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An Inactivated Hepatitis A Virus Vaccine Prepared from Infected Marmoset Liver (40314)

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The isolation of the CR326 strain of human hepatitis A virus in mystax marmosets was reported from these laboratories (1, 2) in 1973 and the virus was shown to be inactivated by formaldehyde (3). It was demonstrated, subsequently, that the livers of white-moustached and rufiventer marmosets (*S. mystax* and *S. labiatus*, respectively) infected with CR326 virus contained large amounts of hepatitis A viral antigen (3–6). This made possible the development of the first practical assays for hepatitis A virus antigen and antibody by the complement fixation and immune adherence (IA) methods. The present studies showed that CR326 strain hepatitis A virus vaccine, purified from infected marmoset liver and inactivated with formaldehyde, induced homologous IA antibody and protected marmosets against hepatitis A virus challenge.

Materials and methods. Marmosets. Wild-caught rufiventer (*S. labiatus*) marmosets were used. The animals were conditioned and maintained as described previously (1).

Assays. Isocitric dehydrogenase (ICD) assays were performed, as previously described (1), on the marmoset plasmas collected at weekly intervals. Values of 1500 Sigma units or greater obtained for two or more consecutive weeks were considered to be indicative of infection. Assays for hepatitis A antibody in the marmoset sera were by the IA procedure described earlier (5).

Hepatitis A vaccine. A rufiventer marmoset was injected intravenously with 25th rufiventer marmoset passage CR326 hepatitis A virus. The liver was perfused *in situ* with phosphate buffered saline solution (PBS) and removed from the marmoset at the time that the marmoset first showed pronounced ICD elevation on the 14th day after inoculation. The infected liver was homogenized with PBS using a mortar and pestle with alundum to give a 20% suspension. The supernate was collected following low speed centrifugation and was diluted further to give a 5% liver extract. The extract was then heated at 60° for 30 min after which it was further clarified by centrifugation at 2500 rpm for 30 min yielding an amber-colored supernate that was slightly opalescent. Formalin in a final concentration of 1:4000 was added to the supernate, and the mixture was incubated with continuing agitation at 35.5° for 4 days. The formalin was then partially neutralized with sodium bisulfite to give a final concentration of 10 μg/ml formaldehyde. This was the vaccine, and it was stored at 4°. The viral particle content per ml was 1.4×10^{10} as measured by electron microscopy and the hepatitis A antigen titer was 1:8 by IA. The liver from a noninfected rufiventer marmoset was processed in an identical way to produce vaccine for control purpose.

Vaccination. Rufiventer marmosets were employed, and all were initially devoid of human hepatitis A virus antibody. Eight marmosets were each given 1 ml amounts of hepatitis A vaccine subcutaneously at biweekly intervals for 14 weeks (eight injections). An additional eight animals were injected subcutaneously at the same time with normal marmoset liver vaccine. Six more animals were each given an intravenous injection of 1 ml of hepatitis A vaccine on a single occasion for testing for absence of live hepatitis A virus in the vaccine.

Marmoset challenge. All marmosets were challenged intravenously 17 weeks after the first vaccine injection, with 1 ml of a 10^{-6} dilution of CR326 hepatitis A virus containing approximately 10^3 fifty percent marmoset infectious doses of virus.

Results. IA antibody responses. Serum antibody titrations were performed on plasma samples collected at weekly intervals during the 17-week immunization regimen and the 9-week period following challenge. Figure 1

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shows that all eight animals displayed IA antibody after the sixth vaccine dose had been given (by 12 weeks), at least three of the animals having responded after the fifth dose. The titers ranged from 1:20 to 1:320. None of the animals given control vaccine developed hepatitis A antibody. One of the six animals in the viral inactivation test group that received a single dose of vaccine intravenously developed antibody by the 12th week after injection. Table I shows that none of the animals in any group developed positive ICD enzyme elevations prior to challenge indicating that the materials given to the animals did not contain live hepatitis A virus.

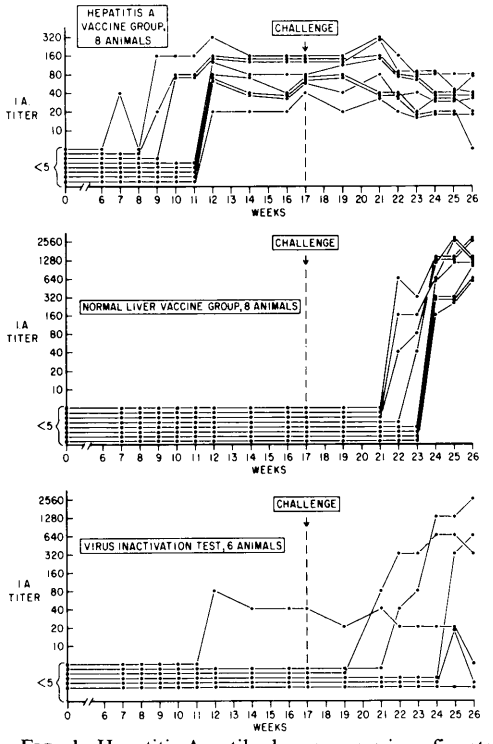

FIG. 1. Hepatitis A antibody responses in rufiventer marmosets as measured by immune adherence during the immunization and challenge regimens.

Protective efficacy. The marmosets in all three groups were challenged intravenously with live CR326 hepatitis A virus during the 17th week after vaccination was initiated, and the findings are shown in Fig. 1 and Table I. All eight of the marmosets that received the normal liver vaccine showed elevations in ICD and all developed IA antibody with titers ranging from 1:640 to 1:2560 or greater. By contrast, none of the animals given hepatitis A vaccine showed ICD elevations and none showed more than twofold increase in antibody titer. Interestingly, only two of the six animals that were given a single dose of vaccine intravenously showed elevations in ICD, and these two animals developed IA antibody. One other animal developed pronounced IA antibody, without an ICD elevation. All of the three remaining animals appeared to be protected even though only one had IA antibody prior to challenge. These findings indicated that the vaccine given subcutaneously in multiple injections was highly effective in preventing experimental hepatitis on challenge in marmosets and that even a single dose of vaccine given intravenously afforded protection to live virus challenge in some animals.

Discussion. The work on which the present findings are based represents the first demonstration that inactivated hepatitis A virus can afford protection against live hepatitis A virus challenge. Vaccine was given in eight divided aqueous doses, and it seems likely that protection might have been afforded following fewer doses, especially if an immmunologic adjuvant had been employed. This vaccine might prove equally effective in preventing hepatitis A in man and might, therefore, be of extreme importance in the control of the disease. The limited availability of marmosets and the lack of ability, to date, to achieve practical replication of the virus in

TABLE I. ANTIBODY AND ENZYME DETERMINATIONS IN A CONTROLLED STUDY OF HUMAN HEPATITIS A VACCINE IN MARMOSETS.

	Time period				
	Before hepatitis A virus challenge		After hepatitis A virus challenge		
Marmoset group	Antibody response No. Pos./Total	Enzyme elevation No. Pos./Total	Antibody response No. Pos./Total	Enzyme elevation No. Pos./Total	Protective efficacy of vaccine
Normal liver vaccine	0/8	0/8	8/8	8/8	–
Hepatitis A vaccine	8/8	0/8	0/8	0/8	100%
Virus inactivation test	1/6	0/6	3/6	2/6	(partial protection by i.v. vaccine administration)

the laboratory precludes any substantial progress toward routine immunization in man at the present time.

Summary. Human hepatitis A virus, partially purified from the liver of a rufiventer marmoset infected with CR326 strain virus, was inactivated with formalin and was shown to be highly potent in stimulating homologous antibody in marmosets when administered subcutaneously at bi-weekly intervals in eight divided doses. The vaccine was shown to prevent hepatitis A in all marmosets when challenged with live hepatitis A virus in a controlled study.

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