanguinated and portions of liver were quickly removed and fixed in Bouin's fixative for 2 hr. Specimens of other tissues were fixed in 10% buffered, neutral formalin. All tissues were stained with hematoxylin-eosin. Histologic evaluations were performed by one person without any knowledge of the inoculation histories or enzyme levels of the animals.

TABLE I. Plasma Glutamic-Pyruvic Transaminase Levels (Frankel units/ml) in *S. mystax* for the First 6 Weeks Following Inoculation with Deinhardt's Marmoset Agent.

	Postinoculation (days)						
	7	14	19	23	28	37	42
Control animals							
1	12	32	32	—	—	—	—
2	18	32	62	74	44	—	—
3	16	46	41	32	36	27	27
4	16	20	44	32	24	27	18
5	14	18	38	17	20	17	24
6	12	41	38	20	27	135	18
7	12	18	18	50	27	17	13
Inoculated animals							
8	14	280	665	—	—	—	—
9	27	284	≥ 1500	100	—	—	—
10	15	41	180	520	205	—	—
11	100	710	360	90	53	75	—
12	18	100	310	340	160	24	22
13	17	22	154	90	370	370	27
14	16	310	≥ 1500	310	250	150	160
15	24	390	790	280	265	310	160

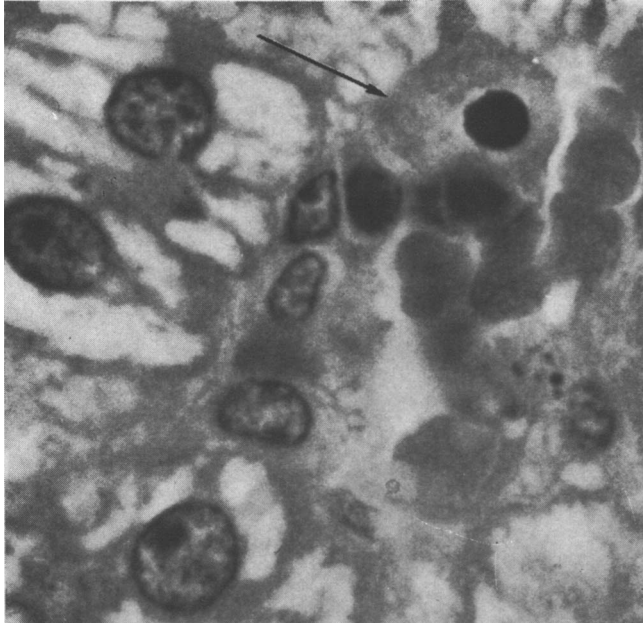


FIG. 1. Section of liver from marmoset No. 8 in Expt. 1: A single hepatocyte with hyaline eosinophilic cytoplasm and a basophilic nucleus is indicated by the arrow; hematoxylin and eosin; $\times 325$.

Results. Transaminase levels were determined in 85 plasma samples from 30 normal *S. mystax*. The means and standard deviations were as follows: SGOT, 97 ± 26 units/ml; SGPT, 31 ± 10 units/ml. Among the 85 SGPT determinations only two were found to be greater than two standard deviations from the mean, *i.e.*, 104 and 138.

Sections of liver biopsies obtained prior to the start of the experiments did not show significant inflammatory infiltrates nor hepatic necrosis. There was minimal evidence of extramedullary hematopoiesis.

During the experiments the animals remained in good condition. Weight, temperature, and microhematocrit values did not fluctuate significantly. Some deaths resulted from hematomas which developed in the groin areas and thighs.

Expt. 1. The SGPT levels are shown in Table I. Results are shown for only seven uninoculated animals because one animal died of unknown causes 1 week after the experiment began. The fluctuations of the SGOT values were similar to the SGPT values in

time of occurrence and duration, although the relative magnitude of increases of SGOT levels in inoculated animals was less than the increases in SGPT. The greatest SGOT level was 645 units/ml, while the highest SGPT level recorded was 1500 units/ml. All eight inoculated animals had elevated enzyme levels between 14 and 37 days postinoculation.

Among the eight animals inoculated with infectious marmoset serum pool H118, the liver biopsy obtained from animal No. 8, inoculated 21 days previously, showed an occasional single hepatocyte with smudgy eosinophilic cytoplasm (Fig. 1) and a dense nucleus. A slight increase in periportal lymphocytic infiltrate was noted. Animal No. 9 died 23 days postinoculation. Sections of its liver showed marked cellular necrosis with destruction of individual hepatocytes and some liver cords. The cells in many areas appeared to be "ghost-like." There was a prominence of Kupfer cells and a more marked lymphocytic infiltrate in the periportal areas. Also, animal No. 10, sacrificed 28

days postinoculation, had large areas of cellular damage of its liver as indicated by marked eosinophilia and smudginess of the cytoplasm of hepatocytes. In some areas lymphocytic infiltrates were also seen as well as Kupfer cell prominence. The livers of animals (No. 11, 12, 13) sacrificed 37, 47, and 52 days postinoculation showed no abnormalities. Biopsy specimens were not taken from animals 14 and 15. Thus, in three of the six inoculated animals from which liver sections were available there were changes consistent with viral hepatitis.

HAA was not found in any of the sera by the complement-fixation tests. Electron microscopic examination of acute phase sera from animals 1, 2, 8, 10, 12, and 13 for 20-m μ virus-like particles was negative.

Expt. 2. None of the 12 animals inoculated with the HAA positive whole serum or the HAA containing fraction of the same serum from a patient with serum hepatitis developed HAA. One animal developed an SGPT level of 149 units/ml 64 days after inoculation. Sections of its liver showed no histologic changes. The remaining marmosets did not develop enzyme elevations during the 14-week observation period.

Expt. 3. Results are shown in Table II. Neither of the two animals which had been inoculated 6 months previously with the H118 pool of marmoset sera showed histologic changes during the 10 weeks of observation. One animal had SGPT levels of 112 and 129 units during the eighth and ninth weeks, and died during the tenth week. No histologic changes were found in this animal at autopsy.

Of the four animals previously inoculated with either whole serum containing HAA or partially purified HAA, one animal did not have a rise in SGPT or SGOT level or abnormal liver histology. That animal had been inoculated previously with whole serum. The other animal which had been inoculated with whole serum, demonstrated peak SGPT and SGOT levels of 408 and 540 units, respectively, during the seventh week with return to normal levels by the ninth week. Biopsy sections of the liver obtained during the sev-

TABLE II. Inoculation of Marmosets with Human Serum from Patient with Short Incubation Hepatitis (Expt. 3).

Group of marmosets	First inoculum	Second inoculum	Enzyme elevation (no. of animals with elev./no. inoc.)	Time of elev. (weeks)	Pathologic changes in liver	
					N.o. with changes/no. inoc.	Type of change
A	Marmoset serum H118 ^a	Human serum M-3 ^b (short incub. hepatitis)	0/2	—	0/2	—
B	Whole serum containing HAA	Human serum M-3 (short incub. hepatitis)	1/2	7	1/2	± ^c
C	CsCl fractions of serum containing HAA	Human serum M-3 (short incub. hepatitis)	2/2	7	2/2	± ^d
D	None	Human serum M-3 (short incub. hepatitis)	1/2	7	1/2	+

^a Received from Dr. F. Deinhardt.

^b Received from Col. M. Conrad.

^c Equivocal changes.

^d Changes consistent with histological picture of viral hepatitis.

enth week showed moderate amounts of periportal infiltration, but other signs of hepatitis were lacking. One of the two animals inoculated with partially purified HAA developed SGPT elevations to a peak level of 136 and SGOT levels of 288 during the seventh week. The other animal demonstrated moderately elevated transaminase levels (SGPT, 64-116; SGOT, 170-452) from the third to the tenth week postinoculation. All sections from liver biopsies obtained during the period of enzyme elevations from these two animals showed periportal infiltration and necrosis of individual hepatocytes consistent with mild viral hepatitis, similar to that seen in Fig. 1.

The two animals which had not been inoculated previously, but were inoculated with a pool of acute phase sera from short incubation hepatitis patients developed elevated SGPT and SGOT values and died. One died during the eighth week. Just prior to death it had slightly elevated SGPT levels and slight periportal infiltration in the liver. The other animal showed significantly elevated transaminase levels (peak SGPT 264, SGOT 400) as well as extensive periportal infiltration and necrosis of hepatocytes.

Discussion. We have confirmed the findings of Deinhardt *et al.* (1) and Holmes *et al.* (2) using a species of marmoset, *S. mystax*, which has not been commonly used before. Inoculation of a pool of fifth passage marmoset sera into *S. mystax* has resulted in liver changes compatible with viral hepatitis. Furthermore, in three of six marmosets inoculated with pooled sera from three patients with short-incubation (infectious) hepatitis, changes consistent with viral hepatitis developed. Thus, it appears from our study and the work of others that marmosets of the three species *S. mystax*, *S. nigricollis*, and *S. oedipus* can serve as animal models to study the pathogenesis of infectious hepatitis. The two marmosets inoculated previously with the pooled marmoset sera obtained from Deinhardt did not develop evidence of hepatitis when challenged with the pooled sera from patients with short-incubation hepatitis. Unfortunately, this very limited study cannot be

repeated, at present, since *S. mystax* are unavailable.

A difficult problem in experiments of this kind is that of establishing a basis for judging signs of disease. Our first approach was to hold the animals long enough to allow them to adjust to confinement and handling, and to investigate their natural flora. At the time of experimentation we felt we had a stable group of animals and adequate knowledge about their care.

Among 85 determinations of SGPT and SGOT levels performed on marmoset sera before hepatitis materials were introduced into the laboratory, only two SGPT concentrations were greater than two standard deviations above the mean of 31 units/ml, *i.e.*, 50 units/ml. We considered an SGPT concentration of 100 or greater as a definite sign of liver damage. Parks *et al.* (3) used 50 units as the upper limit of normal SGPT levels in *S. oedipus*. During Expt. 1, using the marmoset serum pool H118 obtained from Deinhardt, peak SGPT concentrations from 370 to 1500 units/ml developed in inoculated animals. These concentrations represent a greater than 10-fold increase over the mean value and correlated with histopathologic evidence of hepatic damage 21 to 28 days postinoculation.

The time at which peak transaminase levels were reached in our experiments with the H118 pool of marmoset sera agrees with the reports of Deinhardt *et al.* (1) and Parks *et al.* (3). The duration of the SGPT elevation compares favorably with the data of Parks *et al.* (3) and the duration of SGOT elevation is similar to that described by Deinhardt *et al.* (1) and Liu *et al.* (14). However, the durations of elevations of the two transaminases are different. SGPT reaches greater levels than SGOT and the return to normal appears to take longer.

The characteristics of changes in transaminase levels in inoculated animals were different in Expts. 1 and 3. In normal animals the SGPT levels were usually lower than the SGOT levels. In Expt. 1, the SGPT levels of the inoculated marmosets reached higher values than the highest SGOT levels. In Expt. 3

the SGPT values were always less than the SGOT values.

The histologic changes in the livers seen in our experiments were comparable to descriptions in previous reports (1, 2). These changes were also similar to those seen in acute human hepatitis (15, 16). In our experiments with the H118 pool we found that the hepatic changes were no longer seen 5 days after peak SGPT levels were detected. Thus the liver specimens from animals 11, 12, and 13 in Table I were studied after the enzyme levels had returned to normal.

Of great importance is the fact that marked enzyme elevations and histopathologic changes typical of acute viral hepatitis were not seen in any uninoculated, control animals nor in animals inoculated with HAA positive sera. This is in contradistinction to the report of Parks and Melnick (17).

None of the inocula, including the two which contained HAA, induced HAA in any marmoset. Several explanations for this are possible. Marmosets may not be susceptible to the virus of long incubation (serum) hepatitis and thus neither hepatic changes or induction of the antigen occurred. Further, Millman *et al.* (18) have been unable to detect nucleic acid in preparations of HAA. Very recently, Dane *et al.* (19) have postulated that HAA may not be an infectious virus but that preparations of HAA may consist of large numbers of antigenic particles which are noninfectious portions of a hepatitis virus and a few larger intact virions. If this is so, our preparations of HAA may not contain sufficient viral particles to be infectious. Further studies in marmosets to clarify these problems will be undertaken.

Although there was considerable variation among animals, it appears that the serum pool from three patients with short incubation, infectious hepatitis induced hepatic damage in *S. mystax* which had not been inoculated previously or had been inoculated with HAA containing human serum. Hillis (4) described experiments with *S. mystax* but did not specify the characteristics of the disease produced. Holmes *et al.* (2) reported that individual acute phase sera, but

not preinoculation sera from three volunteers with infectious hepatitis, induced hepatitis in *S. nigricollis*. Parks and Melnick (17) reported that these same sera did not induce hepatitis in *S. oedipus*. Our acute phase serum pool M-3, was prepared from the same three volunteers and did cause changes in the livers of three of six *S. mystax*. It is thus clear that more experience with human hepatitis materials is needed to evaluate the significance of the results reported here as well as by others (1-4, 14, 17).

Summary. The marmoset, *Saguinus mystax*, developed signs of disease similar to human hepatitis when inoculated with marmoset sera containing Deinhardt's hepatitis agent, or sera from patients with infectious hepatitis but not when inoculated with serum containing hepatitis-associated antigen or with partially purified antigen. The evidence of disease were 3- to 10-fold increase above normal SGPT levels and histologic changes of single liver cell necrosis with periportal lymphocytic infiltration. Hepatitis-associated antigen was not found in any marmoset sera by complement-fixation tests or electron microscopy.

1. Deinhardt, F., Holmes, A. W., Capps, R. B., and Popper, H., *J. Exp. Med.* **125**, 673 (1967).
2. Holmes, A. W., Wolfe, W., Rosenblate, H., and Deinhardt, F., *Science* **165**, 816 (1969).
3. Parks, W. P., Melnick, J. L., Voss, W. R., Singer, D. B., Rosenberg, H. S., Alcott, J., and Casazza, A. M., *J. Inf. Dis.* **120**, 548 (1969).
4. Hillis, W. D., *Mil. Med.* **133**, 343 (1968).
5. Blumberg, B. S., Sutnick, A. I., and London, W. T., *Bull. N.Y. Acad. Med.* **44**, 1566 (1968).
6. Prince, A. M., *Proc. Nat. Acad. Sci. U.S.A.* **60**, 814 (1968).
7. London, W. T., Sutnick, A. I., and Blumberg, B. S., *Ann. Intern. Med.* **70**, 55 (1969).
8. Barker, L. F., Smith, K. O., Gehle, W. D., and Shulman, N. R., *J. Immunol.* **102**, 1529 (1969).
9. Krugman, S., Giles, J. P., and Hammond, J., *J. Amer. Med. Ass.* **200**, 365 (1967).
10. Shulman, N. R., and Barker, L. F., *Science* **165**, 304 (1969).
11. Sigma Technical Bulletin No. 505, Sigma Chemical Company, St. Louis, Mo., revised May, 1964.
12. Menghini, G., *Gastroenterology* **35**, 190 (1958).

13. Bowling, M. C., "Histopathology Laboratory Procedures." Pub. Health Serv. Pub. 1595. Superintendent of Documents, U.S. Gov. Printing Office, Washington, D.C. (1967).
14. Liu, C., Thompson, L., Voth, D. W., and Svoboda, D. J., Abstr. Intersci. Conf. Antimicrobial Agents and Chemotherapy, 9th, Washington, D.C., October, 1969.
15. Smetana, H. F., Keller, T. C., and Dubin, I. N., *Rev. Gastroenterol.* **20**, 227 (1953).
16. Smetana, H. F., *Gastroenterology* **26**, 612 (1954).
17. Parks, W. P., and Melnick, J. L., *J. Inf. Dis.* **120**, 539 (1969).
18. Millman, I., Loeb, L. A., Bayer, M. E., and Blumberg, B. S., *J. Exp. Med.*, in press.
19. Dane, D. S., Cameron, C. H., and Briggs, M., *Lancet* **1**, 695 (1970).

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