Etiologic Relationship of Marmoset-Propagated CR326 Hepatitis A Virus to Hepatitis in Man (37220)

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Since the original report by Deinhardt *et al.* (1), at least 3 other groups of workers (2–4) have recorded the development of hepatitis in various species or subspecies of marmosets that were inoculated with blood specimens from human cases of hepatitis A. The etiologic relationship of these marmoset-propagated hepatitis viruses to human hepatitis was not established beyond the demonstration that blood from human hepatitis A was the necessary element for initiating hepatitis in the marmosets, and that similar disease occurred rarely if at all in marmosets given materials for control purpose. Parks *et al.* (5, 6) to the contrary, suggested that the phenomenon represented the activation of latent "marmoset hepatitis" rather than the transmission of human disease and reported an immunologic relationship between "marmoset hepatitis virus" and Deinhardt's GB strain of hepatitis virus propagated in marmosets.

The present report deals with the CR326 strain of hepatitis that was recovered (4) in this laboratory from a patient with hepatitis A in Costa Rica and with the etiologic relationship of the marmoset-propagated CR326 virus to human hepatitis A based on the findings in neutralization tests performed with paired sera from human hepatitis cases and with human immune globulin. Additionally, information is presented concerning the physical-chemical characteristics and size of the CR326 virus and the relative susceptibility of *Saguinus mystax* and *S. nigricollis* species of marmosets to the agent.

Materials and Methods. Procedures used earlier. The recovery of the CR326 virus, the handling of marmosets, and the enzyme and serologic tests of marmoset and human sera were described earlier (4).

Patients' sera. Natural hepatitis cases in Costa Rica. These were cases of hepatitis A or B that occurred among persons who resided in the province of Alejuela, Costa Rica and who were subjects in the large-scale epidemiologic studies of hepatitis that were conducted there (7, 8). They bear 8 digit identification (*e.g.*, 206-033-02). The presera or pre-illness sera were individual sera taken from the subjects shortly following the initial appearance of a case of hepatitis in their families. The postsera or convalescent sera were individual sera or pools of sera from the same subjects who had subsequently developed and recovered from hepatitis; their samples were taken at various time periods at least 29 days after onset of illness. The exact times that the specimens were collected are shown in Figs. 1–3.

Experimental hepatitis cases. The prebleed and post specimens from human subject *F. Kirk* (Test No. 6, Fig. 2) were taken 3 days prior to inoculation with MS1 strain hepatitis A virus of human origin and 60 days after first enzyme elevation, respectively (9). The post-"hyperimmunized" specimen was taken after multiple subsequent parenteral injections into Kirk of Kirk's acute phase plasma. These sera/plasmas were kindly furnished to us by M. Conrad, Col., M.C., U.S.A. Subjects 1, 2, 3, and 4 (Tests No. 7 and 8, Fig. 2) had been infected with human hepatitis A (MS-1) or hepatitis B (MS-2) (10) at Willowbrook State School in studies carried out by Dr. Saul Krugman and his

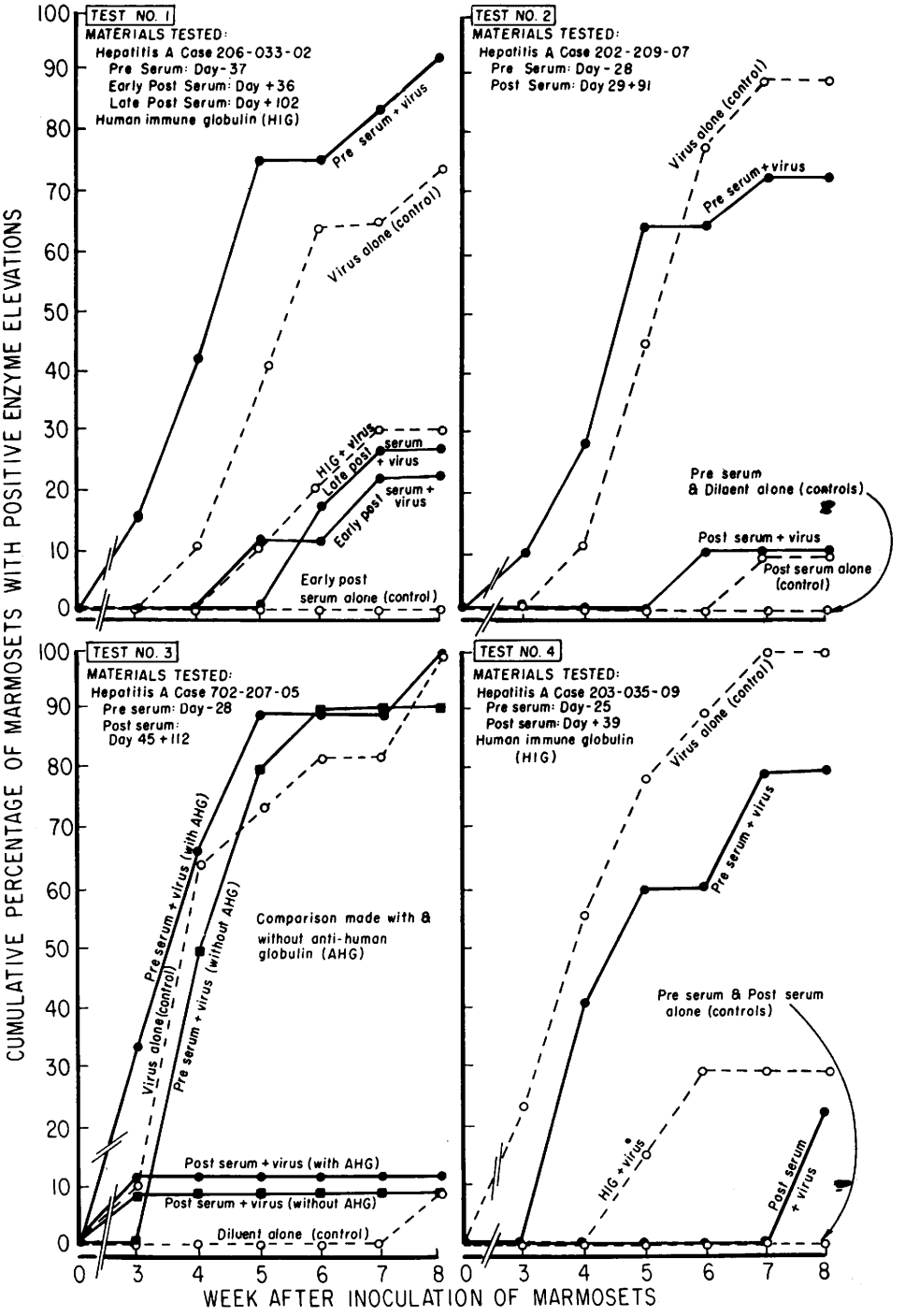

FIG. 1. Serum neutralization tests in marmosets of sera from hepatitis A cases in Costa Rica and of human immune globulin.

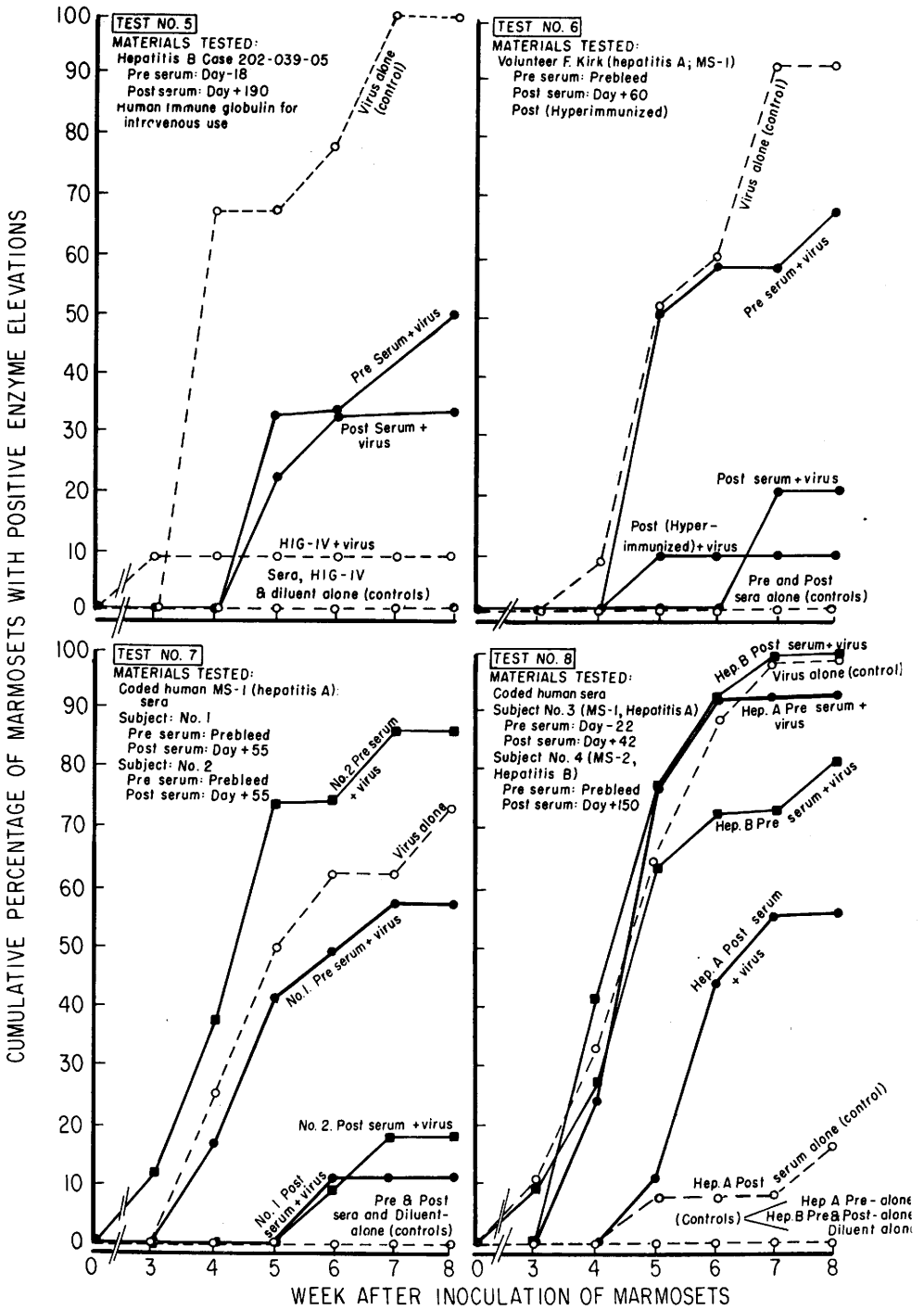

FIG. 2. Serum neutralization tests in marmosets of sera from hepatitis A and B cases in Costa Rica and in human volunteers.

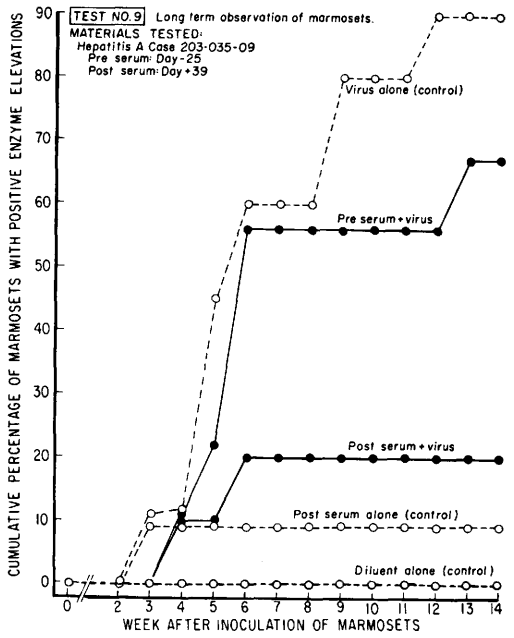

FIG. 3. Long-term holding of serum neutralization tests in marmosets.

associates.[1] The blood specimens were taken at times as indicated in Tests 7 and 8, Fig. 2. Prebleed specimens were taken prior to inoculation of the subjects. Other specimens are designated by days pre- or post-onset of disease.

The human immune globulins (HIG) were prepared from dried lots 65832 (Test 1, Fig. 1) and 71297 (Test 4, Fig. 1) intended for commercial distribution. The powder was reconstituted to 15% solution in phosphate buffered saline solution (PBS) at pH 7.2 and contained no preservative. Lot PR2176 (Cutter; Test 5, Fig. 2) was a special lot of human immune globulin, intravenous, used by Dr. R. Ward and his associates in studies of prevention of post-transfusion hepatitis in Chile (11). The globulin was kindly furnished to us by Dr. R. McAllister.

Serum neutralization tests. Strain CR326 hepatitis virus (4) was used. This was a virus-containing pool of sera (fourth passage) from mystax marmosets that had been inoculated with virus previously passed 3 times in marmosets. In the tests, the noninactivated human serum samples were incubated with virus at 37° for 1 hr in a final mixture containing 1:7 to 1:15 serum dilution and virus at about 200 marmoset ID_{50}/ml. The human immune globulins were tested in a final dilution of 1:3 to 1:6 of the globulin solutions. Controls consisted of serum or HIG comparably diluted and with no added virus (serum controls; HIG controls), of virus alone containing no added serum or HIG (virus controls), and of PBS to which nothing was added (diluent controls). After one hour the mixtures were diluted 1:2 in chilled PBS and injected via femoral artery or vein in 1.0 ml volume into groups of 12 *S. mystax* marmosets/sample. In an early experiment, goat antihuman globulin (AHG) was added to the serum–virus mixtures after the 1 hr incubation to determine if neutralization was enhanced (12) (Fig. 1, Test 3). The goat antiserum was added in proper amount and the mixtures were further incubated at 37° for 15 min before chilling. *The marmosets* used in the tests had been held in isolation for at least 4 wk, had shown normal serum enzyme values for at least 2 wk prior to use, and were assigned to test groups by statistical randomization. The animals were bled at weekly intervals and determinations for serum isocitric dehydrogenase (SICD) and serum glutamic oxaloacetic transaminase (SGOT) enzymes were performed. The definition for a positive test was given earlier (4). Liver biopsies were not performed during the observation periods because of the hazard of killing the animal and because of the demonstrated reliability of the tests for serum enzyme elevation in detecting hepatitis (4). However, all animals that showed enzyme elevations for 2 wk were sacrificed at that time and liver samples were examined histopathologically for viral hepatitis lesions by qualified pathologists. All the other animals were killed and autopsied at the end of each experiment and histologic examination was made of their livers. The histologic findings were as described earlier (4) and were consistent with those of the tests for enzyme elevation. The *findings in the marmosets* were expressed as the cumulative percentages

[1] These sera were furnished to us by Dr. Saul Krugman under code and the code was not revealed to us until the tests had been completed and the results were reported to him.

of animals that showed significant enzyme elevations (4) at each successive weekly bleeding (Figs. 1 and 2). In one experiment, the marmosets were held for 14 wk to determine whether the animals that received "neutralized" virus developed hepatitis on long-term holding (Fig. 3). In another experiment (Test 1, Fig. 1), some of the marmosets were held for 5 mo and were challenged at that time with about 100 marmoset ID_{50} of CR326 virus to examine further the significance of neutralization of the virus by antibody (Table III). Marmoset inoculations and bleedings and the testing of the marmoset sera for enzymes were carried out blind by persons who were without knowledge of the identity of the materials being tested.

Physical-chemical characteristics of CR326 virus. Strain CR326 hepatitis virus of fourth passage in marmosets was used. Strains Greer of parainfluenza 2 and Gregory of ECHO 11 viruses were employed in control tests and were grown and assayed in grivet monkey kidney cell cultures. These as well as the CR326 virus preparations were diluted 1:100 for treatment. Thus, about 500 marmoset ID_{50}/ml of CR326 virus were employed and the initial titers of the diluted parainfluenza 2 and ECHO 11 virus preparations were $10^{3.5}$ and $10^{4.5}$ $TCID_{50}$/0.2 ml, respectively.

Heating was for 1 hr at 60° in sealed glass containers immersed in a water bath employing viruses diluted in distilled water. Controls were held for 1 hr at 4°.

Ether treatment consisted of adding 20% by volume of diethyl ether to virus diluted 1:100 in PBS (pH 7.2) with subsequent vigorous mixing in a sealed container and storage for 18 hr at 4°. Controls were held under the same conditions without ether. Flowing nitrogen gas was used to remove the ether from the aqueous specimen prior to marmoset inoculation.

To test for acid stability, the virus samples were diluted 1:100 in lactalbumin hydrolysate medium in Hanks' salt solution at pH 3.0 or 7.2 (control) and held for 3 hr at room temperature after which the pH of the acidified sample was adjusted to 7.2. Tests for strain CR326 hepatitis virus were carried out in marmosets by intravenous inoculation of 1.0 ml of the treated preparations to each of 12 animals/sample. The tests for parainfluenza and ECHO viruses consisted of titrations of the treated preparations in grivet monkey kidney cell cultures by ordinary procedures.

Size determination. CR326 strain hepatitis virus diluted 1:50 in distilled water (about 1000 marmoset ID_{50}/ml) was passed sequentially through Millipore filters of 220, 100, 50 and 25 nm average pore diameter. All filters were pretreated with 1% veal infusion broth that had been prefiltered through a 25 nm Millipore filter. Tests for infectivity of the CR326 filtrates were carried out at each successive step by intravenous inoculation of 1.0 ml of filtrate into each of 12 marmosets/filtrate.

Comparative susceptibility of marmoset species. Strain CR355 of hepatitis virus was described in an earlier report (4, Table II) and was the isolate from patient 068-330-08. All evidence to date indicates that strain CR355 is similar to strain CR326. Marmosets of the *Saguinus mystax* and *S. nigricollis* species were compared. In the tests, 1.0 ml of strain CR355 virus in marmoset serum diluted in PBS to contain about 100 marmoset ID_{50} was inoculated via femoral artery or vein into groups of 10 or 12 marmosets of each species.

Results. Figures 1–3 summarize the results of serum neutralization experiments carried out in *S. mystax* marmosets. Several different kinds of information were obtained and these are discussed in the designated paragraphs below:

Tests of paired sera from natural hepatitis cases in Cost Rica. Four cases of hepatitis A and 1 case of hepatitis B were selected for study. The important clinical–laboratory findings in each case are shown in Table I. Tests 1–4 of Fig. 1 show the cumulative percentages, according to time, of animals developing serum enzyme elevations (hepatitis) in tests of the neutralizing capacity of sera from cases of hepatitis A. In all 4 tests, the percentages of marmosets that developed hepatitis following administration of CR326 virus mixed with preserum were the same or only slightly less than the percentages in the virus control. By contrast, there was a marked reduction in the percentages of animals with hepatitis and there was a slowed

TABLE I. Clinical Histories of Costa Rican Patients Whose Sera Were Used in Neutralization Experiments.

Case no.	Age (yr)	Clinical findings[a]	Laboratory findings[b]	Tests for HBAg and anti-HBAg
Hepatitis A				
206-033-02	11	1-3-5-6-8-9	c-d-e-f	Negative
203-035-09	8	1-2-3-4-6-8	a-b-c-d-e-f	Negative
702-207-05	4	1-2-3-4-5-6-8	a-b-c-d-e-f	Negative
202-209-07	10	1-2-3-5-6-7-8	a-b-c-d-e-f	Negative
Hepatitis B				
202-039-05	10	1-2-3-4-5-6-7-8	a-b-c-d-e-f	Positive

[a] 1 = Anorexia; 2 = asthenia; 3 = nausea; 4 = vomiting; 5 = jaundice; 6 = choluria; 7 = acholia; 8 = hepatomegaly; 9 = fever.

[b] Positive laboratory test results for: a = Direct bilirubin; b = total bilirubin; c = SGOT; d = SGPT; e = cephalin cholesterol flocculation; f = thymol turbidity.

rate of hepatitis development in the animals that received CR326 virus mixed with the postsera. This indicated absence of significant amounts of CR326 neutralizing antibody in the 4 presera and the development of such antibody during convalescence from the disease. Animals that received the serum control mixtures did not develop hepatitis, with the few exceptions as in Tests 2 and 9. These were postserum specimens and might have contained a small amount of nonneutralized virus. The sera from patient 702-207-05 (Test 3) were retested in Test 10 (no figures; see Table II) and essentially identical results were obtained. Figure 2, Test 5, shows the results of the tests of pre- and postsera from a case of hepatitis B whose blood contained hepatitis B antigen (HBAg) during illness and who developed antibody to HBAg in convalescence. Both the pre- and postsera contained, by chance, neutralizing antibody against CR326 but there was no change in the amount of antibody in the 2 specimens. This indicated no development of antibody to CR326 following hepatitis B infection. This patient quite likely had contracted hepatitis A at an earlier stage of life.

Tests of sera from cases of experimental hepatitis A and B. Test charts 6, 7 and 8 of Fig. 2 present the findings in tests of pre- and postsera from human subjects 1, 2, and 3 and subject F. Kirk that were fed the MS-1 strain hepatitis A or were inoculated with MS-2 strain hepatitis B virus (subject 4) of human origin. The findings show that there was increase in neutralizing antibody against CR326 virus in the paired sera from the human cases of hepatitis A but not hepatitis B, as was the case with the Costa Rica sera. The serum control samples all failed to induce hepatitis with exception of convalescent serum from subject 3.

Tests of human immune globulin. The 2 lots of human immune globulin (Tests 1 and 4) and the special lot of human immune globulin for intravenous use (Test 5) all neutralized the CR326 virus strongly.

Influence of the addition of goat antihuman globulin on the serum neutralization test results. Notkins *et al.* (12) noted that certain "nonneutralizable" viruses could be neutralized by further addition to the serum–virus mixture of antiserum against globulin of the same species. Test 3 (Fig. 1) compares the results in tests in which goat antihuman globulin (AHG) was added or not added to the virus–serum mixtures. It is seen that AHG was not necessary for neutralization to occur and there was no apparent effect of the AHG on the test results.

Influence of long-term holding of marmosets on the neutralization test results. Figure 3, Test 9, shows the findings in a repeat test of sera from human hepatitis A patient 203-035-09 (see Test 4, Fig. 1) except that the observation period was extended to 14 wk. There were small increases beyond 8 wk in the percentages of animals with hepatitis in those groups given virus alone or virus with preserum. Importantly, however, there was no increase in the percentage of positives in the test of the postserum with virus.

Mathematical expression of the findings in tests 1–10. The findings in the neutralization tests were reduced to simple mathematical expressions for purpose of further examination of the data, and the results are given in Table II. The differences in the percentages

TABLE II. Serum Neutralization Values Obtained in Tests with Hepatitis A Virus Strain CR326.

Test no.	Serum sample		Neutralization[a] value	Difference[b]
1.	Hepatitis A. Patient 206-033-02	Preserum	—26	—
		Postserum 1	70	96
		Postserum 2	63	89
	Human immune globulin Lot 65832		59	
2.	Hepatitis A. Patient 202-209-07	Preserum	18	—
		Postserum	89	71
3.	Hepatitis A. Patient 702-207-05	Preserum	10	—
		Postserum	91	81
10.	Repeat: Patient 702-207-05	Preserum	11	—
		Postserum	71	60
4.	Hepatitis A. Patient 203-035-09	Preserum	20	—
		Postserum	78	58
	Human immune globulin Lot 71297		71	
9.	Repeat: Patient 203-035-09[c]	Preserum	11	—
		Postserum	71	60
6.	Hepatitis A. Subject Kirk (MS-1)	Preserum	27	—
		Postserum 1	78	51
	"Hyperimmune"	Postserum 2	90	63
7.	Hepatitis A. Subject No. 1 (MS-1)	Preserum	23	—
		Postserum	85	63
	Hepatitis A. Subject No. 2 (MS-1)	Preserum	—16	—
		Postserum	76	92
8.	Hepatitis A. Subject No. 3 (MS-1)	Preserum	8	
		Postserum	44	36
	Hepatitis B. Subject No. 4 (MS-2)	Preserum	18	—
		Postserum	0	(—18)
5.	Hepatitis B. Patient 202-039-05	Preserum	50	
		Postserum	67	17
	Human immune globulin (intravenous) Lot PR2176		92	

[a] Neut. value = [(virus alone % minus serum plus virus %)/(virus alone %)] × 100.
[b] Difference = Postserum value minus preserum value.
[c] Analysis based on values obtained at 8 wk.

of positive animals (at 8 wk) given CR326 virus alone compared with those given virus plus serum were expressed in Table II as the *neutralization value*. Positive neutralization values were often obtained with the presera but these were small and did not exceed 27. The only exception was the preserum from the Costa Rican hepatitis B case (Test 5) in which there was antibody against hepatitis A in the patient prior to his hepatitis B episode. The postserum neutralization values in cases of hepatitis A all exceeded 50 except for subject 3 (Test 8) whose value was 44. The neutralization values for the 3 human immune globulins were in the range of 59–92. For routine interpretation, neutralization values of 30 or less were regarded as not significant and those with values of 50 or more were considered significant; the value of 44 (subject 3) was probably real, but less definitive. Increase in neutralizing capacity of sera from hepatitis A cases was expressed numerically as the *difference* between the neutralization values obtained with

the pre- and postsera. A difference in value of at least 50 was obtained with the paired sera from all cases of hepatitis A except for subject 3 (Test 8) whose difference was only 36 and was regarded as being of likely borderline significance. Importantly, findings in the repeat tests (Tests 3 and 10; Tests 4 and 9) were confirmatory of the earlier results.

Additional evidence for neutralization of hepatitis A virus. Part of the marmosets in Test 1 (Fig. 1) were held for 5 mo at which time animals that had received neutralized virus or virus alone were challenged with CR326 virus. The results given in Table III show that animals that had received neutralized virus were susceptible to the hepatitis virus on second challenge in contrast to those animals that became infected initially and were solidly immune to reinfection.

Susceptibility of S. mystax *and* S. nigricollis *species of marmosets.* Table IV shows that 80% of the *S. mystax* marmosets developed hepatitis by the eighth week at which time only 33% of the *S. nigricollis* animals had hepatitis. The greater susceptibility of the *S. mystax* species compared with other species of marmosets was commonly seen in other experiments (not presented here) in which no purposely direct comparisons were being made.

Physical-chemical properties of CR326 virus. As shown in Table V, the CR326 virus was heat, ether, and acid stable. Its stability at 60° for 1 hr, in dilute solution, was greater than any previously described for hepatitis A, though hepatitis A was known previously to be stable to 56° for 30 min (13). The infectivity of ECHO 11 virus was destroyed by heating but not by ether or acid treatment. The infectivity of the parainfluenza 2 virus was destroyed by all 3 treatments. The filtration data showed that the particle size of the CR326 virus was less than 50 nm and greater than 25 nm. This size is similar to that obtained by Parks *et al.* (6) with the GB agent. Previous filtration studies with known hepatitis A in human volunteers had defined only that hepatitis A virus passed Seitz EK and Chamberlain No. 2 filters (13).

TABLE III. Challenge with CR326 Hepatitis A Virus of Marmosets That Had Been Given Neutralized or Nonneutralized CR326 Virus 5 mo Earlier.

Initial inoculum	Results on rechallenge: (no. marmosets positive[a]/ total tested)
CR326 virus alone	0/6
CR326 virus + post serum[b]	6/8
Nil	4/7

[a] Animals with hepatitis.

[b] Convalescent serum from case 206-033-02. (see Fig. 1, test 1.)

TABLE IV. Comparison of Susceptibility of *S. mystax* and *S. nigricollis* Species to Hepatitis A Strain CR355.

After virus inoculated (wk)	Species—no. positive according to time	
	S. mystax (10 animals)	*S. nigricollis* (12 animals)
0	0	0
4	4	0
5	4	0
6	5	1
7	7	3
8	7	4
9	8	4
10	8	4

Discussion. For decades, workers have sought to propagate and study human hepatitis viruses outside the human volunteer system. Deinhardt *et al.* (1, 14–16) provided the breakthrough in their demonstration that viral hepatitis developed in *S. nigricollis* and *S. oedipus* marmosets following injection of specimens from cases of human hepatitis A but not following administration of other materials. Though confirmed (2–4) or challenged (5, 6) by others, there was no proof of etiologic relationship of marmoset-propagated hepatitis to human hepatitis.

The present investigations were designed to provide definitive evidence as to whether marmoset-propagated hepatitis virus does have an etiologic relationship to the human disease. For the studies, the CR326 hepatitis A virus strain (4) recovered in marmosets in this laboratory from a case of hepatitis A in Costa Rica was employed. Fulfillment of Koch's postulates, in terms of reinoculation of marmoset-grown virus into human subjects, was not attempted. Instead, indirect

TABLE V. Determination of Physical-Chemical Properties and Size of Strain CR326 Hepatitis A Virus.

Treatment of virus[a]	(°)	(hr)	No. marmosets positive/ total tested	Conclusion
Heat	60	1	11/12	Heat stable
Control	4	1	12/12	
Ether (20%)	4	18	11/11	Ether stable
Control (no ether)	4	18	10/11	
	pH			
Acid	3.0	3	12/12	Acid stable
Control	7.2	3	10/11	
Filtration (Millipore filters) (nm)				
No filter			10/11	
220			9/10	
100			7/12	Size: <50 nm but
50			9/11	>25 nm
25			0/10	diam
Diluent control			0/10	

[a] In control tests conducted simultaneously, ECHO 11 virus was shown to be heat labile, and ether and acid stable; parainfluenza 2 virus was heat, ether and acid labile.

approaches were used and a newly described serum neutralization test carried out in marmosets was of prime importance for this purpose.

The evidences to relate CR326 virus to human hepatitis A are several: As shown in the recent report from our laboratories (4): (a) the essential factor needed to initiate a serially propagable hepatitis infection in marmosets was serum or blood from a case of human hepatitis A; (b) serial passage in marmosets of sera from uninfected marmosets did not cause hepatitis though spontaneous hepatitis of undefined cause was found in marmosets on rare occasion; (c) the release of enzymes into the blood stream and the histopathologic findings in marmosets inoculated with the marmoset-propagated hepatitis agent(s) resembled those of human viral hepatitis. Added to this, as shown in the present report: (d) the CR326 virus is of small size and is heat, ether and acid stable. This is consistent with the defined properties of human hepatitis A virus based on studies in human volunteers (13, 17). Its stability to acid is consistent with infectivity of hepatitis A by the oral route; (e) patients with hepatitis A were without neutralizing antibody against CR326 virus in their pre-illness sera but developed antibody to CR326 during convalescence; sera from cases of hepatitis B did not show this. This quite clearly establishes that CR326 is immunologically related to hepatitis A. Added credence was given to the neutralization test results because of the general blind conduct of the tests and because of the inclusion of coded serum pairs.[1] The relationship of CR326 virus to hepatitis A was especially well defined by tests of paired sera from subjects who had been infected with the well-defined MS-1 strain hepatitis A or the MS-2 strain hepatitis B virus (10); (f) the CR326 virus was neutralized by human immune globulin which is known to be immunologically active in preventing or suppressing human hepatitis A in man (18); and (g) the CR326 virus was completely neutralized by convalescent serum from human hepatitis A since marmosets that received the neutralized virus mixture retained susceptibility to the virus while animals that had been given the nonneutralized virus were solidly immune to reinfection.

The newly described marmoset serum neutralization test is unlikely to become a tool for routine diagnosis or seroepidemiologic in-

vestigation of hepatitis A because of the high cost of the animals and of their maintenance and handling in special isolation facilities. The marmoset model and the neutralization test will, however, find practical importance in special studies of the pathogenesis of the disease, in detecting and identifying the agent, and in eventual studies to evaluate the safety and protective efficacy of vaccines, eliminating or reducing to a large extent the need for human volunteers. For studies of CR326 virus, at least, there would appear to be an advantage in using *S. mystax* species marmosets which appear to be the most susceptible to this particular virus.

The conduct of the neutralization tests was based on use of a single dilution of serum and a single virus dose that had to be carefully adjusted to be adequate to infect and not so excessive as to exceed the neutralizing capacity of the serum. These limitations were imposed by economic necessity and it was necessary to use 12 marmosets/test inoculum so that at least 10 would survive the full test period. Also, it was necessary to use only those animals in which there was no intercurrent infection as indicated by normal serum enzyme levels prior to inoculation. The following, at weekly intervals, of the serum enzyme levels in the individual marmosets provided a dynamic view of the evolution of hepatitis in the test groups and gave an added basis for judging the credibility of the readings taken at the eighth week and considered final. It is worth noting that the incubation period in marmosets (about 5 wk) closely parallels that of hepatitis A in humans. The infrequent development of hepatitis in marmosets between weeks 8 and 14 did not alter the results significantly and the longer observation period could not be justified on economic grounds.

Though some viruses are not neutralized by antiviral antibody (12), this was not the case for hepatitis A. The addition of goat antihuman globulin gave no demonstrable effect and serves no useful purpose in the routine test.

Precise definition of the limits of sensitivity and reliability of the serum neutralization test must await the conduct of larger numbers of assays. By inspection, however, it was evident in the present series that a reduction by 30% or less of animals developing hepatitis (neutralization value) compared with the virus control was not significant whereas a reduction of 50% or more had true meaning. Similarly, a difference in the neutralization values obtained in simultaneous tests of paired sera from a hepatitis A case of 50% or more was clearly indicative of the development of neutralizing antibody and an immunologic response to infection. The lesser numerical values obtained in the tests are considered of borderline significance Their reliability and reproducibility remain to be assessed.

Summary. Studies were conducted to define the etiologic relationship of CR326 hepatitis virus recovered in marmosets to hepatitis A in man. CR326 virus exhibited physical-chemical properties considered characteristic of human hepatitis A virus, *viz,* small size and heat, ether and acid stability. A serum neutralization test carried out with CR326 virus in *S. mystax* marmosets is described and the factors influencing the results are given. Tests of paired sera from 8 cases of hepatitis A and 2 cases of hepatitis B were carried out including coded paired sera from 3 human subjects given MS-1 strain hepatitis A and 1 given MS-2 strain hepatitis B virus of human source. All subjects with hepatitis A developed antibody that neutralized CR326 virus; there was no such antibody response in persons with hepatitis B. All of 3 samples of human immune globulin neutralized the CR326 agent. Neutralization was highly effective since marmosets given the neutralized virus remained susceptible to reinfection with the agent; by contrast recovered animals that had been given non-neutralized virus were immune to reinfection. All evidences to establish the relationship of CR326 virus to human hepatitis A are summarized.

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