

Continuing Studies of Transmission of Hepatitis B Virus
to Gibbons by Exposure to Saliva Containing
Hepatitis B Surface Antigen

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OBJECTIVE : To determine if human saliva containing HB_sAg is capable of infecting gibbons by the oral route.

BACKGROUND : Previous attempts to demonstrate transmission of hepatitis B virus (HBV) to gibbons by the oral route, using saliva from HB_sAg carriers were unsuccessful (1). Investigations conducted in our laboratory and others (2) indicated that the risk of human infants acquiring HBV was greater for the offspring of mothers that possess high titers of hepatitis B surface antigen (HB Ag) and hepatitis B e-antigen (HB_eAg). In view of this observation, attempts were made to demonstrate transmission of HBV by the oral route to gibbons, employing saliva from HB_sAg carriers who had high levels of HB_sAg and HB_eAg in their serum.

METHODS : Saliva was collected in a manner similar to that reported previously. One ml. of saliva was removed for testing. The remainder was promptly frozen at 70°C and pooled for transmission trials. Each one ml. sample was tested for occult blood using paper strips impregnated with a buffered mixture of organic peroxide and ortholidine (Labstix, Ames Company, Elkhart, Indiana).

The first day of exposure to the saliva pool was designated Day 0. On every subsequent day, each animal was observed for altered behavior and the rectal temperature was recorded. Once a week, each gibbons was weighed and examined by a veterinarian. Following sedation with a rapidly acting intramuscular anesthetic (phencyclidine hydrochloride or ketamine hydrochloride), a blood sample was drawn for a complete blood count, serum transaminase level (SGOT, SGPT), and hepatitis B serological tests.

All serum and saliva samples were tested for HB Ag by solid phase radio-immune assay (AUSRIA II, Abbott Laboratories, Inc., North Chicago, Illinois) without preliminary treatment. Positive reactions were confirmed by a 50% or more reduction of test serum reactivity after incubation with a human serum containing anti-HB_s. HB Ag subtypes were detected by radioimmunoassay adsorption using subtype-specific rabbit antisera. Anti-HB_e was detected by a solid phase radioimmuno assay (AUSRAB, Abbott Laboratories, Inc.) interpreted according to the manufacturer's recommendations. Positive reactions were confirmed whenever possible by a 50% or more reduction in reactivity after incubating the test sera with human serum containing HB_sAg/adw.

RESULTS : HB Ag was detected in the 2 gibbons (PC-26, B-40) inoculated with the saliva pool (Figures 1 and 2). PC-26 developed detectable antigenemia by the 10th week and carried it for 4 weeks (week 10-13). The saliva used for transmission trials consisted of a pool of 10 specimens that were shown to contain HB_s Ag radio-immune assay. These specimens were collected from two HB_s Ag positive donors who carried HB Ag/adw at a level \geq 1:128. One female had transmitted HBV to her offspring (Table 1). Specimens were thawed rapidly, mixed together and centrifuged at 1,200 x g for 30 minutes at 4°C. After pooling the supernatant fluid of each specimen, tests were performed to determine if the mixture contained HB Ag, occult blood and/or bacteria. Antibiotics, penicillin (1000 units/ml) and streptomycin (1000 mcg/ml), were added to saliva that was inoculated subcutaneously into monkeys. Results showed the saliva to contain occult blood, and to be positive for HB Ag by radioimmune assay. All saliva specimens were maintained at sub-zero temperatures until used.

Gibbons management has been described earlier (1). Seven gibbons, 3 males and 4 females, with no detectable HB Ag or antibody to HB Ag were selected for exposure to the saliva pool (Table 2). Two, (B-40 and PC-26) received two subcutaneous injections of 2.5 ml on consecutive days. Two (P-16 and PC-16) of the five other gibbons were exposed on two consecutive days to 2.5 ml of the saliva pool over the same 2 day period. It may be assumed that the majority of the saliva administered by the oral and nasal routes was swallowed. Two additional gibbons, had anti-HB_s resulting from previous inoculation with saliva that contained HB_s Ag. These 2 gibbons were employed as controls for the antibody detection assay. The antibody control gibbons were not exposed to the saliva.

The SGPT rose towards the end of this period (week 13) and remained above background levels for 2 weeks. Anti-HB_s was detected in samples taken on weeks 15-17 and from week 22 until the end of the study. HB Ag was detectable in B-40 by the 16th week of the study. SGOT and SGPT were slightly elevated above background from week 13-18. HB Ag was not detected after week 18. No anti-HB_s developed despite continued follow up for 12 weeks. HB Ag in both cases was adw by radioimmune assay adsorption. None of the gibbons exposed to saliva by the oral or nasal routes developed HB_s Ag or anti-HB_s through the thirty weeks of the study.

The laboratory is planning further transmission experiments employing semen. This semen will be obtained by Dr. Harvy Alter from humans known to have transmitted HBV (3).

Table 1. Saliva Samples from Carriers of HB_sAg/adw Used in the Saliva Pool

Donors	Transmit to Offspring	Serum			Saliva Samples		
		HB _e Ag (+)	HB _s Ag Titer	Occult Blood Reaction	HB _s Ag (+)	Contribution to Pool	
					Amt. (ml)	Percent (%)	
Females (FM317)	+	+	1:181*	+	+	56	(68)
Males (SH)	?	+	1:128	+	+	25.5	(32)

* Geometric mean titer over 4 samples

Table 2. Routes of Exposure of Gibbons to Human Saliva Pool from HB_sAg/adw Donor's

Exposure Category	Gibbon	Age (year)	Sex	Daily Dose	Day of Dosage	Total
Subcutaneous Inoculation	B-40	10	M	2.5	D1,2	5.0
	PC-26	1.5	F	2.5	D1,2	5.0
Tooth brush & oral aerosol	P-16	10	M	2.5	D1,2	5.0
	PC-16	2.5	F	2.5	D1,2	5.0
Oral & nasal aerosol	PC-21	2	F	2.5	D1,2	5.0
	PC-20	3	M	2.5	D1,2	5.0
	S-81	12	F	2.5	D1,2	5.0

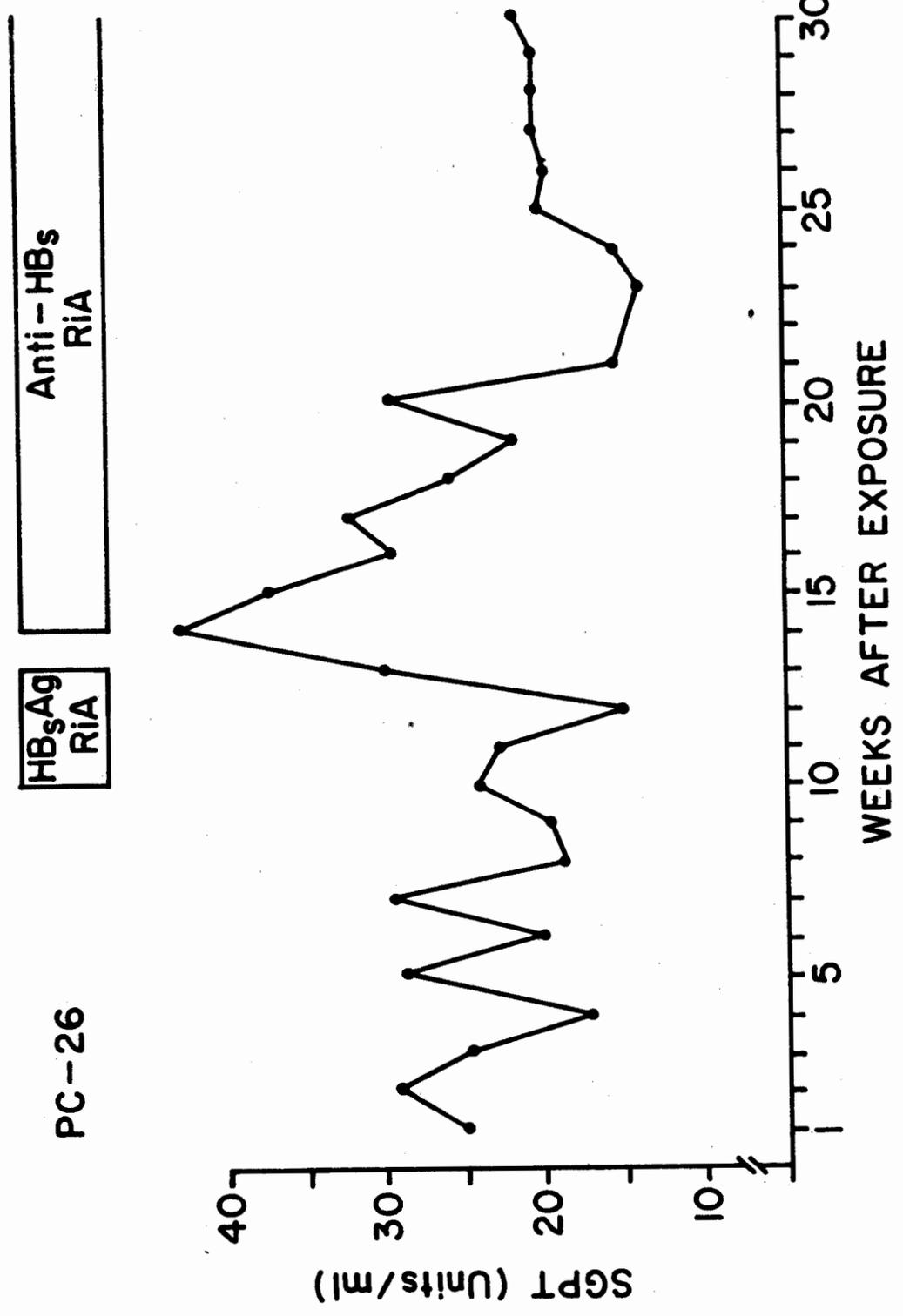


Figure 1. Response of gibbon PC-26 to the subcutaneous injection of a pool of human saliva containing HBsAg.

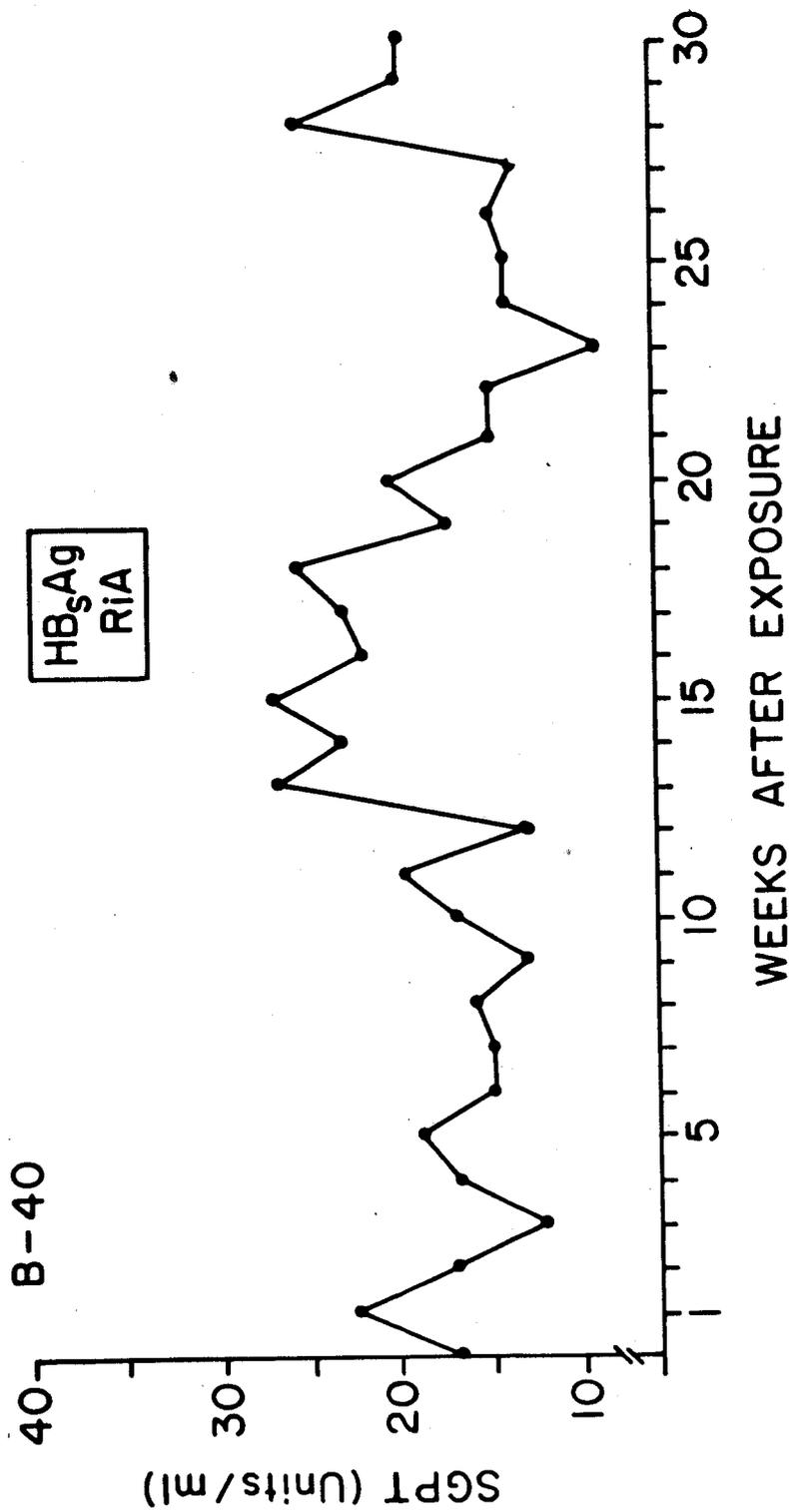


Figure 2. Response of gibbon B-40 to the subcutaneous injection of a pool of human saliva containing HBsAg.

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