PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 155, 283-286 (1977)

Suitability of the Rufiventer Marmoset as a Host Animal for Human Hepatitis A Virus (39790)

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The white-moustached marmoset, *Saguinus mystax*, has been the most suitable of several marmoset species for studies of human hepatitis A virus (1-3) and for preparation of hepatitis A antigen for serologic testing for hepatitis A antibody in human and animal sera (4, 5). Embargoes placed on export of these marmosets from the Amazon basin in 1974 precluded their further use and a suitable substitute has been sought. Another marmoset species of poor taxonomic description and which is often referred to as the rufiventer marmoset has proved equal to *S. mystax* for research purposes. The findings in comparative tests in rufiventer and other marmoset species are described here.

Materials and methods. Marmosets. All animals were legally wild-caught and were purchased through licensed importers. Marmoset species *S. mystax, S. weddelli, S. oedipus, Callithrix jacchus,* and *C. argentata* were described by Napier and Napier (6). A rufiventer-like marmoset has been designated by Hershkovitz (7) as *Marikina labiata* (*Jacchus rufiventer*). A similar animal was designated *S. labiatus* by Napier and Napier (6). Because of imprecise definition, we refer to the animal used in the present work simply as the rufiventer marmoset.

Tests for susceptibility. A 10% extract was prepared from liver of an *S. mystax* marmoset inoculated with fourth-passage *S. mystax*-adapted CR326 human hepatitis A virus according to methods described earlier (4). The virus was stored frozen over liquid nitrogen and was injected intravenously into marmosets at further 1:150 dilution (phosphate-buffered saline; PBS) in 1.0-ml amounts. For serial passage of CR326 virus in rufiventer marmosets, the livers were perfused at the time of first pronounced enzyme elevations, processed as 20% liver extracts as described above, and further diluted 1:20 in PBS prior to intravenous inoculation. Primary isolation of hepatitis A virus from human specimens was made using serum and clot extract (diluted 1:5 in PBS) from Costa Rican patient 033-03 and using serum (diluted 1:12 in PBS) from volunteer Kirk who had been infected with MS-1 strain human hepatitis virus (8). The latter was kindly provided by Dr. M. Conrad.

Assays. Serum glutamic oxaloacetic transaminase (SGOT) and serum isocitric dehydrogenase (SICD) enzyme assays (1) were performed on plasmas obtained from the marmosets at weekly intervals. Immune adherence (IA) assays (5, 9) for hepatitis A antibody were performed using 2 units of CR326 hepatitis A antigen obtained from infected *S. mystax* liver. To test for hepatitis A antigen, livers of infected marmosets were harvested (4) at the time of the first pronounced serum enzyme elevation. The 20% liver extracts were processed by cesium chloride density gradient separation (3). Six 3-ml fractions from the bottom of each gradient were dialyzed against PBS and assayed for hepatitis A antigen content versus 3-6 units of hepatitis A antibody in the IA test. Antigen titers of each fraction were determined as the highest dilution producing complete hemagglutination. The best antigen preparations had titers of 1:4 or higher ($\geq$4 units).

Results. Comparison of susceptiblity of marmoset species to S. mystax-adapted virus. Figure 1 shows the percentage of animals with enzyme elevations, according to time, following injection with the CR326 hepatitis A marmoset liver extract. Greatest susceptibility was indicated by the shortest incubation period and the largest percentage of

ISSN 0037-9727

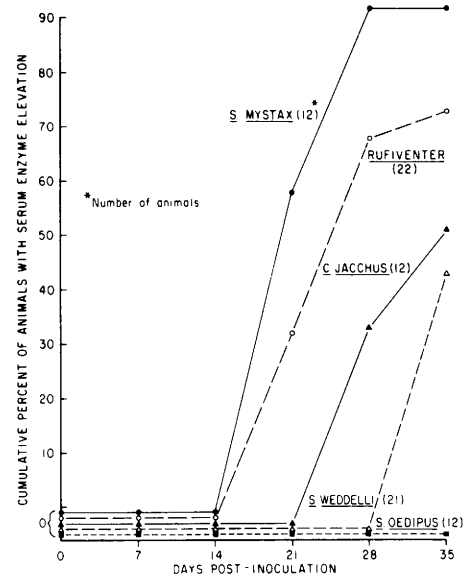

FIG. 1. Comparison according to time and percentage of serum enzyme elevations in five species of marmosets injected with CR326 strain human hepatitis A virus.

animals with enzyme elevation. *S. mystax* and rufiventer were the most susceptible species. *C. jacchus* and *S. weddelli* were less susceptible and *S. oedipus* gave no evidence of infection by this method of detection. *C. argentata* marmosets, not shown in the figure, behaved similarly to *C. jacchus* animals. The difference between *S. mystax*–rufiventer and the other marmoset species with respect to percentage of animals infected at Days 21, 28, and 35 was statistically significant ($P < 0.01$ by χ^2 test). Importantly, even though 50% of *C. jacchus* animals showed enzyme elevations through Day 35, these were generally at low level and were less than the 200 and 2000 SGOT and SICD values, respectively, considered indicative of infection in *S. mystax* marmosets (1).

Hepatitis A virus in human specimens. Table I shows that rufiventer marmosets were very susceptible to hepatitis A virus in human specimens. Of 11 animals, 9 showed enzyme elevations within 42 to 113 days and all but 1 of the animals (033-03) developed hepatitis A antibody. Importantly, patient 033-03 was the same individual from whom the CR326 strain of hepatitis A virus was originally isolated (1).

Hepatitis A antigen titers in livers of marmosets infected with S. mystax-adapted CR326 virus. Livers of infected marmosets from the experiments shown in Fig. 1 were perfused and harvested at the time of first pronounced serum enzyme elevation. Most livers were harvested within the 35-day period shown in Fig. 1. Table II shows that antigen detectable by IA was present only in infected livers from *S. mystax* and rufiventer marmosets. The antigen was most commonly found and was present in highest titer ($\geq$1:4) in *S. mystax* liver.

Adaption of CR326 virus to rufiventer marmosets. The *S. mystax*-adapted CR326 virus was passed serially for five passages in rufiventer marmosets and the IA antigen titer was measured in individual marmoset livers at each passage level. As shown in Table III, the *S. mystax*-adapted CR326 strain hepatitis A virus was readily adapted to rufiventer marmosets on serial passage. This was evidenced by (a) increase in the percentage of animals infected, (b) progressive shortening of the incubation period for disease (time of first enzyme elevation), and

TABLE I. PRIMARY ISOLATION OF HEPATITIS A VIRUS IN RUFIVENTER MARMOSETS.

Inoculum	No. of animals/total: Serum enzyme elevations (no. of days to first elevation)	No. of animals/total: IA antibody[a]
Saline solution	0/6	0/6
Patient 033-03	5/5 (42–113)	4/5
Kirk Day 30 (MS-1)	4/6 (63–103)	6/6

[a] Through 21 weeks postinoculation.

TABLE II. RESULTS OF ASSAYS OF LIVER EXTRACTS FROM VARIOUS MARMOSET SPECIES INFECTED WITH *S. mystax*-ADAPTED CR326 VIRUS FOR HEPATITIS A IA ANTIGEN.

Type of marmoset	No. of livers assayed	IA antigen content (units): 0	2	4
S. mystax	12	3	2	7
Rufiventer	14	9	5	0
C. jacchus	8	8	0	0
C. argentata	9	9	0	0
S. weddelli	5	5	0	0

(c) progressive increase in the percentage of liver extracts with an IA titer of ≥1:4.

Use of rufiventer-adpated CR326 virus in diagnostic tests. Serum samples from hepatitis A and B cases and from normal persons were tested for hepatitis IA antibody content using *S. mystax*- or rufiventer-adapted CR326 virus as antigen. Table IV shows that essentially identical results were obtained, indicating that the virus retained its original antigen specificity and hepatitis A antibody-measuring capacity on serial passage in rufiventer marmosets.

Discussion. The present findings show that the rufiventer marmoset is a suitable substitute species for *S. mystax* in studies of human hepatitis A both for initial virus recovery from human specimens and for production of antigen for use in the IA assay. Additional studies, not recorded in the text, showed the presence in infected rufiventer marmoset liver of typical 27-nm particles previously demonstrated by us in infected *S. mystax* marmosets (3) and by Feinstone *et al*. in human stools (10). Additionally, the density of the rufiventer-adapted CR326 virus was found to be in the range of 1.32 to 1.36 g/cm^3 based on cesium chloride density gradient centrifugation, being identical with our previous findings with *S. mystax* liver-derived hepatitis A antigen (3, 5).

The early appearance of serum enzyme elevation in rufiventer marmosets, within 7 days following inoculation at passage levels three, four and five, was earlier than seen with *S. mystax* marmosets in our previous

TABLE III. EVIDENCE FOR ADAPTATION TO RUFIVENTER MARMOSETS OF *S. mystax*-ADAPTED CR326 HEPATITIS A VIRUS.

Passage level in rufiventer marmoset	Inoculum (liver dilution)	No. of infected/total (%)	No. of days to first enzyme elevation	No. of livers with ≥ 4 units of IA antigen/ total (%)
1	1:1500 (*mystax*)	18/22 (82)	21	0/14 (0)
2	1:100	20/24 (80)	14	1/12 (8)
3	1:100	23/24 (96)	7	3/18 (17)
4	1:100	23/23 (100)	7	7/16 (44)
5	1:100	12/12 (100)	7	6/12 (50)

TABLE IV. HEPATITIS A ANTIBODY TITERS OF HUMAN SERA TESTED USING *S. mystax*-ADAPTED AND RUFIVENTER-ADAPTED CR326 HEPATITIS A VIRUS ANTIGEN.

Subjects	Case	Time of specimen (days)	IA Antibody Titer	
			S. mystax antigen[a]	Rufiventer antigen[b]
Hepatitis A cases	033-02	−21	<5	<5
		+36	20,480	≥2,560
		+102	40,960	≥2,560
	056-08	−57	<5	<5
		−28	<5	<5
		+185	10,240	≥2,560
	343-09	−6	<5	<5
		+3	800	160
		+104	12,800	≥2,560
Hepatitis B cases	039-01	−108	<5	<5
		+10	5	<5
		+149	<5	<5
	039-03	−27	2,560	≥2,560
		+7	5,120	≥2,560
		+192	2,560	≥2,560
Normal controls	2	–	1,280	640
	16	–	<5	<5
	20	–	<5	<5

[a] Tests performed September 1974.
[b] Tests performed March 1976.

studies (1–3). However, this is probably due to the use of more potent inocula in the present work. Livers from such "early onset" animals in a number of cases gave excellent yields of hepatitis A IA antigen (titer $\geq$ 1:4). These findings, in the marmoset at least, remove hepatitis A from the category of "slow-growing" virus and indicate that hepatitis A can be the result of an acute liver infection by the virus probably without need for a secondary factor, such as an autoimmune response (11) to liver tissue antigens. A number of additional passages of CR326 virus have been made in rufiventer marmosets with no important changes noted either with respect to time of onset of disease or content of antigen in the liver. Such passages are continuing to be made.

Patient 033-03 from whom hepatitis A virus was isolated in rufiventer marmosets in the present study was the same individual from whom the original CR326 virus isolation was made in *S. mystax* marmosets. Thus, two CR326 strains have been derived, one by original passage in *S. mystax* marmosets and the other by original passage in rufiventer marmosets.

Though none of the five additional marmoset species tested other than rufiventer was a satisfactory replacement for *S. mystax*, it should be recorded that all species presented evidence of infection with human hepatitis A. Even though serum enzyme elevations were low or absent, a portion of animals of all species developed hepatitis A IA antibody.

Summary. The rufiventer marmoset, which closely resembles marmosets designated as *S. labiatus, Marikina labiata,* and *Jacchus rufiventer*, proved to be an equally satisfactory host animal as *S. mystax* for studies of human hepatitis A virus. *C. jacchus, C. argentata, S. weddelli,* and *S. oedipus* marmosets were not satisfactory. Rufiventer marmosets were highly susceptible to infection with hepatitis A virus. Following viral adaptation, livers of rufiventer marmosets produced satisfactory CR326 virus antigen for IA tests in terms of both amount and specificity. Importantly, rufiventer marmosets inoculated with rufiventer-adapted CR326 virus showed enzyme elevations and high titers of viral antigen in their livers as early as 7 days after virus inoculation. These findings may be of importance in understanding the pathogenesis of hepatitis A, indicating that a primary viral infection can cause hepatitis without need for a secondary autoimmune response to liver tissue.

We gratefully acknowedge the expert technical assistance of F. S. Banker, Dr. P. A. Conti, W. P. M. Fisher, J. R. Gehret, P. A. Giesa, B. J. Harder, Dr. O. L. Ittensohn, M. Johnston, and R. A. Machlowitz.

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Received January 31, 1977. P.S.E.B.M. 1977, Vol. 155.