Table 4 (cont.)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0889
TREATMENT :
LOT NUMBER : CK937
DOSE : 20 MCG
PATIENT CLASS: RETARDED

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (100 PATIENTS) - DOSE 2 — DAYS POST VACCINATION 0	1	2	3	4	5			NUMBER WITH MAX TEMP
< 99	93 (93.0%)	94 (94.9%)	87 (87.0%)	87 (87.9%)	89 (91.8%)	86 (87.8%)			67 (67.0%)
99 - 99.9	5 (5.0%)	5 (5.1%)	13 (13.0%)	11 (11.1%)	8 (8.2%)	10 (10.2%)			29 (29.0%)
100 - 100.9	2 (2.0%)	0 (0.0%)	0 (0.0%)	1 (1.0%)	0 (0.0%)	2 (2.0%)			4 (4.0%)
TEMPERATURE TAKEN	100 (100.0%)	99 (99.0%)	100 (100.0%)	99 (99.0%)	97 (97.0%)	98 (98.0%)			100 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	1 (1.0%)	0 (0.0%)	1 (1.0%)	3 (3.0%)	2 (2.0%)			0 (0.0%)

00841

THALASSEMICS/
HEMOPHILIACS

Hemophiliacs and Thalassemics

Two studies have been initiated to assess antibody and clinical responses to recombinant hepatitis B vaccine in persons with hemophilia or thalassemia.

Study 799 - New York, New York - Dr. C. Stevens

Thirty-one thalassemic children, less than 16 years of age, who are negative for hepatitis B serologic markers, are receiving either 5 mcg doses or 2.5 mcg doses of vaccine lot 972/C-K444 at 0, 1, and 6 months. The vaccine is administered intramuscularly.

Fifteen children have received three 5 mcg injections. At 7 months, seroconversion was 89% (8/9) (S/N $\geq$2.1) with a GMT for all vaccinees of 88 S/N. When the cut-off was S/N $\geq$10, the seroconversion rate was 78% (7/9).

Sixteen children have received two 2.5 mcg injections of vaccine and 12 of these have received the third injection. The seroconversion rate at 7 months was 100% (5/5) whether the cut-ff was S/N $\geq$2.1 or S/N $\geq$10. The GMT for all vaccinees at 7 months was 200.0 S/N.

Twenty of the children enrolled in the study had pre-vaccination elevated ALT levels which is characteristic of the clinical disease process of thalassemia. One recipient of 5 mcg doses who had a normal pre-vaccination ALT level developed an elevation of ALT which was approximately 2.5 times the upper limit of normal one month after receiving the first injection of vaccine. This elevation was transient and returned to normal within a month. No serious adverse experiences attributable to vaccine have been reported.

Study 861 - Milwaukee, Wisconsin - Dr. S. Gill

The study population consists of persons with hemophilia who are negative for hepatitis B serologic markers. Participants under 20 years of age are receiving 5 mcg doses while those who are 20 years of age or older are receiving 10 mcg doses of vaccine at 0, 1, and 6 months from lot 979/C-K564. The vaccine is administered subcutaneously in this population.

Twelve hemophiliacs <20 years of age have received two 5 mcg injections and 5 of these have received the third injection. At three months, seroconversion by either cut-off (S/N $\geq$2.1 or mIU/ml $\geq$10) was 100% (8/8). The geometric mean titer was 143.2 mIU/ml.

Three hemophiliacs $\geq$20 years of age have received two 10 mcg doses of vaccine and one has received all three injections. Serologic data at 3 months are available for two vaccine recipients. Both participants seroconverted (S/N $\geq$2.1) at three months. Neither developed protective levels of anti-HBs (mIU/ml $\geq$10) at that time. The geometric mean titer was 6.7 mIU/ml. No serious or alarming adverse experiences attributable to vaccine (either dose regimen) have been reported.

wva/3137I
1/21/86

Study 799

PROGRAM:	Yeast Recombinant Hepatitis B Vaccine, Study 799
PURPOSE:	To evaluate antibody and clinical responses to the vaccine among thalassemic children who are negative for hepatitis B virus serologic markers.
VACCINE:	Yeast Recombinant Hepatitis B Vaccine Lot #972/C-K444 (10 mcg/ml)
PRIMARY INVESTIGATOR:	Cladd Stevens, M.D. Lindsley F. Kimball Research Institute New York Blood Center 310 East 67th Street New York, New York 10021
SECONDARY INVESTIGATOR:	Patricia Giardina, M.D. New York Hospital - Cornell Medical Center 525 East 68th Street New York, New York 10021 Margaret W. Hilgartner, M.D. New York Hospital - Cornell Medical Center 525 East 68th Street New York, New York 10021 Patricia E. Taylor, Ph.D. Lindsley F. Kimball Research Institute New York Blood Center 310 East 67th Street New York, New York 10021
STUDY LOCATION:	Lindsley F. Kimball Research Institute New York Blood Center 310 East 67th Street New York, New York 10021 New York Hospital - Cornell Medical Center 525 East 68th Street New York, New York 10021
DATE INITIATED:	August 1984
DATE COMPLETED:	In progress.

Study 799

DATE COMPLETED: In progress

STUDY POPULATION: The study population consists of 31 thalassemic children, 16 years of age or less, who are negative for HBsAg, anti-HBc and anti-HBs, and have not previously received any hepatitis B vaccine.

PROCEDURE: Study participants are allocated to one of two groups and receive the vaccine at 0, 1 and 6 months. Group 1 receives 0.5 ml (5 mcg) doses and Group 2 0.25 ml (2.5 mcg) doses. All injections intramuscular. The parent or guardian are asked to record the child's temperature for 5 days after each injection and note any local or systemic complaints. Medically significant events and therapies relating to the child's pre-existing thalassemia will be recorded.

Blood specimens are obtained prior to vaccination, monthly for 3 months and at 6, 7, 9, 12 and 24 months post initial injection.

All samples are assayed for HBsAg, anti-HBs, anti-HBc and ALT by Dr. Steven's laboratory. Samples may also be assayed for yeast antibody at MSDRL.

RESULTS: THALASSEMIC CHILDREN:

5 mcg Lot #972/C-K444 at 0, 1 and 6 months.

2.5 mcg Lot #972/C-K444 at 0, 1 and 6 months.

1. Number Vaccinated:

Dose Level	Injection Number		
	1	2	3
5 mcg	15	15	15
2.5 mcg	16	16	12

Study 799

RESULTS (CONT.):

2. Serologic Results:

Serologic data at 7/8 months are available for 9 and 5 recipients of 5 and 2.5 mcg injections respectively.

Seroconversion was 89% (8/9) when the cutoff was S/N ≥2.1 among those receiving 5 mcg doses, with a GMT of 88.0 for all vaccinees. When the cutoff was S/N ≥10, seroconversion was 78% (7/9).

Among the recipients of 2.5 mcg doses, seroconversion was 100% (5/5) whether the cutoff was S/N ≥2.1 or S/N ≥10. The GMT for all vaccinees was 200.0. Table 1 shows seroconversion rates and GMT's for up to 9 months of follow-up.

3. Clinical Complaints:

Clinical follow-up data are available for 14, 14, and 3 participants following the first, second and third injections of 5 mcg doses; and for 16, 16, and 2 participants following the first, second and third injections of 2.5 mcg doses.

Specific complaints and maximum temperatures reported during the 5 days following each injection are provided in Tables 2 through 5.

Type of Complaint	Dose Level	Frequency in % by Injection No. 1	2	3
Injection Site	5 mcg	14 (2/14)	21 (3/14)	33 (1/3)
	2.5 mcg	19 (3/16)	19 (3/16)	0 (0/2)
Systemic	5 mcg	36 (5/14)	14 (2/14)	0 (0/3)
	2.5 mcg	6 (1/16)	13 (2/16)	0 (0/2)

There were no serious or alarming adverse reactions attributable to vaccine.

ALT Elevations

Twenty of the 31 children enrolled in this study had prevaccination ALT levels ranging from 1.5-9

Study 799

RESULTS (CONT.):

times the upper limit of normal. Most of these remained at an elevated level during the course of follow-up. Thalassemia is characterized by increased serum alanine aminotransferase, reflecting hepatic damage secondary to hemosiderosis.

One recipient of 5 mcg doses who had a normal prevaccination ALT level developed an elevation of ALT which was approximately 2.5 times the upper limit of normal one month after receiving the first injection. This elevation was transient and returned to normal within a month.

Adverse Experiences:

Two of the children enrolled in this study experienced episodes of vomiting accompanied by fever within a day of receiving their first dose of recombinant vaccine. One of the children was a two-year-old who received a 5 mcg dose. His temperature was 104°F the day following the injection. The child was examined by the investigator who diagnosed a possible viral infection. The second child was a one-year-old who received a 2.5 mcg dose. A fever of 100.2°F was recorded on the day of injection. A diagnosis was made of a probable concurrent respiratory infection.

TABLE 1

Antibody Responses Among Thalassemic Children Following Vaccination with 5 or 2.5 mcg Doses of Yeast Recombinant Hepatitis B Vaccine Lot # 972/C-K444 at 0, 1, and 6 Months in Study # 799.

	5 mcg					2.5 mcg				
Time (Months)	% with Anti-HBs		GMT (S/N)			% Anti-HBs		GMT (S/N)		
			All Vaccinees	Responders				All Vaccinees	Responders	
	S/N ≥ 2.1	S/N ≥ 10		S/N ≥ 2.1	S/N ≥ 10	S/N ≥ 2.1	S/N ≥ 10		S/N ≥ 2.1	S/N ≥ 10
1	25 (4/16)	6 (1/16)	1.5	4.7	11.5	33 (5/15)	13 (2/15)	2.2	12.4	42.8
2	93 (13/14)	71 (10/14)	21.6	27.8	45.2	78 (7/9)	67 (6/9)	13.2	29.4	45.7
3	93 (14/15)	73 (11/15)	24.2	29.2	46.4	83 (10/12)	67 (8/12)	16.1	29.7	48.7
6	75 (9/12)	58 (7/12)	13.6	35.5	64.6	82 (9/11)	64 (7/11)	13.8	25.2	46.5
7	89 (8/9)	78 (7/9)	88.0	144.0	248.1	100 (5/5)	100 (5/5)	200.0	200.0	200.0
9	90 (9/10)	90 (9/10)	91.4	146.5	146.5	100 (5/5)	100 (5/5)	150.1	150.1	150.1

2472I-5/cfs
12/20/85

Table 2

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT :
LOT NUMBER : CK444
DOSE : 5 MCG
PATIENT CLASS: THALASSEMICS

CLINICAL COMPLAINTS	TOTAL VACCINEES (15 PATIENTS) - DOSE 1 — DAYS POST VACCINATION 0	1	2	3	4	5	NUMBER WITH COMPLAINTS
REACTION, LOCAL (INJECT. SITE)	1 (7.1%)	2 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (14.3%)
SORENESS	1 (7.1%)	2 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (14.3%)
SYSTEMIC	1 (7.1%)	2 (14.3%)	0 (0.0%)	1 (7.1%)	2 (15.4%)	0 (0.0%)	5 (35.7%)
INTEGUMENTARY SYSTEM	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	1 (7.7%)	0 (0.0%)	2 (14.3%)
RASH, NOS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	1 (7.7%)	0 (0.0%)	2 (14.3%)
RESPIRATORY	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)
PHARYNGITIS (SORE THROAT)	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)
MUSCULOSKELETAL	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	1 (7.7%)	0 (0.0%)	1 (7.1%)
ARM PAIN	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	1 (7.7%)	0 (0.0%)	1 (7.1%)
DIGESTIVE SYSTEM	1 (7.1%)	1 (7.1%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	3 (21.4%)
NAUSEA	1 (7.1%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	2 (14.3%)
VOMITING	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)

Table 2 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT :
LOT NUMBER : CK444
DOSE : 5 MCG
PATIENT CLASS: THALASSEMICS

CLINICAL COMPLAINTS	TOTAL VACCINEES (15 PATIENTS) - DOSE 1							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
ORGANS OF SPECIAL SENSE	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	1 (7.7%)	0 (0.0%)		1 (7.1%)
EARACHE	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	1 (7.7%)	0 (0.0%)		1 (7.1%)
PERSONS WITH COMPLAINTS	2 (14.3%)	4 (28.6%)	0 (0.0%)	1 (7.1%)	2 (15.4%)	0 (0.0%)		7 (50.0%)
PERSONS WITH NO COMPLAINTS	12 (85.7%)	10 (71.4%)	14 (100.0%)	13 (92.9%)	11 (84.6%)	1 (100.0%)		7 (50.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 2 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT :
LOT NUMBER : CK444
DOSE : 5 MCG
PATIENT CLASS: THALASSEMICS

CLINICAL COMPLAINTS	TOTAL VACCINEES (15 PATIENTS) - DOSE 2 — DAYS POST VACCINATION 0	1	2	3	4	5		NUMBER WITH COMPLAINTS
REACTION, LOCAL (INJECT. SITE)	2 (14.3%)	2 (14.3%)	2 (14.3%)	1 (7.1%)	1 (7.7%)	0 (0.0%)		3 (21.4%)
SORENESS	2 (14.3%)	2 (14.3%)	2 (14.3%)	1 (7.1%)	1 (7.7%)	0 (0.0%)		3 (21.4%)
ECCHYMOSIS	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (7.1%)
SYSTEMIC	1 (7.1%)	1 (7.1%)	2 (14.3%)	2 (14.3%)	1 (7.7%)	0 (0.0%)		2 (14.3%)
WHOLE BODY/GENERAL	0 (0.0%)	0 (0.0%)	1 (7.1%)	1 (7.1%)	0 (0.0%)	0 (0.0%)		1 (7.1%)
HEADACHE	0 (0.0%)	0 (0.0%)	1 (7.1%)	1 (7.1%)	0 (0.0%)	0 (0.0%)		1 (7.1%)
INTEGUMENTARY SYSTEM	0 (0.0%)	1 (7.1%)	2 (14.3%)	1 (7.1%)	1 (7.7%)	0 (0.0%)		2 (14.3%)
RASH, NOS	0 (0.0%)	1 (7.1%)	2 (14.3%)	1 (7.1%)	1 (7.7%)	0 (0.0%)		2 (14.3%)
DIGESTIVE SYSTEM	1 (7.1%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (7.1%)
DIARRHEA	1 (7.1%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (7.1%)
PERSONS WITH COMPLAINTS	3 (21.4%)	3 (21.4%)	3 (21.4%)	2 (14.3%)	2 (15.4%)	0 (0.0%)		4 (28.6%)
PERSONS WITH NO COMPLAINTS	11 (78.6%)	11 (78.6%)	11 (78.6%)	12 (85.7%)	11 (84.6%)	1 (100.0%)		10 (71.4%)

Table 2 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT :
LOT NUMBER : CK444
DOSE : 5 MCG
PATIENT CLASS: THALASSEMICS

CLINICAL COMPLAINTS	TOTAL VACCINEES (15 PATIENTS) - DOSE 2							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)		0 (0.0%)

Table 2 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT - :
LOT NUMBER : CK444
DOSE : 5 MCG
PATIENT CLASS: THALASSEMICS

CLINICAL COMPLAINTS	TOTAL VACCINEES (15 PATIENTS) - DOSE 3							
	DAYS POST VACCINATION							NUMBER WITH COMPLAINTS
	0	1	2	3	4	5		
REACTION, LOCAL (INJECT. SITE)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	0 (0.0%)		1 (33.3%)
SORENESS	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	0 (0.0%)		1 (33.3%)
PERSONS WITH COMPLAINTS	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	0 (0.0%)		1 (33.3%)
PERSONS WITH NO COMPLAINTS	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	0 (0.0%)		2 (66.7%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 3

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT :
LOT NUMBER : CK444
DOSE : 5 MCG
PATIENT CLASS: THALASSEMICS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (15 PATIENTS) - DOSE 1 — DAYS POST VACCINATION							NUMBER WITH MAX TEMP
	0	1	2	3	4	5		
NORMAL	4 (28.6%)	4 (28.6%)	4 (28.6%)	4 (28.6%)	4 (30.8%)	0 (0.0%)		4 (28.6%)
< 99	4 (28.6%)	4 (28.6%)	7 (50.0%)	5 (35.7%)	6 (46.2%)	1 (100.0%)		2 (14.3%)
99 - 99.9	4 (28.6%)	4 (28.6%)	1 (7.1%)	4 (28.6%)	2 (15.4%)	0 (0.0%)		4 (28.6%)
100 - 100.9	2 (14.3%)	1 (7.1%)	2 (14.3%)	1 (7.1%)	1 (7.7%)	0 (0.0%)		3 (21.4%)
103 - 103.9	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (7.1%)
TEMPERATURE TAKEN	14 (93.3%)	14 (93.3%)	14 (93.3%)	14 (93.3%)	13 (86.7%)	1 (6.7%)		14 (93.3%)
TEMPERATURE NOT TAKEN	1 (6.7%)	1 (6.7%)	1 (6.7%)	1 (6.7%)	2 (13.3%)	14 (93.3%)		1 (6.7%)

Table 3 (cont.)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT :
LOT NUMBER : CK444
DOSE : 5 MCG
PATIENT CLASS: THALASSEMICS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (15 PATIENTS) - DOSE 2							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
NORMAL	6 (46.2%)	6 (42.9%)	6 (50.0%)	6 (42.9%)	6 (46.2%)	0 (0.0%)		6 (42.9%)
< 99	4 (30.8%)	3 (21.4%)	5 (41.7%)	5 (35.7%)	5 (38.5%)	1 (100.0%)		3 (21.4%)
99 - 99.9	3 (23.1%)	3 (21.4%)	1 (8.3%)	3 (21.4%)	1 (7.7%)	0 (0.0%)		3 (21.4%)
100 - 100.9	0 (0.0%)	2 (14.3%)	0 (0.0%)	0 (0.0%)	1 (7.7%)	0 (0.0%)		2 (14.3%)
TEMPERATURE TAKEN	13 (86.7%)	14 (93.3%)	12 (80.0%)	14 (93.3%)	13 (86.7%)	1 (6.7%)		14 (93.3%)
TEMPERATURE NOT TAKEN	2 (13.3%)	1 (6.7%)	3 (20.0%)	1 (6.7%)	2 (13.3%)	14 (93.3%)		1 (6.7%)

Table 3 (cont.)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT :
LOT NUMBER : CK444
DOSE : 5 MCG
PATIENT CLASS: THALASSEMICS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (15 PATIENTS) - DOSE 3 — DAYS POST VACCINATION								NUMBER WITH MAX TEMP
	0	1	2	3	4	5			
NORMAL	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	0 (0.0%)			1 (33.3%)
< 99	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	0 (0.0%)			2 (66.7%)
TEMPERATURE TAKEN	3 (20.0%)	3 (20.0%)	3 (20.0%)	3 (20.0%)	3 (20.0%)	0 (0.0%)			3 (20.0%)
TEMPERATURE NOT TAKEN	12 (80.0%)	12 (80.0%)	12 (80.0%)	12 (80.0%)	12 (80.0%)	15 (100.0%)			12 (80.0%)

00855

Table 4

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT :
LOT NUMBER : CK444
DOSE : 2.5 MCG
PATIENT CLASS: THALASSEMICS

CLINICAL COMPLAINTS	TOTAL VACCINEES (16 PATIENTS) - DOSE 1						
	DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
REACTION, LOCAL (INJECT. SITE)	2 (20.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (18.8%)
SORENESS	2 (20.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (18.8%)
SYSTEMIC	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)
RESPIRATORY	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)
RHINITIS	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)
PHARYNGITIS (SORE THROAT)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)
PERSONS WITH COMPLAINTS	3 (30.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (25.0%)
PERSONS WITH NO COMPLAINTS	7 (70.0%)	15 (93.8%)	16 (100.0%)	16 (100.0%)	16 (100.0%)	6 (100.0%)	12 (75.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 4 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT :
LOT NUMBER : CK444
DOSE : 2.5 MCG
PATIENT CLASS: THALASSEMICS

CLINICAL COMPLAINTS	TOTAL VACCINEES (16 PATIENTS) - DOSE 2						NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION						
	0	1	2	3	4	5	
REACTION, LOCAL (INJECT. SITE)	3 (23.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (18.8%)
SORENESS	3 (23.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (18.8%)
SYSTEMIC	2 (15.4%)	1 (6.7%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (12.5%)
ORGANS OF SPECIAL SENSE	1 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)
OTHER	1 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)
PSYCHIATRIC/BEHAVIORAL	1 (7.7%)	1 (6.7%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)
IRRITABILITY	1 (7.7%)	1 (6.7%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)
PERSONS WITH COMPLAINTS	4 (30.8%)	1 (6.7%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (25.0%)
PERSONS WITH NO COMPLAINTS	9 (69.2%)	14 (93.3%)	15 (93.8%)	16 (100.0%)	15 (100.0%)	3 (100.0%)	12 (75.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)

00857

Table 4 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT :
LOT NUMBER : CK444
DOSE : 2.5 MCG
PATIENT CLASS: THALASSEMICS

CLINICAL COMPLAINTS	TOTAL VACCINEES (12 PATIENTS) - DOSE 3							
	DAYS POST VACCINATION							NUMBER WITH COMPLAINTS
	0	1	2	3	4	5		
PERSONS WITH COMPLAINTS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)
PERSONS WITH NO COMPLAINTS	1 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	1 (100.0%)		2 (100.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

00858

Table 5

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT :
LOT NUMBER : CK444
DOSE : 2.5 MCG
PATIENT CLASS: THALASSEMICS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (16 PATIENTS) - DOSE 1								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
NORMAL	1 (10.0%)	1 (6.3%)	1 (6.3%)	1 (6.3%)	1 (6.3%)	0 (0.0%)			1 (6.3%)
< 99	4 (40.0%)	10 (62.5%)	8 (50.0%)	10 (62.5%)	11 (68.8%)	5 (83.3%)			8 (50.0%)
99 - 99.9	4 (40.0%)	4 (25.0%)	6 (37.5%)	4 (25.0%)	4 (25.0%)	1 (16.7%)			6 (37.5%)
100 - 100.9	1 (10.0%)	0 (0.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			0 (0.0%)
101 - 101.9	0 (0.0%)	1 (6.3%)	0 (0.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)			1 (6.3%)
TEMPERATURE TAKEN	10 (62.5%)	16 (100.0%)	16 (100.0%)	16 (100.0%)	16 (100.0%)	6 (37.5%)			16 (100.0%)
TEMPERATURE NOT TAKEN	6 (37.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	10 (62.5%)			0 (0.0%)

Table 5 (cont.)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT :
LOT NUMBER : CK444
DOSE : 2.5 MCG
PATIENT CLASS: THALASSEMICS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (16 PATIENTS) - DOSE 2								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
NORMAL	6 (46.2%)	6 (40.0%)	6 (37.5%)	6 (37.5%)	6 (40.0%)	0 (0.0%)			6 (37.5%)
< 99	4 (30.8%)	6 (40.0%)	6 (37.5%)	6 (37.5%)	6 (40.0%)	3 (100.0%)			5 (31.3%)
99 - 99.9	3 (23.1%)	2 (13.3%)	3 (18.8%)	3 (18.8%)	2 (13.3%)	0 (0.0%)			2 (12.5%)
100 - 100.9	0 (0.0%)	1 (6.7%)	1 (6.3%)	1 (6.3%)	1 (6.7%)	0 (0.0%)			3 (18.8%)
TEMPERATURE TAKEN	13 (81.3%)	15 (93.8%)	16 (100.0%)	16 (100.0%)	15 (93.8%)	3 (18.8%)			16 (100.0%)
TEMPERATURE NOT TAKEN	3 (18.8%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	1 (6.3%)	13 (81.3%)			0 (0.0%)

00860

Table 5 (cont.)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0799
TREATMENT :
LOT NUMBER : CK444
DOSE : 2.5 MCG
PATIENT CLASS: THALASSEMICS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (12 PATIENTS) - DOSE 3							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
< 99	1 (100.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	2 (100.0%)	1 (100.0%)		0 (0.0%)
99 - 99.9	0 (0.0%)	2 (100.0%)	1 (50.0%)	2 (100.0%)	0 (0.0%)	0 (0.0%)		2 (100.0%)
TEMPERATURE TAKEN	1 (8.3%)	2 (16.7%)	2 (16.7%)	2 (16.7%)	2 (16.7%)	1 (8.3%)		2 (16.7%)
TEMPERATURE NOT TAKEN	11 (91.7%)	10 (83.3%)	10 (83.3%)	10 (83.3%)	10 (83.3%)	11 (91.7%)		10 (83.3%)

PROGRAM:	Alum-Adsorbed Yeast Recombinant Hepatitis B Vaccine, Study 861.
PURPOSE:	To assess antibody and clinical responses to vaccine in persons with hemophilia or homozygous sickle cell disease who are negative for hepatitis B serologic markers.
VACCINE:	Yeast Recombinant Hepatitis B Vaccine Lot #979/C-K564 (10 mcg HBsAg/ml)
PRINCIPAL INVESTIGATOR:	Joan Gill, M.D., Medical Director Great Lakes Hemophilia Foundation 1701 W. Wisconsin Avenue Milwaukee, WI 53233
SECONDARY INVESTIGATORS:	Robert R. Montgomery, M.D. Great Lakes Hemophilia Foundation 1701 W. Wisconsin Avenue Milwaukee, WI 53233 Kathleen M. Marquardt, R.N. Great Lakes Hemophilia Foundation 1701 W. Wisconsin Avenue Milwaukee, WI 53233 Denise Lockhart, R.N. Nurse Coordinator, Sickle Cell Clinic Milwaukee Children's Hospital 1701 W. Wisconsin Avenue Milwaukee, WI 53233
STUDY LOCATION:	Great Lakes Hemophilia Foundation Milwaukee Children's Hospital 1701 West Wisconsin Avenue Milwaukee, WI 53233
DATE INITIATED:	November 8, 1984
DATE COMPLETED:	In progress

Study 861

STUDY POPULATION:

The study population will consist of 25-30 hemophiliacs of any age and either sex (pregnant women excluded), who are negative for HBsAg, anti-HBc and anti-HBs, have a normal ALT level and have not previously received any hepatitis B vaccine.

Under an addendum to the study, an additional population of approximately 10 persons (< 20 years of age) with homozygous sickle cell disease, who are undergoing chronic blood transfusion, and are negative for hepatitis B serologic markers, will also be included in the study.

PROCEDURE:

Each participant receives an injection of vaccine at 0, 1, and 6 months. The vaccine is administered subcutaneously to the hemophiliacs and intramuscularly to the subjects with homozygous sickle cell disease. Persons under 20 years of age are given a 0.5 ml (5 mcg HBsAg) injection of vaccine, while those 20 years of age and older receive a 1.0 ml (10 mcg HBsAg) injection of vaccine. Vaccine recipients (or their parents/guardians in the case of minors) will be asked to record their temperature for 5 days after each injection and to note any local or systemic complaints.

Blood specimens will be obtained prior to vaccination and 1, 3, 6, and 8 months post-initial injection. Samples will be assayed for HBsAg, anti-HBc, anti-HBs and ALT at MSDRL. Samples with an anti-HBs titer $\geq$25 mIU/ml will be further tested to determine the relative proportions of anti-a and anti-d activity. Samples may be assayed for yeast antibody.

RESULTS:

<u>HEMOPHILIACS:</u>

5 mcg (<20 years of age)
Lot #979/C-K564 at 0, 1, and 6 Months

10 mcg ($\geq$20 years of age)
Lot #979/C-K564 at 0, 1, and 6 Months

Study 861

RESULTS: (Cont.)

1. Number Vaccinated:

Dose Level	Injection No. 1	2	3
5 mcg	9	9	0
10 mcg	2	2	0

2. Serologic Results:

Serologic data are available for 8 participants at 3 months who received 5 mcg injections and 2 participants who received 10 mcg injections. 7/8 month data are available for one participant from each dose level.

At three months, all eight participants (100%) who received 5 mcg injections seroconverted (S/N $\geq$ 2.1) and developed protective levels of anti-HBs (mIU/ml $\geq$ 10). The GMT for those responders was 143.2 mIU/ml.

Both participants who received 10 mcg injections seroconverted for anti-HBs (S/N $\geq$2.1) at three months. Neither developed protective levels of anti-HBs (mIU/ml $\geq$ 10) at that time. The GMT for those participants was 6.7 mIU/ml.

Refer to Table 1 for anti-HBs responses and GMTs for other time intervals.

3. Clinical complaints:

Clinical follow-up data for participants who received 5 mcg injections are available for 10, 8, and 5 vaccinees after injection 1,, 2, and 3, respectively. Among vaccinees who received 10 mcg injections, clinical follow-up data are available for 3, 2, and 1 participants after injection 1, 2 and 3, respectively.

Study 861

RESULTS (CONT.): The overall frequencies of complaints are presented below.

Type of Complaint	Dose Level	Frequency in % by Injection No. 1	2	3
Injection Site	5 mcg	9 (1/11)	13 (1/8)	0 (0/5)
	10 mcg	33 (1/3)	50 (1/2)	0 (0/1)
Systemic	5 mcg	9 (1/11)	13 (1/8)	0 (0/5)
	10 mcg	33 (1/3)	0 (0/2)	100 (1/1)

Refer to Tables 2 and 3 for listings of specific clinical complaints by injection number. Maximum temperature data are provided in Tables 4 and 5.

There were no serious or alarming reactions attributable to vaccine.

HBV Markers (anti-HBc)

One subject with hemophilia (case (b) (6) became seropositive for anti-HBc 2 months after the third injection of yeast recombinant hepatitis B vaccine. At the time the subject was also seropositive for anti-HBs with reported titers of 42769.8 S/N and 118121.4 mIU/ml. The vaccinee was well; serum samples were negative for HBsAg and ALT levels were normal. Attempts will be made to obtain additional serum samples.

Reactions Reported to the OOBRR

One patient (case (b) (6)) was hospitalized for a bleeding telangiectasic site in the distal atrum of the stomach (b) (6) after administration of the third injection of vaccine. This 42 year old male with hemophilia had a medical history significant for recurrent GI bleeding, duodenal and antral gastric ulcer, and hemarthroses. The patient was adminstered whole blood and cryoprecipitate and was discharged after 5 days in stable condition. The investigator stated the patient's experience was not related to vaccination with yeast recombinant hepatitis B vaccine.

Study 861

RESULTS (CONT.): PATIENTS WITH SICKLE CELL ANEMIA

5 mcg Lot #979/C-K564 at 0, 1, and 6 months

1. Number vaccinated:

Injection No.		
1	2	3
4	4	0

2. Serologic Results:

Serologic results are not yet available.

3. Clinical Complaints:

There have been no serious or alarming reactions attributed to vaccination. Detailed data on clinical complaints and temperatures following vaccination are not yet available.

Table 1

Antibody Responses Among Hemophiliacs Following Vaccination with
5 mcg (Hemophiliacs < 20 years) or 10 mcg (Hemophiliacs ≥ 20 years) Injections of
Yeast Recombinant Hepatitis B Vaccine Lot # 979/C-K564 at 0, 1, and 6 Months in Study #861

Time (Months)	5 mcg (Hemophiliacs < 20 Years of Age)					10 mcg (Hemophiliacs > 20 Years of Age)				
	% with Anti-HBs		GMT (mIU/ml)			% with Anti-HBs		GMT (mIU/ml)		
	S/N ≥ 2.1	mIU/ml ≥ 10	All Vaccinees	Responders S/N ≥ 2.1	Responders mIU/ml ≥ 10	S/N ≥ 2.1	mIU/ml ≥ 10	All Vaccinees	Responders S/N ≥ 2.1	Responders mIU/ml ≥ 10
1	18 (2/11)	18 (2/11)	0.9	15.9	15.9	0 (0/3)	0 (0/3)	0.3	---	----
3	100 (8/8)	100 (8/8)	143.2	143.2	143.2	100 (2/2)	0 (0/2)	6.7	6.7	----
6	100 (2/2)	100 (2/2)	223.7	223.7	223.7	0 (0/1)	0 (0/1)	1.3	---	----
7/8	100 (1/1)	100 (1/1)	3878.3	3878.3	3878.3	0 (0/1)	0 (0/1)	1.6	---	----

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00867

Table 2

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0861
TREATMENT :
LOT NUMBER : CK564
DOSE : 5 MCG
PATIENT CLASS: HEMOPHILIACS

CLINICAL COMPLAINTS	TOTAL VACCINEES (12 PATIENTS) - DOSE 1							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
REACTION, LOCAL (INJECT. SITE)	1 (9.1%)	1 (9.1%)	1 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (9.1%)
SORENESS	1 (9.1%)	1 (9.1%)	1 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (9.1%)
SWELLING	1 (9.1%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (9.1%)
SYSTEMIC	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (9.1%)		1 (9.1%)
WHOLE BODY/GENERAL	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (9.1%)		1 (9.1%)
SWEATING	1 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (9.1%)
FATIGUE/WEAKNESS	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (9.1%)		1 (9.1%)
PERSONS WITH COMPLAINTS	2 (18.2%)	2 (18.2%)	2 (18.2%)	1 (9.1%)	1 (9.1%)	1 (9.1%)		2 (18.2%)
PERSONS WITH NO COMPLAINTS	9 (81.8%)	9 (81.8%)	9 (81.8%)	10 (90.9%)	10 (90.9%)	10 (90.9%)		9 (81.8%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 2 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0861
TREATMENT :
LOT NUMBER : CK564
DOSE : 5 MCG
PATIENT CLASS: HEMOPHILIACS

CLINICAL COMPLAINTS	TOTAL VACCINEES (12 PATIENTS) - DOSE 2							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
REACTION, LOCAL (INJECT. SITE)	1 (12.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (12.5%)
SORENESS	1 (12.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (12.5%)
SYSTEMIC	0 (0.0%)	0 (0.0%)	1 (12.5%)	1 (12.5%)	1 (12.5%)	1 (12.5%)		1 (12.5%)
DIGESTIVE SYSTEM	0 (0.0%)	0 (0.0%)	1 (12.5%)	1 (12.5%)	1 (12.5%)	1 (12.5%)		1 (12.5%)
DIARRHEA	0 (0.0%)	0 (0.0%)	1 (12.5%)	1 (12.5%)	1 (12.5%)	1 (12.5%)		1 (12.5%)
PERSONS WITH COMPLAINTS	1 (12.5%)	0 (0.0%)	1 (12.5%)	1 (12.5%)	1 (12.5%)	1 (12.5%)		2 (25.0%)
PERSONS WITH NO COMPLAINTS	7 (87.5%)	8 (100.0%)	7 (87.5%)	7 (87.5%)	7 (87.5%)	7 (87.5%)		6 (75.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 2 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0861
TREATMENT :
LOT NUMBER : CK564
DOSE : 5 MCG
PATIENT CLASS: HEMOPHILIACS

CLINICAL COMPLAINTS	TOTAL VACCINEES (5 PATIENTS) - DOSE 3							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
PERSONS WITH COMPLAINTS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)
PERSONS WITH NO COMPLAINTS	5 (100.0%)	5 (100.0%)	5 (100.0%)	5 (100.0%)	5 (100.0%)	5 (100.0%)		5 (100.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 3

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0861
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: HEMOPHILIACS

CLINICAL COMPLAINTS	TOTAL VACCINEES (3 PATIENTS) - DOSE 1							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
REACTION, LOCAL (INJECT. SITE)	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (33.3%)
SORENESS	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (33.3%)
SYSTEMIC	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (33.3%)
WHOLE BODY/GENERAL	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (33.3%)
SWEATING	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (33.3%)
PERSONS WITH COMPLAINTS	2 (66.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		2 (66.7%)
PERSONS WITH NO COMPLAINTS	1 (33.3%)	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)		1 (33.3%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

00871

Table 3 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0861
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: HEMOPHILIACS

CLINICAL COMPLAINTS	TOTAL VACCINEES (3 PATIENTS) - DOSE 2						
	DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
REACTION, LOCAL (INJECT. SITE)	1 (50.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)
SORENESS	1 (50.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)
PERSONS WITH COMPLAINTS	1 (50.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)
PERSONS WITH NO COMPLAINTS	1 (50.0%)	1 (50.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	1 (50.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

00872

Table 3 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0861
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: HEMOPHILIACS

CLINICAL COMPLAINTS	TOTAL VACCINEES (1 PATIENTS) - DOSE 3							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
SYSTEMIC	0 (0.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)		1 (100.0%)
DIGESTIVE SYSTEM	0 (0.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)		1 (100.0%)
GI BLEEDING	0 (0.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)		1 (100.0%)
PERSONS WITH COMPLAINTS	0 (0.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)		1 (100.0%)
PERSONS WITH NO COMPLAINTS	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

00873

Table 4

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0861
TREATMENT :
LOT NUMBER : CK564
DOSE : 5 MCG
PATIENT CLASS: HEMOPHILIACS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (12 PATIENTS) - DOSE 1 — DAYS POST VACCINATION 0	1	2	3	4	5			NUMBER WITH MAX TEMP
< 99	8 (100.0%)	6 (75.0%)	7 (87.5%)	7 (87.5%)	7 (87.5%)	7 (87.5%)			6 (75.0%)
99 - 99.9	0 (0.0%)	2 (25.0%)	1 (12.5%)	1 (12.5%)	1 (12.5%)	1 (12.5%)			2 (25.0%)
TEMPERATURE TAKEN	8 (66.7%)	8 (66.7%)	8 (66.7%)	8 (66.7%)	8 (66.7%)	8 (66.7%)			8 (66.7%)
TEMPERATURE NOT TAKEN	4 (33.3%)	4 (33.3%)	4 (33.3%)	4 (33.3%)	4 (33.3%)	4 (33.3%)			4 (33.3%)

00874

Table 4 (Contd)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0861
TREATMENT :
LOT NUMBER : CK564
DOSE : 5 MCG
PATIENT CLASS: HEMOPHILIACS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (12 PATIENTS) - DOSE 2							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
< 99	4 (80.0%)	4 (100.0%)	3 (60.0%)	3 (75.0%)	4 (80.0%)	4 (80.0%)		2 (40.0%)
99 - 99.9	1 (20.0%)	0 (0.0%)	2 (40.0%)	1 (25.0%)	1 (20.0%)	1 (20.0%)		3 (60.0%)
TEMPERATURE TAKEN	5 (41.7%)	4 (33.3%)	5 (41.7%)	4 (33.3%)	5 (41.7%)	5 (41.7%)		5 (41.7%)
TEMPERATURE NOT TAKEN	7 (58.3%)	8 (66.7%)	7 (58.3%)	8 (66.7%)	7 (58.3%)	7 (58.3%)		7 (58.3%)

00875

Table 4 (Contd)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0861
TREATMENT :
LOT NUMBER : CK564
DOSE : 5 MCG
PATIENT CLASS: HEMOPHILIACS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (5 PATIENTS) - DOSE 3								
	DAYS POST VACCINATION								NUMBER WITH MAX TEMP
	0	1	2	3	4	5			
< 99	1 (100.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)			1 (100.0%)
TEMPERATURE TAKEN	1 (20.0%)	1 (20.0%)	1 (20.0%)	1 (20.0%)	1 (20.0%)	1 (20.0%)			1 (20.0%)
TEMPERATURE NOT TAKEN	4 (80.0%)	4 (80.0%)	4 (80.0%)	4 (80.0%)	4 (80.0%)	4 (80.0%)			4 (80.0%)

Table 5

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0861
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: HEMOPHILIACS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (3 PATIENTS) - DOSE 1							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
< 99	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	1 (100.0%)		2 (100.0%)
TEMPERATURE TAKEN	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	1 (33.3%)		2 (66.7%)
TEMPERATURE NOT TAKEN	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	2 (66.7%)		1 (33.3%)

Table 5 (Contd)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0861
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: HEMOPHILIACS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (3 PATIENTS) - DOSE 2								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
< 99	1 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)			2 (100.0%)
TEMPERATURE TAKEN	1 (33.3%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)			2 (66.7%)
TEMPERATURE NOT TAKEN	2 (66.7%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)			1 (33.3%)

NONRESPONDERS/
HYPORESPONDERS

SUMMARY - NONRESPONDERS, HYPORESPONDERS AND TRANSIENT RESPONDERS

Nonresponders

A total of 81 persons, all of whom failed to develop antibody after receiving three injections of plasma-derived hepatitis B vaccine, have received one or more injections of yeast recombinant vaccine in six studies. This population includes both healthy adults and patients with chronic renal insufficiency who are receiving dialysis treatment (dialysis patients) . Healthy adults receive three 10 mcg doses and dialysis patients receive three 20 mcg or 40 mcg doses of yeast recombinant vaccine at 0, 1, and 6 months.

Fifty-five healthy adults have received one or more 10 mcg doses of yeast recombinant vaccine. Twenty-four persons have completed the three injection regimen. At 7-9 months, 79% (11/14) seroconverted (S/N $\geq$2.1) and 50% (7/14) developed protective levels of antibody (mIU/ml $\geq$10). Geometric mean titers among responders with a titer of S/N $\geq$10 or mIU/ml $\geq$10 were 39.3 S/N and 245.1 mIU/ml in each of the two studies where such data are available.

Twenty-six dialysis patients have received an initial injection of vaccine. Twenty-one of these received a 40 mcg dose and five received a 20 mcg dose. Six participants have received three injections of vaccine. At 2-3 months, 25% (1/4) and 35% (6/17) have titers of S/N $\geq$2.1 after two 20 or 40 mcg doses of vaccine, respectively. Protective levels of antibody developed in 25% (20 mcg dose) and 18% (40 mcg dose). Geometric mean titers among responders with an antibody level of mIU/ml $\geq$10 were 53.0 mIU/ml (20 mcg dose) and 43.2 mIU/ml (40 mcg dose). At 7-8 months the single individual measured after three 20 mcg doses and one of three persons monitored after three 40 mcg doses have protective levels of antibody (mIU/ml $\geq$10). These two responders had titers of 136.9 mIU/ml (20 mcg dose) and 49.4 mIU/ml (40 mcg dose).

Two hemodialysis patients withdrew from a study due to clinical complaints which were considered possibly related to vaccine. A 32-year old subject developed a swollen, stiff and sore left arm after administration of vaccine. The symptoms persisted for one week and then subsided. A 72-year old male subject developed generalized achiness and a headache three days after administration of the first injection of vaccine. Forty-eight hours after onset of these symptoms, he developed a flu-like syndrome with a temperature of 100°F. He did not receive any further vaccine injections.

Hyporesponders and Transient Responders

Two hyporesponders and three transient responders to plasma-derived hepatitis B vaccine have received a single 10 mcg dose of yeast recombinant vaccine. No serious or alarming adverse reactions attributable to vaccine have been reported.

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Hyporesponders and Transient Responders (Cont.)

At one month post-vaccination, one hyporesponder displayed a marked boost in HBs antibody. A protective level of antibody has been maintained over 6 months of follow-up in this individual. The other individual has not responded to the vaccine. One month after vaccination, 100% (2/2) of transient responders have protective levels of antibody with a geometric mean titer of 67.9 mIU/ml.

NONRESPONDERS, HYPORESPONDERS AND TRANSIENT RESPONDERS

Study 794 - Bethesda, MD - Dr. H. Alter

The study population consists of seronegative nonresponders to plasma-derived vaccine and health care personnel who have not previously received any hepatitis B vaccine. Health care personnel receive either 5 mcg of 10 mcg injections of vaccine and nonresponders receive 10 mcg injections. All participants are administered vaccine lot C-K444 at 0, 1, and 6 months.

Eleven nonresponders have received two 10 mcg injections of vaccine and eight of these have received the third dose. At 7/8 months, 88% (7/8) of the participants seroconverted (S/N $\geq$2.1) and 63% (5/8) developed protective levels of anti-HBs (S/N $\geq$10). The GMT at that time for all vaccinees was 25.0 S/N and 95.9 for responders (S/N $\geq$10).

No serious or alarming adverse reactions attributable to vaccine have been reported. The study continues in progress. Refer to the summary on health care personnel/healthy adults for data regarding other subjects vaccinated in this study.

Study 816 - Philadelphia, PA - Dr. S. Plotkin and Dr. S. Starr

The study population consists of three groups of adults negative for hepatitis B serologic markers: hemodialysis patients, health care personnel, and hemodialysis patients who were nonresponders to plasma-derived vaccine. Nonresponders receive 20 mcg or 40 mcg injections of vaccine lot C-K444 at 0, 1, and 6 months.

Five nonresponders have received two 20 mcg injections of vaccine and three of these have received the third injection. Serology data at 7/8 months is available for one vaccinee only. This subject seroconverted (S/N $\geq$2.1) and developed protective levels of anti-HBs (mIU/ml $\geq$10) with a titer of 136.9 mIU/ml.

Four nonresponders have received two 40 mcg injections of vaccine. Three of these have received the third injection. Seven/eight month serology data are available for three vaccine recipients. One (33%) of the subjects seroconverted (S/N $\geq$2.1) and developed protective levels of anti-HBs (mIU/ml) at that time. The GMT for all vaccinees was 2.1 mIU/ml and 49.4 for responders (mIU/ml $\geq$10).

No serious or alarming reactions attributable to vaccine have been reported. The study continues in progress. Refer to the summaries on health care personnel/healthy adults and dialysis patients for data regarding other subjects vaccinated in this study.

Study 817 - West Point, PA - Dr. R. Bishop

Preimmune healthy adults and nonresponders to plasma-derived vaccine are enrolled in Study 817. Preimmune adults receive a single 10 mcg injection of

Study 817 - West Point, PA - Dr. R. Bishop (Cont.)

vaccine. Nonresponders receive 10 mcg injections of vaccine lot C-K444 at 0, 1, and 6 months.

Four nonresponders have received two 10 mcg injections of vaccine and three of these have received the third injection. Serology data are available for two of the vaccinees at 7/8 months. Neither participant seroconverted for anti-HBs at that time.

No serious or alarming adverse experiences related to vaccine have been reported. The study continues in progress. Refer to the summary on preimmune adults for data regarding other subjects vaccinated in this study.

Study 854 - Boston, MA - Dr. J. Dienstag

The population of Study B54 consists of four groups: chronic carriers of HBsAg, and healthy hyporesponders, nonresponders, and transient responders to plasma-derived hepatitis B vaccine. Hyporesponders and transient responders receive a single 10 mcg injection of vaccine lot C-K564. Nonresponders receive 10 mcg injections of the same vaccine lot at 0, 1, and 6 months.

Two hyporesponders have received a 10 mcg injection of vaccine. One of the vaccinees displayed a marked boost in anti-HBs titer one month after receiving vaccine. The other participant has not responded.

Three transient responders have received a 10 mcg injection of vaccine. At one month, two out of the three (67%) transient responders who were seronegative for anti-HBs prior to vaccination, seroconverted for anti-HBs. The GMT for the two responders was 67.9 mIU/ml.

Fourteen nonresponders have received one 10 mcg injection of vaccine and thirteen of these have been administered the second and third injections. At 6 months, 58% (7/12) of the subjects seroconverted for anti-HBs (S/N $\geq$2.1) and 25% (3/12) developed protective levels of anti-HBs (mIU/ml $\geq$10). The GMT for all vaccinees at 6 months was 3.2 mIU/ml and 45.8 for responders (mIU/ml $\geq$10).

No serious or alarming adverse experiences attributable to vaccine have been reported. The study continues in progress. Refer to the summary on chronic carriers for data regarding other subjects vaccinated in this study.

Study 874 - Pasadena, CA - Dr. M. Tong

Healthy adults who were nonresponders or hyporesponders to plasma-derived hepatitis B vaccine are enrolled in the study. All participants receive 10 mcg injections of vaccine lot C-K563 at 0, 1, and 6 months.

Twenty-six nonresponders and hyporesponders have received two 10 mcg injections of vaccine. None have received the third injection. At one month, 36% (9/25) of the vaccinees seroconverted for anti-HBs (S/N $\geq$2.1). Further serologic data are not currently available.

Study 874 - Pasadena, CA - Dr. M. Tong (Cont.)

No serious or alarming adverse events attributable to vaccine have been reported. The study continues in progress.

Study 875 - Duluth, MN - Dr. T. Johnson

The study population consists of adult hemodialysis patients who were nonresponders to plasma-derived hepatitis B vaccine. Participants received 40 mcg injections of either yeast recombinant vaccine lot C-K937 or plasma-derived vaccine lot 2277K at 0, 1, and 6 months.

Seventeen nonresponders have received one 40 mcg injection of yeast recombinant vaccine and fifteen of these have been administered the second injection. None have yet received the third injection. Two month serology data are available for 13 recipients of yeast recombinant vaccine. Thirty-eight percent (5/13) seroconverted for anti-HBs (S/N $\geq$2.1) and 15% (2/13) developed protective levels of anti-HBs (mIU/ml $\geq$10). The GMT for responders was 70.7 (mIU/ml $\geq$10).

Eighteen nonresponders have received one 40 mcg injection of plasma-derived vaccine. Seventeen of these have received the second injection and none have received the third. At 2 months, 40% (7/15) of the plasma-derived vaccine recipients seroconverted (S/N $\geq$2.1) and developed protective levels of anti-HBs (mIU/ml $\geq$10). The GMT for responders was 131.6 (mIU/ml $\geq$10).

Two subjects experienced adverse experiences which were considered possibly related to vaccine. A 32-year old male hemodialysis patient received a 20 mcg intramuscular injection of vaccine into each deltoid (total dose 40 mcg). The patient's left arm subsequently became swollen, stiff and sore. These symptoms persisted for one week and then subsided. The patient did not receive any further vaccine injections. A 72-year old male hemodialysis patient developed generalized achiness and a headache three days after administration of the first injection of vaccine. Forty-eight hours after onset of these symptoms, he developed a flu-like syndrome with a temperature of 100°F. The patient did not receive any further vaccine injections.

PROGRAM:	Yeast Recombinant Hepatitis B Vaccine, Study 794
PURPOSE:	To evaluate antibody and clinical responses to the vaccine among: 1. Health care personnel immunized with plasma derived vaccine who were nonresponders (anti-HBs negative). 2. Health care personnel who are negative for hepatitis B virus serologic markers.
VACCINE:	Yeast Recombinant Hepatitis B Vaccine: Lot #972/C-K444 (10 mcg/HBsAg ml)
PRIMARY INVESTIGATOR:	Harvey J. Alter, M.D. Chief, Immunology Section Clinical Center Blood Bank National Institutes of Health Bethesda, Maryland
SECONDARY INVESTIGATORS:	David Henderson, M.D. James Schmitt, M.D. Ms. Deloris Koziol Ms. Beverly Elder
STUDY LOCATION:	Clinical Center Blood Bank National Institute of Health Bethesda, Maryland 20205
DATE INITIATED:	April 12, 1984
DATE COMPLETED:	In progress.
STUDY POPULATION:	The study population consists of 71 health care personnel of either sex (excluding pregnant women) who are negative for HBsAG, anti-HBc and anti-HBs, have a normal ALT level and have not previously received any hepatitis B vaccine. It also includes 11 nonresponders to plasma-derived vaccine.

Study 794

PROCEDURE:

Health care workers receive either 5 mcg or 10 mcg doses of vaccine at 0, 1 and 6 months. Nonresponders receive 10 mcg doses at 0, 1 and 6 months. All injections are intramuscular. Participants are asked to record their temperature for 5 days after each injection and note any local or systemic reactions.

Blood specimens are obtained prior to vaccination, and monthly for 7 months and at 9, 12 and 24 months post initial injection. All samples are assayed for anti-HBs, anti-HBc, and HBsAg and ALT by Dr. Alter. Samples with anti-HBs titers ≥25 mIU/ml may be tested for anti-a and anti-d activity at MSDRL.

RESULTS:

NONRESPONDERS TO PLASMA VACCINE

10 mcg Lot 972/C-K444 at 0, 1 and 6 months

1. Number Vaccinated:

Dose Level	Injection No. 1	2	3
10 mcg	11	11	8

2. Serologic Results:

Serologic data are available for 8 study participants at 7/8 months. Seroconversion was 88% (7/8) when the cutoff was S/N ≥2.1. When the cutoff was S/N ≥10, seroconversion was 63% (5/8). The GMT for all vaccinees was 25.0. Table 1 shows anti-HBs responses through 12 months of follow-up.

Study 794

RESULTS: (Contd)

3. Clinical Complaints:

Clinical follow-up data are available for 11, 10, and 8 vaccinees following injections one, two and three, respectively. Listings of specific complaints and maximum temperatures reported during the five days of follow-up after each injection are provided in Tables 2 and 3.

Type of Complaint	Dose Level	Frequency in % by Injection No. 1	2	3
Injection Site	10 mcg	9(1/11)	0(0/10)	25(2/8)
Systemic	10 mcg	18(2/11)	10(1/10)	0(0/8)

There were no serious or alarming adverse reactions attributable to vaccination.

Table 1

ANTIBODY RESPONSES FOLLOWING VACCINATION WITH RECOMBINANT HEPATITIS B VACCINE

STUDY : 0794
POPULATION : NONRESPONDERS (H)
DOSE : 10 MCG
LOT : CK444
REGIMEN : 0, 1, AND 6 MONTHS
INITIAL SEROLOGY: NEGATIVE

TIME (MONTHS)	% WITH ANTI-HBS				GMT (S/N)		
						RESPONDERS	
	S/N >= 2.1		S/N >= 10		ALL VACCINEES	S/N >= 2.1	S/N >= 10
1 MONTH	45%	(5/11)	18%	(2/11)	3.1	11.1	52.6
2 MONTHS	55%	(6/11)	18%	(2/11)	4.7	15.8	114.2
3 MONTHS	78%	(7/9)	33%	(3/9)	8.0	14.1	60.3
6 MONTHS	60%	(3/5)	60%	(3/5)	12.3	61.5	61.5
7/8 MONTHS	88%	(7/8)	63%	(5/8)	25.0	39.0	95.9
9 MONTHS	80%	(4/5)	60%	(3/5)	12.9	23.8	39.3
12 MONTHS	60%	(3/5)	40%	(2/5)	8.2	31.3	55.9

Table 2

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0796
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (N)

CLINICAL COMPLAINTS	TOTAL VACCINEES (11 PATIENTS) - DOSE 1							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
REACTION, LOCAL (INJECT. SITE)	1 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (9.1%)
SORENESS	1 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (9.1%)
SYSTEMIC	0 (0.0%)	1 (9.1%)	1 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		2 (18.2%)
RESPIRATORY	0 (0.0%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (9.1%)
UPPER RESPIRATORY INFECT., NOS	0 (0.0%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (9.1%)
MUSCULOSKELETAL	0 (0.0%)	1 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (9.1%)
ARTHRALGIA, MONOARTICULAR	0 (0.0%)	1 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (9.1%)
PERSONS WITH COMPLAINTS	1 (9.1%)	1 (9.1%)	1 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		3 (27.3%)
PERSONS WITH NO COMPLAINTS	10 (90.9%)	10 (90.9%)	10 (90.9%)	11 (100.0%)	11 (100.0%)	11 (100.0%)		8 (72.7%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 2 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0794
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (N)

CLINICAL COMPLAINTS	TOTAL VACCINEES (11 PATIENTS) - DOSE 2						
	DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
SYSTEMIC	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)
WHOLE BODY/GENERAL	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)
CHILLS	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)
DIGESTIVE SYSTEM	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)
NAUSEA	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)
PERSONS WITH COMPLAINTS	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)
PERSONS WITH NO COMPLAINTS	10 (100.0%)	9 (90.0%)	10 (100.0%)	10 (100.0%)	10 (100.0%)	9 (100.0%)	9 (90.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 2 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0794
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (H)

CLINICAL COMPLAINTS	TOTAL VACCINEES (8 PATIENTS) - DOSE 3						
	DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
REACTION, LOCAL (INJECT. SITE)	2 (25.0%)	1 (12.5%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (25.0%)
SORENESS	2 (25.0%)	1 (12.5%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (25.0%)
PERSONS WITH COMPLAINTS	2 (25.0%)	1 (12.5%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (25.0%)
PERSONS WITH NO COMPLAINTS	6 (75.0%)	7 (87.5%)	7 (87.5%)	8 (100.0%)	8 (100.0%)	8 (100.0%)	6 (75.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 3

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0794
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (N)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (11 PATIENTS) - DOSE 1 — DAYS POST VACCINATION 0	1	2	3	4	5			NUMBER WITH MAX TEMP
NORMAL	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (10.0%)	1 (11.1%)	1 (11.1%)			1 (9.1%)
< 99	8 (72.7%)	8 (72.7%)	8 (72.7%)	8 (80.0%)	6 (66.7%)	7 (77.8%)			7 (63.6%)
99 - 99.9	1 (9.1%)	2 (18.2%)	2 (18.2%)	0 (0.0%)	2 (22.2%)	1 (11.1%)			2 (18.2%)
100 - 100.9	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)			0 (0.0%)
102 - 102.9	1 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			1 (9.1%)
TEMPERATURE TAKEN	11 (100.0%)	11 (100.0%)	11 (100.0%)	10 (90.9%)	9 (81.8%)	9 (81.8%)			11 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (9.1%)	2 (18.2%)	2 (18.2%)			0 (0.0%)

Table 3 (cont.)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0794
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (N)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (11 PATIENTS) - DOSE 2							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
NORMAL	1 (11.1%)	1 (10.0%)	1 (10.0%)	1 (11.1%)	1 (14.3%)	1 (14.3%)		1 (10.0%)
< 99	7 (77.8%)	7 (70.0%)	6 (60.0%)	7 (77.8%)	5 (71.4%)	5 (71.4%)		7 (70.0%)
99 - 99.9	0 (0.0%)	2 (20.0%)	1 (10.0%)	1 (11.1%)	1 (14.3%)	1 (14.3%)		1 (10.0%)
100 - 100.9	1 (11.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (10.0%)
TEMPERATURE TAKEN	9 (81.8%)	10 (90.9%)	10 (90.9%)	9 (81.8%)	7 (63.6%)	7 (63.6%)		10 (90.9%)
TEMPERATURE NOT TAKEN	2 (18.2%)	1 (9.1%)	1 (9.1%)	2 (18.2%)	4 (36.4%)	4 (36.4%)		1 (9.1%)

Table 3 (cont.)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0794
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (H)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (8 PATIENTS) - DOSE 3							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
NORMAL	3 (37.5%)	4 (50.0%)	4 (50.0%)	5 (62.5%)	5 (62.5%)	5 (62.5%)		3 (37.5%)
< 99	5 (62.5%)	4 (50.0%)	4 (50.0%)	3 (37.5%)	3 (37.5%)	3 (37.5%)		5 (62.5%)
TEMPERATURE TAKEN	8 (100.0%)	8 (100.0%)	8 (100.0%)	8 (100.0%)	8 (100.0%)	8 (100.0%)		8 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

PROGRAM: Alum-Adsorbed Yeast Recombinant Hepatitis B Vaccine, Study 816

PURPOSE: To evaluate antibody and clinical responses to yeast recombinant hepatitis B vaccine among:

1. adult dialysis patients negative for hepatitis B serologic markers.
2. health care personnel negative for hepatitis B serologic markers.
3. adult dialysis patients negative for hepatitis B serologic markers, who previously received plasma-derived hepatitis B vaccine and were nonresponders (anti-HBs negative).

VACCINE: Yeast Recombinant Hepatitis B Vaccine
Lot 974/C-K446 (20 mcg HBsAg/ml)
Lot 986/C-K733 (20 mcg HBsAg/ml)

PRIMARY INVESTIGATOR: Stanley Plotkin, M.D./Stuart Starr, M.D.
Division of Preventive Medicine
Joseph Stokes, Jr. Research Institute
Children's Hospital of Philadelphia
34 Street and Civic Center Boulevard
Philadelphia, Pennsylvania 19104

STUDY LOCATION: Biomedical Applications of Lehigh Valley
2015 Hamilton Avenue
Allentown, Pennsylvania 18104

Dialysis, Inc.
1230 Burmont Road
Drexel Hill, Pennsylvania

The Kidney Center of Delaware Count
15th Street and Upland Avenue
Chester, Pennsylvania 19013

The Kidney Center of Chester County
960 East Lincoln Highway
Downingtown, Pennsylvania 19335

Study 816

DATE STUDY INITIATED: May 14, 1984

DATE STUDY COMPLETED: In progress

STUDY POPULATION: The study population consists of 40-50 adult dialysis patients (including previous nonresponders to plasma-derived vaccine), and 20-25 health care personnel, of either sex (excluding pregnant women), who are negative for HBsAg, anti-HBc and anti-HBs, and have a normal ALT level. Dialysis patients (excluding nonresponders to plasma-derived vaccine) and health care personnel have not previously received any hepatitis B vaccine.

STUDY PROCEDURE: Dialysis patients are assigned to one of two groups, stratified by sex and age, to assure that patients in the two groups are similar. Health care personnel constitute a third group.

Dialysis patients receive 1.0 ml (20 mcg HBsAg) or 2 x 1.0 ml (40 mcg HBsAg) intramuscular injections of vaccine at 0, 1, and 6 months. Health care personnel receive 0.5 ml (10 mcg HBsAg) intramuscular injections of vaccine according to the same regimen. Vaccine recipients record their temperature and any local or systemic complaints for five days after each injection of vaccine.

A blood sample is obtained from each study participant approximately two weeks before the first injection of vaccine. Post-vaccination blood samples are obtained at 1, 3, 6, 8, 12 and 24 months.

All serum samples are assayed for HBsAg, anti-HBs, anti-HBc, and ALT. Samples may be tested for yeast antibody. In addition, samples with an anti-HBs titer $\geq$ 25 mIU/ml may be tested to determine anti-a and anti-d subtype specificity.

Study B16

RESULTS:

NONRESPONDERS (DIALYSIS PATIENTS)

20 mcg Lot 974/C-K446 at 0, 1, and 6 months
40 mcg Lot 974/C-K446 at 0, 1, and 6 months

1. Number Vaccinated:

Dose (mcg)	Injection Number 1	2	3
20	5	5	3
40	4	4	3

2. Serologic Results:

Serologic data at 7/8 months are available for four dialysis patients who were nonresponders to the plasma-derived vaccine.

At 7/8 and 12 months, anti-HBs responses are as follows:

Time (Months)	Dose (mcg)	Positive anti-HBs S/N ≥2.1	Positive anti-HBs mIU/ml ≥10	GMT (mIU/ml) All Vaccinees	GMT (mIU/ml) Responders S/N ≥2.1	GMT (mIU/ml) Responders mIU/ml ≥10
7/8	20	100(1/1)	100(1/1)	136.9	136.9	136.9
	40	33(1/3)	33(1/3)	2.1	49.4	49.4
12	20	50(1/2)	50(1/2)	3.4	38.5	38.5
	40	67(2/3)	33(1/3)	3.0	9.3	22.3

Anti-HBs responses at 1 through 12 months are included in Table 1.

3. Clinical Results:

Clinical follow-up data are available for 3 (20 mcg dose) and 4 (40 mcg dose) dialysis patients who

Study 816

RESULTS (CONT.): were nonresponders to the plasma-derived vaccine following the first injection of vaccine; for 4 dialysis patients following the second 20 or 40 mcg dose and for 3 dialysis patients following the third 20 or 40 mcg dose of vaccine.

Clinical complaints and maximum temperatures reported following each injection are provided in Tables 2-5. In summary:

Clinical Complaint	Dose (mcg)	% Frequency by Injection Number 1	2	3
Injection Site	20	0 (0/3)	0 (0/4)	0 (0/3)
	40	0 (0/4)	0 (0/4)	0 (0/3)
Systemic	20	0 (0/3)	0 (0/4)	0 (0/3)
	40	0 (0/4)	0 (0/4)	0 (0/3)

No serious or alarming adverse reactions attributable to vaccination have been reported.

Events Reported to OoBRR

1. A 53-year old female subject, case no. (b) (6) had a history of hypertension, diabetes mellitus, cirrhosis, severe renal osteodystrophy and end-stage renal disease (3x/week hemodialysis). Approximately five months after receiving a second 20 mcg dose of recombinant hepatitis B vaccine lot 974/C-K446, she died due to congestive heart failure, renal failure, and severe arteriosclerosis. The investigator does not consider the death to be related to vaccination.

2. A 63-year old male dialysis patient, case no. (b) (6) with ESRD and severe peripheral vascular disease, was hospitalized for a left femoral-popliteal bypass and lumbar sympathectomy approximately 2 months after administration of a third injection of recombinant hepatitis B vaccine lot 974/C-K446. His hospital course was complicated by postoperative blood loss, hypotension and hyperkalemia. He subsequently experienced a respiratory arrest requiring resuscitative measures. Post resuscitation, the patient was comatose and decerebrate. His condition further deteriorated and he died (b) (6) days after admission to the hospital.

Table 1

Antibody Responses Among Initially Seronegative Nonresponders to Plasma-Derived Hepatitis B Vaccine (Dialysis Patients) Following Vaccination with 20 or 40 mcg Doses of Yeast Recombinant Hepatitis B Vaccine Lot 974/C-K446 at 0, 1, and 6 Months in Study 816

Time (Months)	20 mcg					40 mcg				
	% with Anti-HBs		GMT (mIU/ml)			% with Anti-HBs		GMT (mIU/ml)		
			All	Responders				All	Responders	
	S/N ≥ 2.1	mIU/ml ≥ 10	Vaccinees	S/N ≥ 2.1	mIU/ml ≥ 10	S/N ≥ 2.1	mIU/ml ≥ 10	Vaccinees	S/N ≥ 2.1	mIU/ml ≥ 10
1	0(0/4)	0(0/4)	0.3	---	---	0(0/3)	0(0/3)	0.3	---	---
3	25(1/4)	25(1/4)	1.1	53.0	53.0	25(1/4)	25(1/4)	0.8	16.1	16.1
6	0(0/2)	0(0/2)	0.3	---	---	33(1/3)	0(0/3)	0.9	9.5	---
7/8	100(1/1)	100(1/1)	136.9	136.9	136.9	33(1/3)	33(1/3)	2.1	49.4	49.4
12	50(1/2)	50(1/2)	3.4	38.5	38.5	67(2/3)	33(1/3)	3.0	9.3	22.3

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Table 2

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0816
TREATMENT :
LOT NUMBER : CK446
DOSE : 20 MCG
PATIENT CLASS: NONRESPONDERS (0)

CLINICAL COMPLAINTS	TOTAL VACCINEES (5 PATIENTS) - DOSE 1							
	DAYS POST VACCINATION							NUMBER WITH COMPLAINTS
	0	1	2	3	4	5		
PERSONS WITH COMPLAINTS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)
PERSONS WITH NO COMPLAINTS	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)		3 (100.0%)
PERSONS WITH NO DATA	2 (40.0%)	2 (40.0%)	2 (40.0%)	2 (40.0%)	2 (40.0%)	2 (40.0%)		2 (40.0%)

Table 2 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0816
TREATMENT :
LOT NUMBER : CK446
DOSE : 20 MCG
PATIENT CLASS: NONRESPONDERS (0)

CLINICAL COMPLAINTS	TOTAL VACCINEES (5 PATIENTS) - DOSE 2						
	DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
PERSONS WITH COMPLAINTS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
PERSONS WITH NO COMPLAINTS	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)
PERSONS WITH NO DATA	1 (20.0%)	1 (20.0%)	1 (20.0%)	1 (20.0%)	1 (20.0%)	1 (20.0%)	1 (20.0%)

00600

Table 2 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0816
TREATMENT :
LOT NUMBER : CK446
DOSE : 20 MCG
PATIENT CLASS: NONRESPONDERS (0)

CLINICAL COMPLAINTS	TOTAL VACCINEES (3 PATIENTS) - DOSE 3							
	DAYS POST VACCINATION							NUMBER WITH COMPLAINTS
	0	1	2	3	4	5		
PERSONS WITH COMPLAINTS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)
PERSONS WITH NO COMPLAINTS	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)		3 (100.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 3

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0816
TREATMENT :
LOT NUMBER : CK446
DOSE : 20 MCG
PATIENT CLASS: NONRESPONDERS (D)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (5 PATIENTS) - DOSE 1								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
NORMAL	1 (33.3%)	1 (50.0%)	1 (33.3%)	1 (33.3%)	1 (50.0%)	1 (50.0%)			1 (33.3%)
< 99	2 (66.7%)	1 (50.0%)	2 (66.7%)	2 (66.7%)	1 (50.0%)	1 (50.0%)			2 (66.7%)
TEMPERATURE TAKEN	3 (60.0%)	2 (40.0%)	3 (60.0%)	3 (60.0%)	2 (40.0%)	2 (40.0%)			3 (60.0%)
TEMPERATURE NOT TAKEN	2 (40.0%)	3 (60.0%)	2 (40.0%)	2 (40.0%)	3 (60.0%)	3 (60.0%)			2 (40.0%)

Table 3 (cont.)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0816
TREATMENT :
LOT NUMBER : CK446
DOSE : 20 MCG
PATIENT CLASS: NONRESPONDERS (D)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (5 PATIENTS) - DOSE 2								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
NORMAL	2 (50.0%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)			2 (50.0%)
< 99	2 (50.0%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)			2 (50.0%)
TEMPERATURE TAKEN	4 (80.0%)	3 (60.0%)	3 (60.0%)	3 (60.0%)	3 (60.0%)	3 (60.0%)			4 (80.0%)
TEMPERATURE NOT TAKEN	1 (20.0%)	2 (40.0%)	2 (40.0%)	2 (40.0%)	2 (40.0%)	2 (40.0%)			1 (20.0%)

00903

Table 3 (cont.)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0816
TREATMENT :
LOT NUMBER : CK446
DOSE : 20 MCG
PATIENT CLASS: NONRESPONDERS (D)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (3 PATIENTS) - DOSE 3								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
NORMAL	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)			2 (100.0%)
TEMPERATURE TAKEN	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)			2 (66.7%)
TEMPERATURE NOT TAKEN	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)			1 (33.3%)

Table 4

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0816
TREATMENT :
LOT NUMBER : CK446
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (D)

CLINICAL COMPLAINTS	TOTAL VACCINEES (4 PATIENTS) - DOSE 1						
	DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
PERSONS WITH COMPLAINTS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
PERSONS WITH NO COMPLAINTS	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 4 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0816
TREATMENT :
LOT NUMBER : CK446
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (D)

CLINICAL COMPLAINTS	TOTAL VACCINEES (4 PATIENTS) - DOSE 2							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
PERSONS WITH COMPLAINTS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)
PERSONS WITH NO COMPLAINTS	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)		4 (100.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 4 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0816
TREATMENT :
LOT NUMBER : CK446
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (0)

CLINICAL COMPLAINTS	TOTAL VACCINEES (3 PATIENTS) - DOSE 3							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
PERSONS WITH COMPLAINTS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)
PERSONS WITH NO COMPLAINTS	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)		3 (100.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 5

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0816
TREATMENT :
LOT NUMBER : CK446
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (D)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (4 PATIENTS) - DOSE 1								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
NORMAL	1 (25.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)			1 (25.0%)
< 99	3 (75.0%)	2 (50.0%)	1 (25.0%)	3 (75.0%)	2 (50.0%)	3 (75.0%)			1 (25.0%)
99 - 99.9	0 (0.0%)	1 (25.0%)	2 (50.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)			2 (50.0%)
TEMPERATURE TAKEN	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)			4 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			0 (0.0%)

Table 5 (cont.)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0816
TREATMENT :
LOT NUMBER : CK446
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (D)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (4 PATIENTS) - DOSE 2								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
NORMAL	1 (25.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)			1 (25.0%)
< 99	2 (50.0%)	2 (50.0%)	2 (50.0%)	2 (50.0%)	2 (50.0%)	2 (50.0%)			1 (25.0%)
99 - 99.9	1 (25.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)			2 (50.0%)
TEMPERATURE TAKEN	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)			4 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			0 (0.0%)

60600

Table 5 (cont.)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0816
TREATMENT :
LOT NUMBER : CK446
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (D)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (3 PATIENTS) - DOSE 3								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
< 99	2 (66.7%)	2 (66.7%)	2 (66.7%)	3 (100.0%)	3 (100.0%)	3 (100.0%)			2 (66.7%)
99 - 99.9	1 (33.3%)	1 (33.3%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			1 (33.3%)
TEMPERATURE TAKEN	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)	3 (100.0%)			3 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			0 (0.0%)

PROGRAM:	Yeast Recombinant Hepatitis B Vaccine, Study 817
PURPOSE:	To evaluate antibody and clinical responses to 10 mcg doses of yeast recombinant vaccine among: 1. healthy adults immunized previously with plasma-derived vaccine who were nonresponders (anti-HBs negative) 2. preimmune healthy adults
VACCINE:	Hepatitis B Vaccine (Recombinant) - Alum Adsorbed: Lot #972/C-K444 (10 mcg/ml)
PRIMARY INVESTIGATOR:	Robert P. Bishop, M.D. Director, Health Services Merck & Co., Inc. West Point, PA 19486
SECONDARY INVESTIGATOR(S):	Edgardo P. Avancena, M.D. Joseph C. Rogers, M.D. Joseph P. Romano, M.D. Merck & Co., Inc. West Point, PA & Rahway, NJ
STUDY LOCATION:	Merck & Co., Inc. West Point, PA 19486 Merck & Co., Inc. Rahway, NJ 07065
DATE INITIATED:	March 21, 1984
DATE COMPLETED:	In progress

Study 817

STUDY POPULATION: The study population will consist of 40-50 healthy adults of either sex (excluding pregnant females), who are employees of Merck & Co., Inc. Half of the population will consist of persons with pre-existing hepatitis B antibody which may be either naturally acquired or plasma vaccine induced. The other half will consist of persons who have been vaccinated with plasma vaccine but failed to develop detectable antibody to hepatitis B. All participants must be negative for anti-HBc and HBsAg, and have a normal ALT level.

PROCEDURE: Study participants are allocated to one of two regimens as shown below. All injections are intramuscular.

Group	No.	Dose	Time of Vaccination
1. Preimmune	5	1.0 ml (10 mcg)	0
2. Nonresponders	4	1.0 ml (10 mcg)	0, 1 & 6 mos.

Vaccinees are asked to record their temperature daily for five days after each injection and also to record any local or systemic complaints they may have during this period.

A blood specimen (10-15 ml) is obtained from each participant approximately 2 weeks before the first vaccination. Post-vaccination blood samples are obtained at 1, 2, 3, 6, 8, 12 and 24 months. The samples are assayed for HBsAg, anti-HBc, anti-HBs, yeast antibody and ALT. Those with anti-HBs titers $\geq$ 25 mIU/ml may be tested for the proportions of anti-a and anti-d activity.

Study 817

RESULTS:

NONRESPONDERS TO PLASMA VACCINE:

10 mcg Lot #972/C-K444 at 0, 1, and 6 months

1. Number Vaccinated:

Injection No.		
1	2	3
4	4	3

2. Serologic Results:

Serologic data are avilable for two study participants at 7/8 months.

At seven months neither of the two vaccinees tested had seroconverted. Table 1 shows seroconversion rates and GMT's for up to 7/8 months of follow-up.

3. Clinical Complaints:

Clinical follow-up data are available for four participants following the first two injections and for three after the third injection. Specific complaints and maximum temperatures reported during the five days of follow-up following each injection are provided in Table 2.

	Frequency in % by Injection No.		
Type of Complaint	1	2	3
Injection Site	25(1/4)	0 (0/4)	0 (0/3)
Systemic	25(1/4)	0 (0/4)	0 (0/3)

There were no serious or alarming adverse reactions attributable to vaccine.

Table 1

ANTIBODY RESPONSES FOLLOWING VACCINATION WITH RECOMBINANT HEPATITIS B VACCINE

STUDY : 0817
POPULATION : NONRESPONDERS (H)
DOSE : 10 MCG
LOT : CK444
REGIMEN : 0, 1, AND 6 MONTHS
INITIAL SEROLOGY: NEGATIVE

TIME (MONTHS)	% WITH ANTI-HBS				GMT (MIU/ML)		
	S/N >= 2.1		MIU/ML >= 10		ALL VACCINEES	RESPONDERS S/N >= 2.1	RESPONDERS MIU/ML >= 10
PRE VAC	0%	(0/4)	0%	(0/4)	0.4		
1 MONTH	0%	(0/3)	0%	(0/3)	0.3		
2 MONTHS	0%	(0/1)	0%	(0/1)	0.3		
3 MONTHS	0%	(0/2)	0%	(0/2)	0.3		
6 MONTHS	0%	(0/1)	0%	(0/1)	1.9		
7/8 MONTHS	0%	(0/2)	0%	(0/2)	0.7		

Table 2

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0817
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (H)

CLINICAL COMPLAINTS	TOTAL VACCINEES (4 PATIENTS) - DOSE 1							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
REACTION, LOCAL (INJECT. SITE)	1 (25.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (25.0%)
SORENESS	1 (25.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (25.0%)
SYSTEMIC	0 (0.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (25.0%)
MUSCULOSKELETAL	0 (0.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (25.0%)
SHOULDER PAIN	0 (0.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (25.0%)
PERSONS WITH COMPLAINTS	1 (25.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		2 (50.0%)
PERSONS WITH NO COMPLAINTS	3 (75.0%)	4 (100.0%)	3 (75.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)		2 (50.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 2 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0817
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (H)

CLINICAL COMPLAINTS	TOTAL VACCINEES (4 PATIENTS) - DOSE 2							
	DAYS POST VACCINATION							NUMBER WITH COMPLAINTS
	0	1	2	3	4	5		
PERSONS WITH COMPLAINTS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)
PERSONS WITH NO COMPLAINTS	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)		4 (100.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 2 (cont.)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0817
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (N)

CLINICAL COMPLAINTS	TOTAL VACCINEES (3 PATIENTS) - DOSE 3						
	DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
PERSONS WITH COMPLAINTS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
PERSONS WITH NO COMPLAINTS	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 3

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0817
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (N)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (4 PATIENTS) - DOSE 1								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
< 99	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)			4 (100.0%)
TEMPERATURE TAKEN	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)			4 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			0 (0.0%)

Table 3 (cont.)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0817
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (H)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (4 PATIENTS) - DOSE 2								DAYS POST VACCINATION								NUMBER WITH MAX TEMP
	0	1	2	3	4	5											
NORMAL	1 (25.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)	1 (25.0%)											1 (25.0%)
< 99	3 (75.0%)	3 (75.0%)	3 (75.0%)	3 (75.0%)	3 (75.0%)	3 (75.0%)											3 (75.0%)
TEMPERATURE TAKEN	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)	4 (100.0%)											4 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)											0 (0.0%)

Table 3 (cont.)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0817
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (H)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (3 PATIENTS) - DOSE 3								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
< 99	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)			2 (100.0%)
TEMPERATURE TAKEN	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)			2 (66.7%)
TEMPERATURE NOT TAKEN	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)			1 (33.3%)

00920

Study 854

PROGRAM:	Alum-Adsorbed Yeast Recombinant Hepatitis B Vaccine, Study 854.
PURPOSE:	To evaluate antibody and clinical responses to yeast recombinant hepatitis B vaccine in the following adult populations: 1. Chronic Carriers of HBsAg 2. Healthy Hyporesponders to Plasma-Derived Vaccine. 3. Healthy Nonresponders to Plasma-Derived Vaccine. 4. Healthy Transient Responders to Plasma-Derived Vaccine.
VACCINE:	Yeast Recombinant Hepatitis B Vaccine Lot #979/C-K564 (10 mcg HBsAg/ml)
PRINCIPAL INVESTIGATOR:	Jules Dienstag, M.D. Associate Professor of Medicine Gastrointestinal Unit Massachusetts Gen. Hosp. Boston, MA 02114
SECONDARY INVESTIGATOR:	Eloise Watkins, R.N., M.P.H. Gastrointestinal Unit Mass. General Hosp. Fruit Street Boston, MA 02114 Lynn F. Butterly, M.D. Clinical & Research Fellow Gastrointestinal Unit Mass. General Hosp. Boston, MA 02114
STUDY LOCATION:	Massachusetts General Hospital Fruit Street Boston, MA 02114
DATE INITIATED:	October 14, 1984
DATE COMPLETED:	In progress

Study 854

STUDY POPULATIONS: The study population will consist of adults of either sex (excluding pregnant women) who can be classified into one of the following groups:

Group	Number of Subjects	Qualifications
Carriers	10-15	Chronic carrier of HBsAg for at least one year, with no signs or symptoms of chronic liver disease, and a stable ALT level less than 3 times the upper limit of normal.
Hyporesponders	15-20	Healthy adults who have had only a low level anti-HBs response (positive titer obtained in at least 2 successive bleedings) to a complete 3 injection regimen of plasma derived hepatitis B vaccine. [maximum antibody titer 8-36 when measured in (b) (4) RIA units, 2.1-9.9 when measured in terms of S/N ratio, or <10 mIU/ml]
Nonresponders	15-20	Healthy adults who had a single post-vaccination blood sample with an anti-HBs titer in the range S/N = 2.1-9.9 followed by additional samples all with S/N less than 2.1 as well as persons whose post-vaccination blood samples all had anti-HBs titers of S/N less than 2.1 after receiving a three injection series of plasma-derived hepatitis B vaccine.
Transient Responders	10-15	Healthy adults who had at least one blood sample with an anti-HBs titer of S/N $\geq$10 following a 3 injection series of plasma derived hepatitis B vaccine but have subsequently lost antibody (S/N <2.1).

Study 854

PROCEDURE:

Prior to vaccination, each participant will be screened for HBsAg, anti-HBc, anti-HBs and ALT level. A serum pregnancy test will also be performed for all women of childbearing age. Vaccine is administered intramuscularly according to the following schedule.

Group	Vaccination Regimen
Carriers	1.0 ml (10 mcg HBsAg) at time 0, 1, 2, 3, 4 and 5 months.
Hyporesponders	1.0 ml (10 mcg HBsAg) at time 0
Nonresponders	1.0 ml (10 mcg HBsAg) at time 0, 1 and 6 months.
Transient Responders	1.0 ml (10 mcg HBsAg) at time 0.

The vaccine recipients are asked to record their temperature for 5 days after each injection and to note any local or systemic complaints. Unexpected or serious reactions will be reported to the study physician immediately.

Follow-up blood samples will be obtained from carriers monthly for 6 months and at 9 and 12 months; from hyporesponders and transient responders at 1, 3, 6, 9, 12 and 24 months and; from nonresponders at 1, 2, 3, 6, 9 months, and at 12 and 24 months from those who have seroconverted by 9 months. Samples are assayed for HBsAg, anti-HBc, anti-HBs, and ALT by Dr. Dienstag. Samples may also be assayed at MSDRL for yeast antibody and for the proportions of anti-HBs specific for the a and d determinants of HBsAg.

RESULTS:

HYPORESPONDERS:

10 mcg Lot #979/C-K564 at time 0.

1. Number Vaccinated: 2

Study 854

RESULTS: (Cont.)

2. Serologic Results:

One of the vaccine recipients displayed a marked boost in anti-HBs titer one month after receiving one injection of vaccine (10 mcg HBsAg). The other vaccine recipient has not responded. The anti-HBs titers for these two subjects are presented below.

Case #	Pre-vaccination anti-HBs Titer	Anti-HBs Titer (mIU/ml) 1 Month	3 Months	6 Months
(b) (6)	6.2	186.8	123.0	39.0
	2.4	1.5	0.1	0.2

3. Clinical Complaints:

Clinical follow-up data are available for both vaccinees. One participant had an injection site complaint and one participant had a systemic complaint. Refer to Table 2 for a listing of specific clinical complaints. Temperature data are provided in Table 3.

There were no serious or alarming reactions attributable to vaccine.

NONRESPONDERS

10 mcg Lot #979/C-K564 at 0, 1, and 6 months.

1. Number Vaccinated:

Injection No.		
1	2	3
14	13	13

Study 854

RESULTS (CONT.):

2. Serologic Results:

Serologic data are available for 12 participants at six months. Fifty-eight percent (7/12) of the subjects seroconverted (S/N ≥2.1) for anti-HBs. Twenty-five percent (3/12) of the vaccinees developed protective levels of anti-HBs (mIU/ml ≥10) at that time. The GMT at 6 months for all vaccinees was 3.2 mIU/ml and 45.8 for responders (mIU/ml ≥10).

Refer to Table 1 for anti-HBs responses and GMTs for other time intervals.

3. Clinical Complaints:

Clinical follow-up data are available for at least thirteen participants after each injection. The overall frequencies of complaints are presented below.

Type of Complaint	----Frequency in % by Injection----- 1	2	3
Injection Site	21 (3/14)	8 (1/13)	15 (2/13)
Systemic	14 (2/14)	8 (1/13)	0 (0/13)

Refer to Table 4 for listings of specific complaints by injection number. Maximum temperature data are provided in Table 5.

There were no serious or alarming adverse reactions attributable to vaccine.

TRANSIENT RESPONDERS

10 mcg Lot #979/C-K564 at time 0

1. Number Vaccinated: 3

2. Serologic Results:

At one month, two of the transient responders who were seronegative for anti-HBs prior to vaccination,

Study 854

RESULTS (CONT.): seroconverted for anti-HBs. The GMT for the two responders was 67.9 mIU/ml. The anti-HBs titers for the three subjects are presented below.

Case #	Pre-vaccination anti-HBs Titer	Anti-HBs Titer (mIU/ml) 1 Month
(b) (6)	0.2	14.8
	0	311.7
	0.4	—

3. Clinical Complaints:

Clinical follow-up data are available for all the participants. No vaccinee had an injection site complaint. One subject had a systemic complaint (Table 6). The maximum reported temperature was 99.9°F (Table 7).

No serious or alarming adverse experiences attributable to vaccine have been reported.

PUBLICATIONS: Butterly L, Watkins E, Hinkle CA, Dienstag JL. Response to recombinant yeast hepatitis B vaccine in nonresponders to plasma-derived hepatitis B vaccine. Hepatology 1985; 5:1007 (Abstract).

Table 1

ANTIBODY RESPONSES FOLLOWING VACCINATION WITH RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
POPULATION : NONRESPONDERS (H)
DOSE : 10 MCG
LOT : CK564
REGIMEN : 0, 1, AND 6 MONTHS
INITIAL SEROLOGY: NEGATIVE

TIME (MONTHS)	% WITH ANTI-HBS				GMT (MIU/ML)		
	S/N >= 2.1		MIU/ML >= 10		ALL VACCINEES	RESPONDERS S/N >= 2.1	RESPONDERS MIU/ML >= 10
1 MONTH	38%	(5/13)	15%	(2/13)	3.3	17.2	76.5
2 MONTHS	67%	(8/12)	58%	(7/12)	18.5	38.9	59.5
3 MONTHS	64%	(7/11)	45%	(5/11)	10.9	35.8	86.2
6 MONTHS	58%	(7/12)	25%	(3/12)	3.2	7.7	45.8
9 MONTHS	100%	(4/4)	50%	(2/4)	36.0	36.0	245.1

Table 2

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: HYPORESPONDERS

CLINICAL COMPLAINTS	TOTAL VACCINEES (2 PATIENTS) - DOSE 1 — DAYS POST VACCINATION 0	1	2	3	4	5	NUMBER WITH COMPLAINTS
REACTION, LOCAL (INJECT. SITE)	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)
SORENESS	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)
SYSTEMIC	1 (50.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)
WHOLE BODY/GENERAL	0 (0.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)
FATIGUE/WEAKNESS	0 (0.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)
RESPIRATORY	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)
RHINITIS	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)
PERSONS WITH COMPLAINTS	2 (100.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (100.0%)
PERSONS WITH NO COMPLAINTS	0 (0.0%)	1 (50.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	0 (0.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 3

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: HYPORESPONDERS

	TOTAL VACCINEES (2 PATIENTS) - DOSE 1							
	DAYS POST VACCINATION							NUMBER
MAX TEMPERATURE (DEG F, ORAL)	0	1	2	3	4	5		WITH MAX TEMP
< 99	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)		2 (100.0%)
TEMPERATURE TAKEN	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)		2 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

62600

Table 4

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (N)

CLINICAL COMPLAINTS	TOTAL VACCINEES (14 PATIENTS) - DOSE 1							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
REACTION, LOCAL (INJECT. SITE)	1 (7.1%)	2 (14.3%)	2 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		3 (21.4%)
INFLAMMATION	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (7.1%)
SORENESS	1 (7.1%)	2 (14.3%)	2 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		3 (21.4%)
SYSTEMIC	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	1 (7.1%)		2 (14.3%)
WHOLE BODY/GENERAL	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)		1 (7.1%)
FATIGUE/WEAKNESS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)		1 (7.1%)
HEADACHE	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)		1 (7.1%)
DIGESTIVE SYSTEM	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)		1 (7.1%)
NAUSEA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)		1 (7.1%)
PERSONS WITH COMPLAINTS	1 (7.1%)	2 (14.3%)	2 (14.3%)	1 (7.1%)	0 (0.0%)	1 (7.1%)		5 (35.7%)
PERSONS WITH NO COMPLAINTS	13 (92.9%)	12 (85.7%)	12 (85.7%)	13 (92.9%)	14 (100.0%)	13 (92.9%)		9 (64.3%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

00930

Table 4 (cont)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (H)

CLINICAL COMPLAINTS	TOTAL VACCINEES (13 PATIENTS) - DOSE 2						
	DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
REACTION, LOCAL (INJECT. SITE)	0 (0.0%)	1 (7.7%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.7%)
SORENESS	0 (0.0%)	1 (7.7%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.7%)
SYSTEMIC	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.7%)	0 (0.0%)	1 (7.7%)
RESPIRATORY	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.7%)	0 (0.0%)	1 (7.7%)
PHARYNGITIS (SORE THROAT)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.7%)	0 (0.0%)	1 (7.7%)
UPPER RESPIRATORY INFECT., NOS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.7%)	0 (0.0%)	1 (7.7%)
PERSONS WITH COMPLAINTS	0 (0.0%)	1 (7.7%)	1 (7.7%)	0 (0.0%)	1 (7.7%)	0 (0.0%)	2 (15.4%)
PERSONS WITH NO COMPLAINTS	13 (100.0%)	12 (92.3%)	12 (92.3%)	13 (100.0%)	12 (92.3%)	13 (100.0%)	11 (84.6%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 4 (cont)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (H)

CLINICAL COMPLAINTS	TOTAL VACCINEES (13 PATIENTS) - DOSE 3 — DAYS POST VACCINATION 0	1	2	3	4	5		NUMBER WITH COMPLAINTS
REACTION, LOCAL (INJECT. SITE)	1 (7.7%)	2 (15.4%)	1 (7.7%)	1 (7.7%)	0 (0.0%)	0 (0.0%)		2 (15.4%)
SORENESS	0 (0.0%)	1 (7.7%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (7.7%)
OTHER	1 (7.7%)	1 (7.7%)	0 (0.0%)	1 (7.7%)	0 (0.0%)	0 (0.0%)		1 (7.7%)
PERSONS WITH COMPLAINTS	1 (7.7%)	2 (15.4%)	1 (7.7%)	1 (7.7%)	0 (0.0%)	0 (0.0%)		2 (15.4%)
PERSONS WITH NO COMPLAINTS	12 (92.3%)	11 (84.6%)	12 (92.3%)	12 (92.3%)	13 (100.0%)	13 (100.0%)		11 (84.6%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

00932

Table 5

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (N)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (14 PATIENTS) - DOSE 1							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
< 99	13 (92.9%)	11 (78.6%)	11 (84.6%)	13 (92.9%)	14 (100.0%)	14 (100.0%)		9 (64.3%)
99 - 99.9	1 (7.1%)	3 (21.4%)	2 (15.4%)	1 (7.1%)	0 (0.0%)	0 (0.0%)		5 (35.7%)
TEMPERATURE TAKEN	14 (100.0%)	14 (100.0%)	13 (92.9%)	14 (100.0%)	14 (100.0%)	14 (100.0%)		14 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 5 (cont)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (H)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (13 PATIENTS) - DOSE 2							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
< 99	9 (81.8%)	12 (100.0%)	11 (91.7%)	8 (66.7%)	9 (75.0%)	10 (83.3%)		6 (50.0%)
99 - 99.9	2 (18.2%)	0 (0.0%)	1 (8.3%)	4 (33.3%)	3 (25.0%)	2 (16.7%)		6 (50.0%)
TEMPERATURE TAKEN	11 (84.6%)	12 (92.3%)	12 (92.3%)	12 (92.3%)	12 (92.3%)	12 (92.3%)		12 (92.3%)
TEMPERATURE NOT TAKEN	2 (15.4%)	1 (7.7%)	1 (7.7%)	1 (7.7%)	1 (7.7%)	1 (7.7%)		1 (7.7%)

00934

Table 5 (cont)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: NONRESPONDERS (H)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (13 PATIENTS) - DOSE 3 — DAYS POST VACCINATION							NUMBER WITH MAX TEMP
	0	1	2	3	4	5		
< 99	10 (76.9%)	12 (92.3%)	11 (91.7%)	12 (92.3%)	12 (92.3%)	11 (91.7%)		9 (69.2%)
99 - 99.9	2 (15.4%)	1 (7.7%)	1 (8.3%)	1 (7.7%)	1 (7.7%)	1 (8.3%)		3 (23.1%)
100 - 100.9	1 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (7.7%)
TEMPERATURE TAKEN	13 (100.0%)	13 (100.0%)	12 (92.3%)	13 (100.0%)	13 (100.0%)	12 (92.3%)		13 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	1 (7.7%)		0 (0.0%)

Table 6 (cont)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: TRANSIENT RESPONDERS

CLINICAL COMPLAINTS	TOTAL VACCINEES (3 PATIENTS) - DOSE 1						
	DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
SYSTEMIC	0 (0.0%)	0 (0.0%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (33.3%)
MUSCULOSKELETAL	0 (0.0%)	0 (0.0%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (33.3%)
HIP PAIN	0 (0.0%)	0 (0.0%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (33.3%)
PERSONS WITH COMPLAINTS	0 (0.0%)	0 (0.0%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (33.3%)
PERSONS WITH NO COMPLAINTS	3 (100.0%)	3 (100.0%)	2 (66.7%)	3 (100.0%)	3 (100.0%)	3 (100.0%)	2 (66.7%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

00936

Table 7

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: TRANSIENT RESPONDERS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (3 PATIENTS) - DOSE 1							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
< 99	2 (66.7%)	3 (100.0%)	3 (100.0%)	2 (100.0%)	3 (100.0%)	3 (100.0%)		2 (66.7%)
99 - 99.9	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (33.3%)
TEMPERATURE TAKEN	3 (100.0%)	3 (100.0%)	3 (100.0%)	2 (66.7%)	3 (100.0%)	3 (100.0%)		3 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (33.3%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

244 RESPONSE TO RECOMBINANT YEAST HEPATITIS B VACCINE IN NONRESPONDERS TO PLASMA-DERIVED HEPATITIS B VACCINE

L Butterly, E Watkins, CA Hinkle and JL Dienstag. Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA.

Preliminary reports suggested that recombinant yeast hepatitis B vaccine (R-HBvac) might be more immunogenic than the triply inactivated plasma-derived hepatitis B vaccine (P-HBvac) (Hepatology 1984;4:1077). Therefore, to test this hypothesis, we administered three 10 µg doses of R-HBvac (Merck Sharp & Dohme Research Laboratories) at time 0, 1, and 6 months to 14 normal adults who had failed to respond to one or more courses (3-6 doses) of P-HBvac. The frequency [# positive/# vaccinated] (%) and geometric mean titer (mIU/ml) of anti-HBs responses were as follows:

Month	1	2	3	6
anti-HBs+	5/13 (39)	8/14 (57)	7/14 (50)	7/13 (54)
GMT ± SD	17 ± 7	39 ± 10	36 ± 23	8 ± 7

For comparison, the same data are charted below for 65 seronegative health workers, never previously vaccinated, after receiving R-HBvac:

Month	1	2	3	6
anti-HBs+	26/65 (38)	53/62 (86)	61/65 (94)	60/62 (97)
GMT ± SD	7 ± 4	38 ± 4	50 ± 4	72 ± 4

The mean ± SD ages of the 8 initial nonresponders who ultimately did respond and the 6 who did not were indistinguishable, 38 ± 8 and 41 ± 15. The response to R-HBvac in almost 60% of nonresponders to P-HBvac appeared promising, especially when compared with a 40% rate of low-level, poorly sustained anti-HBs responses in P-HBvac nonresponders given a second course of P-HBvac (Hepatology 1984;4:1077); however, the level of antibody fell substantially by six months, when measured just prior to the booster injection. Additional follow-up will be necessary to determine whether the antibody response to R-HBvac in nonresponders to P-HBvac increases and is sustained after booster immunization.

Butterly L, Watkins E, Hinkle CH, Dienstag JL. Response to recombinant hepatitis B vaccine in nonresponders to plasma-derived hepatitis B vaccine. Hepatology 1985; 5:1007 (abstract).

PROGRAM:	Alum-Adsorbed Yeast Recombinant Hepatitis B Vaccine, Study 874.
PURPOSE:	To evaluate antibody and clinical responses to yeast recombinant hepatitis B vaccine in healthy adults who failed to develop antibody (nonresponders) or developed only low levels of antibody (hyporesponders) in response to three or four injections of plasma-derived hepatitis B vaccine.
VACCINE:	Yeast Recombinant Hepatitis B Vaccine Lot 978/C-K 563 (10 mcg HBsAg/ml)
PRINCIPAL INVESTIGATOR:	Myron Tong, M.D., Ph.D. Department of Medical Education Huntington Memorial Hospital 100 Congress Street P.O. Box 7013 Pasadena, CA 91105 - 7013
SECONDARY INVESTIGATORS:	Deborah Roskamp, R.N. Liver Center Huntington Memorial Hospital 100 Congress Street P.O. Box 7013 Pasadena, CA 91105 - 7013
STUDY LOCATION:	Liver Center Huntington Memorial Hospital 100 Congress Street P.O. Box 7013 Pasadena, CA 91105 - 7013
DATE INITIATED:	September 1985.
DATE COMPLETED:	In progress.
STUDY POPULATION:	Participants in the study will be healthy adults of either sex (pregnant women excluded) who failed to develop antibody (S/N <2.1) or had very minimal antibody development (S/N 2.1-9.9) after receiving three or four injections of plasma-derived hepatitis B vaccine. Approximately 40 persons will be enrolled.

Study 874

PROCEDURE: Each participant will receive a 1 ml injection of vaccine in the deltoid muscle at 0, 1, and 6 months. Study participants will be asked to take and record their body temperature for five days after each injection of vaccine and to record any local or systemic complaints. They will also be asked to notify the study investigator immediately if an unexpected or serious reaction occurs.

Blood specimens will be obtained prior to vaccination and at 1, 2, 3, 6, and 8 months postvaccination. Additional samples will be obtained at 12 and 24 months from those who have seroconverted by eight months.

All blood samples will be assayed for HBsAg, anti-HBc, anti-HBs, and ALT. Testing will be performed at Huntington Memorial Hospital and the Medical Laboratory Network. Some samples may be assayed for yeast antibody and anti-HBs subtype specificity at MSDRL.

RESULTS: NONRESPONDERS/HYPORESPONDERS TO PLASMA-DERIVED VACCINE

10 mcg lot #978/C-K563 at 0, 1, and 6 months

1. Number Vaccinated:

Injection No.		
1	2	3
26	26	0

2. Serologic Results:

At one month, 36% (9/25) of the vaccinees seroconverted for anti-HBs (S/N $\geq$2.1). Further serologic data are not available.

3. Clinical Complaints

A summary of frequencies of clinical complaints is not yet available. However, no serious or alarming adverse events attributable to vaccine have been reported. Vaccination and follow-up continues in progress.

PROGRAM:	Alum-Adsorbed Yeast Recombinant Hepatitis B Vaccine, Study 875
PURPOSE:	To evaluate antibody and clinical responses to licensed hepatitis B vaccine (Heptavax-B) and yeast recombinant hepatitis B vaccine in renal dialysis patients who have already failed to develop antibody after receiving three injections of HEPTAVAX-B.
VACCINE:	Yeast Recombinant Hepatitis B Vaccine Lot #993/C-K937 (20 mcg HBsAg/ml) Licensed Vaccine (Heptavax-B) Lot #2277K (20 mcg HBsAg/ml)
PRINCIPAL INVESTIGATORS:	Theodore L. Johnson, M.D. The Duluth Clinic, Ltd. 400 E. Third Street Duluth, MN 55805 Richard N. Hellman, M.D. The Duluth Clinic, Ltd. 400 E. Third Street Duluth, MN 55805 Mark R. Eckman, M.D. The Duluth Clinic, Ltd. 400 East Third Street Duluth, MN 55805
SECONDARY INVESTIGATORS:	Pamela Elde, R.N. Miller-Dwan Medical Center 502 East Second Street Duluth, MN 55805 Gayle Gilmore, R.N. Miller-Dwan Medical Center 502 East Second Street Duluth, MN 55805
STUDY LOCATION:	Miller-Dwan Medical Center 502 East Second Street Duluth, MN 55805

Study 875

DATE INITIATED: June, 1985

DATE COMPLETED: In progress.

STUDY POPULATION: Adult patients who are receiving dialysis treatments for end stage renal disease and have failed to develop anti-HBs following administration of plasma-derived hepatitis B vaccine (HEPTAVAX-B) are eligible for the study. Prospective subjects must not be pregnant, must be negative for HBsAg, anti-HBc, and anti-HBs, and must have a normal ALT. Approximately 40 patients will be enrolled in the study.

PROCEDURE: Prior to administration of the 1st injection of vaccine, participants will have a blood specimen obtained and tested for HBsAg, anti-HBc, anti-HBs and ALT.

Participants will be assigned to one of the following groups:

Group	Vaccine
1	Plasma vaccine (HEPTAVAX-B)
2	Yeast Recombinant vaccine

Participants will also be distributed between the groups with respect to sex and 10 year age strata (i.e., 30-39, 40-49, etc.).

Each subject will receive 2 - 1.0 ml (40 mcg HBsAg) intramuscular injections of HEPTAVAX-B (Group 1) or the yeast recombinant vaccine (Group 2) at 0, 1, and 6 months. Vaccinees will be asked to take and record their temperatures for 5 days after each injection and record any local or systemic complaints that they have.

Follow-up blood samples will be obtained at 1, 2, 3, 6 and 8 months following the first injection of vaccine. All samples will be tested for HBsAg, anti-HBc, anti-HBs, and ALT. Assays for ALT will be done in Duluth, Minnesota. All other assays will be

Study 875

PROCEDURE: (Cont.) done by the Merck Sharp and Dohme Research Laboratories (MSDRL).

RESULTS: DIALYSIS PATIENTS (Nonresponders to HEPTAVAX-B):

40 mcg Lot 993/C-K937 (Yeast Recombinant) at 0, 1, and 6 months

40 mcg Lot 2277K (Licensed) at 0, 1, and 6 months

1. Number Vaccinated:

Vaccine	Injection No. 1	2	3
Yeast Recombinant	17	15	0
Plasma-Derived	18	17	0

2. Serologic Results:

Two month serologic data are available for 13 participants who received yeast recombinant hepatitis B vaccine. Seroconversion for anti-HBs (S/N $\geq$2.1) at two months was 38% (5/13). Fifteen percent (2/13) of these vaccinees developed protective levels of anti-HBs (mIU/ml $\geq$10) at that time.

The GMT at two months for all subjects who received yeast recombinant vaccine subjects was 1.4 mIU/ml and 70.7 mIU/ml for responders with a titer of mIU/ml $\geq$10.

Two months serologic data are available for 15 subjects who received plasma-derived hepatitis B vaccine. Forty-seven percent (7/15) of the participants seroconverted (S/N $\geq$2.1) and developed protective levels of anti-HBs (mIU/ml $\geq$10) at two months.

The GMT at two months for all vaccinees was 5.1 mIU/ml and 131.6 mIU/ml for responders with a titer of mIU/ml $\geq$10..

Study 875

RESULTS (CONT.):

Refer to Table 1 for anti-HBs responses and GMTs through two months of follow-up.

Two participants who received yeast recombinant hepatitis-B vaccine and one participant who received plasma-derived vaccine were found to have low positive anti-HBs titers prior to vaccination. All three participants had a >4-fold rise in their anti-HBs titers one month after their first injection of vaccine.

3. Clinical Complaints:

Clinical follow-up data are available for at least 15 participants from each vaccine group after the first and second injections. The overall frequencies of complaints are presented below:

	Type of Complaint	Frequency in % by Injection No. 1	2	3
Yeast Recombinant	Injection Site	12(2/17)	0(0/15)	—
	Systemic	29(5/17)	13(2/15)	—
Plasma-Derived	Injection Site	13(2/16)	0(0/15)	—
	Systemic	31(5/16)	7(1/15)	—

Refer to Table 2 and 3 for listings of specific clinical complaints by injection number. Maximum temperature data are provided in Tables 4 and 5.

ALT Elevations:

Two participants had elevated ALT levels (1.5 to 2.0 times the upper limit of normal) prior to vaccination. They remained elevated at one and two months post the initial vaccine injection. Neither patient was seropositive for HBsAg or anti-HBc.

One subject developed an elevated ALT level (1.5 times the upper limit of normal) one month post the second injection of plasma-derived hepatitis-B vaccine. He was seronegative for anti-HBc, HBsAg and anti-HBs at that time. Additional serum samples are pending.

Study 875

RESULTS (CONT.):

Reactions Reported to the OoBRR:

Three participants withdrew from the study due to clinical complaints following one injection of vaccine.

1. A 32-year old male who received two 20 mcg injections of yeast recombinant vaccine (one injection into each deltoid) developed a swollen, sore and stiff left arm after administration of the vaccine. The swelling and soreness persisted for one week and then subsided. No treatment was necessary. The subject recovered.

2. A 70-year old male reported becoming "ill" after receiving two 20 mcg injections of Heptavax-B (one injection into each deltoid). The participant was hospitalized. The study investigator considered the illness unrelated to vaccine.

3. A 72-year old male developed generalized achiness and a headache three days after administration of his first injections of yeast recombinant vaccine. Forty-eight hours after onset of those symptoms, he developed a flu-like syndrome with a temperature of 100°F.

There have been two deaths among the study participants unrelated to vaccine administration.

1. A 53-year old female hemodialysis patient with an 18 month history of widely metastasized adenocarcinoma of the breast in addition to chronic obstructive pulmonary disease, hypertension, and uremic pericarditis, died (b) (6) days after administration of the second injections of Heptavax-B. Death was due to respiratory failure. The study investigator did not consider the death vaccine related.

2. (b) (6) days after administration of the second injections of yeast recombinant vaccine, a 66-year old female dialysis patient was hospitalized for an infarcted bowel. Exploratory surgery was performed and the (b) (6) the patient expired.

Table 1

Antibody Responses Among Dialysis Patients Following Vaccination with
40 mcg Injections of Yeast Recombinant Hepatitis B Vaccine
Lot #993/C-K937 or Plasma-Derived Hepatitis B Vaccine
Lot #2277K at 0, 1, and 6 Months in Study 875

	40 mcg (Yeast Recombinant)					40 mcg (Plasma-Derived)				
			GMT (mIU/ml)					GMT (mIU/ml)		
Time	% with Anti-HBs		All	Responders		% with Anti-HBs		All	Responders	
(Months)	S/N ≥ 2.1	mIU/ml ≥ 10	Vaccinees	S/N ≥ 2.1	mIU/ml ≥ 10	S/N ≥ 2.1	mIU/ml ≥ 10	Vaccinees	S/N ≥ 2.1	mIU/ml ≥ 10
1	7.7(1/13)	0(0/13)	0.4	7.0	----	50(7/14)	36(5/14)	3.4	37.4	103.5
2	38(5/13)	15(2/13)	1.4	12.0	70.7	47(7/15)	47(7/15)	5.1	131.6	131.6

24071/6
12/31/85

00946

Table 2

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0875
TREATMENT :
LOT NUMBER : CK937
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (0)

CLINICAL COMPLAINTS	TOTAL VACCINEES (17 PATIENTS) - DOSE 1 — DAYS POST VACCINATION 0	1	2	3	4	5		NUMBER WITH COMPLAINTS
REACTION, LOCAL (INJECT. SITE)	2 (11.8%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.9%)		2 (11.8%)
SORENESS	1 (5.9%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.9%)		2 (11.8%)
STIFFNESS/TIGHTNESS	1 (5.9%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.9%)		1 (5.9%)
SYSTEMIC	1 (5.9%)	0 (0.0%)	2 (11.8%)	3 (17.6%)	1 (5.9%)	2 (11.8%)		5 (29.4%)
WHOLE BODY/GENERAL	1 (5.9%)	0 (0.0%)	2 (11.8%)	3 (17.6%)	1 (5.9%)	2 (11.8%)		5 (29.4%)
CHILLS	1 (5.9%)	0 (0.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)		1 (5.9%)
HEADACHE	0 (0.0%)	0 (0.0%)	2 (11.8%)	1 (5.9%)	1 (5.9%)	0 (0.0%)		2 (11.8%)
ILLNESS, NOS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.9%)		1 (5.9%)
ACHINESS	0 (0.0%)	0 (0.0%)	1 (5.9%)	2 (11.8%)	1 (5.9%)	1 (5.9%)		3 (17.6%)
PERSONS WITH COMPLAINTS	3 (17.6%)	1 (5.9%)	2 (11.8%)	3 (17.6%)	1 (5.9%)	3 (17.6%)		6 (35.3%)
PERSONS WITH NO COMPLAINTS	14 (82.4%)	16 (94.1%)	15 (88.2%)	14 (82.4%)	16 (94.1%)	14 (82.4%)		11 (64.7%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

00947

Table 2 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0875
TREATMENT :
LOT NUMBER : CK937
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (D)

CLINICAL COMPLAINTS	TOTAL VACCINEES (15 PATIENTS) - DOSE 2							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
SYSTEMIC	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	1 (6.7%)	2 (13.3%)		2 (13.3%)
WHOLE BODY/GENERAL	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	1 (6.7%)		1 (6.7%)
HEADACHE	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	1 (6.7%)		1 (6.7%)
DIGESTIVE SYSTEM	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	1 (6.7%)		1 (6.7%)
VOMITING	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	1 (6.7%)		1 (6.7%)
PERSONS WITH COMPLAINTS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	1 (6.7%)	2 (13.3%)		2 (13.3%)
PERSONS WITH NO COMPLAINTS	15 (100.0%)	15 (100.0%)	15 (100.0%)	14 (93.3%)	14 (93.3%)	13 (86.7%)		13 (86.7%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

00948

Table 3

PATIENT COUNT CLINICAL COMPLAINTS
PLASMA-DERIVED HEPATITIS B VACCINE

STUDY : 0875
TREATMENT :
LOT NUMBER : 2277K
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (D)

CLINICAL COMPLAINTS	TOTAL VACCINEES (16 PATIENTS) - DOSE 1 — DAYS POST VACCINATION 0	1	2	3	4	5		NUMBER WITH COMPLAINTS
REACTION, LOCAL (INJECT. SITE)	0 (0.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	1 (6.3%)	1 (6.3%)		2 (12.5%)
SORENESS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)	1 (6.3%)		1 (6.3%)
ECCHYMOSIS	0 (0.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (6.3%)
SYSTEMIC	2 (12.5%)	0 (0.0%)	3 (18.8%)	1 (6.3%)	2 (12.5%)	2 (12.5%)		5 (31.3%)
WHOLE BODY/GENERAL	2 (12.5%)	0 (0.0%)	2 (12.5%)	0 (0.0%)	1 (6.3%)	0 (0.0%)		4 (25.0%)
FATIGUE/WEAKNESS	2 (12.5%)	0 (0.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		2 (12.5%)
HEADACHE	0 (0.0%)	0 (0.0%)	1 (6.3%)	0 (0.0%)	1 (6.3%)	0 (0.0%)		2 (12.5%)
RESPIRATORY	0 (0.0%)	0 (0.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (6.3%)
UPPER RESPIRATORY INFECT., NOS	0 (0.0%)	0 (0.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (6.3%)
MUSCULOSKELETAL	0 (0.0%)	0 (0.0%)	1 (6.3%)	1 (6.3%)	1 (6.3%)	1 (6.3%)		1 (6.3%)
WRIST PAIN	0 (0.0%)	0 (0.0%)	1 (6.3%)	1 (6.3%)	1 (6.3%)	1 (6.3%)		1 (6.3%)
DIGESTIVE SYSTEM	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)		1 (6.3%)

Table 3 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
PLASMA-DERIVED HEPATITIS B VACCINE

STUDY : 0875
TREATMENT :
LOT NUMBER : 2277K
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (D)

CLINICAL COMPLAINTS	TOTAL VACCINEES (18 PATIENTS) - DOSE 1 — DAYS POST VACCINATION 0	1	2	3	4	5		NUMBER WITH COMPLAINTS
NAUSEA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)		1 (6.3%)
PERSONS WITH COMPLAINTS	2 (12.5%)	1 (6.3%)	3 (18.8%)	1 (6.3%)	3 (18.8%)	3 (18.8%)		6 (37.5%)
PERSONS WITH NO COMPLAINTS	14 (87.5%)	15 (93.8%)	13 (81.3%)	15 (93.8%)	13 (81.3%)	13 (81.3%)		10 (62.5%)
PERSONS WITH NO DATA	2 (11.1%)	2 (11.1%)	2 (11.1%)	2 (11.1%)	2 (11.1%)	2 (11.1%)		2 (11.1%)

Table 3 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
PLASMA-DERIVED HEPATITIS B VACCINE

STUDY : 0875
TREATMENT :
LOT NUMBER : 2277K
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (D)

CLINICAL COMPLAINTS	TOTAL VACCINEES (17 PATIENTS) - DOSE 2							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
SYSTEMIC	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (6.7%)
MUSCULOSKELETAL	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (6.7%)
MUSCLE STIFFNESS	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (6.7%)
PERSONS WITH COMPLAINTS	0 (0.0%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (6.7%)
PERSONS WITH NO COMPLAINTS	15 (100.0%)	14 (93.3%)	15 (100.0%)	15 (100.0%)	15 (100.0%)	15 (100.0%)		14 (93.3%)
PERSONS WITH NO DATA	2 (11.8%)	2 (11.8%)	2 (11.8%)	2 (11.8%)	2 (11.8%)	2 (11.8%)		2 (11.8%)

00951

Table 4

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0875
TREATMENT :
LOT NUMBER : CK937
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (D)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (17 PATIENTS) - DOSE 1 — DAYS POST VACCINATION							NUMBER WITH MAX TEMP
	0	1	2	3	4	5		
NORMAL	1 (5.9%)	1 (5.9%)	1 (5.9%)	1 (6.3%)	1 (5.9%)	2 (12.5%)		1 (5.9%)
< 99	10 (58.8%)	12 (70.6%)	13 (76.5%)	11 (68.8%)	10 (58.8%)	12 (75.0%)		6 (35.3%)
99 - 99.9	5 (29.4%)	4 (23.5%)	2 (11.8%)	4 (25.0%)	5 (29.4%)	1 (6.3%)		7 (41.2%)
100 - 100.9	1 (5.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)		2 (11.8%)
102 - 102.9	0 (0.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	1 (5.9%)	0 (0.0%)		1 (5.9%)
TEMPERATURE TAKEN	17 (100.0%)	17 (100.0%)	17 (100.0%)	16 (94.1%)	17 (100.0%)	16 (94.1%)		17 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	1 (5.9%)		0 (0.0%)

00952

Table 4 (Contd)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0875
TREATMENT :
LOT NUMBER : CK937
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (D)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (15 PATIENTS) - DOSE 2							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
NORMAL	2 (14.3%)	2 (13.3%)	2 (13.3%)	2 (14.3%)	2 (15.4%)	2 (14.3%)		2 (13.3%)
< 99	9 (64.3%)	10 (66.7%)	9 (60.0%)	10 (71.4%)	10 (76.9%)	10 (71.4%)		7 (46.7%)
99 - 99.9	3 (21.4%)	3 (20.0%)	4 (26.7%)	1 (7.1%)	1 (7.7%)	2 (14.3%)		5 (33.3%)
100 - 100.9	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)		1 (6.7%)
TEMPERATURE TAKEN	14 (93.3%)	15 (100.0%)	15 (100.0%)	14 (93.3%)	13 (86.7%)	14 (93.3%)		15 (100.0%)
TEMPERATURE NOT TAKEN	1 (6.7%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	2 (13.3%)	1 (6.7%)		0 (0.0%)

00953

Table 5

PATIENT COUNT MAXIMUM TEMPERATURES
PLASMA-DERIVED HEPATITIS B VACCINE

STUDY : 0875
TREATMENT :
LOT NUMBER : 2277K
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (D)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (18 PATIENTS) - DOSE 1							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
NORMAL	2 (12.5%)	2 (13.3%)	2 (12.5%)	2 (13.3%)	2 (13.3%)	2 (13.3%)		2 (12.5%)
< 99	13 (81.3%)	7 (46.7%)	9 (56.3%)	8 (53.3%)	9 (60.0%)	8 (53.3%)		5 (31.3%)
99 - 99.9	1 (6.3%)	5 (33.3%)	3 (18.8%)	4 (26.7%)	3 (20.0%)	3 (20.0%)		5 (31.3%)
100 - 100.9	0 (0.0%)	1 (6.7%)	2 (12.5%)	1 (6.7%)	1 (6.7%)	1 (6.7%)		3 (18.8%)
102 - 102.9	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.7%)		1 (6.3%)
TEMPERATURE TAKEN	16 (88.9%)	15 (83.3%)	16 (88.9%)	15 (83.3%)	15 (83.3%)	15 (83.3%)		16 (88.9%)
TEMPERATURE NOT TAKEN	2 (11.1%)	3 (16.7%)	2 (11.1%)	3 (16.7%)	3 (16.7%)	3 (16.7%)		2 (11.1%)

00954

Table 5 (Contd)

PATIENT COUNT MAXIMUM TEMPERATURES
PLASMA-DERIVED HEPATITIS B VACCINE

STUDY : 0875
TREATMENT :
LOT NUMBER : 2277K
DOSE : 40 MCG
PATIENT CLASS: NONRESPONDERS (D)

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (17 PATIENTS) - DOSE 2							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
NORMAL	1 (6.7%)	1 (6.7%)	1 (6.7%)	1 (7.1%)	1 (7.1%)	1 (7.7%)		1 (6.7%)
< 99	9 (60.0%)	10 (66.7%)	9 (60.0%)	10 (71.4%)	8 (57.1%)	10 (76.9%)		6 (40.0%)
99 - 99.9	4 (26.7%)	3 (20.0%)	4 (26.7%)	2 (14.3%)	5 (35.7%)	2 (15.4%)		7 (46.7%)
102 - 102.9	1 (6.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)
103 - 103.9	0 (0.0%)	1 (6.7%)	1 (6.7%)	1 (7.1%)	0 (0.0%)	0 (0.0%)		1 (6.7%)
TEMPERATURE TAKEN	15 (88.2%)	15 (88.2%)	15 (88.2%)	14 (82.4%)	14 (82.4%)	13 (76.5%)		15 (88.2%)
TEMPERATURE NOT TAKEN	2 (11.8%)	2 (11.8%)	2 (11.8%)	3 (17.6%)	3 (17.6%)	4 (23.5%)		2 (11.8%)

00955

PREIMMUNE ADULTS

PREIMMUNE ADULTS - POPULATION SUMMARY

Preimmune adults are included in the populations of two studies (Study 817 and Study 813, addendum 6 and 7). The pre-existing hepatitis B antibody in this population may be naturally acquired or due to previous administration of either plasma-derived or yeast recombinant hepatitis B vaccine. The studies are designed to assess antibody and clinical responses of preimmune adults to a single 10 or 5 mcg booster injection of hepatitis B yeast recombinant vaccine.

To date, 63 preimmune adults have received a 10 mcg dose of yeast recombinant vaccine. Anti-HBs responses 1-2 months after the booster injections have been measured in mIU/ml for 31 subjects. All 31 participants demonstrated a boost in anti-HBs titer at that time. The GMT at 1-2 months post-vaccination was 1110.6 mIU/ml versus a prevaccination GMT of 62.0 mIU/ml. Anti-HBs responses expressed in S/N ratio units are also available for an additional 31 subjects whose antibody response was measured 2-4 weeks after a single 10 mcg booster injection. Ninety-seven percent (30/31) of these participants demonstrated a boost in antibody titer at 2-4 weeks. One vaccinee who was seronegative at the time of vaccination but antibody positive at an earlier time failed to develop detectable antibody four weeks after vaccination.

Twenty-eight preimmune adults have received a single 5 mcg booster injection of vaccine. All 25 participants tested at 1-2 months after the booster injection demonstrated a boost in anti-HBs titer. The GMT 1-2 months post-vaccination was 1275.2 mIU/ml versus a pre-vaccination GMT of 59.9 mIU/ml.

The vaccine has been well tolerated in this population. No serious reactions attributable to vaccination have been reported.

PREIMMUNE ADULTS

Study 813 - New York, NY - Dr. M. Davidson

The population for study 811 addenda 6 and 7 consists of two groups of pre-immune health care personnel. Group 1 (addendum 6) includes personnel who received plasma-derived hepatitis B vaccine at 0, 1, 2, and 9 months, 5 to 7 years previously. These subjects receive a 10 mcg booster injection of yeast recombinant hepatitis B vaccine lot C-M126. Group 2 (addendum 7) includes subjects who previously received 2.5 mcg injections of yeast recombinant vaccine at 0, 1, and 6 months in study 813. These participants receive either a 5 mcg or 10 mcg booster injection of yeast recombinant vaccine lot C-M126.

Thirty-one group 1 participants have received a 10 mcg injection of vaccine. At one month post the booster injection, 21 of 30 (70%) subjects had a greater than four-fold rise in anti-HBs titer.

In group 2, 28 participants have received a 5 mcg injection and 28 have received a 10 mcg injection of vaccine. At 1-2 months after receipt of the booster injection, 21 of 25 (84%) subjects, who received a 5 mcg dose, had a greater than four-fold rise in anti-HBs titer. The GMT for all vaccinees was 59.9 mIU/ml prior to the booster dose and 1275.2 mIU/ml 1-2 months post the booster injection.

Twenty-three of 27 (85%) participants, who received a 10 mcg booster dose, had a greater than four-fold rise in anti-HBs titer 1-2 months post the vaccine injection. Prior to the booster injection, the GMT for all vaccinees was 96.5 mIU/ml. The GMT rose to 1337.0 mIU/ml 1-2 months after the booster dose.

No serious adverse experiences attributable to vaccine have been reported. Refer to the summary on health care personnel/healthy adults for data regarding other subjects vaccinated in this study.

Study 817 - West Point, PA - Dr. R. Bishop

The study population consists of 2 groups of healthy adults. Group 1 includes pre-immune adults (naturally acquired anti-HBs or plasma-derived vaccine induced) who receive a single 10 mcg dose of yeast recombinant vaccine lot C-K444. Group 2 includes healthy adults who were nonresponders to previously administered plasma-derived vaccine. These participants receive a 10 mcg injection of yeast recombinant hepatitis B vaccine lot C-K444 at 0, 1, and 6 months.

Five healthy pre-immune adults (group 1) have received a 10 mcg injection of vaccine. All five subjects showed a greater than four-fold rise in anti-HBs titer three months post the booster injection. The GMT for all the vaccinees prior to the booster dose was 5.7 mIU/ml. At three months after the booster injection, the GMT for all vaccinees was 402.5 mIU/ml.

Study 817 - West Point, PA - Dr. R. Bishop (Cont.)

There were no serious or alarming adverse experiences attributable to vaccine. Refer to the summary on non-responders/hyporesponders for data regarding other subjects vaccinated in this study.

Study 813

PROGRAM:	Alum-Adsorbed Yeast Recombinant Hepatitis B Vaccine, Study 813
PURPOSE:	To evaluate antibody and clinical responses to several dose levels of yeast recombinant hepatitis B vaccine among the following populations: 1. Health Care Personnel (Seronegative) 2. Preimmune Adults
VACCINE:	Yeast Recombinant Hepatitis B Vaccine Lot 972/C-K444 (10 mcg HBsAg/ml) Lot 81954I/18071/C-L220 (10 mcg HBsAg/0.5 ml) Lot 85860/22123/C-M125 (20 mcg HBsAg/ml) Lot 85861/22124/C-M126 (10 mcg HBsAg/ml)
PRINCIPAL INVESTIGATOR:	Morton Davidson, M.D. New York University Medical Center University Hospital 560 First Avenue New York, NY 10016
SECONDARY INVESTIGATOR:	Saul Krugman, M.D. Professor Department of Pediatrics New York University Medical Center 550 First Avenue New York, NY 10016
STUDY LOCATION:	New York University Medical Center University Hospital 560 First Avenue New York, NY 10016
DATE INITIATED:	February 1, 1984
DATE COMPLETED:	In progress.

Study B13

STUDY POPULATIONS: Under the original protocol and subsequent addenda, the following groups of health care personnel are included in the study. Participants may be of either sex, but pregnant women are excluded. Initially seronegative subjects have not previously received any hepatitis B vaccine.

Addendum No.	Characteristics	Number	Vaccine Lot No.#	Regimen
Initial protocol	Initially seronegative	50	972/C-K444	10 mcg (1.0 ml) at 0, 1, and 6 months
Add. #1	Initially seronegative	50	972/C-K444	5 mcg (0.5 ml) at 0, 1, and 6 months
Add. #2	Initially seronegative	50	972/C-K444	2.5 mcg (0.25 ml) at 0, 1, and 6 months
Add. #3	Initially seronegative	50	81954I/18071/ C-L220	10 mcg (0.5 ml) at 0, 1, and 6 months
Add. #4	Initially seronegative	50	81954I/18071/ C-L220	5 mcg (0.25 ml) at 0, 1, and 6 months
Add. #5	Initially seronegative; ≥40 years of age	50	85860/22123/ C-M125	20 mcg (1.0 ml) at 0, 1, and 6 months
Add. #5	Initially seronegative; ≥40 years of age	50	85861/22124/ C-M126	10 mcg (1.0 ml) at 0, 1, and 6 months
Add. #6	Vaccinated 3-5 yrs previously with plasma derived hepatitis B vaccine (HEPTAVAX-B)	100	85861/22124/ C-M126	10 mcg (1.0 ml) at time 0
Add. #7	Vaccinated previously with three 2.5 mcg doses of recombinant vaccine under Add. #2.	50	85861/22124/ C-M126	5 mcg (0.5 ml) or 10 mcg (1.0 ml) at time 0

Study 813

PROCEDURE:

Participants receive intramuscular injections of vaccine according to the regimens outlined above under STUDY POPULATIONS. Those enrolled under addendum #5 who fail to develop antibody following 3 injections of vaccine or have only a transient response that becomes negative by 12 months after the first dose may receive a fourth injection of vaccine.

Participants will be asked to record their temperature for 5 days after each injection of vaccine and to note any local or systemic complaints. Unexpected or serious reactions are to be reported immediately to the study physician.

Blood samples will be obtained from the initially seronegative groups prior to and on the day of the first vaccination. Follow-up samples will be obtained 1, 2, 3, 6, 8, 12 and 24 months after the initial injection of vaccine (initial protocol and addenda #1-5). Follow-up samples from persons vaccinated under addendum #6 are only taken 1 month after vaccination while persons enrolled under addendum #7 have blood samples taken 2 weeks, 4 weeks, and 6 months after vaccination.

Blood samples will be assayed for HBsAg, anti-HBc, anti-HBs and ALT by Dr. Krugman's laboratory and may be assayed for yeast antibody by the Merck Sharp and Dohme Research Laboratories. Samples with an anti-HBs titer $\geq$25 mIU/ml may be tested to determine the relative proportions of anti-a and anti-d activity.

RESULTS:

PREIMMUNE ADULTS (Previously Vaccinated with plasma-derived hepatitis B vaccine):

10 mcg lot 85861/22124/C-M126 at time 0

1. Number Vaccinated: 31

2. Serologic Results:

   At one month following administration of the booster injection of yeast recombinant vaccine, 21 of 30 (70%) participants had a greater than 4-fold rise in anti-HBs titer.

Study 813

RESULTS: (Contd)

Refer to Table 1 for anti-HBs titers prior to and post the booster injection.

3. Clinical Complaints:

Clinical follow-up data are available for 19 participants after the booster injection of vaccine. The overall frequencies of complaints are presented below:

	Frequency in %
Injection Site	32 (6/19)
Systemic	21 (4/19)

Refer to Table 4 for listing of specific complaints. Temperature data are provided in Table 5.

No serious or alarming reactions attributable to vaccine have been reported.

PREIMMUNE ADULTS (Previously Vaccinated with Yeast Recombinant Hepatitis B Vaccine:

5 mcg lot 85861/22124/C-M126 at time 0
10 mcg lot 85861/22124/C-M126 at time 0

1. Number Vaccinated:

Dose Level	
5 mcg	28
10 mcg	28

2. Serologic Results:

Serologic data are available for 25 participants who received a 5 mcg injection of vaccine and 27 participants who received a 10 mcg injection.

Study 813

RESULTS: (Contd)

At 1-2 months after administration of the booster injection, 21 of 25 (84%) participants who received a 5 mcg dose had a greater than 4-fold rise in anti-HBs titer. The GMT for all vaccinees was 59.9 mIU/ml prior to receipt of the booster injection and 1275.2 mIU/ml 1-2 months after the booster dose.

Refer to Table 2 for a listing of anti-HBs titers prior to and post the booster injection.

Twenty-three of 27 (85%) participants who received a 10 mcg booster dose of vaccine, had a greater than 4-fold rise in anti-HBs titer at 1-2 months post the injection. The GMT for all vaccinees was 96.5 mIU/ml prior to receipt of the booster injection and 1337.0 mIU/ml 1-2 months after the booster dose.

Refer to Table 3 for a listing of anti-HBs titers prior to and post the booster injection.

3. Clinical Complaints:

Clinical follow-up data are available for 11 participants who received a 5 mcg injection and 14 participants who received a 10 mcg injection of vaccine. The overall frequencies of complaints are presented below:

Type of Complaint	Dose Level	Frequency in %
Injection site	5 mcg	40 (4/10)
Systemic		10 (1/10)
Injection site	10 mcg	21 (3/14)
Systemic		0 (0/14)

Refer to Table 6 for a listing of specific clinical complaints by dose level. Maximum temperature data are provided in Table 7.

There were no serious or alarming adverse reactions attributable to vaccine.

Study 813

PUBLICATIONS:

Davidson M, Krugman S. Immunogenicity of recombinant yeast hepatitis B vaccine. Lancet 1985; 1:108-9.

Davidson M, Krugman S. Recombinant yeast hepatitis B vaccine: Side effects and immunogenicity compared with plasma-derived hepatitis B vaccine. Submitted for publication to Hepatitis Scientific Memoranda.

Table 1

Anti-HBs Response Following Primary Immunization with Plasma-Derived Hepatitis B Vaccine* and a Subsequent Booster Dose (10 mcg) of Yeast Recombinant Hepatitis B Vaccine** 5 to 7 Years Later

No.	Age	Sex	Anti-HBs Response S/N					
			Years After Initial Immunization				Weeks after Booster	
			1	5	6	7	2	4
(b) (6)								
	51	M	69			<2.1	101	101
	45	F	72			<2.1	22	12
	37	F	3.2			<2.1	5	6.5
	36	M	26			<2.1	165	115
	28	M	27			<2.1	22	16
	74	M	23			<2.1	<2.1	<2.1
	47	M	93			2.3	82	95
	54	M	34			2.6	226	165
	44	M	103			4	165	78
	59	M	24			4	60	33
	39	M	40			6	40	28
	64	F	160			9	179	179
	46	F	73			9	225	158
	49	M	145			12	157	99
	41	F	177			13	192	183
	45	F	144			18	205	250
	74	F	214			20	177	238
	49	F	205			38	288	209
	43	F	100			41	168	145
	31	F	64			49	146	173
	34	M	206			87	154	151
	35	M	266			192	195	144
	52	F	128		2.8		106	83
	41	M	10		<2.1		14	88
	34	M	8		13		118	120
	30	M	125		22		112	
	35	M	168		36		190	203
	33	F	217		66		147	167
	28	F	28	3.4			112	153
	34	M	101	29			138	98
	34	M	204	112			173	160

* Plasma-derived vaccine: Lot #C-E575, 20 mcg dose at 0,1,2, and 9 months.
** Yeast recombinant vaccine: Lot #C-M126, 10 mcg dose.

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Table 2

Antibody Responses to a 5 mcg Booster Injection of Yeast Recombinant Hepatitis B Vaccine Lot C-M126 in Health Care Personnel Who Previously Received 2.5 mcg Injections of Yeast Recombinant Vaccine at 0, 1, and 6 Months

	Anti-HBs Titer in mIU/ml	
Case #	Prior to Booster Injection	1-2 Months After Booster Injection
(b) (6)	142	9275
	115	2473
	157	944
	33	2145
	5.5	153
	318	4140
*	Neg.	218
	13	940
	19	832
	6.9	244
	25	274
	7.6	301
	489	811
**	20	1100
	70	1662
	59	228
	241	3645
	90	6360
	551	7278
	19	1553
	3390	4116
	23	277
***	1559	2876
	394	5192
	45	2865
GMT in mIU/ml	59.9	1275.2

* Subject was antibody positive at an earlier time.
** Titer determined 4 months after booster injection.
*** Titer determined 3 months after booster injection.

Table 3

Antibody Responses to a 10 mcg Booster Injection of Yeast Recombinant Hepatitis B Vaccine Lot C-M126 in Health Care Personnel Who Previously Received 2.5 mcg Injections of Yeast Recombinant Vaccine at 0, 1, and 6 Months

	Anti-HBs Titer in mIU/ml	
Case #	Prior to Booster Injection	1-2 Months After Booster Injection
(b) (6)	73	800
	812	5828
	150	651
	115	953
	55	3732
	3.6	18
	358	215
	1778	574
	86	2789
	94	2543
	7	1635
	231	3837
	128	2410
	104	3136
	212	9161
	288	490
	Neg.	245
	15	169
	2498	1837
	95	5716
	84	1784
	56	6188
	300	1611
	759	4514
	93	3508
	18	606
	145	948
GMT in mIU/ml	96.5	1337.0

* Subject was antibody positive at an earlier time.

Table 4
PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0813
TREATMENT :
LOT NUMBER : CM126
DOSE : 10 MCG
PATIENT CLASS: PREIMMUNE ADULTS (Previously vaccinated with plasma-derived hepatitis B vaccine)

CLINICAL COMPLAINTS	TOTAL VACCINEES (21 PATIENTS) - DOSE 1 — DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
REACTION, LOCAL (INJECT. SITE)	3 (15.8%)	2 (10.5%)	2 (10.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (31.6%)
SORENESS	3 (15.8%)	2 (10.5%)	2 (10.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (31.6%)
STIFFNESS/TIGHTNESS	1 (5.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)
SYSTEMIC	1 (5.3%)	4 (21.1%)	2 (10.5%)	2 (10.5%)	3 (15.8%)	2 (10.5%)	4 (21.1%)
WHOLE BODY/GENERAL	1 (5.3%)	2 (10.5%)	2 (10.5%)	2 (10.5%)	1 (5.3%)	1 (5.3%)	2 (10.5%)
FLUSH	1 (5.3%)	0 (0.0%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)
FATIGUE/WEAKNESS	1 (5.3%)	1 (5.3%)	1 (5.3%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	2 (10.5%)
MALAISE	1 (5.3%)	1 (5.3%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (10.5%)
HEADACHE	0 (0.0%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)
ACHINESS	0 (0.0%)	1 (5.3%)	0 (0.0%)	1 (5.3%)	1 (5.3%)	1 (5.3%)	1 (5.3%)
INTEGUMENTARY SYSTEM	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)	0 (0.0%)	1 (5.3%)
RASH, NOS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)	0 (0.0%)	1 (5.3%)

Table 4 (cont)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0813
TREATMENT :
LOT NUMBER : CM126
DOSE : 10 MCG
PATIENT CLASS: PREIMMUNE ADULTS

CLINICAL COMPLAINTS	TOTAL VACCINEES (21 PATIENTS) - DOSE 1						
	DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
CARDIOVASCULAR	0 (0.0%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)
ARRHYTHMIA, OTHER	0 (0.0%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)
MUSCULOSKELETAL	0 (0.0%)	1 (5.3%)	0 (0.0%)	1 (5.3%)	1 (5.3%)	1 (5.3%)	2 (10.5%)
ARTHRALGIA (OTHER)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)	1 (5.3%)	1 (5.3%)	1 (5.3%)
MYALGIA	0 (0.0%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)
DIGESTIVE SYSTEM	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)	1 (5.3%)	1 (5.3%)	2 (10.5%)
NAUSEA	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)	1 (5.3%)	1 (5.3%)	2 (10.5%)
ORGANS OF SPECIAL SENSE	0 (0.0%)	0 (0.0%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)
CONJUNCTIVITIS	0 (0.0%)	0 (0.0%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.3%)
PERSONS WITH COMPLAINTS	4 (21.1%)	5 (26.3%)	4 (21.1%)	2 (10.5%)	3 (15.8%)	2 (10.5%)	8 (42.1%)
PERSONS WITH NO COMPLAINTS	15 (78.9%)	14 (73.7%)	15 (78.9%)	17 (89.5%)	16 (84.2%)	17 (89.5%)	11 (57.9%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 5
PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0813
TREATMENT :
LOT NUMBER : CH126
DOSE : 10 MCG
PATIENT CLASS: PREIMMUNE ADULTS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (21 PATIENTS) - DOSE 1							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
NORMAL	8 (42.1%)	8 (44.4%)	8 (42.1%)	8 (42.1%)	9 (47.4%)	11 (57.9%)		8 (42.1%)
< 99	9 (47.4%)	8 (44.4%)	9 (47.4%)	10 (52.6%)	9 (47.4%)	8 (42.1%)		7 (36.8%)
99 - 99.9	2 (10.5%)	1 (5.6%)	1 (5.3%)	0 (0.0%)	1 (5.3%)	0 (0.0%)		3 (15.8%)
100 - 100.9	0 (0.0%)	1 (5.6%)	1 (5.3%)	1 (5.3%)	0 (0.0%)	0 (0.0%)		1 (5.3%)
TEMPERATURE TAKEN	19 (90.5%)	18 (85.7%)	19 (90.5%)	19 (90.5%)	19 (90.5%)	19 (90.5%)		19 (90.5%)
TEMPERATURE NOT TAKEN	2 (9.5%)	3 (14.3%)	2 (9.5%)	2 (9.5%)	2 (9.5%)	2 (9.5%)		2 (9.5%)

Table 6

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0813
TREATMENT :
LOT NUMBER : CM126
DOSE : 5 MCG
PATIENT CLASS: PREIMMUNE ADULTS (Previously vaccinated with yeast recombinant hepatitis B vaccine)

CLINICAL COMPLAINTS	TOTAL VACCINEES (11 PATIENTS) - DOSE 1 — DAYS POST VACCINATION 0	1	2	3	4	5		NUMBER WITH COMPLAINTS
REACTION, LOCAL (INJECT. SITE)	4 (40.0%)	1 (10.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		4 (40.0%)
SORENESS	4 (40.0%)	1 (10.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		4 (40.0%)
SYSTEMIC	0 (0.0%)	1 (10.0%)	1 (10.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)		1 (10.0%)
INTEGUMENTARY SYSTEM	0 (0.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (10.0%)
PRURITIS/ITCHING	0 (0.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (10.0%)
RESPIRATORY	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)		1 (10.0%)
PHARYNGITIS (SORE THROAT)	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)		1 (10.0%)
PERSONS WITH COMPLAINTS	4 (40.0%)	2 (20.0%)	2 (20.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)		4 (40.0%)
PERSONS WITH NO COMPLAINTS	6 (60.0%)	8 (80.0%)	8 (80.0%)	10 (100.0%)	9 (90.0%)	10 (100.0%)		6 (60.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 6 (cont)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0813
TREATMENT :
LOT NUMBER : CM126
DOSE : 10 MCG
PATIENT CLASS: PREIMMUNE ADULTS

CLINICAL COMPLAINTS	TOTAL VACCINEES (14 PATIENTS) - DOSE 1							
	DAYS POST VACCINATION							NUMBER WITH COMPLAINTS
	0	1	2	3	4	5		
REACTION, LOCAL (INJECT. SITE)	2 (14.3%)	1 (7.1%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		3 (21.4%)
SORENESS	2 (14.3%)	1 (7.1%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		3 (21.4%)
PERSONS WITH COMPLAINTS	2 (14.3%)	1 (7.1%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		3 (21.4%)
PERSONS WITH NO COMPLAINTS	12 (85.7%)	13 (92.9%)	13 (92.9%)	14 (100.0%)	14 (100.0%)	14 (100.0%)		11 (78.6%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 7

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0813
TREATMENT :
LOT NUMBER : CM126
DOSE : 5 MCG
PATIENT CLASS: PREIMMUNE ADULTS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (11 PATIENTS) - DOSE 1							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
NORMAL	7 (70.0%)	9 (90.0%)	10 (100.0%)	10 (100.0%)	10 (100.0%)	10 (100.0%)		7 (70.0%)
< 99	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (10.0%)
99 - 99.9	2 (20.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		2 (20.0%)
TEMPERATURE TAKEN	10 (90.9%)	10 (90.9%)	10 (90.9%)	10 (90.9%)	10 (90.9%)	10 (90.9%)		10 (90.9%)
TEMPERATURE NOT TAKEN	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (9.1%)	1 (9.1%)		1 (9.1%)

Table 7 (cont)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0813
TREATMENT :
LOT NUMBER : CM126
DOSE : 10 MCG
PATIENT CLASS: PREIMMUNE ADULTS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (14 PATIENTS) - DOSE 1							NUMBER WITH MAX TEMP
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
NORMAL	13 (92.9%)	13 (92.9%)	12 (85.7%)	12 (85.7%)	12 (85.7%)	12 (85.7%)		12 (85.7%)
< 99	1 (7.1%)	1 (7.1%)	1 (7.1%)	2 (14.3%)	2 (14.3%)	2 (14.3%)		1 (7.1%)
99 - 99.9	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (7.1%)
TEMPERATURE TAKEN	14 (100.0%)	14 (100.0%)	14 (100.0%)	14 (100.0%)	14 (100.0%)	14 (100.0%)		14 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

IMMUNOGENICITY OF RECOMBINANT YEAST HEPATITIS B VACCINE

SIR,—In Dr Jilg and colleagues' study (Nov 24, p 1174) in thirty recipients of recombinant hepatitis B vaccine "the immune response in the recombinant vaccine group was less pronounced during the first months than in the plasma vaccine group, as shown by lower seroconversion rates and lower mean anti-HBs levels". They compared a 10 μg dose of recombinant vaccine with a 20 μg dose of plasma-derived vaccine.

As indicated in the table, our results in a similar study in one hundred and seven seronegative health professionals, 21–30 years of age, revealed essentially the same immune response in recipients of 5 μg and 10 μg doses of recombinant yeast hepatitis B vaccine when compared with a comparable group who received 20 μg doses of plasma-derived vaccine.

Valid conclusions cannot be drawn from studies in thirty or a hundred vaccinees. More extensive studies will be required to evaluate anti-HBs response and its persistence in recipients of recombinant hepatitis B vaccine. In the meantime, our initial results are encouraging.

NYU Medical Center,
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MORTON DAVIDSON
SAUL KRUGMAN

THE LANCET, JANUARY 12, 1985

SEROCONVERSION RATES AND GEOMETRIC MEAN TITRES (GMT) OF SERONEGATIVE INDIVIDUALS, ADULTS GIVEN RECOMBINANT OR PLASMA-DERIVED HEPATITIS B VACCINE

	Recombinant vaccine†						Plasma derived (20 μg)‡	
	10 μg			5 μg				
Time* (mo)	Anti-HBs response	mIU/ml (GMT)	S/N ratio (GMT)	Anti-HBs response	mIU/ml (GMT)	S/N ratio (GMT)	Anti-HBs response	S/N ratio (GMT)
0	..	..	..	..	..	..	..	..
1	22/51 (43%)	42	10	21/56 (37%)	99	25	18/47 (38%)	20
2	48/51 (94%)	88	37	51/56 (91%)	69	38	34/47 (72%)	37
3	50/51 (98%)	149	52	52/56 (93%)	128	51	45/47 (96%)	70
6	49/50 (98%)	321	63	53/56 (95%)	184	42	44/47 (94%)	94
7/8	45/46 (98%)	1911	164	49/50 (98%)	890	124	46/47 (98%)	141

*Vaccines given at 0, 1, and 6 months. Follow-up at 7 months (plasma derived) or 8 months (recombinant). †Above the 97th C.I. cont. ‡Above the 75th.

Davidson M, Krugman S. Immunogenicity of recombinant yeast hepatitis B vaccine. Lancet 1985; 1:108-9.

RECOMBINANT YEAST HEPATITIS B VACCINE: SIDE EFFECTS AND IMMUNOGENICITY COMPARED WITH PLASMA-DERIVED HEPATITIS B VACCINE.

Morton Davidson and Saul Krugman
NYU Medical Center, New York, N.Y.

A yeast recombinant hepatitis B vaccine (Merck Lot no. 972/C-K444) was evaluated in 107 seronegative health professionals, 21-30 years of age. The clinical and antibody responses were compared with the results of a previous similar study using a plasma-derived hepatitis B vaccine (Merck Lot no. 751).

The vaccine was administered at 0, 1 and 6 months to the following three groups: 1) 51 adults who received a 10 mcg dose of recombinant vaccine; 2) 56 adults who received a 5 mcg dose of recombinant vaccine, and 3) 47 adults who received a 20 mcg dose of plasma-derived vaccine. The three groups included medical students, house staff, and nurses who were of comparable age and sex.

Results

Side effects were negligible in all three groups. They consisted of transient, local soreness at the site of the inoculation in about 25% of the vaccinees in each group. No systemic reactions were observed.

The seroconversion rates and geometric mean titers are summarized in the Table. The results are essentially the same for all three groups. Under the conditions of this study the 5 mcg and 10 mcg doses of recombinant hepatitis B vaccine were just as immunogenic as a 20 mcg dose of plasma-derived hepatitis B vaccine.

Comment

A recent report by Jilg et al (Lancet 1984; 2:1174-75) described a similar study in 30 seronegative medical students and laboratory workers whose age and sex were comparable to those in our groups. They stated that "the immune response in the recombinant vaccine group was less pronounced during the first months than in the plasma vaccine group, as shown by lower seroconversion rates and lower mean anti-HBs levels." Our results in 107 similar recipients of the recombinant hepatitis B vaccine do not support this conclusion.

It is obvious that valid conclusions cannot be drawn from studies involving either 30 or 100 vaccinees. More extensive studies will be required to determine anti-HBs response and its persistence in recipients of recombinant hepatitis B vaccines.

Davidson M, Krugman S. Recombinant yeast hepatitis B vaccine: Side effects and immunogenicity compared with plasma-derived hepatitis B vaccine. Submitted for publication to Hepatitis Scientific Memoranda.

TABLE

Seroconversion Rates and Geometric Mean Titers of Seronegative Adults Who Received Recombinant Yeast Hepatitis B Vaccine (Merck Lot No. 972/C-K444) or Plasma-Derived Hepatitis B Vaccine (Merck Lot No. 751).

Time Interval (Months)	Recombinant Hepatitis B Vaccine					
	10 mcg dose			5 mcg dose		
	anti-HBs response	mIU/ml GMT	S/N Ratio GMT	anti-HBs response	mIU/ml GMT	S/N Ratio GMT
0	-	-	-	-	-	-
1	22/51 (43%)	42	19	21/56 (37%)	55	25
2	48/51 (94%)	88	37	51/56 (91%)	69	38
3	50/51 (98%)	145	52	52/56 (93%)	128	51
6	49/50 (98%)	321	63	53/56 (95%)	184	42
8	45/46 (98%)	1911	164	49/50 (98%)	839	124

Vaccine given at 0, 1 and 6 months.
Age Range: 21 - 30 years

Time Interval (Months)	Plasma-Derived Hepatitis B Vaccine 20 mcg dose	
	anti-HBs response	S/N Ratio GMT
0	-	-
1	18/47 (38%)	20
2	34/47 (79%)	37
3	45/47 (96%)	79
6	44/47 (94%)	94
7	46/47 (98%)	141

Vaccine given at 0, 1 and 6 months.
Age range: 21 - 30 years

PROGRAM:	Yeast Recombinant Hepatitis B Vaccine, Study 817
PURPOSE:	To evaluate antibody and clinical responses to 10 mcg doses of yeast recombinant vaccine among: 1. preimmune healthy adults 2. healthy adults immunized previously with plasma-derived vaccine who were nonresponders (anti-HBs negative.
VACCINE:	Yeast Recombinant Hepatitis B Vaccine Lot #972/C-K444 (10 mcg/ml)
PRIMARY INVESTIGATOR:	Robert P. Bishop, M.D. Director, Health Services Merck & Co., Inc. West Point, PA 19486
SECONDARY INVESTIGATOR(S):	Edgardo P. Avancena, M.D. Joseph C. Rogers, M.D. Joseph P. Romano, M.D. Merck & Co., Inc. West Point, PA & Rahway, NJ
STUDY LOCATION:	Merck & Co., Inc. West Point, PA 19486 Merck & Co., Inc. Rahway, NJ 07065
DATE INITIATED:	March 21, 1984
DATE COMPLETED:	In progress

2547I/cfs
12/20/85

Study 817

STUDY POPULATION: The study population will consist of 40-50 healthy adults of either sex (excluding pregnant females), who are employees of Merck & Co., Inc. Half of the population will consist of persons with pre-existing hepatitis B antibody which may be either naturally acquired or plasma vaccine induced. The other half will consist of persons who have been vaccinated with plasma vaccine but failed to develop detectable antibody to hepatitis B. All participants must be negative for anti-HBc and HBsAg, and have a normal ALT level.

PROCEDURE: Study participants are allocated to one of two regimens as shown below. All injections are intramuscular.

Group	No.	Dose	Time of Vaccination
1. Preimmune	5	1.0 ml (10 mcg)	0
2. Nonresponders	4	1.0 ml (10 mcg)	0, 1 & 6 mos.

Vaccinees are asked to record their temperature daily for five days after each injection and also to record any local or systemic complaints they may have during this period.

A blood specimen (10-15 ml) is obtained from each participant approximately 2 weeks before the first vaccination. Post-vaccination blood samples are obtained at 1, 2, 3, 6, 8, 12 and 24 months. The samples are assayed for HBsAg, anti-HBc, anti-HBs, yeast antibody and ALT. Those with anti-HBs titers $\geq$ mIU/ml may be tested for the proportions of anti-a and anti-d activity.

Study 817

RESULTS:

PREIMMUNE ADULTS:

10 mcg Lot #972/C-K444 at time 0

1. Number Vaccinated: 5

2. Serologic Results:

All five vaccinees showed a large boost in anti-HBs following vaccination. Table 1 shows individual anti-HBs responses for up to 12 months of follow-up.

3. Clinical Complaints:

Clinical follow-up data are available for all 5 vaccinees for the five days of follow-up following vaccination. Specific complaints and maximum temperatures reported during that time are provided in Tables 2 and 3.

Type of Complaint	Frequency in %
Injection Site	20 (1/5)
Systemic	0 (0/5)

There were no serious or alarming adverse reactions attributable to vaccine.

Study 817

Table 1

Antibody Responses Among Preimunne Adults Following Vaccination with a Single 10 mcg Dose of Yeast Recombinant Hepatitis B Vaccine Lot #972/C-K444 in Study 817

Case #	Pre	Anti-HBs (mIU/ml) 1 Mo.	3 mo.	6 mo.	12 mo.
(b) (6)	4*	15.2	105.7	150.0	26.5
	4*	810.9	404.3	99.5	52.5
	8*	475	456.1	355.72	62.3
	11.5	---	350.3	50.7**	
	4*	1734.4	2063.4	1119.3	318.6
GMT (mIU/ml)	5.7	317.4	402.5	197.6	72.5

*Approximate mIU/ml (b) (4) titer ÷ 4)
**Late bleeding at 8 months.

2547I-4/cfs
12/20/85

Table 2

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0817
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: PREIMMUNE ADULTS

CLINICAL COMPLAINTS	TOTAL VACCINEES (5 PATIENTS) - DOSE 1							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
REACTION, LOCAL (INJECT. SITE)	1 (20.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (20.0%)
SORENESS	1 (20.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (20.0%)
PERSONS WITH COMPLAINTS	1 (20.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (20.0%)
PERSONS WITH NO COMPLAINTS	4 (80.0%)	4 (80.0%)	5 (100.0%)	5 (100.0%)	5 (100.0%)	5 (100.0%)		4 (80.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 3

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0817
TREATMENT :
LOT NUMBER : CK444
DOSE : 10 MCG
PATIENT CLASS: PREIMMUNE ADULTS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (5 PATIENTS) - DOSE 1 — DAYS POST VACCINATION								NUMBER WITH MAX TEMP
	0	1	2	3	4	5			
< 99	5 (100.0%)	5 (100.0%)	5 (100.0%)	4 (100.0%)	5 (100.0%)	5 (100.0%)			5 (100.0%)
TEMPERATURE TAKEN	5 (100.0%)	5 (100.0%)	5 (100.0%)	4 (80.0%)	5 (100.0%)	5 (100.0%)			5 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)			0 (0.0%)

CHRONIC CARRIERS

Chronic Carriers - Population Summary

One study (#854) has been initiated to determine the safety of the vaccine for persons who are chronic carriers of HBsAg and to determine whether vaccination can eliminate the carrier state in these persons. Eighteen adult chronic carriers (positive for HBsAg for at least one year) have been scheduled to receive six 10 mcg injections of yeast recombinant hepatitis B vaccine at monthly intervals. Three participants have received all six injections; eighteen have received at least four injections. The study continues in progress.

To date, none of the chronic carriers has become negative for HBsAg. The vaccine has been well tolerated. No serious adverse experiences attributable to vaccine have been reported.

PROGRAM:	Alum-Adsorbed Yeast Recombinant Hepatitis B Vaccine, Study 854.
PURPOSE:	To evaluate antibody and clinical responses to yeast recombinant hepatitis B vaccine in the following adult populations: 1. Chronic Carriers of HBsAg 2. Healthy Hyporesponders to Plasma-Derived Vaccine. 3. Healthy Nonresponders to Plasma-Derived Vaccine. 4. Healthy Transient Responders to Plasma-Derived Vaccine.
VACCINE:	Yeast Recombinant Hepatitis B Vaccine Lot #979/C-K564 (10 mcg HBsAg/ml)
PRINCIPAL INVESTIGATOR:	Jules Dienstag, M.D. Associate Professor of Medicine Gastrointestinal Unit Massachusetts Gen. Hosp. Boston, MA 02114
SECONDARY INVESTIGATOR:	Eloise Watkins, R.N., M.P.H. Gastrointestinal Unit Mass. General Hosp. Fruit Street Boston, MA 02114 Lynn F. Butterly, M.D. Clinical & Research Fellow Gastrointestinal Unit Mass. General Hosp. Boston, MA 02114
STUDY LOCATION:	Massachusetts General Hospital Fruit Street Boston, MA 02114
DATE INITIATED:	October 14, 1984
DATE COMPLETED:	In progress

Study 854

STUDY POPULATIONS: The study population will consist of adults of either sex (excluding pregnant women) who can be classified into one of the following groups:

Group	Number of Subjects	Qualifications
Carriers	10-15	Chronic carrier of HBsAg for at least one year, with no signs or symptoms of chronic liver disease, and a stable ALT level less than 3 times the upper limit of normal.
Hypo-responders	15-20	Healthy adults who have had only a low level anti-HBs response (positive titer obtained in at least 2 successive bleedings) to a complete 3 injection regimen of plasma derived hepatitis B vaccine. [maximum antibody titer 8-36 when measured in (b)(4) RIA units, 2.1-9.9 when measured in terms of S/N ratio, or <10 mIU/ml]
Non-responders	15-20	Healthy adults who had a single post-vaccination blood sample with an anti-HBs titer in the range S/N = 2.1-9.9 followed by additional samples all with S/N less than 2.1 as well as persons whose post-vaccination blood samples all had anti-HBs titers of S/N less than 2.1 after receiving a 3 injection series of plasma derived hepatitis B vaccine.
Transient Responders	10-15	Healthy adults who had at least one blood sample with an anti-HBs titer of S/N $\geq$10 following a 3 injection series of plasma derived hepatitis B vaccine but have subsequently lost antibody (S/N <2.1).

Study 854

PROCEDURE:

Prior to vaccination, each participant will be screened for HBsAg, anti-HBc, anti-HBs and ALT level. A serum pregnancy test will also be performed for all women of childbearing age. Vaccine is administered intramuscularly according to the following schedule.

Group	Vaccination Regimen
Carriers	1.0 ml (10 mcg HBsAg) at time 0, 1, 2, 3, 4 and 5 months.
Hyporesponders	1.0 ml (10 mcg HBsAg) at time 0
Nonresponders	1.0 ml (10 mcg HBsAg) at time 0, 1 and 6 months.
Transient Responders	1.0 ml (10 mcg HBsAg) at time 0.

The vaccine recipients are asked to record their temperature for 5 days after each injection and to note any local or systemic complaints. Unexpected or serious reactions will be reported to the study physician immediately.

Follow-up blood samples will be obtained from carriers monthly for 6 months and at 9 and 12 months; from hyporesponders and transient responders at 1, 3, 6, 9, 12 and 24 months and; from nonresponders at 1, 2, 3, 6, 9 months, and at 12 and 24 months from those who have seroconverted by 9 months. Samples are assayed for HBsAg, anti-HBc, anti-HBs, and ALT by Dr. Dienstag. Samples may also be assayed at MSDRL for yeast antibody and for the proportions of anti-HBs specific for the a and d determinants of HBsAg.

Study 854

RESULTS:

CARRIERS

10 mcg Lot #979/C-K564 at 0, 1, 2, 3, 4, and 5 months.

1. Number Vaccinated:

Injection No.					
1	2	3	4	5	6
18	18	18	18	12	3

2. Serologic Results:

None of the carriers has yet become negative for HBsAg.

3. Clinical Complaints:

Clinical follow-up data are available for 18 participants after injections one through four, 12 participants after injection five, and for 2 subjects after injection six. The overall frequencies of complaints are presented below:

	Frequency in % by Injection					
Complaint	1	2	3	4	5	6
Injection Site	22(4/18)	17(3/18)	22(4/18)	22(4/18)	8(1/12)	0(0/2)
Systemic	17(3/18)	11(2/18)	17(3/18)	11(2/18)	17(2/12)	50(1/2)

Refer to Table 1 for listings of specific complaints by injection number. Maximum temperature data are provided in Table 2.

There were no serious or alarming reactions attributable to vaccine.

Table 1

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

CLINICAL COMPLAINTS	TOTAL VACCINEES (18 PATIENTS) - DOSE 1 — DAYS POST VACCINATION 0	1	2	3	4	5		NUMBER WITH COMPLAINTS
REACTION, LOCAL (INJECT. SITE)	3 (16.7%)	2 (11.1%)	2 (11.1%)	2 (11.1%)	0 (0.0%)	0 (0.0%)		4 (22.2%)
SORENESS	3 (16.7%)	2 (11.1%)	2 (11.1%)	2 (11.1%)	0 (0.0%)	0 (0.0%)		4 (22.2%)
HEMATOMA	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
SYSTEMIC	1 (5.6%)	2 (11.1%)	0 (0.0%)	0 (0.0%)	1 (5.9%)	1 (5.6%)		3 (16.7%)
WHOLE BODY/GENERAL	1 (5.6%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
FATIGUE/WEAKNESS	1 (5.6%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
DIGESTIVE SYSTEM	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	1 (5.9%)	1 (5.6%)		2 (11.1%)
DIARRHEA	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
NAUSEA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.9%)	1 (5.6%)		1 (5.6%)
DIMINISHED APPETITE	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.9%)	0 (0.0%)		1 (5.6%)
PERSONS WITH COMPLAINTS	4 (22.2%)	4 (22.2%)	2 (11.1%)	2 (11.1%)	1 (5.9%)	1 (5.6%)		7 (38.9%)
PERSONS WITH NO COMPLAINTS	14 (77.8%)	14 (77.8%)	16 (88.9%)	16 (88.9%)	16 (94.1%)	17 (94.4%)		11 (61.1%)

Table 1 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

CLINICAL COMPLAINTS	TOTAL VACCINEES (18 PATIENTS) - DOSE 1							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

06600

Table 1 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

CLINICAL COMPLAINTS	TOTAL VACCINEES (18 PATIENTS) - DOSE 2 — DAYS POST VACCINATION 0	1	2	3	4	5		NUMBER WITH COMPLAINTS
REACTION, LOCAL (INJECT. SITE)	2 (11.1%)	3 (16.7%)	2 (11.1%)	1 (5.6%)	0 (0.0%)	0 (0.0%)		3 (16.7%)
SORENESS	1 (5.6%)	2 (11.1%)	1 (5.6%)	1 (5.6%)	0 (0.0%)	0 (0.0%)		2 (11.1%)
TENDERNESS	1 (5.6%)	1 (5.6%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
SWELLING	2 (11.1%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		2 (11.1%)
SYSTEMIC	1 (5.6%)	2 (11.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.6%)		2 (11.1%)
WHOLE BODY/GENERAL	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
SWEATING	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
INFECTIOUS SYNDROMES	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.6%)		1 (5.6%)
INFLUENZA, NOS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.6%)		1 (5.6%)
DIGESTIVE SYSTEM	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
OTHER	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
NERVOUS SYSTEM	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)

16600

Table 1 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

CLINICAL COMPLAINTS	TOTAL VACCINEES (18 PATIENTS) - DOSE 2							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
VERTIGO/DIZZINESS	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
PERSONS WITH COMPLAINTS	3 (16.7%)	5 (27.8%)	2 (11.1%)	1 (5.6%)	0 (0.0%)	1 (5.6%)		5 (27.8%)
PERSONS WITH NO COMPLAINTS	15 (83.3%)	13 (72.2%)	16 (88.9%)	17 (94.4%)	18 (100.0%)	17 (94.4%)		13 (72.2%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 1 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

CLINICAL COMPLAINTS	TOTAL VACCINEES (18 PATIENTS) - DOSE 3							NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION							
	0	1	2	3	4	5		
REACTION, LOCAL (INJECT. SITE)	3 (16.7%)	2 (11.1%)	2 (11.1%)	1 (5.6%)	0 (0.0%)	0 (0.0%)		4 (22.2%)
SORENESS	2 (11.1%)	1 (5.6%)	1 (5.6%)	1 (5.6%)	0 (0.0%)	0 (0.0%)		3 (16.7%)
TENDERNESS	1 (5.6%)	1 (5.6%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
NODULE FORMATION	1 (5.6%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
SYSTEMIC	2 (11.1%)	1 (5.6%)	0 (0.0%)	2 (11.1%)	0 (0.0%)	1 (5.6%)		3 (16.7%)
WHOLE BODY/GENERAL	1 (5.6%)	0 (0.0%)	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)		2 (11.1%)
FATIGUE/WEAKNESS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
HEADACHE	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
INFECTIOUS SYNDROMES	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
INFLUENZA, NOS	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
RESPIRATORY	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.6%)		1 (5.6%)
UPPER RESPIRATORY INFECT., NOS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.6%)		1 (5.6%)

00993

Table 1 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

CLINICAL COMPLAINTS	TOTAL VACCINEES (18 PATIENTS) - DOSE 3 — DAYS POST VACCINATION 0	1	2	3	4	5		NUMBER WITH COMPLAINTS
DIGESTIVE SYSTEM	1 (5.6%)	0 (0.0%)	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)		2 (11.1%)
NAUSEA	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
OTHER	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
PERSONS WITH COMPLAINTS	5 (27.8%)	3 (16.7%)	2 (11.1%)	2 (11.1%)	0 (0.0%)	1 (5.6%)		5 (27.8%)
PERSONS WITH NO COMPLAINTS	13 (72.2%)	15 (83.3%)	16 (88.9%)	16 (88.9%)	18 (100.0%)	17 (94.4%)		13 (72.2%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)

Table 1 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

CLINICAL COMPLAINTS	TOTAL VACCINEES (18 PATIENTS) - DOSE 4 — DAYS POST VACCINATION 0	1	2	3	4	5		NUMBER WITH COMPLAINTS
REACTION, LOCAL (INJECT. SITE)	3 (16.7%)	4 (22.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		4 (22.2%)
SORENESS	2 (11.1%)	3 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		3 (16.7%)
TENDERNESS	1 (5.6%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
SYSTEMIC	1 (5.6%)	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		2 (11.1%)
WHOLE BODY/GENERAL	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
FATIGUE/WEAKNESS	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
ITCHING, FACIAL	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
DIGESTIVE SYSTEM	0 (0.0%)	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
OTHER	0 (0.0%)	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
NERVOUS SYSTEM	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
VERTIGO/DIZZINESS	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		1 (5.6%)
PERSONS WITH COMPLAINTS	4 (22.2%)	4 (22.2%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		5 (27.8%)

Table 1 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

CLINICAL COMPLAINTS	TOTAL VACCINEES (18 PATIENTS) - DOSE 4						
	DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
PERSONS WITH NO COMPLAINTS	14 (77.8%)	14 (77.8%)	17 (94.4%)	18 (100.0%)	18 (100.0%)	18 (100.0%)	13 (72.2%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 1 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

CLINICAL COMPLAINTS	TOTAL VACCINEES (12 PATIENTS) - DOSE 5						
	DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
REACTION, LOCAL (INJECT. SITE)	0 (0.0%)	1 (8.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)
SORENESS	0 (0.0%)	1 (8.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)
SYSTEMIC	1 (8.3%)	2 (16.7%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	2 (16.7%)
WHOLE BODY/GENERAL	1 (8.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)
FATIGUE/WEAKNESS	1 (8.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)
INFECTIOUS SYNDROMES	0 (0.0%)	1 (8.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)
INFLUENZA, NOS	0 (0.0%)	1 (8.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)
DIGESTIVE SYSTEM	0 (0.0%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	1 (8.3%)
DYSPEPSIA/HEARTBURN	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)	0 (0.0%)	0 (0.0%)	1 (8.3%)
OTHER	0 (0.0%)	1 (8.3%)	1 (8.3%)	0 (0.0%)	1 (8.3%)	1 (8.3%)	1 (8.3%)
PERSONS WITH COMPLAINTS	1 (8.3%)	2 (16.7%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	2 (16.7%)
PERSONS WITH NO COMPLAINTS	11 (91.7%)	10 (83.3%)	11 (91.7%)	11 (91.7%)	11 (91.7%)	11 (91.7%)	10 (83.3%)

00997

Table 1 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

CLINICAL COMPLAINTS	TOTAL VACCINEES (12 PATIENTS) - DOSE 5						NUMBER WITH COMPLAINTS
	DAYS POST VACCINATION						
	0	1	2	3	4	5	
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 1 (Contd)

PATIENT COUNT CLINICAL COMPLAINTS
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

CLINICAL COMPLAINTS	TOTAL VACCINEES (3 PATIENTS) - DOSE 6						
	DAYS POST VACCINATION						NUMBER WITH COMPLAINTS
	0	1	2	3	4	5	
SYSTEMIC	0 (0.0%)	1 (50.0%)	1 (50.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	1 (50.0%)
RESPIRATORY	0 (0.0%)	1 (50.0%)	1 (50.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	1 (50.0%)
PHARYNGITIS (SORE THROAT)	0 (0.0%)	1 (50.0%)	1 (50.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	1 (50.0%)
ORGANS OF SPECIAL SENSE	0 (0.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)
EARACHE	0 (0.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)
PERSONS WITH COMPLAINTS	0 (0.0%)	1 (50.0%)	1 (50.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	1 (50.0%)
PERSONS WITH NO COMPLAINTS	2 (100.0%)	1 (50.0%)	1 (50.0%)	1 (50.0%)	1 (50.0%)	2 (100.0%)	1 (50.0%)
PERSONS WITH NO DATA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

66600

Table 2

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (18 PATIENTS) - DOSE 1 — DAYS POST VACCINATION 0	1	2	3	4	5			NUMBER WITH MAX TEMP
< 99	15 (83.3%)	17 (94.4%)	18 (100.0%)	16 (88.9%)	17 (94.4%)	18 (100.0%)			12 (66.7%)
99 - 99.9	3 (16.7%)	1 (5.6%)	0 (0.0%)	2 (11.1%)	0 (0.0%)	0 (0.0%)			5 (27.8%)
100 - 100.9	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (5.6%)	0 (0.0%)			1 (5.6%)
TEMPERATURE TAKEN	18 (100.0%)	18 (100.0%)	18 (100.0%)	18 (100.0%)	18 (100.0%)	18 (100.0%)			18 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			0 (0.0%)

01000

Table 2 (Contd)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (18 PATIENTS) - DOSE 2								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
< 99	16 (88.9%)	18 (100.0%)	16 (88.9%)	17 (94.4%)	18 (100.0%)	17 (94.4%)			13 (72.2%)
99 - 99.9	2 (11.1%)	0 (0.0%)	2 (11.1%)	1 (5.6%)	0 (0.0%)	1 (5.6%)			5 (27.8%)
TEMPERATURE TAKEN	18 (100.0%)	18 (100.0%)	18 (100.0%)	18 (100.0%)	18 (100.0%)	18 (100.0%)			18 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			0 (0.0%)

Table 2 (Contd)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (18 PATIENTS) - DOSE 3								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
< 99	15 (83.3%)	16 (88.9%)	15 (83.3%)	15 (83.3%)	17 (94.4%)	15 (83.3%)			9 (50.0%)
99 - 99.9	3 (16.7%)	2 (11.1%)	2 (11.1%)	3 (16.7%)	1 (5.6%)	3 (16.7%)			8 (44.4%)
100 - 100.9	0 (0.0%)	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			1 (5.6%)
TEMPERATURE TAKEN	18 (100.0%)	18 (100.0%)	18 (100.0%)	18 (100.0%)	18 (100.0%)	18 (100.0%)			18 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			0 (0.0%)

01002

Table 2 (Contd)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (18 PATIENTS) - DOSE 4								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
NORMAL	0 (0.0%)	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			0 (0.0%)
< 99	17 (94.4%)	16 (88.9%)	15 (83.3%)	17 (94.4%)	17 (94.4%)	18 (100.0%)			14 (77.8%)
99 - 99.9	1 (5.6%)	2 (11.1%)	2 (11.1%)	1 (5.6%)	1 (5.6%)	0 (0.0%)			4 (22.2%)
TEMPERATURE TAKEN	18 (100.0%)	18 (100.0%)	18 (100.0%)	18 (100.0%)	18 (100.0%)	18 (100.0%)			18 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			0 (0.0%)

Table 2 (Contd)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (12 PATIENTS) - DOSE 5								NUMBER WITH MAX TEMP
	DAYS POST VACCINATION								
	0	1	2	3	4	5			
< 99	9 (75.0%)	10 (83.3%)	9 (75.0%)	9 (81.8%)	10 (83.3%)	9 (75.0%)			6 (50.0%)
99 - 99.9	3 (25.0%)	1 (8.3%)	2 (16.7%)	1 (9.1%)	1 (8.3%)	3 (25.0%)			4 (33.3%)
100 - 100.9	0 (0.0%)	1 (8.3%)	1 (8.3%)	1 (9.1%)	0 (0.0%)	0 (0.0%)			1 (8.3%)
101 - 101.9	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)	0 (0.0%)			1 (8.3%)
TEMPERATURE TAKEN	12 (100.0%)	12 (100.0%)	12 (100.0%)	11 (91.7%)	12 (100.0%)	12 (100.0%)			12 (100.0%)
TEMPERATURE NOT TAKEN	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (8.3%)	0 (0.0%)	0 (0.0%)			0 (0.0%)

Table 2 (Contd)

PATIENT COUNT MAXIMUM TEMPERATURES
RECOMBINANT HEPATITIS B VACCINE

STUDY : 0854
TREATMENT :
LOT NUMBER : CK564
DOSE : 10 MCG
PATIENT CLASS: CHRONIC CARRIERS

MAX TEMPERATURE (DEG F, ORAL)	TOTAL VACCINEES (3 PATIENTS) - DOSE 6 — DAYS POST VACCINATION							NUMBER WITH MAX TEMP
	0	1	2	3	4	5		
< 99	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0%)		2 (100.0%)
TEMPERATURE TAKEN	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	2 (66.7%)		2 (66.7%)
TEMPERATURE NOT TAKEN	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)		1 (33.3%)

EFFICACY

EFFICACY SUMMARY

Hepatitis B vaccine derived from the plasma of chronically infected individuals was previously shown to be effective in preventing hepatitis B infection among adult male homosexuals and staff members in dialysis units. This vaccine also proved to be effective in preventing chronic hepatitis B infection among infants born to mothers who are positive for both HBsAg and HBeAg.

The efficacy studies involving plasma-derived hepatitis B vaccine demonstrated that the presence of anti-HBs equated with protection against hepatitis B. Consequently, the high seroconversion rates observed for recipients of the yeast recombinant hepatitis B vaccine (e.g. 96% of healthy adult vaccinees develop anti-HBs titers of mIU/ml $\geq$10) suggest that these individuals should be protected against hepatitis B. Ongoing in vitro studies to demonstrate the equivalence of anti-HBs raised to yeast recombinant hepatitis B vaccine and plasma-derived hepatitis B vaccine are described in Appendix 1.

The feasibility of conducting efficacy studies of the yeast recombinant hepatitis B vaccine in various populations was considered. Such studies are reasonable only in populations known to experience relatively high rates of infection. Control groups are also a problem. Since a proven preventive therapy (plasma-derived hepatitis B vaccine) is now available in most parts of the world, it is no longer ethical to conduct a study with untreated controls. In some instances, notably with infants of Asian mothers who are positive for HBsAg and HBeAg, there have been very high rates of infection documented among untreated individuals, and it is reasonable to use these rates as a basis for estimating protective efficacy in contemporary studies lacking untreated controls. However, historical data on the incidence of infection in various candidate adult population are no longer applicable. Rates of hepatitis B infection are probably declining among homosexuals, due to changed sexual practices since the AIDS epidemic. The incidence of hepatitis B infection has also been declining for a number of years in dialysis units. We have concluded that an efficacy study of the yeast recombinant hepatitis B vaccine in an adult population is not feasible. However, studies involving infants born to mothers who are carriers of the hepatitis B virus are feasible.

Four studies have been initiated to evaluate the efficacy of yeast recombinant hepatitis B vaccine in preventing chronic hepatitis B infection in infants born to mothers who carry the virus:

Study	Study Population/Regimen
862	Healthy infants born to mothers who are positive for HBsAg and either positive or negative for HBeAg receive a single 0.5 ml injection of HBIG at birth following by 5 mcg doses of yeast recombinant hepatitis B vaccine or 10 mcg doses of plasma-derived hepatitis B vaccine at 0, 1, and 6 months. The study is being conducted in Hong Kong.

Study	Study Population/Regimen
864	Healthy infants born to mothers of Asian descent, who are positive for both HBsAg and HBeAg, receive a single 0.5 ml injection of HBIG at birth following by 5 mcg doses of the yeast recombinant hepatitis B vaccine at 0 (within the first few days of birth), 1, and 6 months. The study is being conducted in the United States.
878	Healthy infants born to mothers who are positive for both HBsAg and HBeAg receive either a single 0.5 ml injection of HBIG at birth followed by 5 mcg doses of the yeast recombinant hepatitis B vaccine at 0 (within 12 hours of birth), 1, and 6 months, or vaccine alone. The study is being conducted in China.
892	Healthy infants born to mothers who are positive for both HBsAg and HBeAg receiving yeast recombinant hepatitis B vaccine (5 or 10 mcg dose) or plasma-derived hepatitis B vaccine (10 or 20 mcg dose) at 0 (within 12 hours of birth), 1, and 6 months. This study is being conducted in China.

A total of 412 infants have been enrolled to date in the four studies, 289 of these in groups receiving the yeast recombinant hepatitis B vaccine. Postvaccination follow-up data are currently available from studies 862 and 864 only. Comments regarding efficacy will be restricted to infants of mothers positive for both HBsAg and HBeAg who are receiving passive-active prophylaxis (HBIG at birth plus 5 mcg doses of yeast recombinant hepatitis B vaccine at 0, 1, and 6 months). The numbers of infants who have received the first, second, and third injections of vaccine together with their antigen status at various times are tabulated below:

Number Vaccinated	Study 862	Study 864	Both Studies
First Injection	40	134	174
Second Injection	37	120	157
Third Injection	12	61	73

Proportion HBsAg Positive	Study 862	Study 864	Both Studies
Birth	4/40	4/134	8/174
3 Months	1/25	3/85	4/110
6 Months	0/12	1/47	1/59
9 Months	---	0/19	0/19

Eight (8) infants were positive for HBsAg at birth. Four (4) of the eight have been tested at 3 months and all were still positive for HBsAg. One of these infants has been followed through 6 months, is still HBsAg positive, and is now classified as a chronic carrier. The infants who are positive for

HBsAg at birth may have been infected in utero and such infections cannot be prevented through postnatal vaccination. To date, there have been no persistent infections appearing after birth.

The efficacy of HBIG and yeast recombinant vaccine in preventing chronic hepatitis B infection may be estimated with the following formula:

$$\% \text{ Efficacy} = \left[1 - \left(\frac{\text{Incidence of infection in vaccinated}}{\text{Incidence of infection in unvaccinated}}\right)\right] \times 100$$

The single chronic carrier among the vaccinated infants followed for six months represents an incidence of 1.7%. A number of previous studies have estimated the incidence of chronic infection among untreated infants born to Asian mothers positive for HBsAg and HBeAg at 60-92%.[1-10] In addition, the investigators in Study 864 have recently obtained follow-up serology on 13 children born in the United States during the past several years to mothers positive for HBsAg and HBeAg who did not receive HBIG or hepatitis B vaccine. Nine of the 13 children (69.2%) had become positive for HBsAg. This rate is similar to those cited above and was used as our estimate for the incidence of chronic infection in unvaccinated infants. Estimates of the efficacy of the HBIG-yeast recombinant vaccine regimen at 6 and 9 months are tabulated below:

Efficacy in %	Study 862	Study 864	Both Studies
6 Months	100	97	98
9 Months	---	100	--

No serious adverse experiences related to vaccine have been reported. These data suggest that passive-active prophylaxis involving a single dose of HBIG and three 5 mcg doses of yeast recombinant hepatitis B vaccine is safe and will provide a high level of protection against chronic hepatitis B virus infection.

References:

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EFFICACY

Study 862 - Hong King - Dr. E. K. Yeoh

The study population consists of two groups of healthy infants. Group 1 includes infants born to mothers positive for HBsAg and HBeAg. The infants receive HBIG at birth and then either 5 mcg injections of yeast recombinant hepatitis B vaccine lot C-K734 or 10 mcg injections of plasma-derived vaccine at 0, 1, and 6 months. Group 2 includes infants born to mothers positive for HBsAg and negative for HBeAg. These infants also receive HBIG at birth and then either yeast recombinant or plasma-derived vaccine according to the same dose and schedule as the infants in group 1. The initial injection of vaccine is administered within 12 hours after birth.

Twenty-eight infants in group 1 (HBeAg positive mothers) have received one dose of HBIG and the first injection of plasma-derived vaccine. Twenty-seven and eight of these infants have received the second and third injections of vaccine, respectively. Forty infants in group 1 have received one dose of HBIG and the first injection of yeast recombinant vaccine. Thirty-seven and five of these participants have been administered the second and third injections of vaccine, respectively.

At three months, 100% (19/19) of the infants (group 1) who received plasma-derived vaccine and 100% (24/24) of the infants who received yeast recombinant vaccine developed protective levels (mIU/ml $\geq$10) of anti-HBs (excludes infants HBsAg positive at birth).

Two infants (group 1) who received plasma-derived vaccine were HBsAg positive at birth. Both infants were negative for HBsAg at one month of follow-up. One infant, who was HBsAg negative at one and three months, tested HBsAg positive at six months. Her serum was anti-HBc IgM negative. Four infants who received yeast recombinant vaccine were HBsAg positive at birth. At one month, two of these were negative for HBsAg and two of the infants remained positive. Three month serology data is available for one of the HBsAg positive infants. This subject remained positive at that time.

Eighty-five infants in group 2 (HBeAg negative mothers) have received one dose of HBIG and the first injection of plasma-derived vaccine. Seventy-nine and 18 of these have received the second and third injections, respectively. Seventy-five infants in group 2 have received one dose of HBIG and the first injection of yeast recombinant vaccine. Seventy-three and 15 of these infants have been administered the second and third injections, respectively.

At three months, 100% (42/42) of the infants (group 2) who received plasma-derived vaccine and 100% (41/41) of the infants who received yeast recombinant vaccine developed protective levels (mIU/ml $\geq$10) of anti-HBs (excludes infants HBsAg positive at birth).

Two infants who received plasma-derived vaccine were HBsAg positive at birth. They tested negative at one month. Another infant was positive for HBsAg at one month. Additional serology data are not available for this

Study 862 - Hong King - Dr. E. K. Yeoh (Cont.)

infant. Three additional infants, who were negative for HBsAg at one and three months, tested positive for HBsAg at six months. In two of these cases, the six month sera were anti-HBc IgM negative. All three infants seroconverted for anti-HBs by three months post-entry into the study.

Two infants (group 2) who received yeast recombinant vaccine were HBsAg positive at birth. Both infants were negative at one month. An additional infant, who was HBsAg negative at one and three months, tested HBsAg positive at six months. The six month serum was anti-HBc IgM negative. The infant seroconverted for anti-HBs by three months post-entry into the study.

No serious or alarming adverse experiences related to vaccine have been reported. The study continues in progress.

Study 864 - New York, NY - Dr. C. Stevens

Healthy infants born to women of Asian descent who are positive for HBsAg and HBeAg, are enrolled in Study 864. The study is designed to evaluate rates of chronic hepatitis B antigenemia in infants at extremely high risk of infection. The infants are scheduled to receive one dose of HBIG within the first few hours of birth. Yeast recombinant vaccine (5 mcg injections) lot C-K732 is administered within the first few days after birth and at one and six months of age.

One hundred thirty-four infants have received one dose of HBIG and the first injection of vaccine. One hundred twenty and 61 infants have been administered the second and third injections of vaccine, respectively. All of 46 antigen negative infants followed for 6 months had developed anti-HBs (S/N $\geq$2.1).

Four infants were positive for HBsAg at birth. One of these has been followed for 1 month only and remains positive. Three of the infants have been followed for at least 3 months and were still antigen positive. One of the three has been followed for 6 months and is still positive.

No serious or alarming adverse experiences related to vaccine have been reported. Vaccination and follow-up continues in progress.

Study 878 - China - Dr. T. Sun

Healthy infants, born to women who are positive for HBsAg and HBeAg, are enrolled in Study 878. The first 30 infants entered in the study receive one dose of HBIG and a 5 mcg injection of vaccine lot C-K564 at birth. Subsequent 5 mcg injections of vaccine are administered at one and six months of age. All additional infants enrolled in the study receive no HBIG at birth and 5 mcg injections of vaccine according to the same schedule (0, 1 and 6 months).

Study 878 - China - Dr. T. Sun (Cont.)

Thirty infants have received one dose of HBIG and their first 5 mcg injection of vaccine. Serologic data are not yet available. There have been no reports of serious or alarming adverse experiences related to vaccine. Vaccination and follow-up continues in progress.

Study 892 - China - Dr. Z. H. Hu

The study population consists of healthy infants born to mothers who are positive for HBsAg and HBeAg. The study is designed to compare the efficacy of yeast recombinant vaccine and plasma-derived vaccine in preventing chronic hepatitis B antigenemia among infants at high risk for infection. Infants are randomly assigned to receive either 5 mcg or 10 mcg injections of recombinant vaccine lot C-K564 or 10 mcg or 20 mcg injections of plasma-derived vaccine lot 0027L. All injections are administered within 12 hours after birth and at one and six months of age.

Five infants have receive the first injection of vaccine in each dose and vaccine regimen. Serology data are not yet available. No serious adverse experiences related to vaccine have been reported. Vaccination and follow-up continues in progress.

PROGRAM:	Yeast Recombinant Hepatitis B Vaccine, Study 862
PURPOSE:	To evaluate the efficacy of 5 mcg doses of the yeast recombinant hepatitis B vaccine, as compared with 10 mcg doses of plasma derived vaccine H-B-VAX, both given in conjunction with HBIG at birth in preventing chronic hepatitis B infection among: 1. Infants born to mothers positive for HBsAg and HBeAg. 2. Infants born to mothers positive for HBsAg and Negative for HBeAg.
VACCINE:	Yeast Recombinant Hepatitis B Vaccine Lot 987/C-K734 H-B-VAX, Plasma Derived Hepatitis B Vaccine Lot 1032K Lot 2455J Lot 0027L Lot 1507J Hep-B-Gammagee Lot 0031L Lot 1120K
PRIMARY INVESTIGATOR:	E. K. Yeoh, M.D., B.S., M.R.C.P. Consultant Physician Medical "A" Unit Queen Elizabeth Hospital Kowloon, Hong Kong
SECONDARY INVESTIGATORS:	W. K. Chang, M.P., B.S., F.R.C. Path. Consultant Microbiologist Queen Mary Hospital Hong Kong Patricia Ip, M.B., B.S., M.R.C.P. Consultant Pediatrician United Christian Hospital Kowloon, Hong Kong

Study B62

SECONDARY INVESTIGATORS: (Contd)	Enid Chan, M.B., B.S., F.R.C.O.G. Consultant Obstetrician United Christian Hospital Kowloon, Hong Kong K. H. Chan, M.B., B.S., M.R.C.P. Consultant Pediatrician Caritas Medical Centre Kowloon, Hong Kong Charles Fung, M.D., B.S., M.R.C.O.G. Consultant Obstetrician Caritas Medical Center Kowloon, Hong Kong
STUDY LOCATION:	United Christin Hospital Hip Wo Street Kowloon, Hong Kong Caritas Medical Centre Wing Hong Street Kowloon, Hong Kong Queen Mary Hospital Pokfulam Road Hong Kong
DATE INITIATED:	February, 1985
DATE COMPLETED:	In progress.
STUDY POPULATION:	The 300 population consists of approximately 150 infants born to mothers who are positive for HBsAg and HBeAg and 150 infants born to mothers who are HBsAg positive and HBeAg negative. Other criteria for eligibility of the infants include the following: 1) Birth weight $\geq$2000 grams. 2) Apgar score $\geq$7 (taken at 5 mins.) 3) Good health

Study 862

PROCEDURE:

At the first prenatal visit, a blood specimen is obtained from prospective mothers and assayed for HBsAg. Women who are detected to be positive for HBsAg are recruited into the study. A second prenatal blood specimen will be obtained from women who wish to participate and assayed for HBsAg. A third blood specimen will be obtained from the women at parturition and assayed for HBsAg and HBeAg. Eligible infants born to HBsAg, HBeAg positive women will be randomized into Groups 1 and 2. Infants of HBsAg positive and HBeAg negative women will be randomized into Groups 3 and 4.

Infants in all four groups receive HBig and hepatitis B vaccine within 12 hours after birth in different sites (anterior thighs). The second and third doses of vaccine are administered one and six months after birth. Infants in Groups 1 and 3 receive recombinant vaccine (5 mcg) and those in Groups 2 and 4 received plasma-derived vaccine (10 mcg).

Blood specimens are obtained ffrom the infants prior to vaccination and 1, 3, 6, 9, 12, 18 and 24 months post initial injection. All specimens are assayed for HBsAg, and anti-HBs. Anti-HBc is also tested in the infants' sera at 18 months. A follow-up blood sample is also obtained from the mother at six months. Assays are performed by W. K. Chang using RIA kits.

RESULTS:

HEALTHY INFANTS

HEP-B-GAMMAGEE Lot #0031L or 1120K at time 0
5 mcg Lot 987/C-K734 at 0, 1, and 6 months
10 mcg H-B-VAX Lot #1032K, 2455J, 0027L or 1507J at 0, 1, and 6 months

A. HEALTHY INFANTS BORN TO HBsAg-POSITIVE and HBeAg-POSITIVE MOTHERS

1. Number Vaccinated:

Dose Level	Injection No. 1	2	3
5 mcg Recombinant	40	37	12
10 mcg Plasma	28	27	10

Study 862

RESULTS (Contd)

B. HEALTHY INFANTS BORN TO HBsAg-POSITIVE, HBeAg-NEGATIVE MOTHERS:

1. Number Vaccinated:

Dose Level	Injection No. 1	2	3
5 mcg Recombinant	75	73	30
10 mcg Plasma	85	79	25

2. Serologic Results:

A. Healthy Infants Born to HBsAg-Positive and HBeAg-Positive Mothers

At three months, 100% (24/24) of the infants who received yeast recombinant vaccine and 100% (19/19) of the infants who received plasma-derived vaccine developed protective levels (mIU/ml $\geq$10) of anti-HBs (excludes infants who were HBsAg-positive at birth). Table 1 gives the range of antibody titers observed at 1 and 3 months.

Four infants who received yeast recombinant vaccine were HBsAg-positive at birth. At one month, two of these were negative for HBsAg. Of the two who remained positive for HBsAg, one has been followed through three months and has remained positive at that time.

Two infants who received plasma-derived vaccine were HBsAg-positive at birth. Both were negative for HBsAg at one onth. One infant, who was negative for HBsAg at one and three months became positive at six months. The serum sample at that time was anti-HBc IgM-negative.

Refer to Figure 1 for a summary of HBsAg positivity in these infants.

Study 862

RESULTS (Contd)

B. Healthy Infants Born to HBsAg-Positive, HBeAg-Negative Mothers

At three months, 100% (41/41) of the infants who received yeast recombinant vaccine and 100% (42/42) of those who received plasma-derived vaccine developed protective levels (mIU/ml $\geq$10) of anti-HBs (excluding infants HBsAg positive at birth). Table 2 gives the range of antibody titers observed at 1 and 3 months.

Two infants who received yeast recombinant vaccine were HBsAg-positive at birth. Both were negative at one month. An additional infant, who was HBsAg-negative at one and three months was HBsAg-positive at six months. The six month serum was anti-HBc IgM-negative. This infant seroconverted for anti-HBs by three months.

Two infants who received plasma-derived vaccine were HBsAg positive at birth. Both were negative at one month. Another infant tested positive for HBsAg at one month. Additional serology is not yet available for this infant. Three additional infants, who were negative for HBsAg at one and three months, were positive at six months. In two of these, the six month sera were anti-HBc IgM-negative. All three of these had seroconverted for anti-HBs at three months.

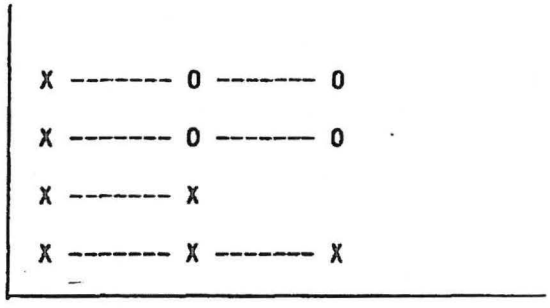
Figure 1 presents a summary of HBsAg positivity in these infants.

3. Clinical Complaints:

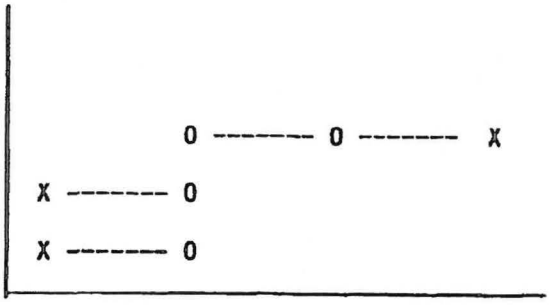
Currently, only a preliminary summary of clinical complaint data is available. The investigator has reported that there have been no clinical complaints among the recipients of either vaccine other than one infant who had a fever of 37.8°C on the day following the first injection. This infant received yeast recombinant vaccine.

Figure 1

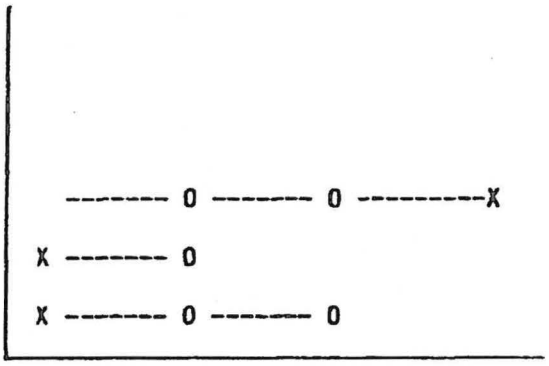
HBsAg Positive Infants in Study 862



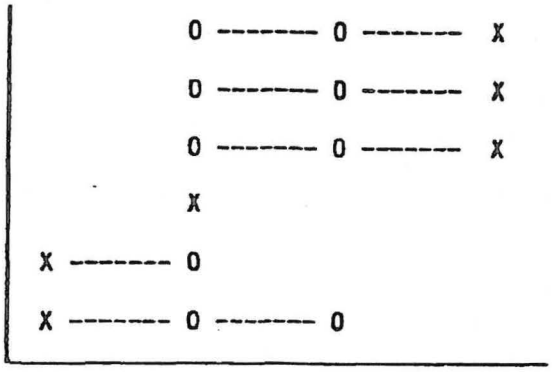
Infants born to HBeAg Positive Mothers who Received Yeast Recombinant Vaccine



Infants born to HBeAg Positive Mothers who Received Plasma-Derived Vaccine



Infants born to HBeAg Negative Mothers who Received Yeast Recombinant Vaccine



Infants born to HBeAg Negative Mothers who Received Plasma-Derived Vaccine

X = HBsAg positive
0 = HBsAg negative

3246I/1
1/23/86

Table 1

Anti-HBs in HBsAg Negative Infants* Born to HBeAg Positive Mothers
Who Received Yeast Recombinant Vaccine

Infants Age at Testing	Number Tested	% (Proportion) Anti-HBs mIU/ml			
		2.1-10	11-49	50-99	≥100
1 month	31			3.2 (1/31)	96.7 (30/31)
3 months	24		41.7 (10/24)	41.7 (10/24)	16.7 (4/24)

*Excludes four who were HBsAg positive at birth

Anti-HBs in HBsAg Negative Infants* Born to HBeAg Positive Mothers
Who Received Plasma-Derived Vaccine

Infants Age at Testing	Number Tested	% (Proportion) Anti-HBs mIU/ml			
		2.1-10	11-49	50-99	≥100
1 month	25			12.0 (3/25)	88.0 (22/25)
3 months	19		52.6 (10/19)	15.8 (3/19)	31.6 (6/19)

*Excludes two infants who were HBsAg positive at birth

3246I/2
1/23/86

Table 2

Anti-HBs in HBsAg Negative Infants* Born to HBeAg Negative Mothers
Who Received Yeast Recombinant Vaccine

Infants Age at Testing	Number Tested	% (Proportion) Anti-HBs mIU/ml			
		2.1-10	11-49	50-99	≥100
1 month	68		1.5 (1/68)	4.4 (3/68)	94.1 (64/68)
3 months	41		41.5 (17/41)	36.6 (15/41)	21.9 (9/41)

*Excludes two who were HBsAg positive at birth

Anti-HBs in HBsAg Negative Infants* Born to HBeAg Negative Mothers
Who Received Plasma-Derived Vaccine

Infants Age at Testing	Number Tested	% (Proportion) Anti-HBs mIU/ml			
		2.1-10	11-49	50-99	≥100
1 month	76			2.6 (2/76)	97.4 (74/76)
3 months	42		40.5 (17/42)	23.8 (10/42)	35.7 (15/42)

*Excludes two infants who were HBsAg positive at birth

3246I/3
1/23/86

PROGRAM: Alum-Adsorbed Yeast Recombinant Hepatitis B Vaccine, Study 864

PURPOSE: This study is designed to evaluate rates of chronic hepatitis B antigenemia in infants at extremely high risk of infection who are treated with a combination of HBIG and yeast recombinant hepatitis B vaccine.

VACCINE: Yeast Recombinant Hepatitis B Vaccine
Lot # 985/C-K732 (5 mcg HBsAg/ml)
Lot # 987/C-K734 (5 mcg HBsAg/ml)

Hep-B-GAMMAGEE
Lot # 1120K
2745J
2660J
0031L

PRINCIPAL INVESTIGATOR: Cladd E. Stevens, M.D.
The Lindsley F. Kimball Research Institute
New York Blood Center
New York, New York 10021

SECONDARY INVESTIGATORS: Rita H. Fisher, M.D.
Chief of Neonatology
St. Vincent's Hospital
New York, New York 10011

Myron Tong, M.D.
Director, Liver Research Center
Huntington Memorial Hosp.
Pasadena, CA 91109

Pearl Toy, M.D.
San Francisco General Hosp.
San Francisco, CA 94110

STUDY LOCATIONS: New York University Hospital
550 First Avenue
New York, NY 10016

Beekman Downtown Hospital-
New York Infirmary
170 William Street
New York, NY 10038

Study 864

STUDY LOCATIONS:
(Contd)

Huntington Memorial Hospital
100 Congress Street
Pasadena, CA 91105

French Hospital
532 W. College St.
Los Angeles, CA 90012

University of California S.F.
Medical Center
Parnassus Avenue
San Francisco, CA 94143

St. Mary's Hospital Medical Center
450 Stanyan
San Francisco, CA 94117

Columbia Presbyterian Medical Center
622 West 168th Street
New York, NY

Saint Vincent's Hospital
153 West 11th Street
New York, NY 10011

California Hospital
1414 S. Hopr St.
Los Angeles, CA 90015

Garfield Hospital
150 Hampton
Monterey Park, CA 91754

San Francisco General Hospital
1001 Portrero Avenue
San Francisco, CA 94110

Santa Clara Valley Medical Center
751 South Bascom Avenue
San Jose, CA 95128

Highland General Hospital
1411 E. 31st Street
Oakland, CA 94553

Kaiser Foundation Hospital
2425 Geary Blvd.
San Francisco, CA 94115

Study 864

STUDY LOCATIONS: (Contd)

Children Hospital, S.F.
3700 California St.
San Francisco, CA 94118

Contra Costa County Health Services
2500 Alhambra Avenue
Martinez, CA 94553

Kaiser Permanente Hospital
280 West MacArthur Blvd.
Oakland, CA 94611

DATE INITIATED: September 1, 1984.

DATE COMPLETED: In progress.

STUDY POPULATION: The study population consists of healthy infants (i.e., weigh $\geq$ 2000 gms at birth and have an apgar score $\geq$ 7 at 5 minutes) born to mothers of Asian descent who are positive for both HBsAg and HBeAg. Enrollment of at least 80 infants is planned.

STUDY PROCEDURE: Infants, whose parents consent to their enrollment in the study, receive a single intramuscular injection of HBIG (0.5 cc) within the first few hours after birth. Pregnant women of Asian descent are screened for hepatitis infection prior to delivery to identify potential study candidates within the first few hours of birth (infants). The initial 1.0 ml (5 mcg HBsAg) intramuscular injection of recombinant hepatitis B vaccine is given in the first few days after birth. The second injection of vaccine is administered at one month of age, and the third injection is received at six months. If an infant becomes HBsAg positive prior to completing the immunization schedule, no further vaccine injections will be administered.

A cord blood specimen is obtained at the time of delivery and just prior to administration of the HBIG. A venous blood sample is also to be taken from the infant at this time. The cord sample is tested for HBsAg and the venous sample for HBsAg and ALT (SGPT).

Study 864

STUDY PROCEDURE: (Contd)

Follow-up venous blood samples are obtained from the infant at 1, 3, 6, 9, 12, and 18 months of age. These sera are tested for HBsAg, anti-HBc, anti-HBs and ALT. A follow-up blood sample is also obtained from the mother at or near the time of delivery to verify her HBsAg and HBeAg positive status.

Sera are being tested at the New York Blood Center by radioimmunoassay using (b) (4) kits. Some sera may be tested for yeast antibody at MSDRL.

RESULTS:

INFANTS OF HBsAg$^+$/HBeAg$^+$ MOTHERS

5 mcg Lot 985/C-K732 at 0, 1, and 6 months

1. Number Vaccinated:

Injection #		
1	2	3
134	120	61

2. Serologic Results:

Four infants were positive for HBsAg at birth. One of these has been followed for 1 month only and remains positive. Three of the infants have been followed for at least 3 months and were still antigen positive. One of the three has been followed for 6 months and is still positive. This infant is now classified as a chronic carrier (Figure 1).

At present, only a preliminary summary of antibody response data is available. According to the study investigator, all of 46 antigen negative infants followed for 6 months had developed anti-HBs (S/N $\geq$2.1). Refer to Table 1 for anti-HBs responses through 9 months of follow-up.

Study 864

RESULTS: (Contd)

3. Clinical Complaints:

Currently, only a preliminary summary of clinical complaint data is available. The study investigator has reported the following overall frequencies of complaints:

Type of Complaint	Frequency in % by Injection # 1	2	3
Fever ≥100°F	3.2 (3/95)	1.3 (1/77)	9.7 (3/31)
Local redness or swelling	2.1 (2/95)	1.3 (1/77)	19.4 (6/31)
Rash	1.1 (1/95)	5.2 (4/77)	3.2 (1/31)
Other	3.2 (3/95)	2.6 (2/77)	0 (0/31)

There have been no serious or alarming reactions attributable to vaccine.

Reactions Reported to the DoBRR:

A neonatal male received HBIG and his first injection of vaccine at birth (b)(6) . On the fifth and sixth days post-vaccination he had a temperature of 38°C. The infant received Tylenol and his temperature returned to normal. He received his second and third injections of vaccine without temperature elevation.

A male neonate received 1 dose of HBIG at birth (b)(6) . He developed physiologic jaundice on day 4 (b)(6) after birth. The jaundice resolved by day 7. The first injection of vaccine was administered on (b)(6) The infant received the second and third injections of vaccine without local or systemic complaints.

On the first day of life, a female neonate had a fever of 101.7°F. The child received one dose of HBIG at birth. The following day her temperature was normal and she received her first injection of vaccine. Her temperature remained within normal limits after the first, second, or third injections of vaccine.

Study 864

RESULTS: (Contd)

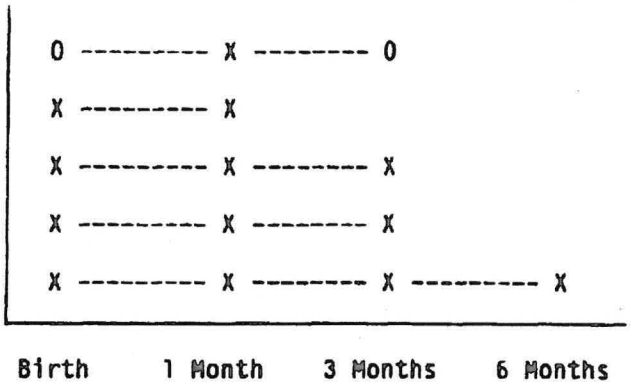
A male neonate was reported to have developed jaundice during the post-natal period. He had received one dose of HBIG at birth (b)(6) and his first injection of vaccine three days later. The second injection of vaccine was administered on (b)(6)

There has been one death among study participants unrelated to vaccine.

A one-day old full term male infant with Apgar scores of 9 at both 1 and 5 minutes was entered into the study. He received one dose of Hep-B-Gammagee on the day of birth and his first dose of vaccine on the following day. The infant did well until 2 days post delivery when poor feeding was noted. A cardiac evaluation revealed a murmur and possible atrial septal defect. His clinical condition deteriorated requiring intubation and administration of pressor and diuretic agents. The infant died 7 days after birth after circulatory collapse and the onset of arrhythmias. An autopsy revealed intracranial, renal and hepatic hemorrhage, hypoplasia of the left auricle and ventricle, a patent foramen ovale, an atrial septal defect, and aspiration pneumonia.

Figure 1

HBsAg Positive Infants in Study 864



X = HBsAg^{+}

O = HBsAg^{-}

Table 1

Yeast Recombinant Hepatitis B Vaccine in
Perinatal Transmission: Anti-HBs Response in HBsAg Negative Infants
Study 864

Infant's Age at Testing	Number Tested	% Anti-HBs (Titer in S/N)		
		2.1-19.9	20-49.9	≥50
3 Months	82	36	43	21
6 Months	46	7	29	64
9 Months	19	0	16	84

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PROGRAM:	Alum-Adsorbed Yeast Recombinant Hepatitis B Vaccine, Study 878
PURPOSE:	To evaluate the efficacy of yeast recombinant hepatitis B vaccine, given in conjunction with hepatitis B immune globulin at birth, or alone, in preventing chronic hepatitis B infection among infants born to mothers positive for HBsAG and HBeAg.
VACCINE:	Yeast Recombinant Hepatitis B Vaccine Lot 979/C-K564 (10 mcg HBsAg/ml)
IMMUNE GLOBULIN:	Hepatitis B Immune Globulin HEP-B-GAMMAGEE Lot 0031L
PRIMARY INVESTIGATOR:	Sun Tsung-tang, M.D. Chairman, Department of Immunology Cancer Institute (Hospital) Chinese Academy of Medical Sciences Panjiaynan, Beijing People's Republic of China
SECONDARY INVESTIGATOR:	Dr. Chu Yuan Yun Qidong Liver Institute Qidong People's Republic of China
STUDY LOCATION:	Qidong Liver Institute Qidong, Jiangsu Province People's Republic of China
DATE STUDY INITIATED:	July, 1985
DATE STUDY COMPLETED:	In progress
STUDY POPULATION:	The study population consists of 70-150 healthy infants born to mothers who are positive for HBsAg and HBeAg.

Study 878

STUDY PROCEDURE:

Prior to enrollment of an infant in this study, a prenatal blood sample is obtained from each prospective mother. A follow-up blood sample is also obtained from the mother at the time of delivery to verify the eligibility of infants for the study.

Eligible infants receive a single 0.5 ml intramuscular injecton of hepatitis B immune globulin in the anterior thigh within 12 hours of birth, followed by a 0.5 ml (5 mcg HBsAg) intramuscular injection of yeast recombinant hepatitis B vaccine in the contralateral anterior thigh at 0 (within 12 hours of birth), 1 and 6 months, or vaccine alone according to the same regimen.

The parent or guardian will be asked to record the child's temperature and any local or systemic complaints for five days after each injection of vaccine.

A blood sample is obtained from each infant prior to vaccination and, if possible, at 3, 6, 12, and 24 months.

All serum samples obtained from each mother are assayed for HBsAg, anti-HBs, anti-HBc, and ALT.

All serum samples obtained from each infant are assayed for HBsAg, anti-HBs, and when indicated for anti-HBc and ALT. Samples may be tested for yeast antibody. In addition, samples with an anti-HBs titer $\geq$25 mIU/ml may be tested to determine anti-a and anti-d subtype specificity.

RESULTS:

To date, 20 infants have received one injection of vaccine in conjunction with HBIG. No serious or alarming reactions attributable to vaccination have been reported. Clinical follow-up data and serologic results are not yet available. The study continues in progress.

PROGRAM:	Alum-Adsorbed Yeast Recombinant Hepatitis B Vaccine, Study 892
PURPOSE:	To compare the efficacy of yeast recombinant hepatitis B vaccine and plasma-derived hepatitis B vaccine in preventing chronic hepatitis B infection among infants born to mothers positive for HBsAg and for HBeAg.
VACCINE:	1. Yeast Recombinant Hepatitis B Vaccine Lot 81954I/18071/C-L220 (10 mcg HBsAg/ml) 2. Plasma-Derived Hepatitis B Vaccine Lot 0027L (20 mcg HBsAg/ml)
PRIMARY INVESTIGATOR:	Dr. Hu Zong-Han Department of Biological Products Inspection Bureau of Pharmaceutical and Biological Inspection Ministry of Health Temple of Heaven, West Gate Beijing, People's Republic of China
SECONDARY INVESTIGATOR:	Dr. Meng Lingxian
STUDY LOCATIONS:	The Third Hospital Chinese Medical University Shen Yang, People's Republic of China Shen Yang Municipal Anti-Epidemic Station Shen Yang, People's Republic of China Fujian Provincial Anti-Epidemic Station Fujian, People's Republic of China Guang Dong Provincial Anti-Epidemic Station Guang Dong, People's Republic of China Si Chuan Provincial Anti-Epidemic Station Si Chaun, People's Republic of China
DATE STUDY INITIATED:	December, 1985
DATE STUDY COMPLETED:	In progress

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Study 892

STUDY POPULATION: The study population consists of 200 healthy infants of either sex, born to mothers who are positive for HBsAg and for HBeAg.

STUDY PROCEDURE: Prior to enrollment of an infant in this study, a prenatal blood sample is obtained from each prospective mother. A follow-up blood sample is also obtained from the mother at the time of delivery to verify the eligibility of infants for the study.

Infants are randomly assigned to receive yeast recombinant or plasma-derived hepatitis B vaccine as follows:

Group	Vaccine	Dose	Number	Regimen
1	Recombinant	5 mcg	50	0.5 ml intramuscular injection of vaccine within 12 hours of birth and at 1 and 6 months
		10 mcg	50	1.0 ml intramuscular injection of vaccine within 12 hours of birth and at 1 and 6 months
2	Plasma	10 mcg	50	0.5 ml intramuscular injection of vaccine within 12 hours of birth and at 1 and 6 months
		20 mcg	50	1.0 ml intramuscular injection of vaccine within 12 hours of birth and at 1 and 6 months

The parent or guardian will be asked to record the child's temperature and any local or systemic complaints for five days after each injection of vaccine.

A blood sample is obtained from each infant prior to vaccination and at 1, 3, 6, 7 or 8, 12, and 24 months of age.

Study 892

STUDY PROCEDURE: (Contd)

All serum samples obtained from each mother are assayed for HBsAg, HBeAg, anti-HBe and ALT.

All serum samples obtained from each infant are assayed for HBsAg and anti-HBs, and when indicated for anti-HBc and ALT.

RESULTS:

To date, 20 infants have received one injection of yeast recombinant or plasma-derived hepatitis B vaccine. No serious or alarming reactions attributable to vaccination have been reported. Clinical follow-up data and serologic results are not yet available. The study continues in progress.

APPENDIX 1

EQIVALENCE OF ANTIBODY RAISED TO YEAST RECOMBINANT HEPATITIS B VACCINE AND TO PLASMA-DERIVED HEPATITIS B VACCINE

Antibodies and Protective Efficacy

Clinical studies with the plasma-derived vaccine established the relationship between antibody to the hepatitis B surface antigen (anti-HBs) and protection against hepatitis B infection.

To support the protective efficacy studies that have been done in chimpanzees (with yeast-derived hepatitis B vaccine) and those ongoing in neonates, serological studies designed to demonstrate the equivalence of anti-HBs antibodies raised to yeast-derived hepatitis B vaccine and to plasma-derived hepatitis B vaccine are being carried out.

These are:

A. Cross-Adsorption of Antibodies Raised to Plasma-Derived Vaccine and to Yeast-Derived Vaccine

(b) (4) assays (b) (4) showed that anti-HBs raised in plasma vaccinees completely reacted with yeast-derived vaccine antigen and, conversely, antibodies raised to the yeast-derived vaccine were completely cross-reactive with plasma-derived vaccine antigen (see Table 1). This demonstrates that both vaccines raise essentially identical antibodies. Had either vaccine raised substantially different antibodies, incomplete cross-reactivity would have been observed with the converse antigens. This did not occur.

B. Binding of Anti-HBs to Synthetic Peptide (affinity constants)*

An important common antibody is elicited in recipients of both vaccines as demonstrated by (b) (4) (b) (4) (an important amino acid sequence in HBsAg). Binding of this antibody to this peptide can be used to derive affinity constants by (b) (4)

(b) (4)

Affinity constants are shown in Table 2. It will be noted that the average affinity constant for antibodies induced in plasma vaccinees is 4 X 10^{7} and that in the yeast vaccinees is also 4 X 10^{7}.

* Affinity constant defines the binding strength of the antibody to its respective antigen.

C. Inhibition Assay with "Protective" Monoclonal anti-HBs Antibody

Using the "protective" monoclonal antibody (b) (4) (b) (4) in an inhibition assay, the presence of antibodies to the identical HBsAg epitope can be detected and quantitated in the sera of plasma and yeast vaccinees (see Table 3). It has been clearly shown that recipients of our plasma and yeast hepatitis B vaccines make such antibodies in equivalent amounts.

D. Avidity Constants

If the assay described under Affinity Constants is used with the entire hepatitis B surface antigen against sera from plasma and yeast vaccinees, a property can be derived which is called the avidity constant (see Table 4). The similarity of these constants for the anti-HBs antibodies in plasma and yeast vaccinees further demonstrates the qualitative similarity of antibodies elicited in recipients of both vaccines.

E. IgM/IgG Antibody Pattern

Comparisons of IgM and IgG anti-HBs in plasma and yeast vaccinees revealed similar patterns; i.e., initial production of IgM anti-HBs changes over to IgG anti-HBs as the vaccination regimen progresses in recipients of each vaccine (see Table 5).

F. D Antibody, A Antibody Pattern

The plasma and yeast vaccinees sera show similar patterns with respect to the formation of antibody specific for the subtype determinants of HBsAg (type ad HBsAg used as immunogen). D antibody is initially high and as the vaccine regimen progresses, this converts to A antibody and is nearly 100% A at the completion of the 3-dose regimen (see Table 6). [A is the broadly reactive and protective antibody in anti-HBs.]

TABLE 1

CROSS NEUTRALIZATION OF ANTIBODIES

YEAST HBsAB (CL934)	(b) (4)	% NEUTRALIZATION WITH AY PLASMA	AD PLASMA	AD YEAST
(b) (6) (4 MOS.)		98	100	100
(4 MOS.)		98	100	100
(4 MOS.)		98	100	99
(4 MOS.)		94	100	99
(4 MOS.)		97	100	99
(4 MOS.)		87	100	100

PLASMA HBSAB (LOT 820)	(b) (4)	% NEUTRALIZATION WITH AY PLASMA	AD PLASMA	AD YEAST
(b) (6) (3 MOS.)		86	100	99
(3 MOS.)		97	99	95
(3 MOS.)		94	100	97
(3 MOS.)		50	100	93
(3 MOS.)		86	100	97
(3 MOS.)		87	100	87

ASSAYS PERFORMED AT MSDRL BY W. MILLER ET AL.

TABLE 2

AFFINITY CONSTANTS OF HBsAb IN HUMANS RECEIVING
RECOMBINANT OR PLASMA DERIVED VACCINE

(b) (4)

TABLE 3

INHIBITION OF THE PROTECTIVE MONOCLONAL HBsAb BY HUMAN HBsAb FROM RECOMBINANT OR PLASMA-DERIVED VACCINE

PLASMA VACCINEES

SAMPLE	(b) (4)	% INHIBITION (b) (4)	% INHIBITION MSDRL
(b) (6)		19	17
		18	--
		46	--
		77	74
		97	--
		23	--
		38	--
		99	97
		37	--
		86	79

YEAST VACCINEES

SAMPLE	(b) (4)	% INHIBITION (b) (4)	% INHIBITION MSDRL
(b) (6)		66	59
		19	--
		14	18
		65	--
		13	--
		38	44
		68	--
		13	--
		10	8
		69	--
		--	83
		--	79
		--	77
		--	81

METHOD (b) (4)

TABLE 4

AVIDITY CONSTANTS OF HBsAb

YEAST RECOMBINANT HBs VACCINEES

BLEEDING NUMBER		AVIDITY CONSTANT
(b) (6)	(4 MOS.)	4×10^{10}
	(4 MOS.)	1×10^{10}
	(4 MOS.)	16×10^{10}
	(4 MOS.)	5×10^{10}
	(4 MOS.)	1×10^{10}
	(4 MOS.)	14×10^{10}

(b) (4)

PLASMA DERIVED HBs VACCINEES

BLEEDING NUMBER		AVIDITY CONSTANT
(b) (6)	(3 MOS.)	4×10^{10}
	(3 MOS.)	8×10^{10}
	(3 MOS.)	4×10^{10}
	(3 MOS.)	4×10^{10}
	(3 MOS.)	7×10^{10}
	(3 MOS.)	8×10^{10}

(b) (4)

ASSAYS PERFORMED AT MSDRL BY W. MILLER ET AL.

TABLE 5

RELATIVE PROPORTIONS OF (b) (4) ANTI-HBs IN SERUM

STUDY	CASE	TYPE*	PERCENT OF TOTAL (b) (4) MONTHS POST INITIAL VACCINATION					
			PRE	1	2	3	4	6
779 (YEAST)	(b) (6)	(b) (4)	0	100	0	-	-	-
			0	0	100	-	-	-
			0	-	25	-	0	-
			0	-	75	-	100	-
			0	-	0	-	0	-
			0	-	100	-	100	-
			0	-	-	0	-	-
			0	-	-	100	-	-
			0	100	-	1	-	-
			0	0	-	99	-	-
542 (PLASMA)			0	-	0	-	4	-
			0	-	100	-	96	-
			0	-	37	-	-	0
			0	-	63	-	-	100
			0	-	8	-	12	-
			0	-	92	-	88	-
639 (PLASMA)			0	-	-	0	-	0
			0	-	-	100	-	100
			0	-	-	0	-	0
			0	-	-	100	-	100

ASSAY (b) (4) (b) (4)

TABLE 6

PERCENTAGES OF ANTI-HBs SPECIFIC FOR A AND D DETERMINANTS OF HBsAg IN POST-VACCINATION SERA

YEAST VACCINEES

Months After First Injection	Number of Samples	% Anti-A Range	% Anti-A Mean	% Anti-D Range	% Anti-D Mean
1	20	0-100	65	0-100	34
3	69	33-100	91	0-63	9
6	44	58-100	93	0-37	7
7	27	81-100	95	0-19	5
8	12	94-100	97	0-6	3

PLASMA VACCINEES

Months After First Injection	Number of Samples	% Anti-A Range	% Anti-A Mean	% Anti-D Range	% Anti-D Mean
1	0	-	-	-	-
3	3	87-89	88	9-13	12
6	6	79-95	89	5-18	10
7	8	74-97	93	2-26	7
12	7	87-96	94	4-13	6

ASSAYS PERFORMED AT MSDRL BY W. MILLER ET AL. (b) (4) METHOD (b) (4)

(b) (4)

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244 RESPONSE TO RECOMBINANT YEAST HEPATITIS B VACCINE IN NONRESPONDERS TO PLASMA-DERIVED HEPATITIS B VACCINE

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Preliminary reports suggested that recombinant yeast hepatitis B vaccine (R-HBvac) might be more immunogenic than the triply inactivated plasma-derived hepatitis B vaccine (P-HBvac) (Hepatology 1984;4:1077). Therefore, to test this hypothesis, we administered three 10 µg doses of R-HBvac (Merck Sharp & Dohme Research Laboratories) at time 0, 1, and 6 months to 14 normal adults who had failed to respond to one or more courses (3-6 doses) of P-HBvac. The frequency [# positive/# vaccinated] (%) and geometric mean titer (mIU/ml) of anti-HBs responses were as follows:

Month	1	2	3	6
anti-HBs+	5/13 (39)	8/14 (57)	7/14 (50)	7/13 (54)
GMT ± SD	17 ± 7	39 ± 10	36 ± 23	8 ± 7

For comparison, the same data are charted below for 65 seronegative health workers, never previously vaccinated, after receiving R-HBvac:

Month	1	2	3	6
anti-HBs+	26/65 (38)	53/62 (86)	61/65 (94)	60/62 (97)
GMT ± SD	7 ± 4	38 ± 4	50 ± 4	72 ± 4

The mean ± SD ages of the 8 initial nonresponders who ultimately did respond and the 6 who did not were indistinguishable, 38 ± 8 and 41 ± 15. The response to R-HBvac in almost 60% of nonresponders to P-HBvac appeared promising, especially when compared with a 40% rate of low-level, poorly sustained anti-HBs responses in P-HBvac nonresponders given a second course of P-HBvac (Hepatology 1984;4:1077); however, the level of antibody fell substantially by six months, when measured just prior to the booster injection. Additional follow-up will be necessary to determine whether the antibody response to R-HBvac in nonresponders to P-HBvac increases and is sustained after booster immunization.

Butterly L, Watkins E, Hinkle CH, Dienstag JL. Response to recombinant hepatitis B vaccine in nonresponders to plasma-derived hepatitis B vaccine. Hepatology 1985; 5:1007 (abstract).

Journal of Medical Virology 17:57–62 (1985)

Safety and Immunogenicity of a Recombinant Hepatitis B Vaccine

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A hepatitis B vaccine produced in yeast by recombinant DNA technology was evaluated using 5-μg and 10-μg doses in a randomized trial lasting 7 months in 110 male armed forces recruits aged 17–19 years. Results were compared to those of an identical trial of a plasma-derived vaccine. No allergic reactions were observed, and the rate of mild side effects was similar to the plasma-derived vaccine. Seroconversion rates in the first month were 60% (33/55) and 67% (37/55) with the 5-μg and 10-μg doses of the recombinant vaccine, respectively. All participants seroconverted by 3 months, and none lost antibody. These results are very similar to those for plasma-derived vaccine. Comparison of titres of antibody to hepatitis B surface antigen (anti-HBs) showed a slightly higher level with the 10-μg than with the 5-μg dose of the recombinant vaccine. Geometric mean titres of anti-HBs after the booster dose were similar in the 5-μg and 10-μg dose recombinant vaccine groups (2,620 and 2,748 IU/l, respectively) and in the 5-μg plasma-derived vaccine group (3,591 IU/l) but significantly higher (9,227 IU/l) with the 10-μg dose of the plasma-derived vaccine. These results confirm the safety and immunogenicity of the recombinant vaccine, although further study is needed on the duration of immunity.

Key words: active immunoprophylaxis, hepatitis B, plasma-derived hepatitis B vaccine, recombinant hepatitis B vaccine

INTRODUCTION

The safety and immunogenicity of plasma-derived hepatitis B vaccines have been amply demonstrated by clinical trials in various high-risk groups in different parts of the world [Szmuness et al, 1980; Maupas et al, 1981; Beasley et al, 1983]. However, the high cost and limited availability have prevented widespread use of these vaccines, especially in the less developed areas where they are needed most. Vaccination programmes are at present generally limited to groups at high risk of infection, such as hospital personnel. Within these programmes, acceptance may have been affected by unfounded loss of confidence in the safety of the vaccine, following

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isons at each time point. All analyses were carried out after logarithmic transformation of anti-HBs titres.

RESULTS

The trial was completed in all but two recruits, both the losses being from the group receiving the 10-μg dose. One was lost from the study after receiving the second dose and the other after the booster dose. No participant developed either clinical or asymptomatic viral hepatitis, and neither anaphylactoid nor other allergic reactions were observed. Mild side effects were reported, but no case of fever above 37.5°C was noted, and no local discomfort or pain lasting for more than 1 day. The overall frequency of side effects was very similar to that reported for the plasma-derived vaccine in the earlier study (Table I).

The two groups receiving recombinant vaccine showed a similar and rapid immune response (Table II). Both of the recruits who did not complete follow-up had already seroconverted in the first month. All participants had seroconverted by 3 months, and none lost antibody. These rates are very similar to those recorded in the trial of the plasma-derived vaccine. Differences in seroconversion rates at 1 month between the four groups in Table II are not significant ($\chi^2_3 = 5.26$; $P = 0.15$).

Geometric mean titres (GMT) of anti-HBs are shown in Table III. Multivariate comparison between the two recombinant vaccine groups shows that they do not differ in rates of increase of anti-HBs ($F_{3,104} = 1.99$; $P > 0.1$). The 10-μg group had significantly higher GMT of antibody overall than the 5-μg group ($t_{106} = 2.08$; $P < 0.05$), although the difference appears to be small after the booster dose.

Multivariate comparisons of the anti-HBs profiles in the 5-μg and 10-μg recombinant vaccine groups against the corresponding plasma-derived vaccine groups show

TABLE I. Frequency of Side Effects by Type of Vaccine (Summed Over Administrations of Vaccine)

Side effect	Recombinant vaccine (%)	Plasma-derived vaccine (%)
Local pain	6.0	9.0
Fever < 37.5°C	16.3	11.1
Other	2.3	2.3
Total	24.6	22.4

TABLE II. Number (%) of Seroconverted (anti-HBs > 2.1 IU/l) by Month and Type of Vaccine

	Recombinant vaccine		Plasma-derived vaccine	
Month	5 μg (N = 55)	10 μg (N = 55)	5 μg (N = 50)	10 μg (N = 50)
1	33 (60)	37 (67)	40 (80)	32 (64)
3	55 (100)	54 (100)[a]	49 (98)	49 (98)
6	55 (100)	54 (100)[a]	49 (98)	49 (98)
7	55 (100)	53 (100)[b]	49 (98)	50 (100)

[a]One person lost to follow-up.
[b]Two persons lost.

population, with all participants in both the trials of recombinant and plasma-derived vaccines being males of similar age living under exactly similar conditions.

Comparison of the 5-μg and 10-μg doses of recombinant vaccine shows a small advantage to the 10-μg dose overall in terms of GMT anti-HBs, although any final difference is slight. Davidson and Krugman [1985], with older vaccinees of both sexes, reported a final (8 months) GMT anti-HBs in the 10-μg group more than double that in the 5-μg group, although the statistical significance is not stated. Irrespective of dose, all participants in our trial reached the 10 IU/l generally regarded as protective. Only five (4.6%; two from the 5-μg group and three from the 10-μg group) had titres lower than 100 IU/l.

Our results confirm reports of the safety and immunogenicity of the Merck Sharp and Dohme recombinant yeast hepatitis B vaccine [Jilg et al, 1984b; Davidson and Krugman, 1985]. The minor differences observed in the immune response stress the need for more extensive studies in various population groups under consideration for vaccination, before the appropriate dose and vaccination scheme are decided. Similarly, further follow-up is required to establish the duration of protective levels of antibody [Jilg et al, 1984a; Davidson and Krugman, 1985]. Finally, in assessing the efficacy of the vaccine, information concerning the quality of the anti-HBs induced should complement the data on the anti-HBs levels achieved [Brown et al, 1984].

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IMMUNOGENICITY OF RECOMBINANT YEAST HEPATITIS B VACCINE

SIR,—In Dr Jilg and colleagues' study (Nov 24, p 1174) in thirty recipients of recombinant hepatitis B vaccine "the immune response in the recombinant vaccine group was less pronounced during the first months than in the plasma vaccine group, as shown by lower seroconversion rates and lower mean anti-HBs levels". They compared a 10 μg dose of recombinant vaccine with a 20 μg dose of plasma-derived vaccine.

As indicated in the table, our results in a similar study in one hundred and seven seronegative health professionals, 21–30 years of age, revealed essentially the same immune response in recipients of 5 μg and 10 μg doses of recombinant yeast hepatitis B vaccine when compared with a comparable group who received 20 μg doses of plasma-derived vaccine.

Valid conclusions cannot be drawn from studies in thirty or a hundred vaccinees. More extensive studies will be required to evaluate anti-HBs response and its persistence in recipients of recombinant hepatitis B vaccine. In the meantime, our initial results are encouraging.

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SEROCONVERSION RATES AND GEOMETRIC MEAN TITRES (GMT) OF SERONEGATIVE INDIVIDUALS, ADULTS GIVEN RECOMBINANT OR PLASMA-DERIVED HEPATITIS B VACCINE

Test* (mo)	Recombinant vaccine†						Plasma derived (20 μg)‡	
	10 μg			5 μg				
	Anti-HBs response	mIU/ml (GMT)	S/N ratio (GMT)	Anti-HBs response	mIU/ml (GMT)	S/N ratio (GMT)	Anti-HBs response	S/N ratio (GMT)
0	..	..	..	..	..	..	..	..
1	22/51 (43%)	42	19	21/56 (37%)	95	25	18/47 (38%)	20
2	48/51 (94%)	88	37	51/56 (91%)	69	30	34/47 (72%)	37
3	50/51 (98%)	145	52	52/56 (93%)	120	51	45/47 (96%)	70
6	49/50 (98%)	321	83	53/56 (95%)	184	42	44/47 (94%)	94
7/8	45/44 (98%)	1011	164	49/50 (98%)	899	124	46/47 (98%)	141

*Vaccine given at 0, 1, and 6 months. Follow-up to 7 months (plasma derived) or 8 months (recombinant). †Above lot 972/C-8 only. ‡Above lot 751.

Davidson M, Krugman S. Immunogenicity of recombinant yeast hepatitis B vaccine. Lancet 1985; 1:108-9.

RECOMBINANT YEAST HEPATITIS B VACCINE: SIDE EFFECTS AND IMMUNOGENICITY COMPARED WITH PLASMA-DERIVED HEPATITIS B VACCINE.

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A yeast recombinant hepatitis B vaccine (Merck Lot no. 972/C-K444) was evaluated in 107 seronegative health professionals, 21-30 years of age. The clinical and antibody responses were compared with the results of a previous similar study using a plasma-derived hepatitis B vaccine (Merck Lot no. 751).

The vaccine was administered at 0, 1 and 6 months to the following three groups: 1) 51 adults who received a 10 mcg dose of recombinant vaccine; 2) 56 adults who received a 5 mcg dose of recombinant vaccine, and 3) 47 adults who received a 20 mcg dose of plasma-derived vaccine. The three groups included medical students, house staff, and nurses who were of comparable age and sex.

Results

Side effects were negligible in all three groups. They consisted of transient, local soreness at the site of the inoculation in about 25% of the vaccinees in each group. No systemic reactions were observed.

The seroconversion rates and geometric mean titers are summarized in the Table. The results are essentially the same for all three groups. Under the conditions of this study the 5 mcg and 10 mcg doses of recombinant hepatitis B vaccine were just as immunogenic as a 20 mcg dose of plasma-derived hepatitis B vaccine.

Comment

A recent report by Jilg et al (Lancet 1984; 2:1174-75) described a similar study in 30 seronegative medical students and laboratory workers whose age and sex were comparable to those in our groups. They stated that "the immune response in the recombinant vaccine group was less pronounced during the first months than in the plasma vaccine group, as shown by lower seroconversion rates and lower mean anti-HBs levels." Our results in 107 similar recipients of the recombinant hepatitis B vaccine do not support this conclusion.

It is obvious that valid conclusions cannot be drawn from studies involving either 30 or 100 vaccinees. More extensive studies will be required to determine anti-HBs response and its persistence in recipients of recombinant hepatitis B vaccines.

Davidson M, Krugman S. Recombinant yeast hepatitis B vaccine: Side effects and immunogenicity compared with plasma-derived hepatitis B vaccine. Submitted for publication to Hepatitis Scientific Memoranda.

TABLE

Seroconversion Rates and Geometric Mean Titers of Seronegative Adults Who Received Recombinant Yeast Hepatitis B Vaccine (Merck Lot No. 972/C-K444) or Plasma-Derived Hepatitis B Vaccine (Merck Lot No. 751).

Time Interval (Months)	Recombinant Hepatitis B Vaccine					
	10 mcg dose			5 mcg dose		
	anti-HBs response	mIU/ml GMT	S/N Ratio GMT	anti-HBs response	mIU/ml GMT	S/N Ratio GMT
0	-	-	-	-	-	-
1	22/51 (43%)	42	19	21/56 (37%)	55	25
2	48/51 (94%)	88	37	51/56 (91%)	69	38
3	50/51 (98%)	145	52	52/56 (93%)	128	51
6	49/50 (98%)	321	63	53/56 (95%)	184	42
8	45/46 (98%)	1911	164	49/50 (98%)	839	124

Vaccine given at 0, 1 and 6 months.
Age Range: 21 - 30 years

Time Interval (Months)	Plasma-Derived Hepatitis B Vaccine 20 mcg dose	
	anti-HBs response	S/N Ratio GMT
0	-	-
1	18/47 (38%)	20
2	34/47 (79%)	37
3	45/47 (96%)	79
6	44/47 (94%)	94
7	46/47 (98%)	141

Vaccine given at 0, 1 and 6 months.
Age range: 21 - 30 years

SAT-LA.10

CLINICAL EVALUATION OF A RECOMBINANT HEPATITIS B VACCINE

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Thirty healthy, young volunteers free of any HBV markers were vaccinated with a recombinant hepatitis B vaccine prepared by Merck, Sharp & Dohme, West Point, PA. Ten µg HBsAg were administered intramuscularly at time 0, and one month later. Seroconversion rates and geometric mean concentrations after 1, 2 and 3 months were compared with an age- and sex-matched control group vaccinated with 20 µg of plasma derived vaccine (Merck Sharp & Dohme) (Table 1).

Table 1: Comparison of immune response after recombinant vaccine and plasma derived vaccine.

month	seroconversion %		anti-HBs (geom. mean) mIU	
	recombinant vaccine	plasma vaccine	recombinant vaccine	plasma vaccine
1	27	44	8.6	15.2
2	70	95	37.8	52.5
3	97	95	27.4	164.4

In the recombinant vaccine group, 38% of the total anti-HBs at month 3 was directed against the determinant a of HBsAg, compared to 30% in the control group. No increase in antibody titers against candida albicans was found in recipients of the recombinant vaccine 4 weeks after the second injection as compared to prevaccination levels. No serious side effects were observed in any of the vaccinated individuals.

Deinhardt F, Jilg W, Zoulek G, Lorbeer B, Wilske B. Clinical evaluation of a recombinant hepatitis B vaccine. In: Vyas GN, Dienstag JL, Hoofnagle JH, eds. Viral Hepatitis and Liver Disease. Orlando:Grune and Straton, 1984:699.

RECOMBINANT YEAST HEPATITIS B VACCINE: IMMUNOGENICITY AND SAFETY. JL Dienstag, E Watkins, and CA Hinkle. Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA.

Cumbersome to produce, expensive, and limited in supply, currently available human plasma-derived hepatitis B vaccines are likely to be replaced in the future by "genetically engineered" vaccines. Recently, a recombinant DNA vaccine was prepared in recombinant yeast Saccharomyces cerevisiae strain 2150-2-3 cells transformed with the plasmid pHBS 56-GAP347/33, containing the gene for hepatitis B surface antigen (HBsAg/ad) (Valenzuela et al. Nature 1982; 298:347-50). Purified by biochemical and biophysical methods from the yeast extract, the HBsAg particles synthesized by these yeast cells are not glycosylated but otherwise are indistinguishable from native 22 nm HBsAg particles. Treated with formalin and adsorbed to alum, the recombinant vaccine is immunogenic and protective in experimental animals. We administered three 10 µg doses of the recombinant hepatitis B vaccine (Merck Sharp & Dohme Research Laboratories) at time 0, 1, and 6 months to 60 seronegative adult health workers. The frequency and geometric mean titer (mIU/ml) of anti-HBs responses were as follows:

Month	1	2	3	4	5	6
Number	37	29	30	29	25	16
anti-HBs+	41%	83%	93%	97%	96%	94%
GMT ± SD	7 ± 2	33 ± 5	36 ± 4	46 ± 4	55 ± 4	79 ± 4

94 ± 9 (mean ± SD) % of the anti-HBs was specific for the a determinant of HBsAg. Changes in antibodies to yeast antigens were negligible. The most frequent adverse reaction was transient soreness at the injection site, occurring after 52% of first, 37% of second, and 55% of third injections. No serious adverse effects were encountered, and neither type B nor non-B hepatitis has occurred in any vaccinee. These preliminary results demonstrate that the recombinant yeast hepatitis B vaccine is safe and that 10 µg of the recombinant vaccine is equivalent in immunogenicity to 20 µg of the plasma-derived vaccine.

Dienstag JL, Watkins E, Hinkle CA. Recombinant yeast hepatitis B vaccine: immunogenicity and safety. Hepatology 1984; 4:1077 (Abstract).

SAT-LA-50

SAFETY AND IMMUNOGENICITY OF A RECOMBINANT HEPATITIS B VACCINE

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Currently available, licensed hepatitis B vaccines are prepared from plasma obtained from hepatitis B surface antigen (HBsAg) carriers. Cumbersome to produce, expensive, and available in limited supply, the plasma vaccine is likely to be replaced in the future by one of a number of later generation vaccines. Recently, a recombinant DNA vaccine was prepared in recombinant yeast Saccharomyces cerevisiae strain 2150-2-3 cells transformed with plasmid pHBS56-GAP347/33, which contains the gene for HBsAg (Valenzuela et al, Nature 1982; 298:347-50). The HBsAg synthesized by these yeast cells was purified from the yeast extract by physical and chemical methods and was found to be indistinguishable from native 22 nm HBsAg particles, except that the HBsAg is not glycosylated. Treated with formalin and adsorbed to alum, the recombinant vaccine is comparable in purity to the plasma vaccine and is immunogenic and protective in experimental animals.

We studied the immunogenicity and safety of recombinant hepatitis B vaccine Lot 934, formulated to contain 10 micrograms of HBsAg per 1.0 ml dose (Merck Sharp & Dohme Research Laboratories). Thirty seronegative adult health care workers received three 1.0 ml doses of the recombinant vaccine at time 0, 1 and 6 months. Adverse effects were limited to soreness at the injection site, and immunogenicity was excellent, approximating 50% at one month. Three months of follow-up will be complete by the time of the International Meeting.

Dienstag JL, Watkins E, Hinkle CA. Safety and immunogenicity of a recombinant hepatitis B vaccine (Abstract). In: Vyas GN, Dienstag JL, Hoofnagle JH, eds. Viral Hepatitis and Liver Disease. Orlando:Grune and Stratton, 1984:710.

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A. Billiau, E. De Clercq and H. Schellekens (eds.)

IMMUNE RESPONSE AFTER VACCINATION WITH RECOMBINANT HEPATITIS B VACCINE AS COMPARED TO THAT AFTER PLASMA-DERIVED VACCINE

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SUMMARY

Thirty-one individuals (health care workers) were vaccinated with recombinant hepatitis B vaccine (10 µg dose) and their immune response (anti-HBs) was compared to that of twenty-five health care workers after vaccination with plasma-derived vaccine (20 µg dose). Although the seroconversion rate and the percentage of anti-HBs/a antibodies at month 7 were comparable, the geometric mean titre of anti-HBs at month 7 was considerably lower for the recombinant vaccine group (857.4 vs. 6736.5 IU/l). However, vaccinees from the two groups showing seroconversion at month 1 had comparable titres at month 7. Raising the dose of HBsAg in the recombinant vaccine may favourably influence the seroconversion rate at month 1 and thereby the immune response after three injections.

INTRODUCTION

Only six years ago, a plasma-derived vaccine was introduced to overcome the worldwide problem of hepatitis B infections.[1] General acceptance of the vaccine, however, has been hampered by the high costs and in particular by doubts about the suitability of infectious plasma as its source. Public concern has waned considerably since the discovery of human T-cell leukaemia virus as a possible cause of the acquired immune deficiency syndrome and the possibility of investigating the efficacy of inactivation of this virus in vaccine preparation procedures.[2] Meanwhile, an alternative for the latter objective has been found in the preparation of hepatitis B surface antigen by recombinant DNA technology in the yeast *Saccharomyces cerevisiae*.[3] Although the yeast recombinant DNA produced HBsAg polypeptides, unlike the native HBsAg, are not glycosylated, the vaccine thus prepared has proven to induce protective antibodies during chimpanzee challenge studies.[4] Its safety and immunicity in man has been demonstrated by several groups of investigators.[5 6 7 8] One of these studies is presented here.

Soon after the introduction of the plasma-derived vaccine it was uncertain whether an HBsAg/adw vaccine would protect against HBsAg/ayw virus infections. Nowadays it is generally known from chimpanzee studies as well as experiments in man[3 9 10] that the antibodies directed against the main determinant a provide cross protection for infections with strains not incorporated in the vaccine.

However, in the plasma-derived vaccine studies[11 12] it was found that the relative proportion of anti-HBs antibodies is variable, which may partially account for hepatitis B infections in the first few months after vaccination. Therefore, the need to monitor the development of anti-HBs/a antibodies after vaccination is stressed.

MATERIAL AND METHODS

Population

The study population consisted of 56 health care workers. Recombinant vaccine was given to 31 individuals (17 female, 14 male; mean age 32 ± 2 yr, range 20-59); plasma-derived vaccine was given to 25 individuals (13 female, 12 male; mean age 30 ± 2 yr, range 22-53). Participants to this study were negative for HBsAg, anti-HBc, and anti-HBs and had a normal alanine transferase level at the entrance to the study.

Vaccine

Participants were vaccinated at 0, 1, and 6 months with either a 10 µg HBsAg/adw dose of the recombinant hepatitis B vaccine (Merck, Sharp and Dohme, lot 972/C-K444) or a 20 µg HBsAg/adw dose of the plasma-derived vaccine (Merck, Sharp and Dohme, lot 1510 J). Recombinant HBsAg used here was purified by hydrophobic interaction chromatography.[8 7]

Assays

HBsAg, anti-HBc, anti-HBs were measured in commercially available kits (Ausria II, Corab, and Ausab; Abbott Laboratories, North Chicago, USA). The concentration of anti-HBs was calculated by the method of Hollinger et al.[13] and expressed in IU/l after comparison with the WHO standard preparation (125 IU/l), obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands. Calculations were made for positive results in Ausab only (sample/negative control ratio ≥ 2.1). Samples containing more than 200 IU/l were diluted and retested. Dilutions were made in the negative control serum from Ausab. Estimation of the proportion of anti-HBs/a antibodies was performed according to the method of Hoofnagle et al.[14] In short, undiluted or diluted sera containing 1000-2000 cpm in Ausab were incubated for 2 h at room temperature with pooled HBsAg/ad, HBsAg/ay, and normal human serum, respectively. Pooled sera

included reference sera from Dr.A.M.Couroucé-Pauty as mentioned in an earlier study.[13] Reduction of cpm after incubation with HBsAg/ay strains measured the anti-HBs/a proportion of the total amount of anti-HBs, since the vaccine consisted of HBsAg/adw only. The proportion of anti-HBs/d(w) antibodies was obtained by subtracting the reduction percentage after incubation with HBsAg/ay pooled serum from the reduction percentage after incubation with HBsAg/ad pooled serum.

RESULTS

Table I shows a delayed seroconversion rate for the recombinant vaccine group as compared to the plasma-derived vaccine group in the course of the vaccine study. Similar results were obtained for titres $\geq$ 10 IU/l, the supposed protective level of antibodies.

TABLE I

SEROCONVERSION RATE AFTER VACCINATION WITH RECOMBINANT (10 µg) AND PLASMA-DERIVED (20 µg) VACCINE IN HEALTH CARE WORKERS

Month	Recombinant vaccine	Plasma-derived vaccine	Recombinant vaccine	Plasma-derived vaccine
	Percentage seroconversion		Percentage anti-HBs $\geq$ 10 IU/l	
1	19(6/31)	56(14/25)	13(4/31)	40(10/25)
2	77(24/31)	96(22/23)	39(12/31)	74(17/23)
3	90(28/31)	100(25/25)	74(23/31)	96(24/25)
6	94(29/31)	100(25/25)	87(27/31)	100(25/25)
7	100(31/31)	100(22/22)	100(31/31)	100(22/22)

Geometric mean titres of anti-HBs were significantly lower in the recombinant vaccine group as compared to the plasma-derived vaccine group at month 2, 3, 6, and 7 (Table II).

After three injections females had significantly ($p < 0.05$) higher anti-HBs titres than males in the recombinant vaccine group (1412 vs. 468 IU/l) but not in the plasma-derived vaccine group (6036 vs. 7519 IU/l).

All vaccinees were negative for HBsAg and anti-HBc at 7 months and had normal alanine transferase levels in all sera obtained. Table III illustrates the increase of the relative proportion of anti-HBs/a antibodies from about 60% at month 1 to about 100% at month 7 following the first injection for both vaccine groups as measured by specific absorption. In any sample at

TABLE II

GEOMETRIC MEAN TITRES OF ANTI-HBs AFTER VACCINATION WITH RECOMBINANT VACCINE (10 µg) AND PLASMA-DERIVED VACCINE (20 µg)

Month	Recombinant vaccine GMT in IU/l	Plasma-derived vaccine GMT in IU/l
1	16.8(n= 6)[*]	19.7(n=14)
2	13.7(n=24)	61.8(n=22)[D]
3	34.8(n=28)	177.7(n=25)[D]
6	69.0(n=29)	291.1(n=25)[D]
7	857.4(n=31)	6736.5(n=22)[D]

[*] Responders only [D] $p < 0.05$ Wilcoxon's rank sum test

TABLE III

DETERMINATION OF SUBDETERMINANT SPECIFIC ANTIBODIES AFTER VACCINATION WITH RECOMBINANT VACCINE (10 µg) AND PLASMA-DERIVED VACCINE (20 µg) AS DETERMINED BY SPECIFIC ABSORPTION

Month	Recombinant vaccine			Plasma-derived vaccine		
	No. samples	% anti-HBs/a (range)	% anti-HBs/d	No. samples	% anti-HBs/a (range)	% anti-HBs/d
1	4	60(19- 92)[*]	39	6	57(22- 99)	42
2	9	81(40- 98)	17	15	83(25- 99)	17
3	18	95(74-100)	5	23	88(26-100)	11
6	26	99(89-100)	1	24	94(43-100)	6
7	31	99(90-100)	1	22	97(91-100)	3

[*] Determination of anti-HBs/a and anti-HBs/d was limited by the minimum amount of 25 IU/l anti-HBs.

month 7 the proportion of anti-HBs/a antibodies was at least 90%. In sera with anti-HBs $\geq$ 10 IU/l at month 1, two out of four in the recombinant vaccine group and three out of six in the plasma-derived vaccine group had less than 50% anti-HBs/a. In only two cases, one in each group, the anti-HBs/a percentage at month 1 was above 90, suggesting an anamnestic response. Geometric mean titres for those vaccinees with a positive anti-HBs response

at month 1 increased to 11158 IU/l (n=6) in sera from the recombinant vaccine group and to 13748 IU/l (n=13) in sera from the plasma-derived vaccine group, both at month 7.

DISCUSSION

Table IV compares the results of the immunicity of recombinant hepatitis B vaccine of Merck, Sharp and Dohme in our study with results of others as recently published. [3 6 7 8] Several lots of vaccine with minor differences in the purification procedure were used. Comparison is made in some studies with earlier results using plasma-derived vaccine from the same manufacturer. In our study vaccination with recombinant vaccine and plasma-derived vaccine took place simultaneously. Serum samples could therefore be handled similarly and investigated with the same batch of reagents.

We found anti-HBs development during the first six months following the first injection very similar to Scolnick et al.[3] and Jilg et al.[6]. After the booster injection at month 6 we found a lower geometric mean titre than observed by others. The proportion of anti-HBs/a antibodies, however, was very similar for the two vaccine groups and increased from 60% at month 1 to about 100% at month 7.

Interestingly, we noted high titres of anti-HBs at month 7 for those vaccinees who had already shown seroconversion at month 1. Titres in this subgroup were comparable to those in early responders in the plasma-derived vaccine group. Since we had the lowest seroconversion rate at month 1 observed so far for recombinant vaccine (19%), this may explain the low geometric mean titre at month 7. The reason for the initial low conversion rate in our study is unknown. Sex and age differences with other study groups may have contributed. Sex and age effects may have their most pronounced influence on vaccination of weak responders.[16 17] The highest seroconversion rate (67%) and the highest geometric mean titre (2749 IU/l) at month 7 were observed by Papaevangelou et al.[8] in male recruits aged 17-19 years.

If our observations can be confirmed in more extended studies, equalizing the dose of HBsAg in the recombinant vaccine preparation to that of the plasma-derived vaccine may favourably influence the seroconversion rate at month 1 and the amount of anti-HBs produced after three injections.

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TABLE IV

IMMUNE RESPONSE AFTER VACCINATION WITH RECOMBINANT AND PLASMA-DERIVED HEPATITIS B VACCINE AS COMPARED FROM LITERATURE

Authors	Dose	Geometric mean titres in IU/l Month 1	3	6	7	No.	Mean age	No. of men	No. of women	Lot no.
Recombinant vaccine										
Scolnick et al.[5]	10 µg	8	56	68	1905	15	33,23-53	10	5	934
Jilg et al.[6]	10 µg	9	29	68	2135	30	25,21-34	13	17	934
Papaevangelou et al.[8]	10 µg	11	198	189	2749	55	17-19	55		979
Davidson and Krugman[7]	10 µg	42	145	321	1911	51	21-30			972
Present study	10 µg	17	35	69	857	31	32,20-59	14	17	972
Plasma-derived vaccine										
Jilg et al.[6]	20 µg	15	164	263	4299	41	25,21-32	18	23	
Present study	20 µg	20	177	291	6737	25	30,22-53	12	13	
Papaevangelou et al.[8]	10 µg	4	278	492	9227	50				

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ANTI-HBs/a DETERMINATION AFTER HEPATITIS B VACCINATION

Sir, — The determination of antibodies against the a determinant from HBsAg after vaccination with HBsAg/adw is of interest, since anti-HBs/a antibodies are thought to be protective. Two methods for measurement of these antibodies are in use: 1) Specific absorption of serum anti-HBs with pooled HBsAg/ay.[1] The reduction of anti-HBs, as measured in direct tests for anti-HBs, reflects the proportion of anti-HBs/a antibodies. 2) A radioimmunoassay or ELISA using HBsAg/ay as solid phase antigen.

We applied methods 1 and 2 on sera from our comparative study on the immunogenicity of recombinant and plasma-derived vaccine. Thirty-one health care workers were vaccinated with recombinant vaccine and twenty-five with plasma derived-vaccine, both from Merck, Sharp & Dohme. All participants showed seroconversion at month 7. In all individual sera sampled at month 7 we found that the anti-HBs contained 90–100% anti-HBs/a antibodies by method 1 in both groups of vaccinees, as published elsewhere.[2] The percentage of anti-HBs/a according to method 2 was calculated from the geometric mean anti-HBs concentrations found in Ausab (Abbott Laboratories) using HBsAg/adw,ayw coated beads ($Ausab_t$) and in Ausab using HBsAg/ayw coated beads ($Ausab_{ay}$). All sera were prediluted until the concentration in $Ausab_t$ was less than 200 IU/l. For both assays, $Ausab_t$ and $Ausab_{ay}$, anti-HBs was determined by linear intrapolation of the results from the test samples in between the results from a twofold dilution series of the WHO reference serum. Results in the recombinant vaccine and the plasma-derived vaccine groups showed 80 and 40% anti-HBs/a, respectively. An ELISA (Organon Diagnostics Research Labs, Oss, The Netherlands) using microtitre plates coated with HBsAg/ayw, showed 60 and 45% anti-HBs/a in the recombinant vaccine and plasma-derived vaccine groups, respectively.

The specific absorption method confirmed the findings of Scolnick et al[3] (90–100% anti-HBs/a after recombinant vaccine administration), whereas our results with method 2 (anti-HBs/a "specific" tests) are in accordance with those of Jilg et al.[4] (49% anti-HBs/a after plasma-derived vaccine administration). Our results show, however, that the apparent differences in percentage anti-HBs/a as published by Scolnick

Heijtink RA, Schalm SW. Anti-HBs/a determination after hepatitis B vaccination. Submitted for publication to Lancet.

et al. and Jilg et al. are not primarily related to the differences in the vaccines but to the methodology applied to assess anti-HBs/a antibodies.

Which test system provides the most useful data? In method 1, anti-HBs antibodies are absorbed with an excess of pooled HBsAg/ay, which may contain other epitopes in addition to a- and y-related epitopes. High, but also low affinity antibodies[5] are removed and the reduction of anti-HBs will be optimal. This test will likely overestimate the percentage of neutralizing anti-HBs/a antibodies.

Problems with the determination of anti-HBs/a by method 2 are illustrated in the figure. Anti-HBs/a containing reagents (anti-a monoclonal antibody, positive control serum Ausab$_t$ test, the WHO reference standard containing 200 IU/l) and anti-d monoclonal antibody were tested in various dilutions simultaneously in Ausab$_t$ and Ausab$_{ay}$. Monoclonal anti-a gave almost identical results in both tests, and fitted the line of identity. About 10% (based on cpm) anti-d monoclonal antibody measured in Ausab$_t$ was detected in Ausab$_{ay}$, presumably as a result of non-specific absorption. The line obtained with the Ausab positive control serum was also linear, but not parallel to the line of identity. Results for the WHO reference serum showed a curved line. Quantitation of anti-HBs/a using the Ausab positive control and/or the WHO reference serum as a standard is therefore in fact impossible, although both standards contain more than 90% anti-HBs/a antibodies, according to specific absorption. In our opinion, antibodies with variable affinity and/or reacting with different epitopes must be present to explain the discrepancies.

Detailed description of the anti-HBs response after vaccination is important. The initial interest concerned the quantitative aspects. Many investigators are shifting their interest to the qualitative aspect of the anti-HBs evoked by vaccines.

In our opinion, there is an urgent need for unambiguous test systems for vaccine evaluation, especially when results from vaccines with HBsAg from different sources (plasma, recombinant, synthetic) or with different compositions (with and without pre-s-polypeptides) are to be compared.

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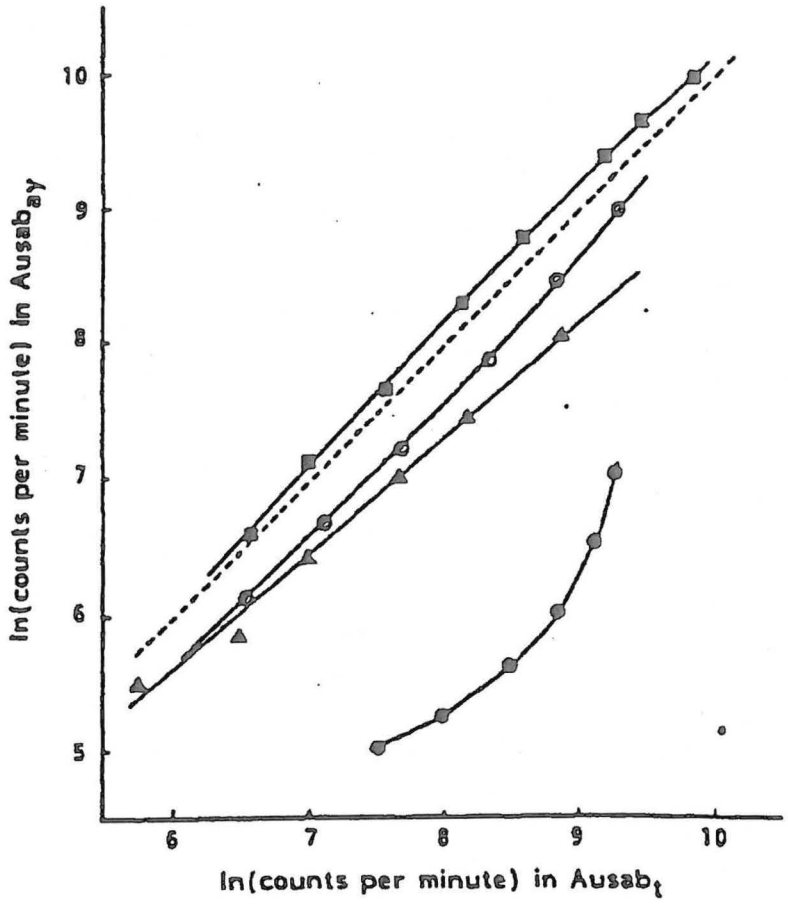
Figure: Comparison between counts per minute (natural logarithm) in $Ausab_t$ (HBsAg/adw,ayw coated beads) and $Ausab_{ay}$ (HBsAg/ayw coated beads) in dilution series of monoclonal anti-a (■——■), monoclonal anti-d (●——●), the WHO standard reference serum (○——○), and the $Ausab_t$ positive control serum (▲——▲). The dashed line represents identical results in $Ausab_t$ and $Ausab_{ay}$.


10
9
8
7
6
5
6
7
8
9
10
ln(counts per minute) in Ausab$_{ay}$
ln(counts per minute) in Ausab$_{t}$

Maurice R. Hilleman, Eugene B. Buynak, Henry Z. Markus,
Robert Z. Maigetter, William J. McAleer, Arlene A. McLean,
William J. Miller, D. Eugene Wampler and Robert E. Weibel

22

Control of Hepatitis B Virus Infection: Vaccines Produced from Alexander Cell Line and from Recombinant Yeast Cell Cultures

Human hepatitis B virus has yet to be successfully grown in cell culture. Current vaccines *(1–5)* against hepatitis B virus employ hepatitis B surface antigen (HBsAg) that is obtained from the plasmas of human carriers of hepatitis B virus infection. The HBsAg stimulates antibody against the virus and prevents infection and illness caused by the agent. Available supply of suitable carrier plasma and the need to apply highly technical procedures to purify HBsAg and to render it safe limit the amount of plasma-derived vaccine that can be made and impose cost restrictions on its use. We have sought to explore alternative sources of HBsAg to prepare hepatitis B vaccine and have prepared and tested vaccines made from HBsAg secreted from carrier hepatocellular carcinoma (HCC) cells *(6)* and from yeast cells carrying an expression vector of HBsAg *(7)*. The properties of such vaccines are the subject of this report.

HEPATITIS B VACCINE DERIVED FROM A HEPATOCELLULAR CARCINOMA CELL LINE

Alexander and co-workers *(8)* recovered a continuous line of HCC cells (PLC/PRF/5: Alexander cell line) in culture from a cancer patient who was also an HBsAg carrier. These cells, grown in vitro, secrete HBsAg but no infectious virus *(9)*. The immortality of such cells offered an alternative source of HBsAg but the yields grown in conventional culture were too small to be considered feasible economically *(10–13)*. McAleer and colleagues *(6,14)*, in our laboratories, adapted the Alexander cells to growth in Vitafiber pseudocapillary units. In this system, the Alexander cells are propagated in the interstices of bundles of semipermeable membrane capillaries through which the growth medium is circulated. Maximal

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Hilleman MR, Buynak EB, Markus HZ, Maigetter RZ, McAleer WJ, McLean AA, et.al. Control of Hepatitis B virus infection: Vaccines produced from alexander cell line and from recombinant yeast cell cultures. In: Vyas GN, Dienstag JL, Hoofnagle JH, eds. Viral Hepatitis and Liver Disease. Orlando:Grune and Statton, 1984:307-14.

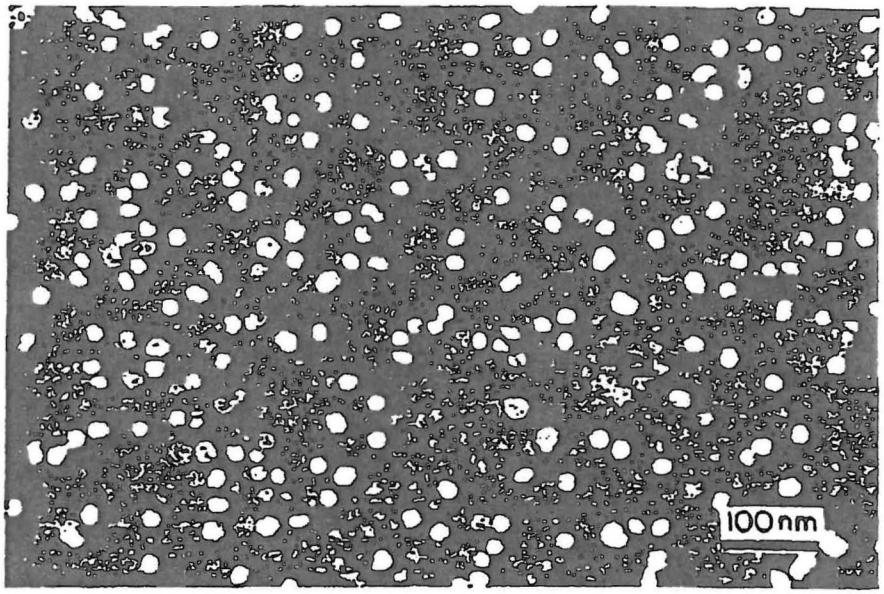

Fig. 22.1. Hepatitis B surface antigen particles purified from Alexander hepatocarcinoma cell culture fluid.

yields of HBsAg were obtained in the fiber bundle units under conditions that lowered cellular metabolism. This consisted of lowering the temperature of incubation to 32°C with the addition of 10^{-4} molar caffeine to the circulating medium. Such cells could be maintained for periods up to a year, with periodic harvest of fluid that contained an amount of HBsAg equal to that of some human plasmas. The HBsAg was readily purified from the cell culture fluid by immune affinity chromatography followed by digestion with pepsin and DNase.

Figure 22.1 shows purified HBsAg particles that were indistinguishable morphologically from those that were purified from human plasma. Particles obtained from plasma were essentially identical in all measurable aspects to those purified from Alexander cell fluids. The particles were 22 nm in diameter; the ultraviolet absorption spectra were the same; and the $E^{1\%}$ and the HBsAg to protein ratios were alike.

Purified HBsAg derived from Alexander cells was treated with formaldehyde and was formulated into vaccine *(6)* by absorbing 20 μg of HBsAg to each ml of aluminum hydroxide suspension containing 0.5 mg of aluminum and adding 1:20.000 concentration of thimerosal as preservative. The vaccine was proved safe in tests in four chimpanzees that were given aqueous material by the intravenous route.

The vaccine was assayed for immunizing potency in mice by a standardized extinction dilution assay and was compared with plasma-derived vaccine. Table 22.1 shows that the 50% extinction dose, ED_{50}, was nearly the same for both vaccines and the geometric mean titers were comparable. It is evident that the HBsAg produced in HCC cells is indistinguishable in potency from that derived from plasma.

Table 22.1
Mouse Potency of HBsAg Vaccine Prepared in Alexander Cell Culture Compared with That Prepared from Human Plasma

	Alexander Cell Vaccine		Plasma Vaccine Lot 799-2	
Vaccine (μg)	No. mice positive/total	G.M. Titer*	No. mice positive/total	G.M. Titer
10	8/10	1431	8/10	1729
2.5	8/10	504	9/10	1204
0.625	7/10	74	4/10	8
0.156	0/9	<8	0/10	<8
ED_{50}†	0.79		0.81	

*Geometric mean titer.
†Dose required to seroconvert 50% of mice.

Two persons who were initially seronegative for hepatitis B virus markers and who had advanced central nervous system cancer were given two primary doses of vaccine a month apart and a booster dose 6 months after the initial injection. The findings shown in Table 22.2 revealed that both patients developed antibody to HBsAg (anti-HBs) in low titer. Three persons, two of whom were given only the primary doses and one of whom was given all three doses of vaccine, but were lost to follow-up, demonstrated no anti-HBs response. The slow and relatively low antibody responses to the HCC cell-derived vaccine were similar to those in other immunosuppressed persons who were given vaccine of human plasma origin. The vaccine was well tolerated in all the subjects.

Table 22.2
Findings in Two Cancer Patients who Received Alexander Cell-Produced Hepatitis B Vaccine at Time 0, 1, and 6 Months

		Titer*					
			Months after Vaccination				
Observation	Patient	Pre-vaccine†	1	2	3	6	7
Anti-HBs	717-4	< 8	< 8	8	16	16	36
	-6	< 8	< 8	< 8	ND	< 8	36
HBsA	717-4	–	–	–	–	–	–
	-6	–	–	–	–	–	–
Anti-HBc	717-4	–	–	–	–	–	–
	-6	–	–	–	–	–	–
AST	717-4	18	25	16	24	16	14
	-6	9	7	8	ND	9	6
ALT	717-4	19	23	24	21	22	18
	-6	24	20	20	ND	17	12

*Titer is expressed in units. ND = no determination; – = below the limit of detection.
†7 days prior to starting vaccination.

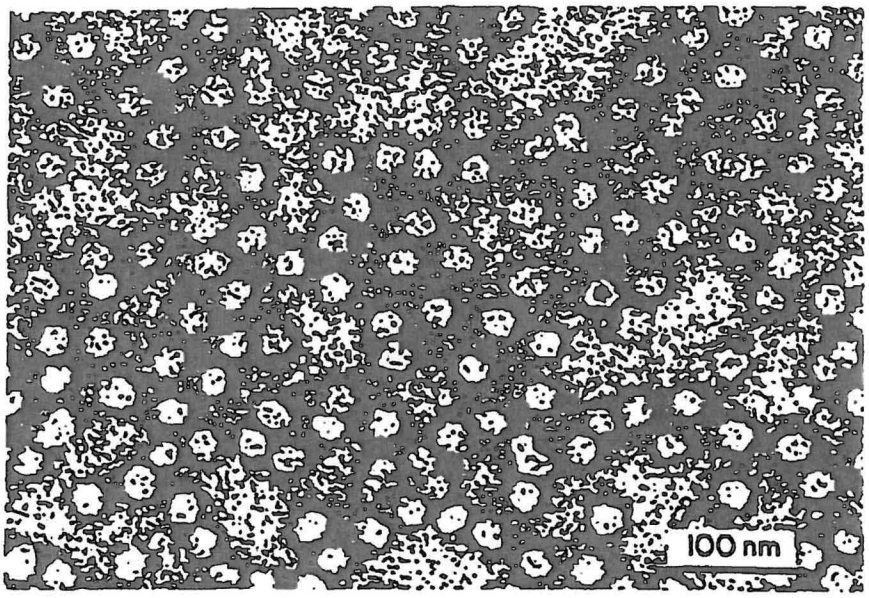

Fig. 22.2. Hepatitis B surface antigen particles purified from recombinant yeast cell culture.

Careful attention was given to the matter of safety of the vaccine, considering its origin from human liver cancer cells. The methods used for treatment in purifying the HBsAg and the DNA-destructive processes aimed at viral and cellular nucleic acids that were used to prepare the vaccine were of such efficiency as to delete any possible oncogenic DNA that might have been theoretically present in the starting fluid.

RECOMBINANT HEPATITIS B VACCINE

Joint efforts between our laboratories and those of W. Rutter and B. Hall led to the preparation of vectors carrying the DNA sequence for HBsAg *(7,15)*. The HBsAg was of subtype *adw* and was produced in fermentation cultures of *Saccharomyces cerevisiae* carrying the vector and employing yeast alcohol dehydrogenase I as the promoter. HBsAg was released from the cells by homogenization and was purified by immune affinity chromatography *(16)*.

Electron microscopy of yeast-derived HBsAg, as shown in Figure 22.2, revealed a homogeneous array of particles free of extraneous morphologic entities. The ultraviolet absorption spectrum was the same as for plasma-derived HBsAg with an $E^{1\%}$ of 45. The SDS-polyacrylamide gel electrophoretic pattern under reducing conditions shown in Figure 22.3 revealed a single band at 23,000 daltons (23K) corresponding to the nonglycosylated polypeptide of HBsAg derived from plasma.

The purified HBsAg was formulated into vaccine by adsorbing to aluminum hydroxide adjuvant to contain 40 μg of HBsAg protein and 0.5 mg aluminum per

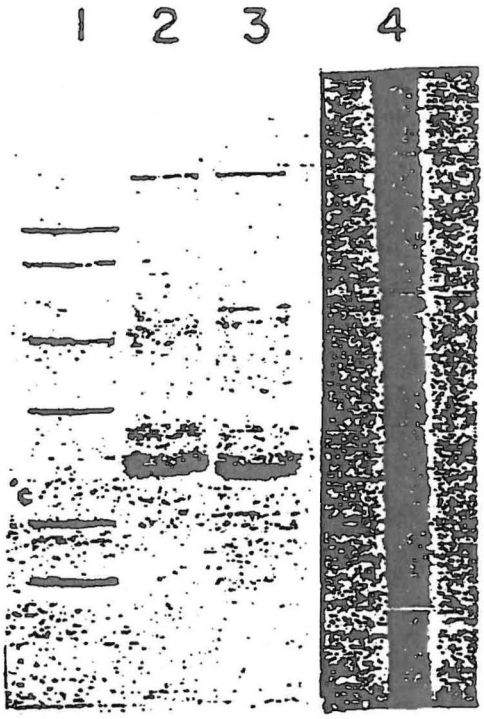

Fig. 22.3. SDS-polyacrylamide gel electrophoresis of purified Alexander cell (lane 2) and yeast-derived (lane 3) hepatitis B surface antigen. Lane 1 contains molecular weight standards and lane 4 contains clarified yeast extract before purification.

dose. The standardized extinction mouse potency test, shown in Table 22.3, demonstrated that the yeast-derived HBsAg was at least as potent as plasma-derived antigen based on the 50% extinction dose (ED_{50}) and the geometric mean titers.

Table 22.4 shows that grivet monkeys also developed antibody following vaccination with the yeast-derived antigen. A single injection at all dose levels resulted in seroconversion of all the animals in both yeast and plasma vaccine groups. High antibody titers were maintained for more than a year.

Protective efficacy was tested in challenge experiments with susceptible chimpanzees. In the tests shown in Table 22.5, four chimpanzees were given three 1-ml injections of the *adw* subtype yeast-derived vaccine 1 month apart and four animals were held as unvaccinated controls. One month after the third dose of vaccine was given, two vaccinated and two control animals were challenged intravenously with *adr* subtype virus and the other four vaccinated or unvaccinated animals were challenged with *ayw* subtype virus. All the vaccinated animals developed antibody following immunization and all were solidly protected against the virus with all serologic and histopathologic markers remaining negative. Protection

Table 22.3
Antigenic Potency in Mice of HBsAg Purified from Yeast and from Human Plasma

Vaccine Source	HBsAg dose per injection (μg protein)	Anti-HBs response after vaccination	
		No. pos./Total	GMT
Human Plasma	10	9/10	563
Lot 799-2	2.5	10/10	2235
	0.625	4/9	32
	0.156	0/10	4
ED_{50}	0.639		
Yeast	40	10/10	5432
Lot 81-4	10	10/10	3400
	2.5	8/10	673
	0.625	8/10	967
ED_{50}	< 0.625		

was afforded irrespective of HBsAg subtype. The finding of subtype cross-protection is consistent with the presence of the common *a* antigen determinant present in all hepatitis B virus subtypes *(17,18)*. That this common *a* antigen suffices to protect against all subtypes was confirmed recently in clinical studies *(19)* in which HBsAg/*ad* vaccine protected renal dialysis staff against type HBsAg/*ay* exposures.

Clinical studies of the yeast cell-derived vaccine have been initiated by our group. The early findings indicate most favorable antibody responses in man that are being reported elsewhere in this symposium (Abstr. SAT. LA 50 and chapter 23).

CONCLUSION

The evolution in our laboratories of a fiber bundle-engineered culture system for production of HBsAg by Alexander HCC cells presents a simple and practical means for hepatitis B vaccine preparation. However, the more recent develop-

Table 22.4
Antigenic Potency in Grivet Monkeys of HBsAg Purified from Yeast and from Human Plasma[a]

Vaccine Source	HBsAg dose per injection (μg protein)	Anti-HBs response after initial vaccine dose (Geometric mean titer)			
		Week 4	Week 8	Week 12	Week 52
Human plasma	10	36	213	170	127
Lot 86016	2.5	343	6227	17348	9924
	0.625	53	4642	3164	5688
	0.156	15	128	83	358
Yeast	40	88	1078	7103	11554
Lot 81-4	10	184	877	8489	4984
	2.5	225	1168	6361	10868
	0.625	109	925	518	313

[a]Vaccine given at time 0 and 4 weeks.

Table 22.5
Protective Efficacy of Purified Yeast HBsAg Vaccine

				Challenge			
				Result			
Vaccine	Chimp	Anti-HBs Titers	HBsAg Subtype	HBsAg	Anti-HBc	AST & ALT Elevations	Liver Pathology
Yeast Vaccine	1	1830	*adr*	0	0	0	0
	2	540	*adr*	0	0	0	0
	3	18300	*ayw*	0	0	0	0
	4	7200	*ayw*	0	0	0	0
Controls	5	< 8	*adr*	+	+	+	+
	6	< 8	*adr*	+	+	+	+
	7	< 8	*ayw*	+	+	+	+
	8	< 8	*ayw*	+	+	+	+

ment, by our group, of HBsAg production in recombinant yeast cells appears to offer advantages that exceed those of the Alexander cell system. The most important advantages of the recombinant vaccine relate to simpler HBsAg production by yeast cells in fermentation tanks and removal of any lingering apprehensions about safety of vaccine derived from a human cancer cell source.

Human plasma-derived hepatitis B virus vaccine is limited by the supply of plasma and the technical complexity of the process to assure safety and efficacy. Alternative technologies developed in our laboratories include production from (a) hepatocellular carcinoma cells (Alexander cell line: PLC/PRF/5) cells in culture, and (b) from recombinant yeast carrying a high expression vector for hepatitis B surface antigen (HBsAg) gene subtype *adw*. HBsAg was purified mainly by affinity chromatography and formulated on alum adjuvant. The polypeptide dimer of HBsAg produced in Alexander cells was identical to that from plasma; the yeast-derived dimer was not glycosylated but was otherwise the same. Both vaccines were as potent as plasma vaccine in mice and both were highly immunogenic when tested in humans. The subtype *adw* yeast vaccine was also highly immunogenic for monkeys and gave solid protection in chimpanzees against challenge with heterologous subtype *adr* and *ayw* viruses. Vaccine prepared from yeast offers a means for simplified production of HBsAg in fermentation tank culture and does not bear the stigma of cancer cell origin of Alexander cell vaccine. Recombinant yeast-derived HBsAg shows great promise for simplified mass production of hepatitis B vaccine.

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Recombinant Yeast Human Hepatitis B Vaccine

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ABSTRACT

The human hepatitis B vaccine of plasma origin prepared by our laboratories has performed well with respect to safety, immunogenicity and protective efficacy. The vaccine has now been used in about 2 million persons worldwide. The recent demonstration of HTLV-III or LAV virus' putative role in AIDS and its ready inactivation by the steps used in vaccine production has removed the last lingering doubts about safety from the standpoints of AIDS in relation to plasma-derived vaccine.

The limit in supply of human hepatitis B carrier plasma and the need to apply highly technical procedures for purification and inactivation stimulated the seeking of an alternative source of antigen from yeast bearing the surface antigen gene. Preliminary data indicate that the recombinant vaccine prepared by our laboratories has shown at least equivalent immunogenicity for animals as well as human adults and children compared with plasma-derived vaccine. The antigen in the vaccine is highly purified and causes no clinically important reactions. Eighteen lots of vaccine have been prepared to date and licensure is expected during late 1985.

Keywords: *Recombinant Hepatitis B vaccine — yeast — immunogenicity — reactions*

PLASMA VACCINE

Human hepatitis B can be readily controlled by prophylactic vaccination. Licensed "first generation" vaccines prepared using surface antigen purified from the plasma of hepatitis B carriers have been produced in several countries (1-5).

Vaccine prepared in our laboratories (see Table 1) was licensed for general distribution in 1981. This vaccine has performed very well. The vaccine consists of essentially pure surface antigen that is treated by 3 different inactivation procedures which are sequentially applied and which are designed to destroy all microbial life forms. The vaccine incorporated into alum adjuvant induces hepatitis B antibody in more than 95% of recipients, overall, and affords more than 95% protection against hepatitis B in exposed normal persons (6). As may be expected, the vaccine is less effective in persons whose immune systems are immunodeficient or are immunosuppressed. Less than expected antibody responses have been reported in some situations of use (7,8). Investigation has revealed that such lower antibody responses may occur in persons in whom the vaccine was injected into the buttocks rather than into the arm. Vaccine given in the buttocks may fail frequently to reach muscle and be deposited instead into fat where it may not be well mobilized (9).

Table 1

Present: Plasma-derived human hepatitis B vaccine

Antigen source:	Plasma of human hepatitis B carriers.
Preparation:	Essentially pure surface antigen.
	Inactivation by 3 different methods, applied sequentially.
	Incorporated in alum adjuvant.
Efficacy:	>95% of normal children and adults develop antibody after 3 doses.
	>95% protection in normal persons.
	Less effective in immunodeficient or immunosuppressed persons.
	Duration of immunity is not known. It is also unknown when late booster doses of vaccine might be needed.
Targets:	Persons of defined high risk. Especially infants born to carrier mothers in high prevalence areas.
	Eventually all persons.
Safety and Extent of Use:	>4,500,000 doses distributed.
	>2,000,000 persons received vaccine to date.
	The vaccine is safe, including AIDS concerns.

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March 24, 1985

The duration of protective efficacy following vaccination is not known but, as shown in Figure 1, the great majority of persons (39/44) retain antibody for at least 4-5 years (see 6). The 3-dose regimen for immunization, giving the booster dose at 6 months after the initial dose, is highly effective in priming the immune system for rapid anamnestic immune recall on later contact with viral antigen on revaccination (10) or on contact in nature (11) as well as in providing resident active immunity. Because immunity against hepatitis B infection may be present at antibody levels less than detectable in the laboratory, and because of the phenomenon of anamnestic recall, it may be premature (12) to project when late booster immunizations might be needed.

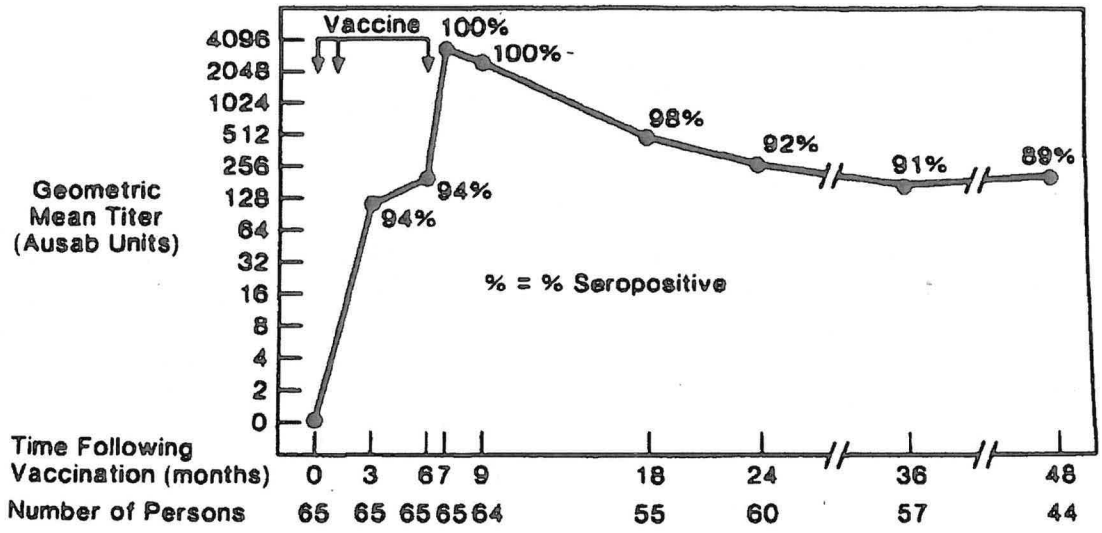

Figure 1
Antibody development and persistence in adults who received 3 doses of hepatitis B vaccine (study 555).

The vaccine has been targeted mainly for use in special groups at high risk to hepatitis B. Presently, added emphasis is being given to preventing infection in newborn infants born to carrier mothers in highly endemic populations such as in Eastern Asia and Africa. Eventually, all susceptible persons may be vaccinated. More than 4.5 million doses of the vaccine have been distributed and more than 2 million persons have received one or more doses of the vaccine to date.

Concern has been expressed for possible transmission of acquired immune deficiency disease (AIDS) by improperly prepared hepatitis B vaccines since the antigen is obtained from human plasma. Retroviruses of the HTLV-III or LAV group have now been shown (13-17) to be the likely cause for this blood and body secretion-transmitted disease. These agents are readily inactivated and destroyed by the process used to prepare the vaccine (18), giving direct evidential proof for the safety of the vaccine from the standpoint of "AIDS virus".

RECOMBINANT YEAST VACCINE

The production of human hepatitis B vaccine from the human plasma source is limited by the available supply of infected plasma and by the need to apply highly technical procedures for purification and inactivation of possible infectious agents that might be present in such plasma. Because of this, alternative sources of antigen were sought and two genetic recombinant antigens have been used in our laboratories (19-21). One of these, the carrier Alexander hepatocarcinoma cell (22) is a recombinant of nature. The other, obtained by cloning the gene of hepatitis B surface antigen into yeast (23), is quite unnatural.

Alexander hepatocarcinoma cell. Vaccine (19) prepared from antigen secreted from the hepatocarcinoma cells grown in culture initially proved very attractive from the standpoint of yield and immunizing potency, but it was evident that a vaccine derived from a non-cancer source would be more acceptable. Hepatitis B vaccine prepared using antigen obtained from either transformed or frankly neoplastic human or animal cells are not likely to be accepted by licensing authorities and the medical profession, especially since the antigens can be made efficiently in recombinant yeast cells.

Hepatitis B surface antigen preparation in recombined yeast. Joint efforts between our laboratories and those of Drs.

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Rutter and Hall of the Universities of California and Washington led to the preparation of a recombinant yeast cell system for producing hepatitis B antigen (23). Figure 2 shows the principal defined areas of the hepatitis B genome The gene region that encodes the hepatitis B surface antigen, but not the "pre-S" (24) or the core antigen, was inserted into a suitable vector and was implanted into ordinary Baker's yeast or *Saccharomyces cerevisiae*. The plasmid construct, shown in Figure 3, consists of the hepatitis B surface antigen gene flanked on one side by a promoter (glyceraldehyde 3-P dehydrogenase I, ADH-I), both being essential to proper translation of the surface antigen. The rest of the nucleic acid in the plasmid is needed to achieve its functions in the yeast cell system and to serve as a marker (yeast leucine gene) for presence of the plasmid in yeast cells. The construct employed subtype Adw surface antigen gene.

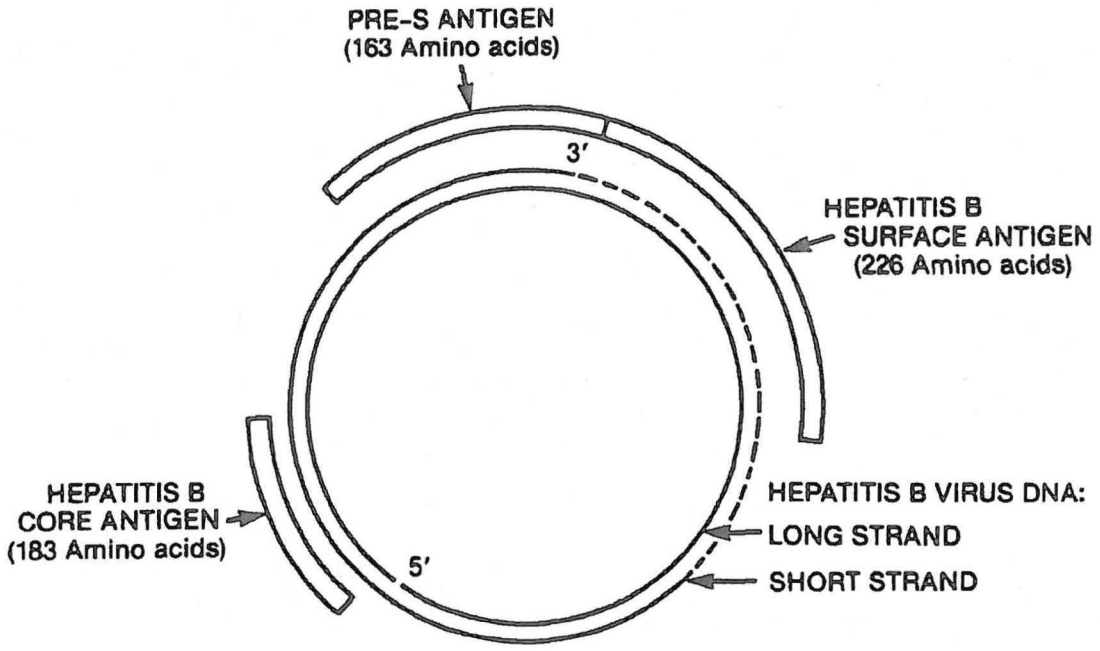

Figure 2
Hepatitis B virus genome and defined antigens that are produced.

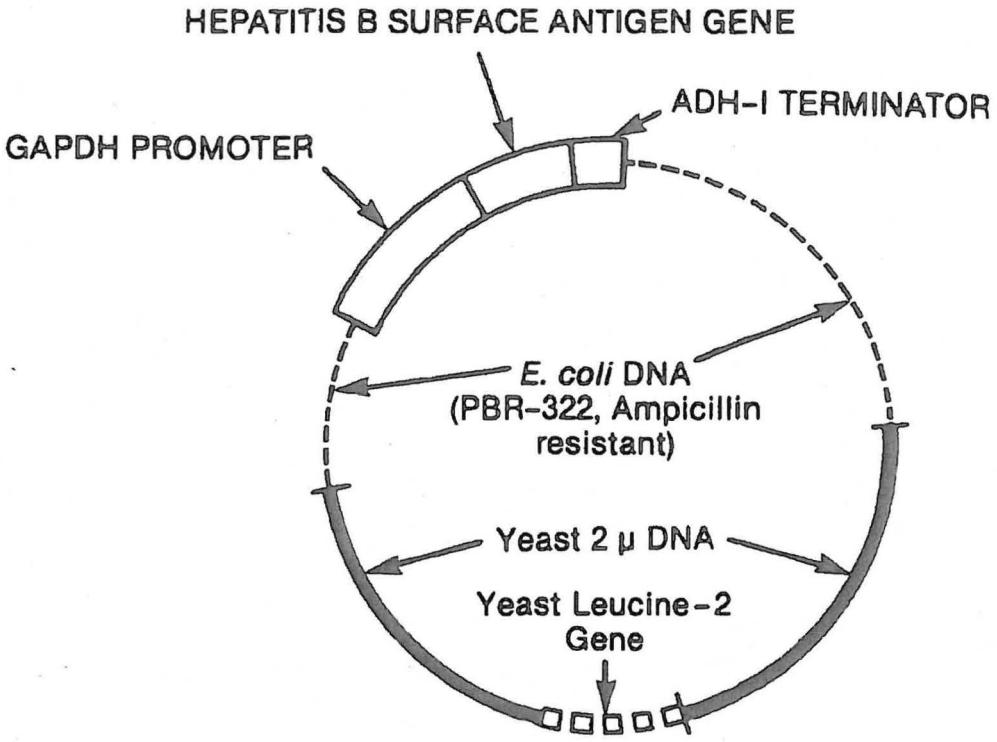

Figure 3
Construct of plasmid (pHBS56-GAP347/33) used to produce hepatitis B surface antigen in yeast.

The hepatitis B surface antigen produced in yeast is cell-associated. Surface antigen was released from the yeast cells by homogenization, the purification was achieved mainly by silica, hydrophobic interaction (butyl agarose) and gel exclusion chromatographies. The hepatitis B surface antigen used in the vaccine consists of polypeptides that are identical in amino acid sequence to those of human plasma source but lacking glycosylation. The glycosyl groups are not required for immunogenicity. Other measurable physical, chemical, and immunological attributes of the yeast-derived vaccine are substantially the same as those of the antigen prepared from human plasma.

As stated above, the recombinant hepatitis B surface antigen vaccine does not contain core antigen, *e* antigen, or antigen from the so-called "pre-S" region (see Figure 2). Antibody against core antigen and perhaps against *e* antigen may provide at least partial protection against hepatitis B virus infection (25-27). Antibody has been demonstrated in infected individuals that reacts with antigen encoded in the pre-S region (28-30) but it is not known whether such antibody may play a role in protective immunization. Though suggestions of the importance of pre-S region in generating full immunity to hepatitis B have been made (31), this statement is not supported by the known scientific evidence and the published literature (32). Indeed, vaccines without pre-S antigen have been proved highly effective in inducing immunity against hepatitis B in the extensive clinical and field studies carried out during the past several years (6,33-34). It is quite clear that there are many immunologic determinants or epitopes within the collection of viral antigens produced under the total of the viral genetic code. The question might be raised of how many different epitopes are needed or ought to be included in the vaccine. The *a* antigen epitopes of the surface antigen are quite adequate to afford solid and lasting protection against hepatitis B and there is presently no evident need or benefit to be derived from increasing the cost or complexity of the vaccine by adding antigens such as those from the pre-S region.

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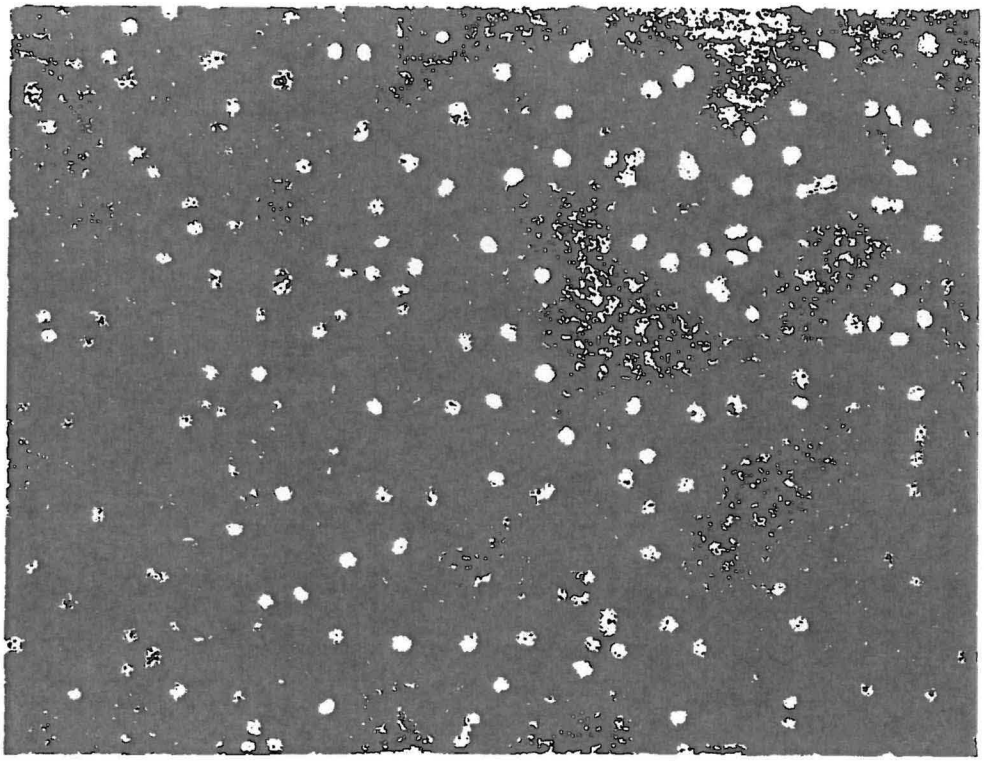

Figure 4
Electron micrograph of purified hepatitis B surface antigen derived from yeast recombinant cells (Lot CL-Y52-1, magnification 156, 750X)

The hepatitis B surface antigen particles produced in yeast cells, shown in Figure 4, are morphologically similar to those isolated from human plasma though the mean particle size of the former may be slightly smaller.

Hepatitis B vaccine prepared from recombinant yeast-derived surface antigen.

The purified antigen was formulated into vaccine by adsorbing to aluminum hydroxide adjuvant to contain 10 μg of antigen and 0.5 mg aluminum per 1 ml vaccine dose. Potency assay by the standard extinction mouse potency assay, as shown in Table 2, showed the yeast-derived vaccine to be at least as potent as plasma-derived antigen based on comparison of the 50% extinction dose (ED_{50}) and the geometric mean titers.

Table 2

Antigenic potency in mice of HBsAg purified from yeast and from human plasma

Vaccine Source	Antigen Dose per injection (μg protein)	Anti-HBsAg response after vaccination	
		no. pos./total	GMT
Human plasma	10	9/10	563
Lot 799-2	2.5	10/10	2,235
	0.625	4/9	32
	0.156	0/10	4
ED_{50}	0.639		
Yeast	40	10/10	5,432
Lot 81-4	10	10/10	3,400
	2.5	8/10	673
	0.625	8/10	967
ED_{50}	<0.625		

Chimpanzees given yeast recombinant hepatitis B vaccine in suitable regimen develop antibodies and are protected against infection on challenge with live hepatitis B virus. In the tests summarized in Table 3, four chimpanzees were given three intramuscular injections of vaccine containing 40 μg of antigen per ml dose at monthly intervals. One month after the third dose was given, 2 vaccinated and 2 control animals were challenged intravenously with heterologous *adr* subtype virus and a similar group of animals were challenged with heterologous *ayw* subtype virus. All the vaccinated animals developed antibody following immunization and all were solidly protected against the virus with all serologic and histopathologic makers remaining negative. The hepatitis B surface antigen contains the *a* antigen common to all subtypes plus the *d* and *w* subtype determinants. Protection was given against heterologous subtypes *adr* and *ayw*, showing the adequacy of the broad spectrum *a* epitopes in the recombinant antigen to protect against the heterologous subtypes. The finding of heterologous subtype protection with recombinant-derived vaccine is consistent with the findings with plasm-derived vaccines obtained in studies in animals (35-36) and in clinical studies (34) in which subtype *ad* vaccine protected renal dialysis staff workers against subtype *ay* challenge.

Table 3

Protective efficacy of purified yeast antigen vaccine

Vaccine	Chimp	Antibody Response to HBsAg	Subtype	Challenge Result			
				Antigenemic (HBsAg)	Anti-HBcAg	Enzyme Elevations	Liver Pathology
Yeast Vaccine	1	1,830	Adr	0	0	0	0
	2	540	Adr	0	0	0	0
	3	18,300	Ayw	0	0	0	0
	4	7,200	Ayw	0	0	0	0
Controls	5	<8	Adr	+	+	+	+
	6	<8	Adr	+	+	+	+
	7	<8	Ayw	+	+	+	+
	8	<8	Ayw	+	+	+	+

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Clinical tests in human beings. Studies in human subjects of the recombinant yeast vaccine have been initiated for purpose of measuring antibody responses and for demonstrating protective efficacy. About 1500 persons of diverse age, sex, and health status, and geographic residence have received vaccine to date. There were no important clinical reactions attributed to vaccination. Mild soreness at the injection site has been reported in 19% of recipients and other minor complaints such as headache, fatigue and malaise have been stated by a small percentage of vaccinees.

Prior studies established (6,33-34) the relationship between antibody response to vaccination and immunity to hepatitis B. Though any new vaccine must stand on its own merits, it is instructive to compare the serologic responses in human beings to the widely used plasma-derived vaccine prepared in our laboratories with those to the new recombinant preparation.

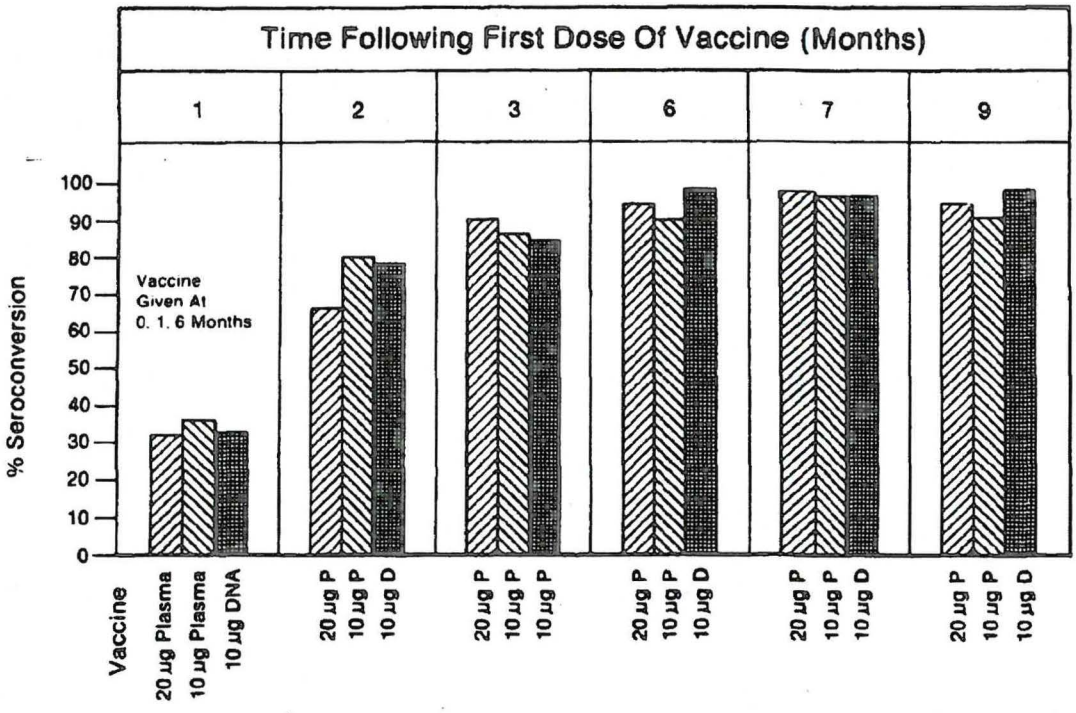

Figure 5
Serologic responses to 3 doses of plasma (20 µg or 10 µg) compared with recombinant (10 µg) vaccine in adults

Figure 5 shows the serologic findings in a composite of studies carried out by our laboratories to compare the antibody responses in adult persons to 3 doses of plasma-derived vaccine at 20 or 10 µg antigen per dose with that of the recombinant vaccine at 10 µg per dose. Data were from 400 to 800 subjects per vaccine group. All vaccines were given intramuscularly by the same regimen at time 0, 1 and 6 months. The rate and rapidity of antibody seroconversion in persons given 10 µg yeast vaccine compared with 10 µg or 20 µg plasma vaccine per dose were nearly alike. Most important, 87% of the subjects had developed antibody within 1 month following injection of the second dose of yeast-derived vaccine (3-month bleeding) and this was increased to 96-99% by 1 or 2 months following the booster dose given at 6 months.

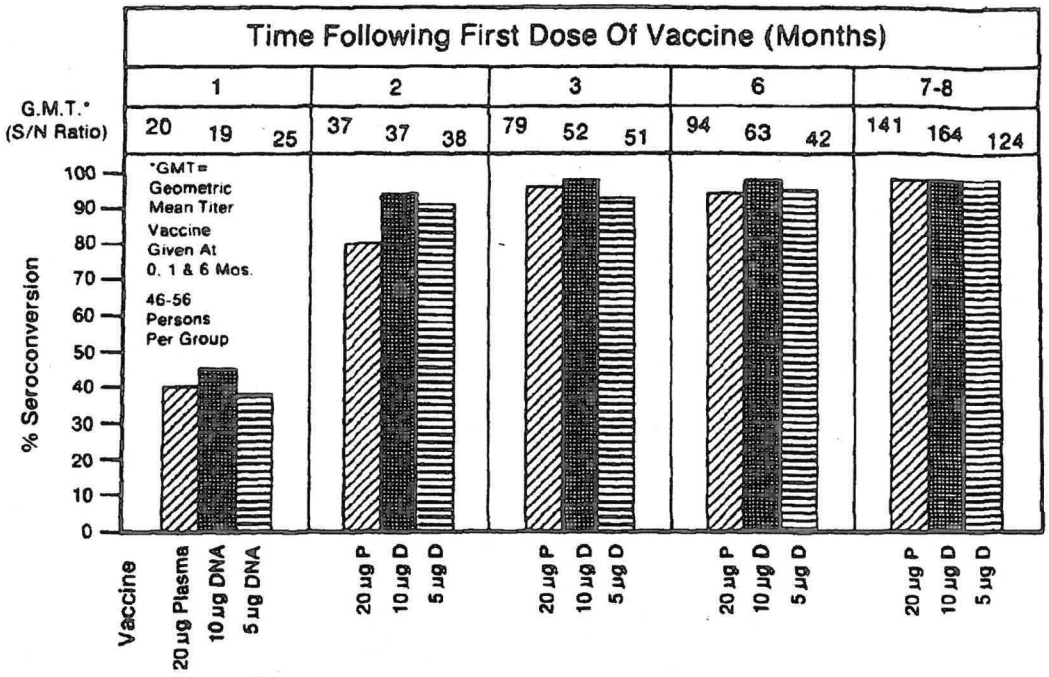

Figure 6
Serologic responses to 3 doses of plasma (20 µg) compared with recombinant (10 µg or 5 µg) vaccines in 21-30 year-old persons (adapted from Davidson and Krugman, Lancet 1: 108, 1985).

Similar findings, summarized in Figure 6, were obtained in studies carried out in 21-30 year old adults by Davidson and Krugman (37) in which rates for seroconversion, and heights of antibody following 20 µg dose plasma vaccine were compared with 10 µg or 5 µg dose yeast vaccine. These authors suggested that the lesser antibody responses to yeast recombinant vaccine reported by Jilg *et al.* (38) might have been related to the small numbers of individuals included in that study.

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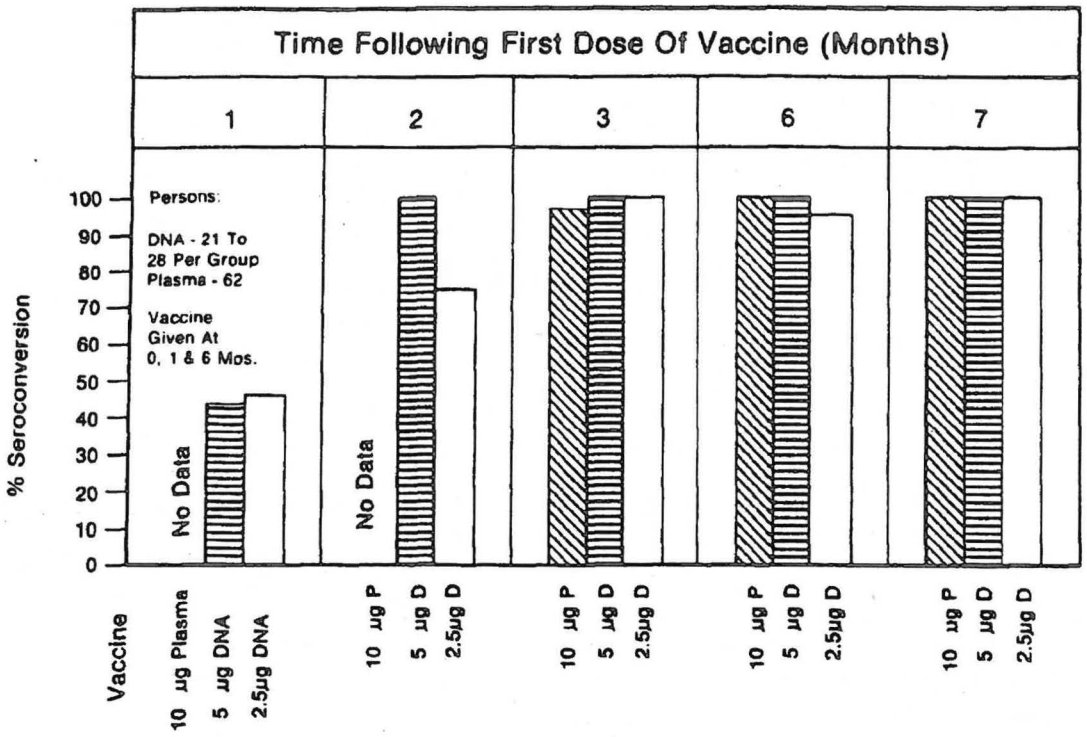

Figure 7
Serologic responses to 3 doses of plasma (10 µg) compared with recombinant (5 µg or 2.5 µg) vaccines in children 1-11 years of age.

Studies to measure antibody responses in children and infants are in progress. Only limited findings are available to date and these are from studies carried out by our group in 1- to 11-year old children. Figure 7 shows the serologic responses in these children, to 3 doses of plasma-derived vaccine given in 10 µg dose (62 children) compared with yeast recombinant vaccine given in 5.0 µg or 2.5 µg dose (21 and 28 children per group, respectively). The responses were essentially equivalent in all groups, though it must be noted that the numbers of individuals given vaccine are small.

CONCLUDING REMARKS

It is clear, we believe, that the plasma-derived hepatitis B vaccine has performed in an exemplary way and has provided a means for inducing immunity, with safety, against human hepatitis B virus infection. The necessity for developing a substitute but equally satisfactory vaccine, free from the need for human plasma and technologically simpler to produce, has been accomplished by the application of yeast recombinant technology. It is anticipated that the yeast vaccine will be licensed in the U.S.A. and other countries by late 1985 and that the vaccine will be available for general distribution in early 1986. In anticipation of the development of such recombinant vaccines, the World Health Organization convened a group of experts during November of 1984 who wrote the provisional requirements for the standardization and control of hepatitis B vaccine made by recombinant DNA techniques in yeast. These requirements should be made final before the end of 1985 and should provide a basis for worldwide regulatory control of hepatitis B vaccine produced in recombinant yeast cells.

Acknowledgement:

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IMMUNOGENICITY AND REACTOGENICITY OF NEW HEPATITIS B VACCINES. FB Hollinger, Y Sanchez, C Troisi, GR Dreesman, and JL Melnick, Baylor College of Medicine, Houston, TX.

An HBsAg/adw polypeptide (PP) vaccine and a recombinant DNA vaccine produced in yeast (MSD) are being evaluated. The PP vaccine was prepared from 22-nm HBsAg particles, packaged in a micellar form and alum-adsorbed. The starting material (NIH/40) contained 300 HBsAg RIA equivalent units (RSU) based on a HEPTAVAX-B standard of 100 HBsAg RSU. 3 lots containing 5, 1, and 0.2 HBsAg RSU were compared to 2 intact particle vaccines. Vaccine was administered at 0, 1, and 6 months to 52 weight-matched adults. RESULTS: Local and systemic reactions were insignificant. The anti-HBs seroconversion rate at 4 weeks for the 5 RSU PP vaccine group (90%) was considerably better than that seen with HEPTAVAX-B. By 12 weeks, all vaccine recipients in the 1 and 5 RSU PP vaccine groups had seroconverted versus 50% of the 0.2 RSU group ($p<0.02$) which reached 100% seroconversion by month 7. Throughout follow-up, geometric mean (GM) anti-HBs levels (mIU/ml) in the 5 RSU PP group were significantly higher than in the other PP vaccine groups. At 1 month the GM anti-HBs level for the 5 RSU PP group was 8.9, whereas the 300 RSU NIH/40 vaccine group had a GM antibody level of 5.2. By 3 months, the respective anti-HBs levels were 202 vs 90, rising to 8910 and 3450 by 7 months. The 1 RSU PP vaccine produced antiHBs responses comparable to the 100 RSU HEPTAVAX-B vaccine. Thus, the polypeptide vaccines, with substantially lower RIA HBsAg reactivity, produced superior anti-HBs responses when compared with 22-nm HBsAg vaccines. These studies confirm our previous findings in chimpanzees that critical antigenic determinants are associated with these polypetides, and they provide a link to future vaccine studies using synthetic HBsAg macromolecules. The rapid anti-HBs response that follows the initial inoculation suggests that such an immunogen may be beneficial in postexposure prophylaxis where the early development of immunity is advantageous. Preliminary data through 6 months also will be presented on the immunogenicity of 3 doses (5, 10, and 20 mcg) of an HBSAg vaccine made by recombinant DNA technology in yeast (MSD).

Hollinger FB, Sanchez Y, Troisi C, Dreesman GR, Melnick JL. Immunogenicity and reactogenicity of new hepatitis B vaccines. Hepatology 1984; 4:1027 (Abstract).

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Anti-HBs Responses to Vaccination with a Human Hepatitis B Vaccine Made by Recombinant DNA Technology in Yeast

In the United States, the currently licensed vaccine against hepatitis B virus (HEPTAVAX-B®; Merck Sharp & Dohme, West Point, Pa) consists of hepatitis B surface antigen (HBsAg) that is purified from the plasma of chronically infected humans. Antibodies to the group *a* determinant of this complex antigen effectively neutralize the various subtypes of hepatitis B virus (HBV), as shown in a number of controlled clinical trials [1–3]. Despite overwhelming evidence that documents the efficacy of this vaccine, widespread acceptance by those who are at greatest risk of contracting hepatitis B has been less than expected because of a number of unrelated factors. The plasma-derived vaccine is expensive to prepare. A number of physical and chemical inactivation steps are used in purification, and extensive safety testings are mandated by the Food and Drug Administration in laboratory animals, cell cultures, and chimpanzees before the product can be marketed. In addition, there are of necessity batch-to-batch variations in human source material. These problems would have been surmountable in the marketing of this vaccine were it not for two recent events that made potential vaccine candidates overly cautious about accepting this new product: the increased incidence of Guillain-Barré syndrome that followed administration of the swine influenza vaccine in 1976 and the emergence of AIDS in the homosexual population. The latter problem was particularly relevant because HEPTAVAX-B is a plasma-derived product obtained from HBsAg-positive individuals, some of whom are in high-risk groups for AIDS. This raised the question whether AIDS might be transmitted to recipients of this vaccine. Unfortunately, despite numerous studies [4, 5] that eventually have refuted this hypothesis (on the basis of the susceptibility of retroviruses to inactivation by the physical and chemical steps used in producing the vaccine and by the lack of cases of AIDs or antibody seroconversions to human T lymphotropic virus type III observed among vaccinees at low risk of exposure to this disease), many members of groups at risk of contracting hepatitis B have been reluctant to accept this vaccine.

Because of these problems, alternate sources of vaccine are being developed. Among the first to become available for human trials was a 25,000–30,000 molecular weight HBsAg polypeptide derived by disrupting the intact 22-nm HBsAg particle with a nonionic detergent [6]. Immunogenicity of this product was superior to that of the human HBsAg source from which it was prepared, especially during the initial stages of antibody development. More recently a number of other vaccines that do not depend on human plasma as their source of HBsAg have been produced [7]. These include chemically synthesized peptides from several antigenic domains of the HBV, products of recombinant DNA technology, and live vaccinia virus recombinants containing the HBsAg gene.

In this paper we report one-year follow-up data on the immunogenicity and reactogenicity of a nonglycosylated HBsAg hepatitis B vaccine, subtype adw, made by recombinant DNA technology (Merck). The vaccine, prepared in the yeast *Saccharomyces cerevisiae* (strain 2150-2-3) [8, 9] was administered in three different doses (5, 10, and 20 μg) to an adult at-risk population.

Subjects and Methods

After screening 359 Emergency Medical Service personnel in Houston, 105 adult men (median age, 29 years; range, 22–40), determined by RIA or enzyme immunoassay to be free of any seromarkers of hepatitis B infection (Abbott Laboratories, North Chicago, Ill), were admitted to the study. All had antibody to HBsAg (anti-HBs) sample-to-negative-mean (S/N) ratios ≤1.4, levels of antibody to hepatitis B core antigen (anti-HBc) ≤39% inhibition, and HBsAg S/N ratios ≤1.2. These values are substantially below the cutoff levels endorsed by the manufacturers. In addition, each participant was required to have serum levels of liver enzyme (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) ≤50 IU/liter, as determined by the Beckman System TR enzyme autoanalyzer (Beckman Instruments, Palo Alto, Calif). Participants were in good health at the time of enrollment, had not been previously vaccinated against hepatitis B, and had signed informed consent releases. The study was approved by the Baylor College of Medicine Human Investigations Committee.

The 105 volunteers were weight matched within 4.5 kg [9a] into three groups of 35. Each member of each group received 5, 10, or 20 μg of an alum-adsorbed, DNA recombinant hepatitis B vaccine (lot no. 974/CK-446) containing 20 μg of HBsAg/ml. The vaccine was purified from

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yeast extract by physical and chemical methods. Hydrophobic-interaction chromatography followed by gel-exclusion chromatography was the major procedure used to prepare the purified antigen. The removal of yeast components was demonstrated in vitro by immunologic methods and in vivo by anaphylactic testing in guinea pigs.

To deliver the inoculum, we used 0.5-ml syringes for the 5 or 10 μg doses and 1.0-ml syringes for the 20 μg dose. All doses were administered by the same person. The vaccine was thoroughly resuspended before use and inoculated im in the deltoid region with a one-inch, 23-gauge needle at months 0, 1, and 6. Blood samples were obtained at one, two, three, six, eight, and 12 months after the initial inoculation (100% participation). A prevaccination oral temperature was obtained, and participants were asked to take and record their temperature with the same calibrated thermometer 4 hr after inoculation and each morning for the next three days. They were also asked to record any local or systemic symptoms experienced during this time. Responses were received by mail from ~90% of the participants.

All blood samples were processed within 24 hr and assayed for liver enzymes. The unit of measurement for anti-HBs was mIU/ml and was determined by the method of Hollinger et al. [10]. On the basis of the statistical analysis of at least 1,000 normal human sera, a value ≥0.7 mIU/ml on replicate samples was considered evidence of the presence of anti-HBs for determination of seroconversion rates. This cutoff level was ≥5 SD above the mean value for the negative control samples. All samples taken at three and eight months were also tested for anti-HBc and HBsAg to rule out unsuspected infection with HBV that might have occurred during the course of the study.

Statistical calculations included Student's *t* test, McNemar's χ^2 test, analysis of variance, and Duncan's multiple range test [11].

Results

No local or systemic reactions of a serious nature were observed by the volunteers. After the first inoculation, 14% of the vaccinees experienced mild discomfort at the site of injection; this figure was 12% after the second and third inoculations. Temperature elevations ≥1.5 F above an individual's baseline level were recorded in 3.8%, 9.3%, and 3.4% of the participants after each of the three injections, respectively. Only four oral temperatures exceeded 100 F, the highest of which was 101.2 F. Among the systemic reactions recorded after the initial inoculation, headaches (10.5%), diarrhea or abdominal complaints (9.5%), and fatigue (7.6%) were noted most frequently. Rates declined substantially after the second and third injections. Such local and systemic reactions are similar to those observed among recipients of placebos in other studies [10].

None of the participants showed serological evidence of infection with HBV during the study. Ten (9.5%) volunteers had aminotransferase levels >50 IU/liter on one or more occasions over the one-year follow-up period. This rate is similar to that observed in a previous study [10]. Muscle trauma caused by excessive physical activity was felt to be the cause of the enzyme elevations in three of these ten participants; this hypothesis was based on an AST value that was higher than the ALT value and on creatine phosphokinase levels of 47,502, 844, and 533 IU/liter. A fourth volunteer sustained a lacerated liver following an auto accident that occurred two weeks before the blood specimen that showed elevated enzyme levels was taken, and three other men were taking medications that have been reported to cause liver damage. In the other three (2.9%) volunteers, the enzyme levels had returned to normal when their blood was retested one week later. There was nothing in their histories to explain these abnormalities.

Seroconversion rates and geometric mean antibody responses for all participants are shown by dose and time in tables 1 and 2. Seroconversion rates were significantly lower in the 5-μg dose group than in the 10- or 20-μg dose

Table 1. Seroconversion rates of anti-HBs by time and dose.

	Time (months)					
Dose	1*	2	3	6*	8	12
5 (n = 35)	8.6	34.3†	45.7†	62.9†	97.1	88.6‡
10 (n = 35)	28.6	80.0	94.3	94.3	97.1	97.1
20 (n = 35)	28.6	82.9	88.6	94.3	100.0	100.0

NOTE. Results are percentages of subjects who were positive at the noted time. Doses are in μg.

* Vaccine was administered at months 0, 1, and 6.

† $P < .002$, 5 μg compared with 10 or 20 μg.

‡ Four persons who were positive for anti-HBs at eight months became seronegative at 12 months, whereas the one person who had not responded by month 8 seroconverted.

Table 2. Geometric mean levels of anti-HBs (mIU/ml) by time and dose.

	Time (months)					
Dose	1*	2	3	6*	8	12
5 (n = 35)	0.1†	0.5‡	0.7‡	2.0‡	45.7‡	10.0‡
10 (n = 35)	0.3	5.1	6.9	14.0	388.6	76.0§
20 (n = 35)	0.4	7.3	9.4	26.4	519.5	184.6

NOTE. Doses are given in μg.

* Vaccine was administered at months 0, 1, and 6.

† $P < .02$, 5 μg compared with 10 or 20 μg.

‡ $P < .001$, 5 μg compared with 10 or 20 μg.

§ $P = .03$, 10 μg compared with 20 μg.

groups at two, three, and six months after the initial inoculation ($P < .002$). By eight months all but two of the participants had produced specific antibodies. One of these two volunteers, who received 5 μg of vaccine, did develop specific anti-HBs at a low level (1.3 mIU/ml) 12 months following his initial inoculation. Therefore, the total seroconversion rate for the 5-μg group through 12 months was 100%, even though four other vaccinees who were positive at eight months were negative at 12 months; this yielded a point prevalance rate of 88.6% (table 1).

Geometric mean concentrations of anti-HBs were considerably lower in the group receiving 5 μg of yeast-derived HBsAg than in the 10- or 20-μg dose groups after the first month ($P < .001$; table 2). Similar differences were observed when weight-matched group members were compared, most notably at six and eight months. No statistically significant differences were seen between the 10- and 20-μg groups during the first eight months in terms of seroconversion rates or geometric mean levels of antibody. At each bleeding interval, however, geometric mean levels of anti-HBs in the 10-μg group were lower than those seen in the 20-μg vaccinees, and a P value of .03 was obtained at 12 months (table 2).

Discussion

The reasons for the significantly larger differences in immune response seen between the 5-μg group and the other two groups in our study are not readily apparent. Lot-to-lot variation is not a factor since the same lot of vaccine was used to inoculate all three groups. The only known variable is the volume of inoculum administered. Thus, the lower doses of vaccine not only contained less HBsAg, but the total amount of alum administered was also reduced even though the protein-to-alum ratio remained constant among the three doses. Whether a finite amount of alum is essential for an optimal response cannot be ascertained in this study, but levels of alum should not vary significantly between batches of vaccine that use identical doses of vaccine. It is interesting that similar muted responses were not seen in another study that compared 5 μg and 10 μg of yeast-derived HBsAg, although a two-fold difference in the geometric mean levels of antibody was reported [12]. Since the RIA activity of equimolar preparations of purified yeast HBsAg has been reported to vary by as much as 2.5 times [8], this might account for the interstudy differences observed at critical threshold levels.

As expected, a decline in anti-HBs concentration was observed in 96% of the subjects between the eighth and 12th months. To examine the slope of this response more completely, we determined the natural logarithms of the differences in the anti-HBs levels after dividing by the number of months between observations for each subject in the three dose groups. Similar data were obtained for adults participating in previous vaccine studies that used 40 μg of an HBsAg plasma-derived vaccine [10] and 20 or 40 μg of HEPTAVAX-B [9a], and the results were compared by analysis of variance. No significant differences in the rate of decline were found between these four groups when equivalent levels of peak anti-HBs responses were evaluated.

When geometric mean levels of anti-HBs at eight months were compared for two different plasma-derived vaccines, values ranged from 2,980 to 3,322 mIU/ml for 40 μg of vaccine to 1,975 mIU/ml for 20 μg of HBsAg [9a, 10] vs. 46 (5 μg), 389 (10 μg), and 520 (20 μg) mIU/ml for the yeast-derived product. These findings lead us to conclude that the lower antibody levels detected in adults receiving the yeast-derived vaccine may be related to the immunogenicity of the product. It is noteworthy that Dandolos et al. [13] reported similar discrepancies in anti-HBs levels between yeast- and plasma-derived vaccines, in which equivalent doses of antigen could be compared, although immune responses were significantly lower with our lot of recombinant vaccine. Since a butyl agarose method was used to remove contaminating yeast antigens from the final product in both of these studies, it is unlikely that this could account for the reduced immunogenicity found in our study. Two other studies [12, 14] did not permit equivalent time and dose comparisons between the two types of vaccines. Variations between lots, dissimilarities in the lipid content of the antigen produced in the yeast as compared with plasma-derived antigen, reduced antigenicity when compared with human HBsAg, and the fact that the yeast-derived HBsAg is not glycosylated [7, 8] may be factors responsible for the relatively lower anti-HBs response seen with the yeast-derived product. Further field trials in different at-risk groups seem appropriate before a specific adult dose of this vaccine is recommended. Nevertheless, several small trials in humans have shown that the vaccine is safe, and we anticipate that durable levels of protection should be achieved if sufficient immunogen is incorporated in the vaccine.

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The Epidemiology of *Clostridium difficile* with Use of a Typing Scheme: Nosocomial Acquisition and Cross-Infection Among Immunocompromised Patients

Gastrointestinal disturbance, particularly diarrhea, is one of the commonest side effects of the use of antibiotics. Up to 20%–25% of antibiotic-associated diarrhea occurs in conjunction with a fecal isolate of *Clostridium difficile* [1]. This organism is the major cause of pseudomembranous colitis and antibiotic-associated colitis but is also carried in the gastrointestinal tract of 2%–4% of the normal adult population and can be isolated from the feces of 30%–75% of asymptomatic neonates [2].

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Clusters of antibiotic-associated colitis have been noted [3], and early animal studies suggested that environmental contamination and cross-infection might be important in the etiology of outbreaks of antibiotic-associated diarrhea [4]. However, convincing evidence for the cross-infective potential of *C. difficile*, as well as its demonstration as a predominantly nosocomial infection, has been prevented due to lack of a reliable typing scheme for this organism [5].

Various typing schemes have been suggested [6–10]. Among these, Tabaqchali et al. [8] reported a well-defined scheme for typing this organism on the basis of the incorporation of [^{35}S]methionine into bacterial proteins and have described to date nine distinct groups within the *C. difficile* species (A–E, W–Z), as demonstrated by the radiolabeled protein profile obtained by using SDS-PAGE followed by autoradiography. We have applied this technique to isolates obtained from a prospective six-month study of immunocompromised and general medical patients in an attempt to assess the carriage and acquisition of *C. difficile* among hospital patients. The effect of isolation and containment procedures on the spread of *C. difficile* was also studied.

CLINICAL EVALUATION OF A RECOMBINANT HEPATITIS B VACCINE

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Summary Recombinant hepatitis B vaccine prepared from antigen expressed in yeast was given to 30 healthy young volunteers. Seroconversion rates and anti-HBs levels were compared with those in a control group matched for age and sex who had received plasma-derived hepatitis B vaccine. 4 weeks after the third immunisation results were similar in the two groups. In the recombinant vaccine group the immune response developed more slowly during the early phase and seroconversion rates and mean anti-HBs levels were slightly lower in males; this probably reflects use of a lower dose of recombinant vaccine (10 μg compared with 20 μg of the plasma vaccine). Side-effects were slight and antibody titres against *Candida albicans* were not increased in recipients of the recombinant vaccine.

Introduction

CURRENT hepatitis B vaccines are effective and safe.[1] However, because they are prepared from plasma of human hepatitis B virus carriers, supply is restricted by the amount of plasma available and by the cost of purifying the hepatitis B surface antigen (HBsAg) to render it free from hepatitis B virus and other possible infectious agents. Thus, to meet the worldwide need for hepatitis B vaccine, new means of preparation are required. Lately, vectors carrying the DNA sequence for HBsAg were prepared[2] and the antigen was expressed in the yeast *Saccharomyces cerevisiae*.[3] Yeast cells assemble the HBsAg polypeptides into particles similar to the 22 nm particles found in human plasma; yeast HBsAg, however, unlike human HBsAg is not glycosylated. A vaccine developed from yeast HBsAg stimulated antibody production in mice, grivet monkeys, and chimpanzees; and when vaccinated chimpanzees were challenged with human hepatitis B virus of different subtypes, they were completely protected.[4] We now report the immunisation of 30 healthy young volunteers with the first hepatitis B vaccine produced by recombinant DNA technology.

Subjects, Materials, and Methods

Subjects

30 healthy medical students and laboratory workers were studied (17 female, 13 male; mean age 25±3 yr, range 21–34). Subjects in the control group had been immunised with plasma-derived vaccine in an earlier study;[5] they were matched by age and sex to the study group (table I). Before vaccination, all subjects were negative for HBsAg, anti-HBs, and antibodies against hepatitis B core antigen (anti-HBc), and their aminotransferase levels were normal (alanine and aspartate aminotransferases ⩽17 and ⩽19 IU/l, respectively).

TABLE I—SEX AND AGE DISTRIBUTION OF THE TWO VACCINATION GROUPS*

	Total		Female		Male	
	No	Age (yr)	No	Age (yr)	No	Age (yr)
Recombinant vaccine	30	24·9±3·1 (21–34)	17	24·6±3·5 (21–34)	13	25·3±2·6 (23–32)
Plasma-derived vaccine	41	25·0±2·7 (21–32)	23	24·7±3·0 (21–32)	18	25·4±2·3 (23–32)

*Means and standard deviations (range).

Vaccines

The recombinant hepatitis B vaccine was prepared by Merck Sharp & Dohme research laboratories (lot 934/C-J 625). It consists of purified HBsAg, subtype *adw*, produced in recombinant *S cerevisiae* and absorbed on aluminium hydroxide. 1 ml of vaccine contained 10 μg of HBsAg. Plasma vaccine was also subtype *adw* (lot 773/801-2/ C-F 732-2 Merck Sharp & Dohme). Subjects in the study group received 10 μg of recombinant vaccine intramuscularly at 0, 1, and 6 months; subjects in the control group received 20 μg of plasma-derived vaccine at the same intervals. (Since the recombinant vaccine was treated with formalin only, and not with pepsin and urea, it was initially thought to be more immunogenic than the plasma vaccine.) Blood samples were taken on the day of the first vaccination and then monthly. Subjects were asked to keep daily records of body temperature and side-effects for 5 days after each injection.

Serology

HBsAg, anti-HBs, and anti-HBc were tested by radioimmunoassay with commercially available kits ('AUSRIA II', 'AUSAB', 'CORAB', Abbott Laboratories). Anti-HBs concentrations in IU/l were calculated by the method of Hollinger et al,[6] the first WHO reference preparation 1977 being used in a dilution of 1:400.[7] Because *S cerevisiae* and *C albicans* have common antigenic determinants,[8] antibodies against *C albicans* were determined by passive haemagglutination in 26 subjects on day 0 and 4 weeks after the second and third injections of recombinant vaccine. Sera were examined for antibodies against the determinant *a* of HBsAg as previously described.[9]

Results

Seroconversion rates and mean anti-HBs levels during the course of immunisation are shown in table II. The immune response in the recombinant vaccine group was less pronounced during the first months than in the plasma vaccine group, as shown by lower seroconversion rates and lower mean anti-HBs levels. These differences became non-significant after the booster dose at month 6 when 29 out of 30 subjects (97%) were anti-HBs positive (control, 41 out of 41) with a geometric mean anti-HBs level of 2135 IU/l (control, 4299 IU/l). All anti-HBs-positive individuals in the recombinant vaccine group had anti-HBs values above 10 IU/l; 2 (6·7%) were low responders (anti-HBs below 100 IU/l), 3 (10%) were intermediate responders (anti-HBs 101–1000 IU/l), and 22 (73·3%) were normal to high responders (anti-HBs greater than 1000 IU/l). Similar values

Jilg W, Schmidt M, Zoulek G, Lorbeer B, Wilske B, Deinhardt F. Clinical evaluation of a recombinant hepatitis B vaccine. Lancet 1984; 2:1174-5.

TABLE II—IMMUNE RESPONSES AFTER VACCINATION

Month	Seroconversion (%)		Anti-HBs (IU/l)*		p†
	Recombinant vaccine (n = 30)	Plasma-derived vaccine (n = 41)	Recombinant vaccine	Plasma-derived vaccine	
1	8 (27)	18 (44)	9	15	<0·05
2	21 (70)	39 (95)	38	53	<0·05
3	28 (93)	39 (95)	29	164	<0·05
4	28 (93)	39 (95)	63	228	<0·05
5	28 (93)	39 (95)	79	273	<0·05
6	28 (93)	39 (95)	68	263	<0·05
7	29 (97)	41 (100)	2135	4299	>0·05

*Anti-HBs is given as the geometric mean in responders only.
†Wilcoxon's rank-sum test.

TABLE III—IMMUNE RESPONSES IN MALES AND FEMALES (AFTER THREE INOCULATIONS)

—	Recombinant vaccine	Plasma-derived vaccine	p*
Males:			
Seroconversion (%)†	12/13 (92)	18/18 (100)	
Anti-HBs (IU/l)‡	911	3895	<0·05
Females:			
Seroconversion (%)†	17/17 (100)	23/23 (100)	
Anti-HBs (IU/l)‡	3282	4640	>0·05

*Wilcoxon's rank-sum test.
†Numbers of anti-HBs-positive subjects divided by the total number.
‡Geometric mean.

were obtained in the control group. Although the immune responses to the two vaccines were similar after the full course of immunisation, responses of male and female subjects differed. In both groups all the women seroconverted and the geometric mean anti-HBs levels did not differ significantly (3282 IU/l vs 4640 IU/l). However, in males receiving recombinant vaccine the seroconversion rate was 92% vs 100%, and the geometric mean anti-HBs was 911 vs 3894 IU/l (table III).

Preliminary tests indicate that recombinant vaccine, like the plasma-derived vaccine, induces antibodies against both the *a* and the *d* components of HBs antigen. After month 3, about 38% of the total anti-HBs was directed against determinant *a*.

No important side-effects were observed after immunisation with the recombinant vaccine. Minor local symptoms such as transient pain, itching, burning, and slight swelling at the injection site were reported after 24 of the 90 injections. On no occasion did body temperature rise above 37·9°C.

Of 26 subjects tested, all had antibodies against *C albicans* on day 0 (titres from 1:80 to 1:320) and titres did not increase after immunisation.

Discussion

Three doses of 10 μg recombinant hepatitis B vaccine gave seroconversion rates and geometric mean anti-HBs levels similar to those induced by three doses of 20 μg plasma-derived vaccine. The results were also comparable with those obtained in large trials of conventional vaccines.[10,11]

The immune response to the recombinant vaccine, however, was less strong during the early phase (1–6 months) in all subjects, and in males mean anti-HBs values were lower in the recombinant group even after the complete course of immunisation. These results are comparable with findings in subjects immunised with a smaller dose (5 μg) of conventional vaccine (Jilg W, Zachoval R, Schmidt M, Deinhardt F, unpublished), and may reflect the use of smaller amounts of antigen. Antigen content of both recombinant vaccine and plasma-derived vaccine is determined as HBsAg protein. The vaccines are produced and treated differently, however;[6,12] therefore similar protein content does not necessarily mean similar immunogenicity. The yeast and plasma derived HBsAg differed in reactivity in radioimmunoassay tests; the reactivity of the HBsAg produced in yeast was only 20–50% of the reactivity of plasma-derived HBsAg.[4] Thus, weight-for-weight the immunogenicity of the recombinant vaccine seems to be less than that of the plasma-derived vaccine. Another explanation for the lower immune response may be that 10 μg of recombinant vaccine was given per single dose compared with 20 μg of plasma-derived vaccine. A higher dose (20 or 40 μg) of the recombinant vaccine would probably give the same results as the plasma-derived vaccine.

Despite the slightly lower immunity achieved with the recombinant vaccine, protection will probably be as good as with the conventional vaccine, in that all 29 subjects with detectable anti-HBs had values above the protection level of 10 IU/l.[13] In 73%, anti-HBs levels after the third vaccination were more than 1000 IU/l; this has been shown to guarantee persistence of anti-HBs above the protective limit for at least 3 years.[14] In addition, all subjects who seroconverted had antibodies against the common determinant *a* of HBsAg, indicating cross-protection against infections with other subtypes of HBsAg. Side-effects after the recombinant vaccine were negligible and did not differ from those observed after plasma-derived vaccine. The absence of a rise in antibodies against *C albicans* indicates that no cross-reacting yeast antigens were present in the vaccine.

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Jilg, W., Zoulek, G., Lorbeer, B., Wilske, B. and Deinhardt, F.
CLINICAL COMPARISON OF A RECOMBINANT AND A PLASMA-DERIVED HEPATITIS B VACCINE
Paper presented at the 24th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C., Oct. 8-10, 1984, Program Abstract No. 292.

Hepatitis B vaccine, yeast recombinant (Merck), hepatitis B vaccine, plasma derived: Thirty healthy young adults were vaccinated IM at 0, 1, and 6 mo with 10 mcGm HBsAg in aluminum hydroxide adjuvants. A comparable group was vaccinated with 20 mcGm HBsAG derived from plasma. Seroconversions following both vaccines were 30-40% after the 1st vaccination and greater than 90% after the 2nd. Antibody titers for both vaccines were comparable. The percentage of antibodies directed against the common antigenic a component of all hepatitis B virus subtypes was greater than 35%. Side effects were minor or absent. Immune reactions to yeast antigens were not reported in any of the subjects.

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Human hepatitis B vaccine from recombinant yeast

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The worldwide importance of human hepatitis B virus infection and the toll it takes in chronic liver disease, cirrhosis and hepatocarcinoma, make it imperative that a vaccine be developed for worldwide application[1]. Human hepatitis B vaccines[2-6] are presently prepared using hepatitis B surface antigen (HBsAg) that is purified from the plasma of human carriers of hepatitis B virus infection. The preparation of hepatitis B vaccine from a human source is restricted by the available supply of infected human plasma and by the need to apply stringent processes that purify the antigen and render it free of infectious hepatitis B virus and other possible living agents that might be present in the plasma. Joint efforts between our laboratories and those of Drs W. Rutter and B. Hall led to the preparation of vectors carrying the DNA sequence[7,8] for HBsAg and antigen expression in the yeast *Saccharomyces cerevisiae*[9]. Here we describe the development of hepatitis B vaccine of yeast cell origin. HBsAg of subtype adw was produced in recombinant yeast cell culture, and the purified antigen in alum formulation stimulated production of antibody in mice, grivet monkeys and chimpanzees. Vaccinated chimpanzees were totally protected when challenged intravenously with either homologous or heterologous subtype adr and ayw virus of human serum source. This is the first example of a vaccine produced from recombinant cells which is effective against a human viral infection.

Several alternative approaches to a hepatitis B vaccine are being developed. HBsAg has been expressed by several transformed mammalian cell lines, such as the human hepatoma line, PLC/PRF/5 (refs 10, 11), simian virus 40-infected monkey kidney cells[12] and mouse L cells[13]. These sources are of some concern, however, because the cell lines may be neoplastic. Although HBsAg has been cloned in bacteria[7,14], expression was very weak. Other laboratories[15-19] have described the synthesis of oligopeptides that carry antigenic determinants of HBsAg but their potency in animals is low and much work will need to be done to potentiate antigenicity. Smith and collaborators[20] have described the construction of a recombinant vaccinia virus which expresses HBsAg and have proposed its use as a live attenuated vaccine; its antigenic potency has been demonstrated but whether such a vaccine would be safe and effective in man is still unknown.

Valenzuela *et al.*[9] originally reported that yeast cells are able not only to express the HBsAg gene but also to assemble the polypeptides into particles that have much the same appearance as particles isolated from human plasma and which are immunogenic in mice. Since then, other laboratories[21,22] have shown that HBsAg produced in yeast is antigenic in rabbits and guinea pigs. With such progress, recombinant yeast has become an attractive alternative to human plasma as a source of antigen for hepatitis B vaccine.

For vaccine preparation, the HBsAg used was of subtype adw and was produced in fermentation cultures of *S. cerevisiae* carrying an expression vector using yeast alcohol dehydrogenase I as a promoter. The yeast strain used in these studies was obtained from G. Ammerer (University of Washington) and is similar to the strain described by Valenzuela *et al.*[9] in which the production of HBsAg in yeast was first reported.

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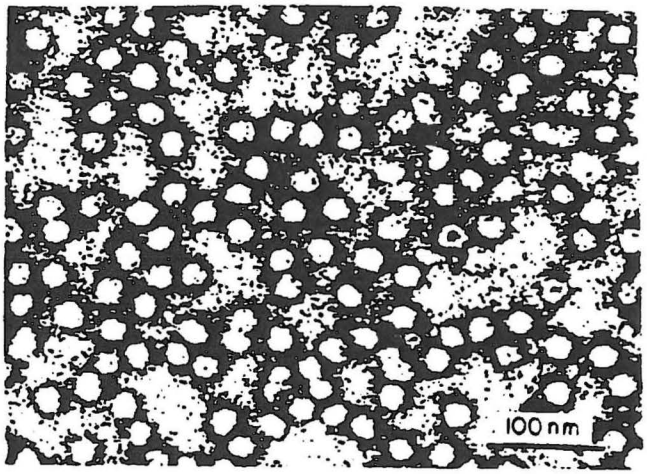

Fig. 1 Electron micrograph of HBsAg particles from recombinant yeast. Cells were grown in a 335-l fermentation vessel, collected by centrifugation, resuspended in an equal volume of 0.01 M sodium phosphate *p*H 7.5, containing 0.01% Triton X-100, and disrupted by rapid stirring with glass beads in a Dyno-Mill (Impandex; see ref. 23). The resulting extract was clarified by centrifugation for 90 min at 10,000*g*. The clarified yeast extract was applied to a column of Sepharose 4B to which had been attached goat antibody to human HBsAg. The column was developed at a flow rate of 2 column vol per h. Extraneous protein was washed away with 5 column vol of buffer A and the HBsAg was eluted with 3 M NH_4SCN. Fractions containing HBsAg were pooled and thiocyanate was removed by dialysis against 0.01 M sodium phosphate *p*H 6.8, containing 0.15 M NaCl. Dialysed antigen was diluted to 40 μg ml^{-1} and visualized by negative staining with 2% phosphotungstic acid.

Cells were collected by centrifugation and broken by homogenization with glass beads[23]. HBsAg particles were purified from the clarified extract by immune affinity chromatography using goat antibody to human HBsAg. Electron microscopy (Fig. 1) revealed a homogeneous array of particles free of extraneous morphological entities. The UV absorption pattern was the same as for the plasma antigen, with an $E^{1\%}$ of 45. SDS-polyacrylamide gel electrophoresis (Fig. 2) in reducing conditions revealed a major band at molecular

Table 1 Antigenic potency in mice of HBsAg purified from yeast and from human plasma

Vaccine source	Antigen dose per injection (μg protein)	Anti-HbsAg response after vaccination No. positive/total	GMT
Human plasma	10	9/10	563
(lot 799-2)	2.5	10/10	2,235
	0.625	4/9	32
	0.156	0/10	4
ED_{50}	0.639		
Yeast	40	10/10	5,432
(lot 81-4)	10	10/10	3,400
	2.5	8/10	673
	0.625	8/10	967
ED_{50}	<0.625		

Groups of 10 5-week-old ICR/Ha mice propagated in our laboratories were given a single 1-ml injection intraperitoneally of serial fourfold dilutions of yeast or human plasma vaccine in alum diluent. The mice were bled individually and tested for serum antibody level 4 weeks later. Human plasma vaccine, lot 799-2, was prepared in these laboratories[2-4]. Yeast-derived vaccine, lot 81-4, was purified as described in Fig. 1 legend and adsorbed to alum. GMT, geometric mean titre, expressed in AUSAB units; ED_{50}, dose required to seroconvert 50% of the mice.

weight 23,000 (23K) corresponding to the non-glycosylated polypeptide which is the major polypeptide of the viral envelope. In this respect it differs from the plasma antigen which has, in addition to the 23K polypeptide, a glycosylated derivative which migrates at 27K. The yeast and plasma antigens differ also in their reactivity in the radioimmunoassay (RIA) (AUSRIA II, Abbott). RIA reactivity of purified yeast-derived HBsAg varied from preparation to preparation in the range 20–50% of the reference human antigen.

Because of this reduced radioimmune reactivity, and because the yeast antigen is not glycosylated, it was important to determine whether the antigen was immunogenic. To test both antigenicity and immunogenicity in animals, purified antigen was formulated into a vaccine by adsorbing on alum adjuvant to contain 40 μg HBsAg protein and 0.5 mg aluminium (hydroxide) per 1 ml dose.

Studies in mice (Table 1) showed the yeast-derived antigen to be at least as antigenic as the antigen purified from human plasma. Grivet monkeys also developed antibody following vaccination with the yeast-derived antigen (Table 2). A single injection of the vaccine at all dose levels resulted in seroconversion of all the animals in both vaccine groups. These results were important as they showed that high antibody titres were maintained for at least a year.

Protective efficacy was tested for by using susceptible chimpanzees. The four chimpanzees that received the recombinant vaccine developed antibody in substantial titre following vaccination (Table 3). Following challenge with infectious human plasma, all four vaccinated animals were protected. By contrast, all four unvaccinated animals developed hepatitis B virus infection with positive antigenaemia, antibody to hepatitis B core antigen (anti-HBcAg), elevation of serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), and liver histopathology. It is important to note that the animals were protected against both subtype adr and ayw challenge even though the vaccine is of the adw subtype.

Yeast fermentation technology is well established and we have shown that HBsAg can be isolated from yeast extracts in a highly purified form by a single application of immune affinity chromatography. Vaccine made from this antigen is equally as potent as human plasma-derived vaccine in stimulating antibodies in mice, and is protective in challenge experiments in chimpanzees. Antibodies raised by yeast-derived vaccine persisted for at least a year in monkeys, showing no important deviation from that of the plasma vaccine.

Human HBsAg is composed of a sequence of 226 amino acids of which the a antigen determinant is dominant. Small differences in amino acid sequence may occur at several positions in the polypeptide chain and are responsible for the subtype specificities[24]. In previous studies, chimpanzees that were cross-challenged with heterologous subtypes of hepatitis B virus after recovery from infection or vaccination with human plasma-derived antigens, were solidly protected due to the common group specificity of the dominant a antigen that is present in all HBsAg subtypes[25]. A protective efficacy trial in man of subtype ad vaccine of human plasma origin has shown strong protection against the homologous subtype[26,27] and, most recently, against the heterologous subtype ay[28] in studies carried out on the staffs of renal dialysis centres where subtype ay hepatitis is most common. The positive cross-protection afforded against heterologous subtype ayw virus challenge in chimpanzee immunized with type adw vaccine of yeast origin, indicates that the a antigen remains dominant in the recombinant-produced antigen obtained from human plasma.

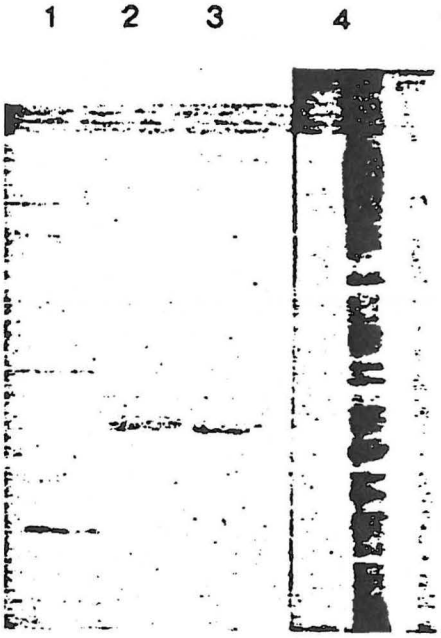

Fig. 2 SDS-polyacrylamide gel electrophoresis of cell culture and yeast-derived HBsAg. All samples were reduced, denatured and electrophoresed as described by Laemmli[30]. After electrophoresis, polypeptides were visualized with Coomassie brilliant blue (lanes 1–3) or with the silver stain procedure described by Morrissey[31] (lane 4). Lane 1, molecular weight standards (3 μg each): phosphorylase *b* (94K), bovine serum albumin (68K), ovalbumin (43K), carbonic anhydrase (30K), soybean trypsin inhibitor (21K) and lysozyme (14.3K). Lane 2, 30 μg of HBsAg from the human hepatoma cell line PLC/PRF/5 (ref. 10), also purified from yeast as described in Fig. 1 legend. Lane 3, 30 μg of HBsAg purified from yeast as described in Fig. 1. Lane 4, 10 μg of clarified yeast extract as described in Fig. 1 legend.

Table 2 Antigenic potency in grivet monkeys of HBsAg purified from yeast and from human plasma

Vaccine source	Antigen dose per injection (μg protein)	Anti-HBsAg response after initial vaccine dose (geometric mean titre) Week 4	Week 8	Week 12	Week 52
Human plasma (lot 86016)	10	36	213	170	127
	2.5	343	6,227	17,348	9,924
	0.625	53	4,642	3,164	5,688
	0.156	15	128	83	358
Yeast (lot 81-4)	40	88	1,078	7,103	11,554
	10	184	877	8,489	4,984
	2.5	225	1,168	6,361	10,868
	0.625	109	925	518	313

A group of four initially seronegative grivet monkeys (*Cercopithecus aethiops*), weighing 3–5 kg, were each given two 1-ml intramuscular (i.m.) doses of yeast or human plasma vaccine 4 weeks apart. Dilutions of antigen were made in alum placebo of the same composition as the vaccine. Animals were bled at biweekly intervals for 1 yr and tested for antibody to HBsAg by using a commercial RIA kit (AUSAB, Abbott). Protein was measured by the method of Lowry[29]. Human plasma lot 86016 was prepared in these laboratories[2–4].

Table 3 Protective efficacy in chimpanzees of HBsAg purified from yeast and from human plasma

Injection	Chimp no.	Before challenge: Anti-HBsAg titre (at 12 weeks)	Before challenge: Antigen subtype	After challenge (week of onset or weeks of duration): HBsAg Onset	HBsAg Duration	Anti-HBcAg Onset	Anti-HBcAg Duration	SGOT elevation Onset	SGOT elevation Duration	SGPT elevation Onset	SGPT elevation Duration	Liver pathology onset
Yeast vaccine (lot 81-4)	110	1,830	adr	–	–	–	–	–	–	–	–	–
	138	540	adr	–	–	–	–	–	–	–	–	–
	103	18,300	ayw	–	–	–	–	–	–	–	–	–
	120	7,200	ayw	–	–	–	–	–	–	–	–	–
Unvaccinated controls	111	<8	adr	10	10	15	9	17	3	17	6	20
	128	<8	adr	8	11	12	12	17	3	16	5	20
	127	<8	ayw	6	14	12	12	13	3	13	7	16
	130	<8	ayw	6	18	10	14	22	1	14	10	24

Eight chimpanzees, each weighing 40–60 kg, were selected for study based on negative findings in tests for HBsAg, anti-HBsAg, anti-HBcAg, elevation in transaminase, liver histopathology and tuberculin reaction. The animals were separated into two groups, four test animals and four controls. Each of the four test animals was given three 40-μg doses of yeast-derived HBsAg vaccine in 1 ml volume i.m. at 4-week intervals. All eight animals were then challenged by intravenous injection of 1,000 chimpanzee infectious doses of subtype adr or ayw virus in 1 ml of human hepatitis B plasma. Antigen and antibody titres were measured by commercial (Abbott) RIA kits (AUSRIA, AUSAB and CORAB for HBsAg, anti-HBsAg and anti-HBcAg, respectively). SGOT and SGPT assays were performed by the Sigma-Frankel (no. 505) and by the UV absorption (Boehringer-Mannheim) procedures, respectively. SGOT titres >40 and SGPT titres >30 were considered elevated. The subtype adr and ayw human plasmas used for challenge were obtained from Drs R. Gerety and E. Tabor of the Office of Biologics, US Food and Drug Administration; they were of measured viral infectiousness for chimpanzees and were subtyped serologically. The animals were bled at weekly intervals during the 36-week period of observation, covering 12 weeks before virus challenge and 24 weeks after. Liver biopsies were taken at 4-week intervals using a Menghini 16T needle. The tissues were fixed in 10% buffered formalin solution and the haematoxylin/eosin-stained sections were prepared by Dr A. Phelps of these laboratories under blind code number. The tests were carried out in animals that were held in isolation in the facilities of Dr William E. Greer at the Gulf South Research Institute, New Iberia, Louisiana. –, All remained negative.

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Human hepatitis B vaccine from recombinant yeast. Nature 1984; 307:178-80.

Erste Erfahrungen mit rekombinanter Hepatitis B-Vaccine bei Patienten unter chronischer Haemodialyse-Behandlung

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Die Immunogenität natürlicher, aus Humanplasma gewonnener Hepatitis B-Vaccine hat sich bei endogen oder exogen immunsupprimierten Patienten beträchtlich schwächer erwiesen als bei gesunden Personen. Es erschien daher interessant zu prüfen, ob nach Impfung mit einer gentechnologisch gewonnenen HB-Vaccine bei chronischen Haemodialyse-Patienten höhere Seronkonversionsraten für anti-HB$_s$ erzielt werden können als mit natürlichem HB-Impfstoff. 51 HBV empfängliche Patienten unter chronischer Haemodialyse-Behandlung erhielten 3 Impfungen mit je 40 ug Hb$_s$Ag Protein, das in einem DNS-rekombiniertem Stamm der Hefe Saccharomyces cerovisiae hergestellt wurde (Hepatitis B-Vaccine [recombinant] MSD, Westpoint USA, Lot 934/C-J625). Die zweite und dritte Impfung erfolgten einen bzw. 6 Monate nach der ersten Impfung. Einen Monat nach der 2. Impfung hatten 20 von 48 (42%) der Patienten anti-HB$_s$ gebildet. Der mittlere Antikörper-Gehalt betrug 24,7 IU/ml. Bei 21 Patienten ist das Impfprogramm abgeschlossen, 13 von ihnen wiesen im 7. Monat nach Impfbeginn eine Serokonversion nach anti-HB$_s$ auf. Der mittlere anti-HB$_s$-Gehalt war auf 151 IU/ml angestiegen. Danach lassen sich bei Dialyse-Patienten mit rekombinat hergestellter HB-Vaccine ähnliche Serokonversionsraten erzielen wie mit HB-Impfstoff, der aus Humanplasma gewonnen wurde.

Muller R, Bommer J, Brass H, Deinhardt A, Jilg W, Kuttler G, et al. Erste erfahrungen mit rekombinanter hepatitis B-vaccine bei patienten unter chronischer haemodialyse-behandlung. Gastroenterol 1985; 23:297.

IMMUNOGENICITY OF RECOMBINANT HEPATITIS B VACCINE

SIR,—Jilg et al[1] have compared the immunogenicity of recombinant[2] and plasma derived hepatitis B vaccines. We report for comparison the results of a similar trial of the recombinant vaccine in a younger age group. 55 male armed forces recruits, aged 17–19, all of whom were susceptible to hepatitis B virus were given

IMMUNE RESPONSES AFTER RECOMBINANT (n=55) OR PLASMA (n=50) HEPATITIS B VACCINATION

Month	Seroconversion		GMT anti-HBs (IU/l)	
	Recombinant	Plasma	Recombinant	Plasma
1	37 (67%)	32 (64%)	11	4
3	54 (100%)*	49 (98%)	198	278
6	54 (100%)*	49 (98%)	189	492
7	53 (100%)†	50 (100%)	2749	9227

*1 lost to follow-up. †2 lost.

10 μg of recombinant vaccine (lot 979/C-K 564, Merck Sharp and Dohme) intramuscularly at 0, 1, and 6 months. The results can be compared with those in another group of recruits of the same age who had been given 10 μg of the same manufacturer's plasma-derived vaccine at 0, 1, and 6 months in an earlier study.[3]

Seroconversion rates and geometric mean antibody titres (GMT) of anti-HBs (see table) were substantially higher than those reported by Jilg et al.[1] The final GMT was 2749 IU/l (95% confidence interval: 1676–4506) compared with 911 IU/l for 12 males reported by Jilg et al.[1] After the booster dose, all vaccines had an anti-HBs titre above the protection level of 10 IU/l; 43 (81%) had titres above 1000 IU/l. The stronger immune response in our study than in Jilg's may be explained by the fact that our vaccines were younger (17–19 vs 21–34). We observed only minor side-effects in 26% of participants; this is as reported by Jilg et al.[1]

The seroconversion rates were the same as those obtained in our earlier trial of a 10 μg dose of the plasma-derived vaccine.[3] In contrast to Jilg et al[1] GMT antibody levels in our recombinant group in the first 3 months were similar ($p>0·05$) to those induced by the plasma-derived vaccine, although levels after the booster dose were significantly lower ($p<0·001$) in the recombinant group (Mann-Whitney tests, separately at each time).

Our results accord with those of Jilg et al in confirming the safety and immunogenicity of the Merck Sharp and Dohme recombinant vaccine. The minor differences in immune responses show the need for further trials in population groups under consideration for vaccination, before a dose and vaccination scheme are decided on. In assessing the efficacy of this vaccine, information on the quality of the anti-HBs induced should complement the anti-HBs levels achieved.[4]

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23

Antibody and Clinical Responses Among Healthy Adults to a Hepatitis B Vaccine Made by Recombinant DNA

Currently, all commercial hepatitis B vaccines are comprised of HBsAg purified from the plasma of human carriers of the virus. However, the use of recombinant DNA technology to effect synthesis of surface antigen by a culture of microorganisms is an attractive alternative to infected human plasma as a source of HBsAg for vaccine. Good expression of the gene for HBsAg has been effected in yeast *(1)*.

Recently, antigen purified from fermentation cultures of a recombinant strain of the yeast, *Saccharomyces cerevisiae* containing the gene for the *adw* subtype of HBsAg was formulated into a vaccine through absorption on alum adjuvant. Two methods were utilized for the purification of the HBsAg. Immune affinity chromatography uses specific antigen-antibody binding to effect purification, while the second method, hydrophobic interaction chromatography followed by gel exclusion chromatography, depends upon the selection of water-immiscible molecules followed by separation on the basis of molecular size.

The physical and chemical characteristics of vaccine made from HBsAg produced in yeast are very similar to those of vaccine prepared with HBsAg purified from human plasma. Furthermore, the yeast recombinant hepatitis B vaccine has been shown to be both immunogenic and protective in animals *(2)*.

We report here the clinical and antibody responses obtained in the first three human clinical studies of the yeast recombinant vaccine involving a total of 101

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vaccinees. Participants were healthy, nonpregnant, adult volunteers. At entry, subjects were negative for all hepatitis B serologic markers, had a normal ALT level, and had not received any other hepatitis B vaccine.

Participants in the studies received a 1.0-ml intramuscular injection of the yeast recombinant hepatitis vaccine containing 10 μg of HBsAg at 0, 1 and 6 months. The vaccine used was from one of two lots. (Lot 934 prepared by the immune affinity chromatography method and Lot 972 prepared by the hydrophobic interaction chromatography method.) Vaccinees were asked to record their temperature daily for 5 days after each injection of vaccine and to report any local or systemic reactions that occurred during that period.

Postvaccination blood samples were taken for the determination of hepatitis B serologic markers and ALT. In addition, a radioimmunoassay for the detection of antibody to antigens in an extract of yeast lacking the gene for HBsAg was applied to pre- and postvaccination samples.

The vaccine was well tolerated. There have been no serious adverse effects attributable to vaccine and no evidence of hepatitis B infection among the vaccinees (i.e., no elevation of ALT and no antigenemia). Local reactions consisting principally of mild soreness at the injection site, generally lasting 1–2 days, have been reported following 20%–80% of injections with vaccine purified by the immune affinity chromatography method (Lot 934) and 16%–25% of injections with vaccine purified by the hydrophobic interaction chromatography method. Systemic complaints including fatigue, headache, elevated temperature (101° F–102° F, oral), gastrointestinal disturbance, symptoms of upper respiratory infection and nosebleed have been reported following 4%–33% of injections (Table 23.1). There have been no significant increases in antibody to antigens in yeast extract associated with vaccination.

Table 23.1
Clinical Responses among Healthy Adults to 10 μg Doses of Recombinant Hepatitis B Vaccine Administered at 0, 1 and 6 Months

			Proportion (%) of Vaccinees with Clinical Complaints within 5 Days of Vaccination		
Study #	Vaccine Lot #	Site	Dose 1 (%)	Dose 2 (%)	Dose 3 (%)
779	934	Local	12/15 (80)	11/15 (73)	11/15 (73)
		Systemic	5/15 (33)	3/15 (20)	1/15 (7)
	972	Local	6/24 (25)	3/19 (16)	
		Systemic	1/24 (4)	3/19 (16)	
792	934	Local	19/28 (68)	11/28 (39)	
		Systemic	5/28 (18)	4/28 (14)	
795	934	Local	5/25 (20)	6/19 (32)	
		Systemic	5/25 (20)	1/19 (5)	

Table 23.2
Seroconversion Frequencies for Anti-HBs among Healthy Adults Receiving 10 μg Doses of Recombinant Hepatitis B Vaccine at 0, 1 and 6 Months

		Proportion (%) of Vaccinees with Antibody				
Study #	Vaccine Lot #	1 Mo.	2 Mo.	3 Mo.	6 Mo.	7 Mo.
779	934	6/15 (40)	14/15 (93)	15/15 (100)	15/15 (100)	14/14 (100)
	972	7/24 (29)	13/19 (68)	12/14 (86)		
792	934	11/28 (39)	21/23 (91)	13/13 (100)		
795	934	8/30 (27)	21/30 (70)	19/22 (86)		

Antibody responses to 10 μg doses of the yeast recombinant vaccine have been comparable to those observed in previous studies with 20 μg doses of vaccine prepared from plasma-derived HBsAg. At 1 month, 27%–40% of the vaccinees were positive for anti-HBs. By 2 months, 68%–93% of the vaccinees had anti-HBs, and at 3 months 86%–100% were antibody positive (Table 23.2). The third dose of vaccine at 6 months has been given to 15 persons in one of the studies, resulting in a more than 25-fold increase in geometric mean titer.

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Original Contributions

Clinical Evaluation in Healthy Adults of a Hepatitis B Vaccine Made by Recombinant DNA

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• A vaccine formulated from hepatitis B surface antigen (HBsAg) produced by a recombinant strain of the yeast *Saccharomyces cerevisiae* was administered to two groups of human volunteers composed of 37 healthy, low-risk adults. Each subject received a 10-μg dose of HBsAg at 0, 1, and 6 months. By one month, 27% to 40% of the vaccinees had antibody to HBsAg, and by three months 80% to 100% were antibody positive. Large boosts in titer followed the third dose at six months. The antibody formed is predominantly specific for the *a* determinant of HBsAg. There have been no serious reactions attributable to the vaccine. The most frequent complaint has been transient soreness at the injection site. As far as we know, this is the first reported use in man of a vaccine prepared by recombinant DNA technology.

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WORLDWIDE, human hepatitis B infection constitutes a major public health problem. In addition to the disability associated with acute clinical disease, chronic liver disease, cirrhosis, and primary hepatocellular carcinoma are now recognized sequelae of unresolved hepatitis B infection. Indeed, in some areas of Asia and sub-Saharan Africa, primary hepatocellular carcinoma ostensibly attributable to hepatitis B infection ranks as a leading cause of cancer deaths among males.[1]

See also p 2765.

The reservoir of hepatitis B virus resides mainly in a population of chronic carriers now estimated to number more than 200 million.[2] Infection is transmitted to susceptible persons through contact with the blood, semen, or saliva of chronic carriers or persons suffering acute infection. In low-incidence countries, such as the United States, the risk of hepatitis B infection is still high among certain groups of health care personnel, patients receiving dialysis treatments or blood products made from large pools, children born to Alaskan Eskimos or to Indochinese or Haitian refugees, residents of institutions for the mentally handicapped, prisoners, users of illicit injectable drugs, and persons who are sexually very promiscuous.[1] In high-incidence areas such as Southeast Asia, transmission from mother to child in the perinatal period is the major mode of infection supplemented by horizontal transmission between other family contacts.[2]

Since there is no effective treatment for hepatitis B infection, prevention is essential. A safe, effective human hepatitis B vaccine is now available. However, it utilizes hepatitis B surface antigen (HBsAg) purified from the plasma of human carriers of hepatitis B virus infection. Consequently, the supply of vaccine is potentially limited by available sources of suitable plasma. In addition, extensive processing and safety testing have been necessary to ensure production of a vaccine antigen that is pure and free of any extraneous living agent that might have been present in the starting plasma. Even though multiple inactivation treatments used in the antigen purification process have been shown to inactivate representatives of all major groups of animal viruses,[4] concern over the theoretical possibility of a living organism such as the etiologic agent of acquired immune deficiency syndrome being present in plasma and surviving the purification and inactivation procedures has slowed acceptance of hepatitis B vaccine.

A promising alternative to infected human plasma as a source of HBsAg for vaccine is the use of recombinant DNA technology to effect synthesis of the surface antigen by a culture of microorganisms. The hepatitis B virus gene coding for HBsAg has been cloned both in *Escherichia coli* and in yeast[5-7]; however, expression of the gene in yeast has been much better than in *E coli*. Furthermore, HBsAg

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produced by recombinant yeast cells has been shown to aggregate into particles closely resembling those isolated from human plasma, and this material was shown to include antibodies in mice and guinea pigs.[9]

Recently, antigen purified from fermentation cultures of a recombinant strain of the yeast *Saccharomyces cerevisiae* containing the gene for HBsAg has been formulated into a vaccine through adsorption on alum adjuvant. Electron microscopy reveals that the purified HBsAg used for this vaccine exists as aggregate particles 20 to 22 nm in diameter, a morphology also characteristic of free surface antigen in infected plasma and of the purified antigen now used in plasma-derived hepatitis B vaccine. In contrast to HBsAg from human plasma, the antigen produced by recombinant yeast is not glycosylated. Under reducing conditions, sodium dodecyl sulfate electrophoresis of the antigen purified from yeast reveals a single band of molecular weight 23,000, which corresponds to the nonglycosylated polypeptide that is the major component of the hepatitis B virus envelope. The vaccine formulated using this material has now been shown to be immunogenic for mice and for monkeys with a potency equal to or superior to that of vaccine made from plasma-derived antigen. In addition, chimpanzees immunized with this yeast recombinant hepatitis B vaccine (HBsAg subtype *adw*) were fully protected when challenged with virus of either type *adr* or *ayw*, while unimmunized animals all showed evidence of infection when challenged.[9]

In this article we describe results of the first human immunogenicity-safety trial of the yeast recombinant hepatitis B vaccine. To the best of our knowledge, this is the first time that a vaccine prepared by recombinant DNA technology has been used in man.

MATERIALS AND METHODS

Population

Participants in this study were healthy, nonpregnant adult employees of Merck and Co, Inc. Subjects had to be negative for hepatitis B serological markers and have a normal level of alanine aminotransferase and must not have received any other hepatitis B vaccine. Written consent was obtained after providing each participant with information on the source of the investigational yeast recombinant hepatitis B vaccine, animal test results obtained with the vaccine, vaccination and bleeding schedules, and the potential risks and benefits of participation in the study.

Vaccine

Hepatitis B surface antigen for the vaccine was produced in fermentation cultures of a recombinant strain of the yeast *S cerevisiae* containing a plasmid carrying the gene for the *adw* subtype of HBsAg, as described previously.[1,10]

Two methods were employed for the purification of HBsAg. Immune affinity chromatography uses specific antigen-antibody binding to effect purification, while the second method, hydrophobic interaction chromatography followed by gel exclusion chromatography, depends on selection of water-immiscible molecules followed by separation by molecular size. Details of the expression of HBsAg in yeast and the purification of the surface antigen will be published elsewhere. Purified HBsAg was treated with formaldehyde to stabilize the material and to kill any extraneous living agents that might be present. The antigen was then formulated into a vaccine through adsorption on alum adjuvant to give 10 μg of HBsAg and 0.5 mg of aluminum (hydroxide) per 1-mL dose. The final formulation also contained 1:20,000 thimerosal as a preservative. Vaccine was maintained at 2 to 8 °C until used.

Procedures

A blood sample was obtained from each subject approximately two weeks prior to the first vaccination and was tested for HBsAg, antibody to HBsAg (anti-HBs), antibody to core antigen (anti-HBc), alanine aminotransferase (ALT), and yeast antibody. Subjects found eligible on the basis of these assays were scheduled to receive a 1.0-mL (10-μg HBsAg) intramuscular injection of the yeast recombinant vaccine at 0, 1, and 6 months. Postvaccination blood samples for the determination of hepatitis B serological markers, ALT, and yeast antibody were scheduled monthly for seven months and at 9, 12, and 24 months following the first injection.

Vaccinees were asked to take their temperature daily for five days after each injection of vaccine and to report any local or systemic reactions that might occur during this period.

Assays

Standard radioimmunoassay test kits were used for the determination of HBsAg, anti-HBs, and anti-HBc. Titers of anti-HBs were expressed in international milliunits per milliliter using the formulation described by Hollinger et al.[11] A serum sample was considered positive for anti-HBs if the ratio of the sample counts per minute to the negative control serum counts per minute was 2.1 or greater.

Estimates of the proportion of anti-HBs in postvaccination sera specific for the *a* or *d* determinants of HBsAg were based on an assay described by Hoofnagle et al.[12] Briefly, aliquots of each serum sample are incubated with a subtype *ad* HBsAg-positive serum, with a subtype *ay* HBsAg-positive serum, and with normal human serum for two hours at room temperature, and then each mixture is carried through a standard radioimmunoassay to measure residual anti-HBs. Based on the percent of neutralization with the two HBsAg subtype sera when compared with the unneutralized normal human serum, an estimate can be made of the relative amounts of anti-*a* and anti-*d* antibodies present. Since the vaccine is a monovalent-type *adw* preparation, sera will contain either anti-*d* antibodies, anti-*a* antibodies, or a combination of both types, and the amount of neutralization with the HBsAg-*ay* serum is therefore a direct assay for the amount of anti-*a* present. Subtracting the amount of neutralization with the HBsAg-*ay* serum from that found for the HBsAg-*ad* serum then gives an estimate of the amount of anti-*d* present.

A radioimmunoassay was developed to detect yeast antibodies in the sera of vaccine recipients. For this assay, an extract of the parent strain of *S cerevisiae* lacking the plasmid containing the gene for HBsAg was prepared by disrupting a 50% suspension of the cells in a homogenizer and then clarified by centrifugation at 9,000 g followed by passage through a 0.45-mμm membrane filter. The clarified, filtered extract was diluted to a final protein concentration of 80 μg/mL with 0.1 M carbonate buffer and pH 9.6 and adsorbed to ¼-in polystyrene beads overnight at 4 °C. Washed, dried beads were maintained at −20 °C. Two hundred-microliter volumes of sera diluted 1:100, 1:1,000, and 1:10,000 in phosphate-buffered saline containing 0.5% bovine serum albumin and 0.5% Tween 20 were incubated with coated beads for three hours at 37 °C. Following three washes with water, the beads were incubated with 200 μL of iodine 125 protein A (specific activity, 100,000 cpm) for 1.5 hours at 37 °C. The protein A binds and labels any antiyeast antibody on the bead that is of the IgG class. After three additional water washes, the beads were counted and titers of yeast antibody were determined by interpolation from a standard curve derived using dilutions of a hyperimmune guinea pig serum having an antibody titer to parent yeast extract of 1 million.

The serum samples of vaccinees were also measured for changes in preexisting specific yeast antibodies or the appearance of new yeast antibodies using a sodium dodecyl sulfate polyacrylamide gel electrophoresis (reducing), Western blot technique. In this procedure, parent yeast extract is separated on a 12.5% polyacrylamide gel. After transfer to a nitrocellulose sheet, polypeptides from the gel are detected by incubation with a 1:50 dilution of the vaccinee's serum, followed by incubation with ^{125}I protein A and exposure to x-ray film (T. Mason, PhD, oral communication, 1982).

RESULTS

The vaccine has been well tolerated. None of the 37 subjects studied to date has experienced a serious adverse effect attributable to vaccine. There has been no evidence of hepatitis B infection among vaccinees, ie, no elevation of ALT values and no antigenemia. Mild soreness at the injection site generally lasting one to two days was reported by 73% to 80% of vaccinees who received vaccine purified by immune affinity chromatography (lot 934) but by a substantially smaller proportion—20% to 24%—of subjects who received vaccine prepared by hydrophobic interaction chromatography (lot 972) (Table 1). Infrequent systemic complaints occurring within a five-day period following vaccination have included elevated temperature (38.3 to 38.8 °C [101 to 102 °F], oral), fatigue, headache, gastrointestinal disturbance, symptoms of upper respiratory tract infection, and nosebleed.

Table 2 summarizes our observations to date on the human immunogenicity of yeast recombinant hepatitis B vaccine. Fifteen persons (ten men, five women; age range, 23 to 53 years; median age, 33 years) have received all three doses of lot 934 vaccine prepared by the immune affinity chromatography method. Forty percent had a detectable titer of anti-HBs within one month of receiving the first dose. By two months, the proportion of seroconverters rose to 93%, and at three months, all recipients of this vaccine were antibody positive. The geometric mean titer following primary immunization reached a plateau at four months, then increased more than 25-fold following the booster dose at six months.

Table 1.—Proportion (%) of Vaccinees With Clinical Complaints During a Five-Day Period Following Injection of Yeast Recombinant Hepatitis B Vaccine

Nature of Complaint	Vaccine Lot No.	Dose 1	Dose 2	Dose 3
Soreness at injection site	934	12/15 (80)	11/15 (73)	11/15 (73)
	972	5/21 (24)	3/15 (20)	
Systemic* complaints	934	5/15 (33)	3/15 (20)	1/15 (7)
	972	1/21 (5)	2/15 (13)	

*Includes persons with one or more episodes of the following: temperature, 38.3 to 38.8 °C (101 to 102 °F) (two), fatigue (three), gastrointestinal disturbance (four), headache (five), symptoms of upper respiratory tract infection (three), and nosebleed (one).

Table 2.—Seroconversion Frequencies and Geometric Mean Titers (GMTs)* for Anti-HBs Among Initially Seronegative Healthy Adults Receiving 10-μg Doses of Yeast Recombinant Hepatitis B Vaccine†

Vaccine Lot No. (Method of Preparation)	No. of Subjects Vaccinated	Time, mo	Seroconversion Proportion (%)	GMT All Vaccinees‡	GMT Responders Only
934 (Immune affinity chromatography)	15	1	6/15 (40)	1.6	6.0
		2	14/15 (93)	31.7	44.2
		3	15/15 (100)	55.5	55.5
		4	15/15 (100)	78.2	78.2
		5	14/14 (100)	77.2	77.2
		6	15/15 (100)	67.9	67.9
		7	12/12 (100)	1,905.1	1,905.1
972 (Hydrophobic interaction chromatography)	22	1	4/15 (27)	1.4	39.6
		2	8/12 (67)	17.8	108.7
		3	4/5 (80)	56.5	218.5

*In international milliunits per milliliter.
†At 0, 1, and 6 months.
‡All serum samples with titers of less than 0.6 ImU/mL were assigned a value of 0.3 ImU/mL for calculating GMTs.

Table 3.—Percentages of Anti-HBs Specific for *a* and *d* Determinants of HBsAg in Postvaccination Sera*

Vaccine Lot No.	Time, mo	No. of Samples	% Anti-*a* Range	% Anti-*a* Mean	% Anti-*d* Range	% Anti-*d* Mean
934	1	1		47		53
	2	7	87-98	93	2-10	6
	3	10	63-98	86	2-37	13
	4	13	65-98	89	2-35	11
	5	12	80-97	92	2-20	6
	6	8	92-97	94	2-8	5
	7	12	89-100	98	0-11	2
972	1	2	56-81	74	8-44	26
	2	6	87-100	94	0-13	6

*Assay done only on serum samples having an anti-HBs titer of 25 ImU/mL or greater.

Twenty-two subjects have received vaccine from lot 972 made from HBsAg purified by the hydrophobic intraction chromatography method. These vaccinees have not been followed up for as long as the lot 934 recipients, and none has yet received a third dose. Preliminary serological results are shown in Table 2 for 15 of these volunteers (12 men, three women; age range, 24 to 63 years; median age, 40 years). The percentage of seroconverters was 27% at one month, 67% at two months, and 80% at three months. Geometric mean titers within the first three months of follow-up were similar to those observed among recipients of lot 934 vaccine.

Postvaccination serum samples with anti-HBs titers of 25 ImU/mL or greater were assayed to determine the percentage of antibody specific for the *a* and *d* determinants of HBsAg. Table 3 shows the results of these assays. Antibody specific for the *a* determinant predominates. In the interval from two to seven

months following the first dose of vaccine, anti-*a* antibody accounted for approximately 90% of the total anti-HBs.

Earlier studies (unpublished) showed that the yeast recombinant hepatitis B vaccine induced a predominantly anti-*a* form of anti-HBs in African green monkeys and that these antibodies have persisted through two years of follow-up.

Analysis of serum samples from participants in this study has revealed no significant postvaccination increases in yeast antibody titers as measured by radioimmunoassay. By Western blot analysis, each human serum sample shows a unique "fingerprint" spectrum of antibodies to yeast components. There may be only a few or as many as 20 different bands present. Analysis of monthly postvaccination serum samples from participants in this study has shown no change in the yeast antibody pattern for any person as compared with his prevaccination pattern. There has been no appearance of new antibodies in postvaccination sera and no significant increases in the intensity of existing antibody bands.

CONCLUSIONS

The results of this study indicate that an alum-adsorbed hepatitis B vaccine formulated using HBsAg of subtype *adw* synthesized by recombinant yeast cells is safe and immunogenic for man. Seroconversion rates and titers of anti-HBs obtained with the yeast recombinant vaccine in this study are comparable with those observed in earlier studies of healthy adults using vaccine derived from human plasma.[13-17]

Previous studies with hepatitis B vaccine of human plasma origin showed that protection from infection is associated with vaccine-induced anti-HBs.[18-20] Furthermore, one of these trials demonstrated that antibody formed in response to vaccine of HBsAg subtype *ad* provided cross-protection against infection caused by heterologous virus of subtype *ay*.[20] Since the antibody formed by recipients of the yeast recombinant hepatitis B vaccine is predominantly anti-*a*, this vaccine should be protective against all hepatitis B virus subtypes. The efficacy of the yeast vaccine against both homologous *ad* and heterologous *ay* virus challenge in chimpanzees has been demonstrated.[10]

Studies are under way to assess antibody persistence and to determine optimal doses of the yeast recombinant hepatitis B vaccine for both healthy and immunocompromised adults and children.

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OVERVIEW OF CLINICAL STUDIES WITH HEPATITIS B VACCINE (RECOMBINANT)

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Clinical studies with the Merck recombinant yeast hepatitis B. vaccine were initiated in July 1983. Over 3000 individuals have received at least one dose of vaccine. Vaccination was carried out at 0, 1 and 6 months and doses ranged from 1.25 mcg to 40 mcg. Seroconversion rates, for various populations, are expressed as the percentage of individuals who, at 7-8 months (1-2 months after the third dose of vaccine) had an anti-HBs titer ≥10 mIU/ml. Geometric mean titers (GMT) are expressed as mIU/ml for responders.

	2.5 mcg Dose		5 mcg Dose		10 mcg Dose	
Population (Age)	Rate(%)	GMT	Rate(%)	GMT	Rate(%)	GMT
Adults (20-69)	97	321	90	335	96	975
Teenagers (16-19)	94	1132	100	2553	100	3059
Children (1-11)	100	4137	100	16000	Not	Tested

The vaccine has been shown to be safe in all populations immunized. The most frequent clinical complaints during a 5-day period following 2179 injections, were soreness, pain and tenderness at the injection site (9%, 4% and 3%, respectively), and fatigue/weakness (5%) or headache (4%).

The recombinant yeast HBsAg is of the ad subtype. In vaccine recipients antibody specific for the a determinant predominates. By 8 months post the first dose of vaccine, the mean percentage of anti-a in all sera tested was 97%.

Sera from 138 vaccine recipients tested for antibodies to yeast antigens showed high antibody titers in both pre and post-vaccination samples. There was no correlation between increased yeast antibody titer and frequency or severity of clinical reactions. The recombinant yeast hepatitis B vaccine has been shown to be safe and immunogenic in all populations studied.

Zajac BA, West DJ, McAleer WJ, Scolnick EM. Overview of clinical studies with hepatitis B vaccine (recombinant). Presented at the fifth biennial scientific meeting, Asian Pacific Association for the Study of the Liver, Symposium on recent advances in the prevention of hepatitis B infection, January 1986, Singapore.