

Anti-Hepatitis B Serum Production in Laboratory Animals

Principal Investigators: Rapin Snitbhan, M. D.
William H. Bancroft, LTC, MC

Associate Investigators: Sumitda Narupitt, B.Sc.
Aree Boriharnvanakett, M. T.

OBJECTIVE: To produce antisera to Hepatitis B surface antigen (HB_sAg) for use in the determination of antigen subtypes.

BACKGROUND: This is a continuation of work which was previously reported (1). The detection of subtypes of Hepatitis B surface antigen requires antisera containing specific antibodies to each subtype determinant. Previously these antisera were prepared using rabbits, who were exsanguinated six weeks after the initiation of the immunization procedure (2). This method of preparation produced antisera with a high titer against homologous antigen; however, because of impurities in the original immunogen, the antisera were often contaminated with anti-human serum protein activity. This anti-human serum activity interfered with immunodiffusion (ID) tests (3) and required absorption with normal human sera. The present study was designed to determine if subtype specific antisera free of anti-human serum activity could be produced in rabbits by selecting the time of bleeding.

DESCRIPTION: Rabbits of 2.5 to 4.0 Kg bodyweight were used. Five milliliters of blood were taken from the peripheral ear vein of each rabbit before incubation and nearly every week post inoculation. Anti-HB_s activity as well as anti-human serum protein activity were tested by immunoelectroosmophoresis (IEOP) and titered by complement fixation test (CF). A cesium chloride purified fraction of HB_sAg/adr (EH-17) prepared by Electronucleonics, Inc., Bethesda, Maryland was emulsified with an equal volume of Freund's complete adjuvant (2). Four rabbits free from anti-HB_s activity were inoculated with 0.25 ml of antigen-adjuvant emulsion, intradermally, into each of four sites on the thighs and back. An identical dose of the same antigen was given four weeks later. Anti-HB_s and anti-human serum protein activities were studied once a week from two to ten weeks.

All blood was tested for anti-HB_s and anti-human serum protein activities by IEOP and CF. The titer of these antibodies was determined by CF and the specificity of the antisera was identified by ID.

PROGRESS: Four rabbits were immunized with HB_sAg/adr (EH-17). Of these four, one died of unknown cause four weeks after immunization.

In the remaining three, antibodies to HB_sAg/adr with CF titers of 1:2 to 1:16 had appeared by the first bleed, two weeks after immunization (Table 1 and Figure 1). CF titers of antibodies increased slowly reaching 1:8 to 1:32 by the fourth week just prior to receiving the booster dose of immunizing antigen. Antisera reached its maximum titer of 1:64 to 1:128 by five weeks, one week after the booster dose. After the fifth week the titer remained stable until the rabbits were exsanguinated at 8-9 weeks.

The specificity of individual rabbit sera were determined by the ID test. Two rabbit sera (R26 and R28) formed definite precipitin lines with reference antigens, but only specific d spurs were observed (Table 1 and Figure 2) suggesting they contained only ad subtype specific determinant antibodies. Another rabbit serum (R29) gave specific reactions with the reference antigens, with both d and r spurs, suggesting it contained subtype specific a, d and r antibodies.

Antibody to normal human serum protein was observed only transiently and at low titer (Table 1 and Figure 1). The interference of anti-human serum protein disappeared in the ID test at 7 to 8 weeks post-inoculation. Anticomplementary activity of all three rabbit antisera was minimal and did not interfere with the interpretation of the tests.

DISCUSSION: The method of immunization with HB_sAg/adr in rabbits proved to be satisfactory for the production of subtype specific antisera in one (R29) out of three rabbits. In the other two, (R27 and R28) a strong d spur was produced but no easily discernible reaction with the r antigen was observed. In R29, anti-human serum activity in ID tests disappeared by the seventh week. This allowed the use of antiserum from this rabbit in the ID test without preabsorption with normal human serum.

REFERENCES:

1. Snitbhan, R. and Bancroft. W.H.: SEATO Medical Research Laboratory Annual Report, March 1974.
2. Bancroft, W.H., Mundon, F.H. and Russell, P.K.: Detection of Additional Determinants of Hepatitis B Antigen. J. Immunol. 109:842, 1972.
3. Ouchterlony, O.: Diffusion-in-gel Methods for Immunological Analysis. Progr. Allerg. 5:1958.

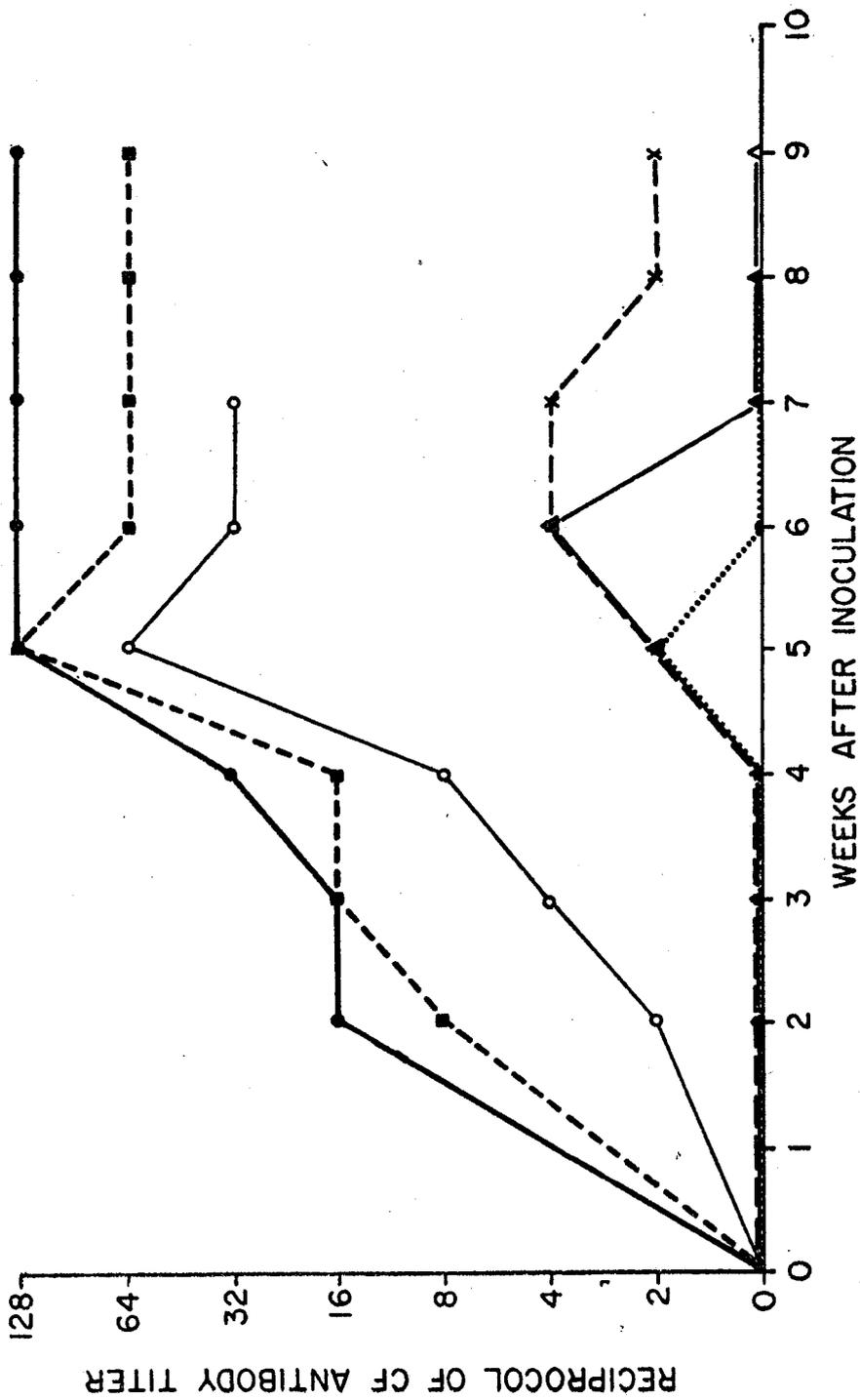


FIGURE 1 Anti-HB_s and anti-human protein responses in rabbits immunized with purified HB_sAg/adr (EH-017).

- Anti-HB_s R26
- Anti-HB_s R28
- Anti-HB_s R29
- ⋯ Anti-human protein R26
- x Anti-human protein R28
- △ Anti-human protein R29

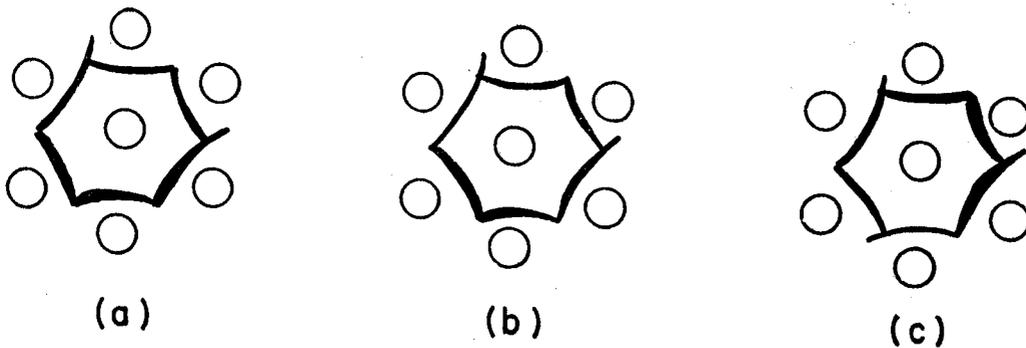


Figure 2. Patterns of immunodiffusion reactions observed with reference HB_sAg. In each pattern, reference HB_sAg/ayw were placed in the top and right upper wells, HB_sAg/adr in right lower and bottom wells and HB_sAg/adw in left upper and left lower wells. The central wells contain: (a) R. 26 anti-adr, (b) R. 28 anti-adr, and (c) R. 29 anti-adr rabbit sera.

Table 1. Production of Anti-HB_sAg/adr in Rabbits

Rabbit No.	Time After Immunization	Anti-HB _s				Anti normal human protein		
		Immunodiffusion		IEOP	CF Titer	Immunodiffusion	IEOP	CF Titer
		d Spur	r Spur					
R 26	Pre-immunization	ND*	ND	—	ND	ND	—	ND
	2 weeks	—	—	+	1:2	—	—	<1:2
	3 weeks	ND	ND	+	1:4	ND	—	<1:2
	4 weeks	ND	ND	+	1:8	ND	—	<1:2
	5 weeks	+	—	+	1:64	+	—	1:2
	6 weeks	+	—	+	1:32	+	—	<1:2
	7 weeks	+	—	+	1:32	+	—	<1:2
R 28	Pre-immunization	ND	ND	—	ND	ND	—	—
	2 weeks	—	—	+	1:8	—	—	<1:2
	3 weeks	ND	ND	+	1:16	ND	—	<1:2
	4 weeks	ND	ND	+	1:16	ND	—	<1:2
	5 weeks	+	—	+	1:128	+	—	1:2
	6 weeks	+	—	+	1:64	+	—	1:4
	7 weeks	+	—	+	1:64	+	—	1:4
	8 weeks	+	—	+	1:64	—	—	1:2
9 weeks	+	—	+	1:64	—	—	1:2	
R 29	Pre-immunization	—	—	—	ND	ND	—	ND
	2 weeks	—	—	+	1:16	—	—	<1:2
	3 weeks	ND	ND	+	1:16	ND	—	<1:2
	4 weeks	ND	ND	+	1:32	ND	—	<1:2
	5 weeks	+	+	+	1:128	+	—	1:2
	6 weeks	+	+	+	1:128	+	—	1:4
	7 weeks	+	+	+	1:128	—	—	<1:2
	8 weeks	+	+	+	1:128	—	—	<1:2
9 weeks	+	+	+	1:128	—	—	<1:2	

Note: ND* = Not done