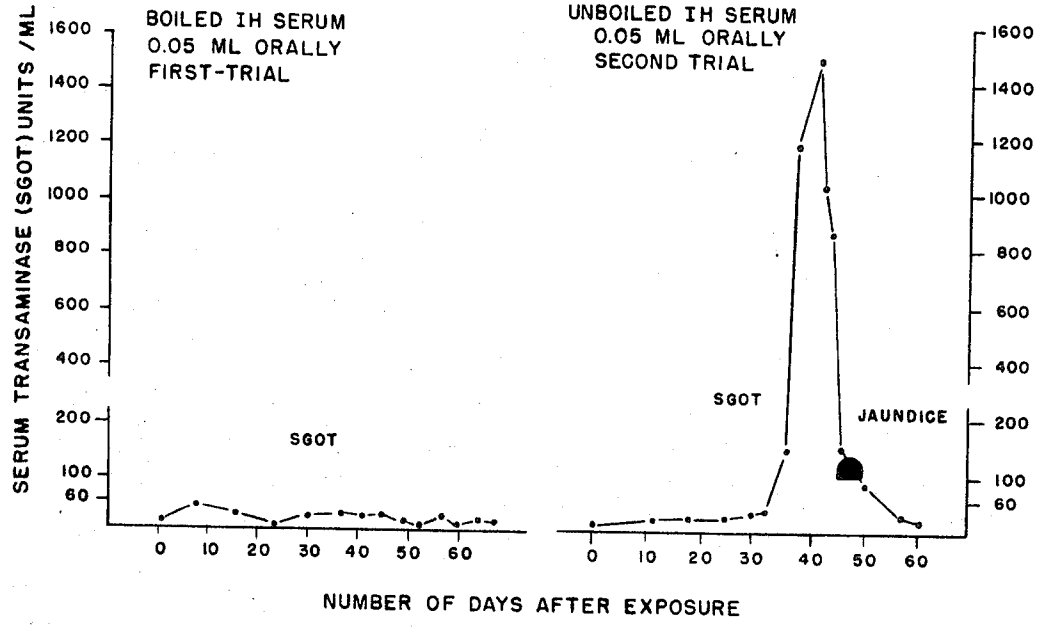
May 22, 1973 S. KRUGMAN ET AL 3,735,004

HEPATITIS VACCINE

Filed Nov. 1, 1971

FIG. 1



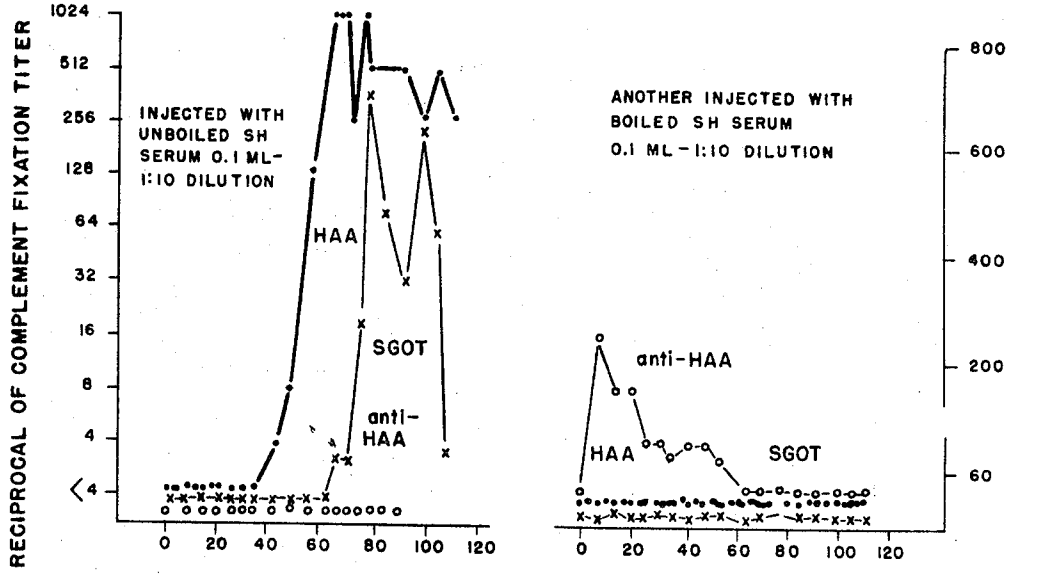


FIG. 2

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HEPATITIS VACCINE

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U.S. Cl. 424—89 15 Claims

ABSTRACT OF THE DISCLOSURE

Production of a vaccine against viral hepatitis, Type B and administration thereof to humans. The vaccine is prepared by boiling viral hepatitis, Type B serum.

BACKGROUND OF THE INVENTION

(1) Field of the invention

This invention relates to preparation and administration to humans of a viral hepatitis, Type B vaccine.

(2) Description of the prior art

Viral hepatitis, Type B, also known as serum hepatitis (SH), has presented a problem in medical circles because of the absence of a vaccine specific thereto. This particular type of hepatitis, as well as viral hepatitis, Type A, also known as infectious hepatitis (IH), has been described by us in an article entitled "Infectious Hepatitis: Evidence for Two Distinctive Clinic, Epidemiological, and Immunological Types of Infection," and JAMA, vol. 200, pp. 365–373 (1967). Prior attempts to find a specific SH vaccine have failed because of the failure to cultivate the causative agent.

SUMMARY OF THE INVENTION

After extended investigation we have found that boiling serum containing viral hepatitis, Type B (SH) destroys the infectivity thereof without affecting its antigenicity. In other words, boiling gives the viral hepatitis, Type B an immunogenic capacity. That is, it does not significantly affect the complement-fixation titer (CFT) of hepatitis-associated antigen (HAA). This is particularly surprising since boiling of viral hepatitis, Type A (IH), on the other hand, while destroying the infectivity thereof, does not result in its becoming immunogenic. We have found that, to insure substantially complete destruction of the infectious nature of SH, it is advantageous to boil for a minimum time of about one minute. To prevent possible loss of immunogenic capacity, we prefer to boil no longer than about 3 minutes. Optimum temperature for boiling is about 98° C., the preferred range being 98–100° C. The dosage may comprises an amount about equivalent to 0.002 to 0.5 ml. of undiluted serum. For best results in immunization of humans with the boiled SH, we administer 2 doses of 0.1 ml. of a 1:10 dilution of MS–2 serum by injection. The immunization appears to be most effective if a second dose is administered about 4 months after a first. A study to evaluate 3 doses at 2 month intervals is currently in progress.

BRIEF DESCRIPTION OF THE DRAWING

For a better understanding of the invention, reference will now be made to the drawing, in which:

FIG. 1 illustrates in graphical form the results of oral administration to one child, first of 0.05 ml. of boiled (98° C. for 1 minute) IH serum, and second of 0.05 ml. unboiled IH serum. The standard serum glutamic-oxaloacetic transaminase (SGOT) test level of less than 100 all the way through the 60-day first-trial period indicates no infectivity. The sharp increase in SGOT after about 35 days of the second trial shows the lack of immunization

by the first-trial oral administration of viral hepatitis, Type A boiled serum.

FIG. 2 is a graphical representation showing the result of intramuscular injection of unboiled SH serum to one child, specifically, development of high Australia or hepatitis-associated antigen (HAA) and high SGOT levels after between about 40 and 80 days, without development of significant amounts of HAA antibody, that is, infection of the child by the unboiled viral hepatitis, Type B. The same figure (FIG. 2), in the second part, shows the effect of intramuscular injection of 0.1 ml. of 1:10 dilution of boiled (here 98° C. for 1 minute) SH on another child, specifically, that the child became asymptomatic as to infection with viral hepatitis, Type B, and developed an antibody response (anti-HAA) from day 7 to day 55.

DESCRIPTION OF PREFERRED EMBODIMENTS

The following examples are intended to better illustrate our invention.

Source of hepatitis viruses

An IH serum pool was prepared from sera obtained from 10 patients (children) with viral hepatitis, Type A. Aliquots of this pool were stored at —20° C. Use of this serum in a series of trials confirmed its capacity to cause viral hepatitis, Type A. The IH serum pool was negative for Australia or hepatitis-associated antigen (HAA).

An SH serum pool was prepared from sera obtained from 6 persons who had viral hepatitis, Type B. Aliquots of this pool were also stored at —20° C. Use of this serum in a series of trials confirmed its capacity to cause viral hepatitis, Type B. The SH serum pool was positive for HAA. The complement-fixation (CF) titer of the HAA was 1:512, a high titer.

Procedure for heating IH and SH serum pools

An aliquot of the serum pool was thawed and a 1:10 dilution prepared by addition of 2 ml. of serum to 18 ml. of sterile distilled water. The dilution was made in a 50-ml. Ehrlenmeyer flask to which previously sterilized glass beads had been added to prevent bumping. The flask was fitted with a 2-holed rubber stopper into which a thermometer and a short glass open tube were inserted. The thermometer was placed in the liquid but did not touch the bottom of the flask, which was held firmly by asbestos tongs and placed directly on the high heat of an electric burner. The liquid began to boil in 43 seconds. The flask was agitated and lifted slightly from the heat at the moment of violent boiling. These actions did not reduce the 98° C. temperature, which was maintained for a minute. The liquid was cooled to room temperature in 25 minutes.

Serum specimens

All sera obtained from patients in the present study were stored in a deep freeze at —20° C. Specimens of sera were tested for the presence of HAA and antibody (anti-HAA) by standard immunodiffusion and by complement fixation (CF) according to the procedure of Purcell et al. described in J. Infect. Dis., vol. 120, pp. 383–386, entitled, "A Complement-Fixation Test for Measuring Australian Antigen and Antibody." The following conventional biochemical tests of liver function were performed: serum bilirubin, thymol turbidity, and serum glutamic oxaloacetic transaminase (SGOT). A result was considered abnormal if the serum bilirubin value was more than 1.0 mg./100 ml., the thymol turbidity more than 6 Maclagan units, and the SGOT 100 units or more.

Studies with IH serum pool

The effect of boiling (in this instance 1 minute at 98° C.) on the infectivity of IH serum pool was evaluated in

a group of 11 children. The study was conducted in two separate trials, the second being 5 months after the first.

First trial.—The boiled IH serum pool was given to 8 children by mouth. The dose was 0.5 ml. of a 1:10 aqueous dilution. Three children were unfed controls (Nos. 9–11). As indicated in Table 1, which follows, all 11 children showed no evidence of having hepatitis during the 5-month period of observation before initiation of the second trial.

Second trial.—Approximately 5 months later the unboiled IH serum pool was given to 6 children who had received the boiled material previously (Patients 1–6). Three children were unfed controls (Patients 9–11). As shown in Table 1, primary viral hepatitis (as indicated by an SGOT of 100) occurred in Patients 1–3 after incubation periods ranging between 28 and 36 days. The hepatitis that occurred in the unfed controls, Patients 9–11, and in Patients 4–6 represents secondary infection due to contact with the first 3 cases.

TABLE 1

[Effect of boiling on the infectivity of IH serum [1] pool]

Patient No.	First trial boiled IH serum, 0.5 ml. of 1:10 dilution given orally: Development of hepatitis	Second trial unboiled IH serum, 0.5 ml. of 1:10 dilution given orally: Type of hepatitis	Incubation period (days)	Peak SGOT (units/ml.)
1	None	Anicteric	28	480
2	do	Icteric	36	1,180
3	do	do	36	1,480
4	do	do	53	1,240
5	do	do	53	1,080
6	do	Anicteric	53	260
7	do			
8	do			
9 [2]	do	Anicteric	42	540
10 [2]	do	do	60	1,480
11 [2]	do	do	63	860

[1] Sera were boiled at 98° C. for 1 minute.
[2] Unfed controls.

The sequence of events following the administration of boiled and unboiled IH serum pool to one child is illustrated in FIG. 1 described hereinabove.

The results of these two trials indicate that the IH strain of viral hepatitis, Type A was inactivated by the boiling. In other words, its infectivity was destroyed. Administration of this inactivated material orally, however, did not have an immunogenic effect. This was demonstrated by subsequent infection of persons upon exposure to unboiled IH serum pool.

Studies with SH serum pool

The effect of boiling (in this instance 1 minute at 98° C.) on the infectivity of the SH serum pool was evaluated in a group of 12 children. Unboiled SH serum was given to Patients 1–4. Patients 5–12 received boiled SH serum pool. The dose of 0.1 ml. of a 1:10 aqueous dilution was injected intramuscularly. The unboiled SH serum pool dilution was mixed with an equal volume of immune serum globulin (ISG) because previous experience revealed that although the ISG did not affect infectivity, there was a suggestion that it might attenuate the disease. Consequently, Patients 1–4 received a 0.2 ml. inoculum containing 0.1 ml. of ISG and 0.1 ml. of a 1:10 dilution of SH serum pool.

The results are shown in Table 2 below and FIG. 2 described hereinabove. Administration of unboiled SH serum pool was followed by typical viral hepatitis, Type B in Patients 1–3. Patient 4 did not develop the disease. HAA was detected between days 41 and 55, and the SGOT became abnormal between days 55 and 69. Two patients were icteric, and one was anicteric.

In contrast, Patients 5–12, who received boiled SH serum, did not develop hepatitis after 140 days. As indicated in Table 2 and FIG. 2, HAA was not detected, and the SGOT was normal. One patient (No. 5) developed antibody (anti-HAA) (as measured by immunodiffusion and CF) on the seventh day after receiving SH serum pool. The anti-HAA persisted until day 55. It was not detectable from day 62 to day 140.

TABLE 2

[Effect of boiling on the infectivity of SH serum pool]

Patient No.	Type of hepatitis	HAA-positive (days after exposure)	Anti-HAA positive (days after exposure)	SGOT abnormal (days after exposure)
	(A) Unboiled SH serum pool—0.1 ml. of 1:10 dilution given parenterally [1]			
1	Anicteric	41–140+	0	66–140+
2	Icteric	41–98	0	55–98
3	do	55–140+	0	69–140
4	None	0	0	0
	(B) Boiled SH serum pool—0.1 ml. of 1:10 dilution given parenterally [2]			
5	None	0	7–55	0
6	do	0	0	0
7	do	0	0	0
8	do	0	0	0
9	do	0	0	0
10	do	0	0	0
11	do	0	0	0
12	do	0	0	0

[1] Unboiled SH serum pool was mixed with an equal volume of gamma-globulin, Squibb Lot No. 7-0. Consequently, Patients 1–4 received a 0.2 ml. inoculum containing 0.1 ml. gamma-globulin and 0.1 ml. of a 1:10 dilution of SH serum pool.
[2] SH serum pool was boiled for 1 minute at 98° C.

The results of tests for the presence of HAA in unboiled and boiled SH serum pool are shown in Table 3. Antigen was detectable after boiling at 98° C. for 1–3 minutes.

TABLE 3

[Effect of boiling on hepatitis-associated antigen (HAA)]

SH serum pool temperature	CF [1] HAA titer	Immunodiffusion test
Unboiled	1:512	Positive.
98° C. for 1 minute	1:128	Do.
98° C. for 2 minutes	1:256	NT. [2]
98° C. for 3 minutes	1:256	NT. [2]

[1] CF=complement fixation.
[2] NT=not tested.

The foregoing results indicate that the IH strain of viral hepatitis, Type A was inactivated by boiling but did not become immunogenic. Boiling had a significant effect on the infectivity of the SH serum pool. Parenteral administration of the boiled serum did not cause hepatitis during the 140-day period of observation. In contrast, inoculation of the unboiled serum pool was followed by hepatitis after an incubation period of 55–69 days. Moreover, the boiled HAA-positive serum pool stimulated the production of anti-HAA in one patient (No. 5, Table 2) possibly because of an unrecognized case of serum hepatitis in the past. We have observed a similar phenomenon following reinfection with the SH strain of viral hepatitis. Thus, it seems clear that the boiled, non-infectious material was antigenic.

Study groups

Thirty-nine children were divided into the following two groups:

Group 1.—25 susceptible children who received SH serum parenterally; and Group 2.—14 susceptible children some of which received one, and some of which two inoculations of boiled SH serum. Certain of these children received an inoculation of unboiled SH serum 4 months later and others 8 months later.

Study plan

The 39 children, 3 to 10 years of age, were admitted directly to a special hepatitis unit in 8 separate groups during a 5-year period. Serum specimens were obtained at weekly intervals and stored at —20° C.

The following tests were performed to detect HAA and anti-HAA: (1) immunodiffusion (ID), as described by B. S. Blumberg in an article entitled, "Polymorphisms of Serum Proteins in the Development of Isoprecipitins in Transfused Patients," in Bull. N.Y. Acad. Med., vol. 40,

pp. 377–386 (1964); (2) complement-fixation (CF), as described by Purcell et al. in an article entitled, "A Complement-Fixation Test for Measuring Australia Antigen and Antibody," in J. Infect. Dis., vol. 120, pp. 383–386 (1969); and (3) hemagglutination (HA), as described by Vyas et al. in an article entitled, "Hemagglutination Assay for Antigen and Antibody Associated with Viral Hepatitis," in Science, vol. 170, pp. 332–333 (1970). In addition, the highly sensitive radioimmunoprecipitation (RIP) test was used for detection of antibody, using the technique of J. J. Lander, H. J. Alter, and R. H. Purcell for measuring frequency of antibody to hepatitis-associated antigen (HAA) by radio-immunoassay.

The following biochemical tests of liver function were performed: serum bilirubin and serum glutamic oxaloacetic transaminase (SGOT). A result was considered abnormal if the serum bilirubin value was more than 1.0 mg./100 ml. and if the SGOT was 100 units or more.

Criteria for determining susceptibility or immunity to viral hepatitis, Type B

Baseline sera were tested for anti-HAA by the aforementioned ID, CF, HA and RIP techniques. The presence of RIP antibody was considered to be indicative of immunity. Absence of anti-HAA indicated susceptibility.

Results.—Group 1

Infectivity of SH serum.—This group of 25 susceptible children received a parenteral inoculation of SH serum in the following doses: 0.25 ml. (8 children), 0.1 ml. (6 children), and 0.1 ml. of a 1:10 dilution in distilled water (11 children). HAA was subsequently detected in the sera of all 25 children. The SGOT was abnormal in 24 of 25 children for a period ranging between 21 days and 5 years (average 8 months). The results of determinations of HAA, SGOT and anti-HAA in a typical case are shown in Table 4. HAA was detected on day 36 and persisted until day 99. SGOT was abnormal from day 46 to 108. Antibody which was first detected by RIP on day 50 persisted for the one year period of observation.

TABLE 4

[Determinations for the presence of hepatitis-associated antigen (HAA), serum glutamic oxaloacetic transaminase (SGOT) and hepatitis-associated antibody (anti-HAA) following a parenteral exposure to SH serum]

Day	HAA CF titer [1]	SGOT units/ml.	Anti-HAA RIP (percent binding) [2]
0	<2	18	0
	0.1 ml. of a 1:10 dilution of MS-2 serum parenterally		
7	<2	24	0
15	<2	20	
22	<2	8	
29	<2	14	0
36	4	28	0
43	64	50	0
46	64	200	0
50	128	200	+ (23%)
53		430	+ (39%)
56	256	640	+ (35%)
60	128	650	+ (37%)
64	256	650	+ (37%)
70		1,500	
81	128	1,280	
91	16	940	
99	4	980	+ (48%)
108	<2	280	
127	<2	44	
148	<2	20	+ (65%)
185	<2	28	+ (78%)
378	<2	16	+ (55%)

[1] Reciprocal of complement fixation HAA titer.
[2] Radioimmunoprecipitation (RIP) test; <15% binding: negative, >15% binding: positive.

Conclusion

This study indicated that SH serum was highly infectious for susceptible individuals. The attack rate, based appearance of HAA, was 100 percent; it was 96 percent when based on abnormal SGOT levels. The one child who had a normal SGOT became HAA-positive 31 days after exposure. The antigen persisted until 5 years later, in spite of no evidence of liver disease.

Group 2.—Active immunization with boiled SH serum

The 14 children in this group were inoculated with 0.1 ml. of a 1:10 dilution of SH serum which had been boiled (98° C.) for one minute. The CF titer of the serum was not significantly affected by boiling. It was 1:512 before and 1:128 after heat treatment. A second inoculation of the boiled SH serum was given to 4 of the 14 children 4 months later.

Of the 10 children who received one inoculation of boiled SH serum, 8 received unboiled SH serum 4 months later, and two received unboiled serum 8 months later. The dose of unboiled SH serum was 0.1 ml. of a 1:10 dilution. The results were as follows: Four children were protected against viral hepatitis, Type B as indicated by (1) no detectable HAA, (2) no abnormal SGOT and (3) detection of anti-HAA 42 to 49 days after receiving unboiled SH serum. The remaining 6 children showed evidence of having hepatitis as indicated by the appearance of HAA and an abnormal SGOT 43 to 57 days after exposure to unboiled SH serum. Preliminary observations indicated that the hepatitis infection of the 6 children had been modified clinically.

Four children received two inoculations of boiled SH serum at 4-month intervals. Four months after the second injection they received unboiled SH serum parenterally, 0.1 ml. of 1:10 dilution. All 4 children had significant protection against hepatitis during the 70-day period of observation. The results were as follows: (1) HAA was not detected and SGOT was normal in 3 children. In the fourth child HAA was detected and the SGOT was abnormal on only one day; and (2) anti-HAA was detected shortly before or after the second inoculation of boiled SH serum in 3 children. Data accumulated from two representative children are shown in Tables 5 and 6.

TABLE 5

[Determinations of HAA, SGOT, and anti-HAA following two inoculations of boiled SH serum and one inoculation of unboiled SH serum]

Day after inoculation	HAA CF titer [1]	SGOT, units/ml.	Anti-HAA RIP (percent binding)
0	<2	16	[2]
	0.1 ml. of 1:10 dilution of boiled SH serum parenterally		
7	<2	10	0
20	<2	12	
25	<2	24	0
34	<2	28	
48	<2	15	
77	<2	16	
91	<2	8	0
125	<2	34	+ (>15%)
140	<2	40	+ (>15%)
	0.1 ml. of 1:10 dilution of boiled SH serum parenterally		
5 (146)	<2	41	+ (>15%)
12 (153)	<2	19	
19 (160)	<2	16	
47 (188)	<2	12	
70 (211)	<2	14	
96 (227)	<2	20	
	0.1 ml. of 1:10 dilution of unboiled SH serum parenterally		
0 (258)	<2	16	
7 (265)	<2	21	
14 (272)	<2	15	+ (18.0)
21 (279)	<2	44	+ (34.3)
28 (286)	<2	22	0(7.1)
35 (293)	<2	17	+ (20.2)
42 (300)	<2	29	+ (62.4)
49 (307)	<2	18	+ (32.4)
56 (314)	<2	16	+ (24.7)
63 (321)	<2	17	+ (21.2)
70 (328)	<2	20	+

[1] Reciprocal of complement fixing HAA titer.
[2] RIP (percent binding)—Radioimmunoprecipitation test; <15% binding: negative, >15% binding: positive.

TABLE 6

[Determinations of HAA, SGOT, and anti-HAA following two inocula tions of boiled SH serum and one inoculation of unboiled SH serum]

Day after inoculation	HAA CF titer [1]	SGOT, units/ml.	Anti-HAA RIP (percent binding) [2]
0	<2	8	
	0.1 ml. of 1:10 dilution boiled SH serum parenterally		
13	<2	16	
20	<2	8	
34	<2	15	
48	<2	10	
77	<2	20	
125	<2	20	
140	<2	40	
	0.1 ml. of 1:10 dilution of boiled SH serum parenterally		
5 (146)	<2	52	
7 (148)	<2	20	0
19 (160)	<2	15	
40 (181)	<2	14	0
47 (188)	<2	12	
70 (211)	<2	10	
	0.1 ml. of 1:10 dilution of unboiled SH serum parenterally		
0 (258)	<2	50	0
7 (265)	<2	28	
14 (272)	<2	18	0
21 (279)	<2	50	0(5.7%)
28 (286)	<2	18	0
35 (293)	4	21	0
42 (300)	<2	120	+ (50.1%)
49 (307)	<2	46	+ (22.7%)
56 (314)	<2	18	0 (10.2%)
63 (321)	<2	28	0
70 (328)	<2	24	

[1] Reciprocal of complement fixation HAA titer.
[2] Radioimmunoprecipitation (RIP) test; <15% binding: negative, >15% binding: positive.

Active

Samples of serum from the group of 10 children who received one inoculation of boiled SH serum were tested for antibody. Anti-HAA was detectable 21 to 49 days after a subsequent inoculation of unboiled SH serum in 4 children, who were protected against hepatitis.

Samples of serum from the 4 children who received two injections of boiled SH serum were tested for anti-HAA before and after the subsequent inoculation of unboiled SH serum. Antibody was detected 125 days after the first inoculation of boiled SH serum in 2 chlidren, 19 days after the second inoculation of boiled SH serum in one child, and 42 days after the inoculation of unboiled SH serum in the fourth child.

Conclusion

Actively acquired antibody was detected after two inoculations of boiled SH serum.

The studies of the foregoing four groups indicate that viral hepatitis, Type B (SH) can be prevented by active immunization. Active immunization was induced by the inoculation of a boiled preparation of SH serum in sterile distilled water. Two inoculations were more effective than one. However, one inoculation gave enough protection to prevent some cases and to modify others. The significance of foregoing results is apparent when one reviews the comparative data in Table 7 below. The absence of hepatitis in all 4 susceptible children who received 2 inoculations of boiled SH serum and its absence in 4 of 10 who received one inoculation is impressive when compared with a 96 to 100 percent attack rate in 25 susceptible children who received SH serum without previous active immunization.

TABLE 7

[Summary of results of studues on active and passive immunization for the prevention of viral hepatitis, Type B (SH)]

Inoculum	Total number inoculated	Number with hepatitis: Positive HAA	Number with hepatitis: Abnormal SGOT
SH serum	25	25	24
(1) Boiled SH serum, one inoculation (2) Unboiled MS-2 serum, 4 months later	10	6	5
(1) Boiled SH serum, two inoculations at 4 months, intervals (2) Unboiled SH serum 4 months after second inoculation	4	*1	*

*HAA was detectable and SGOT was abnormal on only 1 day.

We claim:

1. A process for preparation of a viral hepatitis, Type B vaccine which comprises:
   (a) diluting viral hepatitis, Type B serum with a pharmaceutically acceptable carrier; and
   (b) boiling said viral hepatitis, Type B serum for a sufficient period to destroy the infectivity thereof but insufficient to destroy its antigenicity.
2. The process of claim 1 wherein the boiling is for at least 1 minute.
3. The process of claim 1 wherein the boiling is for from about 1 to about 3 minutes.
4. The process of claim 1 wherein the boiling is at a temperature of about 98° C.
5. The process of claim 1 wherein the boiling is for from about 1 to about 3 minutes at a temperature of about 98° C.
6. The process of claim 1 wherein, after said boiling, the serum is cooled to room temperature and stored at below its freezing temperature.
7. The process of claim 1 wherein said pharmaceutically acceptable carrier is sterile distilled water.
8. The process of claim 1 wherein the serum is diluted with sterile distilled water at a ratio of 1 part of serum to 10 of water prior to the boiling.
9. The product produced by the process of claim 5.
10. A process for immunizing humans against viral hepatitis, Type B which comprises treating humans with at least one dose of an immunizing amount of the product of claim 9.
11. The process of claim 10 wherein the dose is administered parenterally.
12. The process of claim 10 wherein the dose is administered by intramuscular injection.
13. The process of claim 10 wherein the dose comprises an amount about equivalent to 0.002 to 0.5 ml. of undiluted serum.
14. The process of claim 10 wherein humans are treated with two doses of an immunizing amount of the product of claim 9.
15. The process of claim 14 wherein the second dose is administered at least about 3 months following the first.

References Cited

Auerswald: Bibliotheca Haematologica, vol. 12, pp. 232–236, 1961.

RICHARD L. HUFF, Primary Examiner

U.S. Cl. X.R.

195—1.2