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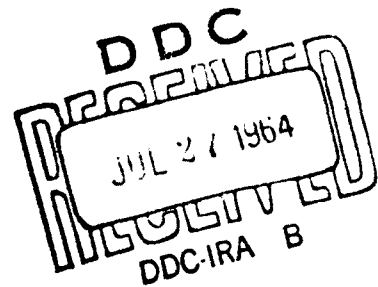
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Chas. Pfizer & Company, Inc.

Brooklyn, N.Y.

Preparation and Evaluation of Staphylococcal Enterotoxoids

Contract No. DA-49-193-MD-2428



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ABSTRACT

1. Preparing Institution: Chas. Pfizer & Company, Inc.,
Brooklyn 6, N.Y.
2. Title of Report: Preparation and Evaluation of
Staphylococcal Enterotoxoids
3. Principal Investigator: Dr. B.A. Sobin
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Thirty Staphylococcal enterotoxoids were prepared and evaluated in rhesus monkeys in a range finding study designed to compare toxicities, antibody response, and protection against intravenous challenge with untreated Staphylococcal enterotoxin.

The immunization schedule consisted of three subcutaneous injections of toxoid with adjuvant spaced at intervals of five weeks. Some toxicity (emesis, diarrhea) was observed with most of the toxoid preparations following the first subcutaneous immunization injection. No toxic reactions followed the second and third immunization injections.

Antibody titers, as measured by hemagglutination and gel-diffusion, were generally low after the first immunization injections. Many of the antibody titers rose sharply after the second and third injections.

A number of the Staphylococcal enterotoxoid preparations provided excellent protection in monkeys against intravenous challenge with untreated Staphylococcal enterotoxin ~~at 10 μ /kg. and 1000 μ /kg.~~ three weeks after the third and final immunization injections.

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The ten most promising toxoid preparations were selected from the range finding studies for expanded toxicity comparisons, antibody titers, and degree and duration of immunity to intravenous challenge with Staphylococcal enterotoxin.

In the extended evaluation studies to date, Sample #10 (0.2% formalin + 0.15% glycine at 25°C. for 2 weeks) is the most impressive of the toxoid preparations on the basis of toxicity and antigenic response. The final evaluation will depend on the degree and duration of immunity to intravenous challenge with Staphylococcal enterotoxin, correlation of immunity with hemagglutination titers, effect of booster shots, and nature of anamnestic response.

7. Protection against intravenous challenge with Staphylococcal enterotoxin based on immunization with enterotoxoid preparations.

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INTRODUCTION

The establishment of the protein and antigenic nature of Staphylococcal enterotoxin, and the availability of a supply of highly purified material provided the background for the following objectives:

- a. To prepare a number of toxoids of Staphylococcal enterotoxin by treatment with a variety of chemical and physical agents.
- b. To test and evaluate the immunological responses of the various Staphylococcal enterotoxoid preparations in rhesus monkeys.
- c. To select a safe and effective Staphylococcal enterotoxoid capable of conferring upon rhesus monkeys immunity of long duration against the action of a large dose of intravenously administered Staphylococcal enterotoxin.

The construction of an animal facility to house two hundred rhesus monkeys was begun in the early part of May, 1963, and was completed August 15, 1963. Animal experimentation was initiated September 3, 1963.

Working supplies of highly purified Staphylococcal enterotoxin, Lot No. 14-31, were made available on May 28 and June 17, 1963.

A search of the literature was made covering the chemical and physical agents used to prepare toxoids, inactivate microorganisms, and alter proteins. Thirty toxoid treatments were selected for evaluation in a preliminary range finding study comparing toxicity, serologic response, and protection against intravenous challenge with untreated Staphylococcal enterotoxin. Ten Staphylococcal enterotoxoid preparations were chosen from this group for evaluation over a twelve month period with a scheduled termination date of May 30, 1965.

MATERIALS AND METHODS

TOXOID PREPARATIONS

Formalin

5 ml. of the various formalin solutions were added to each of a number of vials containing 10 mg. of highly purified freeze-dried Staphylococcal enterotoxin, Lot No. 14-31. After maintenance under the designated experimental conditions, the vials were stored at -20°C .

1. 0.2% formalin at 37°C . for 1 week
2. " " " " " 2 weeks
3. " " " " " 3 weeks

4. 0.2% formalin at 5°C . for 6 weeks

5. 0.8% formalin at 37°C . for 1 week
6. " " " " " 2 weeks
7. " " " " " 3 weeks

8. 0.8% formalin at 5°C . for 6 weeks

9. 0.2% formalin 0.15% glycine at 25°C . for 1 week
10. " " " " " " " 2 weeks
11. " " " " " " " 3 weeks

Hexamethylenetetramine

12. 0.3% hexamethylenetetramine at 37°C . for 3 weeks
13. 1.0% " " " " " " "

Beta-Propiolactone

14. 5 ml. of a chilled 0.2% solution of beta-propiolactone in isotonic disodium hydrogen phosphate was added to each of a number of vials containing 10 mg. of toxin, and held at 37°C . for 2 hours. The reaction was allowed to proceed overnight at room temperature, and then stored at -20°C .

15. Same as above except for change to 1.0% beta-propiolactone.

Diethyl Pyrocarbonate

16. 5 ml. of a chilled 0.2% solution of diethyl pyrocarbonate in ethanol-water (1:1) was added to each of a number of vials containing 10 mg. of toxin, and stored for 1 week at 5°C. The vials were then held overnight at room temperature, and then stored at -20°C.

17. Same as above except for change to 1.0% diethyl pyrocarbonate.

Ethylene Oxide

18. 5 ml. of a chilled 0.2% solution of ethylene oxide was added to each of a number of vials containing 10 mg. of toxin. The vials were held at 5°C. for 2 hours, maintained overnight at room temperature, and stored at -20°C.

19. Same as above except for change to 1.0% ethylene oxide.

20. A number of vials of toxin, 10 mg./vial, were placed in a vacuum desiccator along with 5 ml. of liquefied ethylene oxide contained in a small Erlenmyer flask. The vacuum desiccator was partially evacuated to allow for the expansion of ethylene oxide vapor. After 24 hour exposure in the ethylene oxide atmosphere, the trace amounts of adsorbed ethylene oxide were removed by exhausting the vacuum desiccator for several hours. The vials were stored at -20°C.

Ethyleneimine

21. A number of vials of toxin, 10 mg./vial, were placed in a vacuum desiccator along with 5 ml. of ethyleneimine contained in a small Erlenmyer flask. The vacuum desiccator was partially evacuated to allow for the expansion of ethyleneimine vapor. After 24 hours exposure in the ethyleneimine atmosphere, the vacuum desiccator was exhausted for 24 hours. The vials were stored at -20°C.

Methyl Bromide

22. A number of vials of toxin, 10 mg./vial, were placed in a vacuum desiccator along with 5 ml. of liquefied methyl bromide contained in a small Erlenmyer flask. The vacuum desiccator was partially evacuated to allow for the expansion of methyl bromide vapor. After 24 hours exposure in the methyl bromide atmosphere, the trace amounts of adsorbed methyl bromide were removed by exhausting for several hours.

Propylene Oxide

23. Procedure identical to that described for ethylene oxide and methyl bromide.

Methanol Esterification

24. Two ml. of absolute methanol saturated with dry hydrogen chloride was added to each of a number of vials containing 10 mg. of toxin. After standing for 3 days at room temperature, the vials were taken to dryness in vacuo. Two ml. of sterile distilled water was then added to each vial, the material freeze-dried, and stored at -20°C .

Sodium Gluconate

25. 5 ml. of an aqueous 1.0% solution of sodium gluconate was added to each of a number of vials containing 10 mg. of toxin. After holding for 4 weeks at 37°C ., the vials were stored at -20°C .

Ultraviolet Light Irradiation

26. 250 mg. of toxin was dissolved in water and transferred in a final volume of 125 ml. to a stoppered quartz Erlenmeyer flask fitted with a magnetic stirrer, and cooled by running water. The stirred solution was exposed to the rays from an ultraviolet light lamp, short wave (2537 Angstrom units), fixed at a distance of 20 cm., for 1 hour. Two ml. aliquots were transferred to a number of sterile vials, and stored at -20°C .

27. Same as above except for exposure time of 5 hours.

Photodynamic Action of Methylene Blue

28. 250 mg. of toxin was dissolved in an aqueous solution of methylene blue (1:10,000), and transferred in a final volume of 125 ml. to a stoppered Erlenmeyer flask fitted with a magnetic stirrer, and cooled by running water. The stirred solution was exposed to the light from a 100 Watt incandescent bulb fixed at a distance of 20 cm. for 1 hour. Two ml. aliquots were transferred to a number of sterile vials, and stored at -20°C .

29. Same as above except for exposure time of 8 hours.

Acetic Anhydride

30. To 250 mg. of toxin dissolved in 5 ml. of saturated sodium acetate solution and cooled in an ice bath, was added with stirring 0.3 ml. of acetic anhydride. After stirring for an hour, the material was carefully transferred to a small cellophane bag, and dialyzed against distilled water in the refrigerator for 48 hours with frequent changes of water. Two ml. portions of the opalescent solution were transferred to each of a number of sterile vials, 4 mg./vial, and stored at -20°C .

Adjuvant

A light mineral oil (Drakeol 6VR) and a mannide monoleate (Arlacel A) were mixed in a 9:1 ratio, and sterilized by Seitz filtration.

Emulsification

To each vial containing 1 ml. of toxoid solution, 2 mg./ml. was added an equal volume of the mineral oil - Arlacel A solution. Emulsions of the vaccines were prepared directly in the vials by forcing the contents back and forth through a 1 ml. glass tuberculin syringe fitted with a 20 gauge needle. The process was continued until a drop of emulsion expelled through the hypodermic needle into a beaker of cold water retained its shape.

Immunization Schedule

Range Finding Studies

Pairs of rhesus monkeys were immunized with each of the thirty different toxoid preparations. The immunization schedule consisted of three subcutaneous injections spaced at five week intervals. A total dose of 0.10 mg. was given in the first injection. The second and third injections each consisted of a total dose of 0.25 mg. of toxoid.

The thirty pairs of monkeys, previously immunized with the thirty different enterotoxoid samples, were challenged intravenously intravenously three weeks after the third and final immunization injections with untreated Staphylococcal enterotoxin dissolved in 0.02 M phosphate buffer, pH 7.4. One monkey of each pair was challenged at 10 /kg., and the other at 1000 /kg.

Extended Evaluation Studies

Twelve monkeys were immunized, in the manner already described, with each of the ten selected toxoid samples. Four monkeys from each set are to be challenged intravenously with untreated Staphylococcal enterotoxin six months after the third and final immunization injections. At the same time, a second set of four monkeys from each group is to be given a booster shot of 0.25 mg. of toxoid. These monkeys, along with the third set of four monkeys from each group, are to be challenged intravenously with untreated Staphylococcal enterotoxin one year after the initial immunization period.

Toxicity Determinations

After each injection of Staphylococcal enterotoxin or toxoid, absence or presence of illness (emesis, diarrhea) was noted through a five hour observation period. Death or delayed death with marked weight loss was interpreted as a direct toxic manifestation of the injected material.

Serologic Tests

Antibody titers were determined by hemagglutination and gel-diffusion tests as performed at Fort Detrick.

The lowest dilution of sera in the hemagglutination test was arbitrarily set at 1:10,000. Titers are expressed as the reciprocal of the highest serum dilution showing gross hemagglutination.

In the gel-diffusion assay, a preliminary qualitative test was run in single tubes with sera diluted 1:10 overlaid with Staphylococcal enterotoxin 100 μ /ml. Samples which gave sharp, demonstrable zones after twenty four hours were assayed in the usual eight tube quantitative test. Titers are expressed as reciprocals of the number.

RESULTS

Range Finding Studies

Some toxicity (emesis, diarrhea) was observed following the first subcutaneous immunization injection of the Staphylococcal enterotoxinoid samples. There were no deaths. No toxic reactions followed the second and third immunization injections.

Antibody titers were generally low or negative after the first immunization injections. Many of the antibody titers rose sharply after the second and third subcutaneous immunization injections.

Out of thirty monkeys (each immunized with a different Staphylococcal enterotoxinoid) challenged intravenously with untreated Staphylococcal enterotoxin at 10 μ /kg., there was an illness response in only two monkeys. There were no deaths.

Out of twenty nine monkeys challenged intravenously with untreated Staphylococcal enterotoxin at 1000 μ /kg., there were two deaths and fifteen illness responses. Twelve monkeys had no reactions.

The correlation between antibody titers and protection against intravenous challenge in these range finding studies was suggestive but not clear cut.

Extended Evaluation Studies

The following ten preparations were selected from the range finding studies for more extended comparisons of toxicities, antibody response, and degree and duration of immunity to intravenous challenge with untreated Staphylococcal enterotoxin:

<u>Sample No.</u>	<u>Treatment</u>
2	0.2% formalin at 37°C. for 2 weeks
10	0.2% formalin + 0.15% glycine at 25°C. for 2 weeks
12	0.3% hexamethylenetetramine at 37°C. for 3 weeks
14	0.2% beta-propiolactone at 37°C. for 2 hours
16	0.2% diethyl pyrocarbonate at 5°C. for 1 week
18	0.2% ethylene oxide at 5°C. for 2 hours
22	Exposure to methyl bromide vapor for 24 hours
25	1.0% sodium gluconate at 37°C. for 4 weeks
26	Exposure to ultraviolet light for 1 hour
28	Photodynamic action of methylene blue for 1 hour

Sets of twelve monkeys were immunized with each of the ten toxoid samples. The following table shows the toxicity responses to the first subcutaneous immunization injection of 0.10 mg. of toxoid with adjuvant:

<u>Sample No.</u>	<u>Illness</u>	<u>Death</u>	<u>No Reaction</u>
2	6	0	6
10	0	0	12
12	8	0	4
14	10	1	1
16	8	2	2
18	9	1	2
22	6	0	6
25	11	0	1
26	5	0	7
28	9	1	2

Sample #10 is the most impressive preparation to date. In a separate experiment, three monkeys were injected subcutaneously with 0.25 mg. of toxoid. There were no reactions. In addition to being the least toxic of the preparations tested, Sample #10 produced the highest antibody titers as measured by hemagglutination and gel-diffusion.

SUMMARY AND CONCLUSIONS

The range finding studies concerned with the preliminary evaluation of thirty Staphylococcal enterotoxoid samples have been completed.

Thirty pairs of rhesus monkeys, previously immunized with thirty different toxoid preparations, were challenged intravenously with untreated Staphylococcal enterotoxin three weeks after the third and final subcutaneous immunization injection.

Out of thirty monkeys (each immunized with a different Staphylococcal enterotoxoid) challenged intravenously with untreated enterotoxin at 10 v/mg., there was an illness response in only two monkeys. There were no deaths.

Out of twenty nine monkeys challenged intravenously with enterotoxin at 1000 v/kg., there were two deaths and fifteen illness responses. There were no reactions in twelve monkeys.

On the basis of toxicity, antibody titers, and intravenous protection results, ten Staphylococcal toxoid preparations were selected for more extended evaluation.

Sample #10 (0.2% formalin+0.15% glycine at 25°C. for 2 weeks) is the most impressive of the toxoid preparations on the basis of toxicity and antigenic response. The final evaluation, scheduled for May 30, 1965, will depend on the degree and duration of immunity to intravenous challenge with untreated Staphylococcal enterotoxin.

The attached summary sheets contain all the tabulated data available to date. Additional entries will be made as the experimental studies are extended.

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Sample: Control

Untreated Staphylococcal Enterotoxin, Lot No. 14-31

Date

	9/9/63	9/12/63	9/23/63	10/7/63	10/14/63	11/8/63	11/18/63	12/2/63	12/10/63
<u>Monkey #1</u>									
Immunization									
Dose, mg. (SQ)	0.10						0.10		
Reaction*	I						N.R.		
Antibody Titer									
Hemagglutination									
Gel-Diffusion									
Intravenous Challenge									
Dose, (mcg/kg.)				80,000		80,000		100,000	
Reaction				No Zone		No Zone		No Zone	
									10.0
									N.R.
<u>Monkey #2</u>									
Immunization									
Dose, mg. (SQ)	0.25								
Reaction	X								
<u>Monkey #40</u>									
Immunization									
Dose, mg. (SQ)		0.10							
Reaction			X						

* I, Illness (emesis, diarrhea)
 X Death
 N.R. No Reaction

Sample #1

0.2% formalin at 37°C. for 1 week

Date

	9/18/63	10/15/63	10/23/63	11/25/63	12/9/63	12/17/63	12/23/63
<u>Monkey #47</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose, (mcg./kg.) Reaction	0.10 I	<10,000 No Zone	0.25 N.R.	10,000 No Zone	0.25 N.R.	80,000 No Zone	10.0 N.R.
	0.25 I	<10,000 No Zone	0.25 N.R.	<10,000 No Zone	0.25 N.R.	40,000 No Zone	1000 N.R.
<u>Monkey #48</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose, (mcg./kg.) Reaction							

Sample #2

0.2% formalin at 37° C. for 2 weeks

Date

	9/24/63	10/21/63	10/28/63	11/25/63	12/3/63	12/17/63	12/23/63
<u>Monkey #49</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	160,000 204	0.25 N.R.	320,000 100	0.25 N.R.	1,280,000 400	10.0 N.R.
	0.25 N.R.	320,000 190	0.25 N.R.	320,000 240	0.25 N.R.	1,280,000 205	1000 I
<u>Monkey #50</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	160,000 204	0.25 N.R.	320,000 100	0.25 N.R.	1,280,000 400	10.0 N.R.
	0.25 N.R.	320,000 190	0.25 N.R.	320,000 240	0.25 N.R.	1,280,000 205	1000 I

Sample #3

0.25 formalin at 37°C. for 3 weeks

Date

	10/1/63	10/28/63	11/4/63	12/3/63	12/9/63	12/23/63	12/31/63
<u>Monkey #52</u> Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	160,000 Positive	0.25 N.R.	2,560,000 159	0.25 N.R.	2,560,000 390	10.0 N.R.
	0.25 I	<10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	<10,000 No Zone	1000 I
<u>Monkey #56</u> Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

Sample #4

0.25 formalin at 5°C. for 6 weeks

	<u>Date</u>							
	10/21/63	11/18/63	11/21/63	11/25/63	12/23/63	12/30/63	1/10/64	1/24/64
<u>Monkey #14</u> Immunization Dose, mg. (S.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	160,000 Positive		0.25 N.R.	320,000 50	0.25 N.R.	1,280,000 347	1000 X
	0.25 I	< 10,000 Positive	X					
<u>Monkey #61</u> Immunization Dose, mg. (S.) Reaction Antibody Titer Hemagglutination Gel-Diffusion								

Sample #5

Sample #5

0.8% formalin at 37°C. for 1 week

Date

	9/18/63	10/15/63	10/23/63	11/25/63	12/9/63	12/17/63	12/23/63
<u>Monkey #43</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	<10,000 Positive	0.25 N.R.	40,000 Positive	0.25 N.R.	1,280,000 400	10.0 N.R.
	0.25 N.R.	<10,000 No Zone	0.25 N.R.	20,000 Positive	0.25 N.R.	320,000 160	1000 N.R.
<u>Monkey #44</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	<10,000 Positive	0.25 N.R.	40,000 Positive	0.25 N.R.	1,280,000 400	10.0 N.R.
	0.25 N.R.	<10,000 No Zone	0.25 N.R.	20,000 Positive	0.25 N.R.	320,000 160	1000 N.R.

Sample #6

0.8% formalin at 37°C. for 2 weeks

Date

	9/24/63	10/21/63	10/28/63	11/25/63	12/8/63	12/17/63	12/23/63
<u>Monkey #51</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	10,000 No Zone	0.25 N.R.	80,000 129	0.25 N.R.	640,000 200	10.0 N.R.
<u>Monkey #52</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	40,000 Positive	0.25 N.R.	640,000 160	1000 N.R.

Sample #7

0.8% formalin at 37°C. for 3 weeks

Date

	10/1/63	10/28/63	11/4/63	12/3/63	12/9/63	12/23/63	12/31/63
Monkey #51 Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	20,000 No Zone	10.0 N.R.
	0.25 I	< 10,000 No Zone	0.25 N.R.	80,000 Positive	0.25 N.R.	80,000 No Zone	1000 I
Monkey #58 Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

Sample #8

0.8% formalin at 5°C. for 6 weeks

Date

	10/21/63	11/18/63	11/25/63	12/23/63	12/30/63	1/10/64	1/24/64
<u>Monkey #62</u> Immunization Dose, mg. (S.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	<10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	2,550,000 No Zone	1000 I
<u>Monkey #53</u> Immunization Dose, mg. (S.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.25 I	<10,000 Positive	0.25 N.R.	<10,000 No Zone	0.25 N.R.	90,000 No Zone	10.0 N.R.

Sample #9

0.2% formalin + 0.15% glycine at 25°C. for 1 week

Date

	9/18/63	10/15/63	10/23/63	11/25/63	12/9/63	12/17/63	12/23/63
<u>Monkey #41</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	160,000 No Zone	0.25 N.R.	320,000 Positive	0.25 N.R.	1,256,000 380	10.0 N.R.
	0.25 I	<10,000 No Zone	0.25 N.R.	160,000 Positive	0.25 N.R.	160,000 50	1000 I
<u>Monkey #42</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

Sample #10

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

Date

	9/24/63	10/21/63	10/28/63	11/25/63	12/3/63	12/17/63	12/23/63
<u>Monkey #53</u>							
Immunization							
Dose, mg. (SQ) Reaction	0.10 I		0.25 N.R.	160,000 209	0.25 N.R.	640,000 400	10.0 N.R.
Antibody Titer		10,000 No Zone					
Hemagglutination							
Gel-Diffusion							
Intravenous Challenge							
Dose (mcg./kg.) Reaction							
<u>Monkey #54</u>							
Immunization							
Dose, mg. (SQ) Reaction	0.25 I		0.25 N.R.	40,000 Positive	0.25 N.R.	320,000 50	1000 I
Antibody Titer		10,000 No Zone					
Hemagglutination							
Gel-Diffusion							
Intravenous Challenge							
Dose (mcg./kg.) Reaction							

Sample #11

0.2% formalin + 0.15% glycine at 25°C. for 3 weeks

Date

	10/1/63	10/28/63	11/4/63	12/3/63	12/9/63	12/23/63	12/31/63
<u>Monkey #59</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	<10,000 No Zone	0.25 N.R.	80,000 Positive	0.25 N.R.	320,000 No Zone	10.0 N.R.
	0.25 I	<10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	80,000 No Zone	1000 I

Monkey #60

Immunization
 Dose, mg. (SQ)
 Reaction
 Antibody Titer
 Hemagglutination
 Gel-Diffusion
 Intravenous Challenge
 Dose (mcg./kg.)
 Reaction

Sample #13

1.0% hexamethylenetetramine at 37°C. for 3 weeks

Date

	9/11/63	10/7/63	10/16/63	11/8/63	11/19/63	12/2/63	12/10/63
<u>Monkey #13</u>	0.10 N.R.	80,000 No Zone	0.25 N.R.	320,000 305	0.25 N.R.	80,000 Positive	10.0 N.R.
Immunization Dose, mg. (S ₂) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							
<u>Monkey #15</u>	0.25 I	320,000 No Zone	0.25 N.R.	160,000 Positive	0.25 F.R.	80,000 Positive	1000 N.R.
Immunization Dose, mg. (S ₄) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

Sample #14

0.2% beta-propiolactone at 37°C. for 2 hours

Date

	9/10/63	10/7/63	10/14/63	11/8/63	11/18/63	12/2/63	12/10/63
<u>Monkey #3</u>	0.10 I	20,000 No Zone	0.25 N.R.	320,000 Positive	0.25 N.R.	800,000 174	10.0 N.R.
Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							
<u>Monkey #4</u>	0.25 I	< 10,000 No Zone	0.25 N.R.	80,000 No Zone	0.25 N.R.	160,000 Positive	1000 I
Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

Sample #16

0.2% diethyl pyrocarbonate at 5°C. for 1 week

Date

	9/10/63	10/7/63	10/14/63	11/8/63	11/18/63	12/2/63	12/10/63
<u>Monkey #11</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.)	0.10 I	80,000 213	0.25 N.R.	80,000 Positive	0.25 N.R.	20,000 Positive	10.0 N.R.
<u>Monkey #12</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.25 I	20,000 No Zone	0.25 N.R.	80,000 No Zone	0.25 N.R.	160,000 Positive	1000 N.R.

Sample #17

1.0% diethyl pyrocarbonate at 5°C. for 1 week

Date

	9/18/63	10/15/63	10/23/63	11/25/63	12/9/63	12/17/63	12/23/63
<u>Monkey #45</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	10,000 No Zone	0.25 N.R.	10,000 No Zone	0.25 N.R.	10,000 No Zone	10.0 I
<u>Monkey #46</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.25 N.R.	10,000 No Zone	0.25 N.R.	10,000 No Zone	0.25 N.R.	20,000 No Zone	1000 I

Sample #18

0.2% ethylene oxide at 5°C. for 2 hours

Date

	9/10/63	10/7/63	10/14/63	11/8/63	11/18/63	12/2/63	12/10/63
<u>Monkey #9</u> Immunization Dose, mg. (S ₄) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	20,000 100	0.25 N.R.	160,000 205	0.25 N.R.	320,000 345	10.0 N.R.
<u>Monkey #10</u> Immunization Dose, mg. (S ₄) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.25 I	10,000 No Zone	0.25 N.R.	80,000 Positive	0.25 N.R.	80,000 Positive	1000 N.R.

Sample #19

1.0% ethylene oxide at 5°C. for 2 hours

Date

	9/11/63	10/8/63	10/16/63	11/8/63	11/19/63	12/2/63	12/10/63
<u>Monkey #22</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	<10,000 No Zone	0.25 N.R.	160,000 Positive	0.25 N.R.	160,000 175	10.0 N.R.
<u>Monkey #23</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.25 N.R.	<10,000 No Zone	0.25 N.R.	<10,000 No Zone	0.25 N.R.	20,000 No Zone	1000 I

Sample #20

Exposure to ethylene oxide vapor for 24 hours

Date

	9/11/63	10/8/63	10/16/63	11/8/63	11/19/63	12/2/63	12/10/63
<u>Monkey #20</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	10,000 No Zone	0.50 I	10,000 No Zone	0.50 N.R.	20,000 No Zone	10.0 N.R.
	0.25 N.R.	20,000 No Zone	0.50 N.R.	40,000 No Zone	0.50 N.R.	20,000 No Zone	1000 I
<u>Monkey #21</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

Sample #21

Exposure to ethyleneimine vapor for 24 hours

Date

	9/12/63	10/8/63	10/17/63	11/11/63	11/20/63	12/2/63	12/10/63
<u>Monkey #34</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	10,000 No Zone	0.50 N.R.	< 10,000 No Zone	0.25 N.R.	20,000 No Zone	10.0 N.R.
	0.25 N.R.	< 10,000 No Zone	0.50 N.R.	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	1000 X
<u>Monkey #35</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	10,000 No Zone	0.50 N.R.	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	1000 X
	0.25 N.R.	< 10,000 No Zone	0.50 N.R.	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	1000 X

Sample #22

Exposure to methyl bromide vapor for 24 hours

Date

	9/10/63	10/7/63	10/14/63	11/8/63	11/18/63	12/2/63	12/10/63
<u>Monkey #1</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	<10,000 No Zone	0.25 N.R.	40,000 No Zone	0.25 N.R.	160,000 145	10.0 N.R.
	0.25 N.R.	20,000 No Zone	0.25 N.R.	20,000 Positive	0.25 N.R.	10,000 Positive	1000 I
<u>Monkey #8</u> Immunization Dose, (mg./kg.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	<10,000 No Zone	0.25 N.R.	40,000 No Zone	0.25 N.R.	160,000 145	10.0 N.R.
	0.25 N.R.	20,000 No Zone	0.25 N.R.	20,000 Positive	0.25 N.R.	10,000 Positive	1000 I

Sample #23

Sample #23

Exposure to propylene oxide vapor for 24 hours

Date

	9/11/63	10/8/63	10/16/63	11/8/63	11/19/63	12/2/63	12/10/63
<u>Monkey #24</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	10,000 No Zone	0.25 N.R.	10,000 No Zone	0.25 N.R.	20,000 No Zone	10.0 N.R.
	0.25 I	80,000 No Zone	0.25 N.R.	80,000 Positive	0.25 N.R.	320,000 Positive	1000 I
<u>Monkey #25</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

Sample #24

Methanol esterification

Date

	9/11/63	10/8/63	10/16/63	11/8/63	11/19/63	12/2/63	12/10/63
<u>Monkey #18</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.50 N.R.	< 10,000 No Zone	0.50 N.R.	< 10,000 No Zone	10.0 I
	0.25 N.R.	< 10,000 No Zone	0.50 N.R.	< 10,000 No Zone	0.50 N.R.	< 10,000 No Zone	1.0 N.R.
<u>Monkey #19</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

Sample #26

Exposure to ultraviolet light for 1 hour

Date

	9/12/63	10/8/63	10/17/63	11/11/63	11/20/63	12/3/63	12/10/63
<u>Monkey #36</u> Immunization Dose, mg. (sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	20,000 No Zone	0.25 N.R.	80,000 Positive	0.25 N.R.	320,000 Positive	10.0 N.R.
	0.25 N.R.	40,000 No Zone	0.25 N.R.	10,000 Positive	640,000 No Zone	1000 I	
<u>Monkey #37</u> Immunization Dose, mg. (sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.25 N.R.	40,000 No Zone	0.25 N.R.	10,000 Positive	640,000 No Zone	1000 I	

Sample #26

Exposure to ultraviolet light for 1 hour

Date

	9/12/63	10/8/63	10/17/63	11/11/63	11/20/63	12/3/63	12/10/63
<u>Monkey #36</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	20,000 No Zone	0.25 N.R.	80,000 Positive	0.25 N.R.	320,000 Positive	10.0 N.R.
	0.25 N.R.	40,000 No Zone	0.25 N.R.	10,000 Positive	0.25 N.R.	640,000 No Zone	1000 I
<u>Monkey #37</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.25 N.R.	40,000 No Zone	0.25 N.R.	10,000 Positive	0.25 N.R.	640,000 No Zone	1000 I
	0.25 N.R.	40,000 No Zone	0.25 N.R.	10,000 Positive	0.25 N.R.	640,000 No Zone	1000 I

Sample #27

Exposure to ultraviolet light for 5 hours

Date

	9/12/63	10/8/63	10/17/63	11/11/63	11/20/63	12/2/63	12/10/63
<u>Monkey #28</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	<10,000 No Zone	0.50 N.R.	10,000 No Zone	0.25 N.R.	320,000 No Zone	10.0 N.R.
	0.25 N.R.	80,000 No Zone	0.50 N.R.	40,000 No Zone	0.25 N.R.	320,000 No Zone	1000 I
<u>Monkey #29</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	<10,000 No Zone	0.50 N.R.	10,000 No Zone	0.25 N.R.	320,000 No Zone	10.0 N.R.
	0.25 N.R.	80,000 No Zone	0.50 N.R.	40,000 No Zone	0.25 N.R.	320,000 No Zone	1000 I

sample 7c0

Photodynamic action of methylene blue - 1 hour

Date

	9/12/63	10/8/63	10/17/63	11/11/63	11/20/63	12/2/63	12/10/63
<u>Monkey #30</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	160,000 No Zone	0.25 N.R.	40,000 No Zone	0.25 N.R.	320,000 Positive	10.0 N.R.
	0.25 N.R.	10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	160,000 No Zone	1000 N.R.
<u>Monkey #31</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.25 N.R.	10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	160,000 No Zone	1000 N.R.
	0.25 N.R.	10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	160,000 No Zone	1000 N.R.

Sample #29

Photodynamic action of methylene blue - 8 hours

Date

	9/12/63	10/8/63	10/17/63	11/11/63	11/20/63	12/2/63	12/10/63
<u>Monkey #26</u> Immunization Dose, mg. (sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	<10,000 No Zone	0.25 N.R.	<10,000 No Zone	0.25 N.R.	40,000 No Zone	10.0 N.R.
	0.25 I	<10,000 No Zone	0.25 N.R.	10,000 No Zone	0.25 N.R.	160,000 Positive	1000 I
<u>Monkey #27</u> Immunization Dose, mg. (sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

Sample #30

Acetylation with acetic anhydride

Date

	9/12/63	10/8/63	10/17/63	11/11/63	11/20/63	12/3/63	12/10/63
<u>Monkey #38</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	10,000 No Zone	0.25 N.R.	10,000 No Zone	0.25 N.R.	160,000 Positive	10.0 N.R.
	0.25 I	10,000 No Zone	0.25 N.R.	10,000 No Zone	0.25 N.R.	80,000 No Zone	1000 N.R.
<u>Monkey #39</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

Sample #2

0.2% formalin at 37°C. for 2 weeks

Date

	12/12/63	1/9/64	1/16/64	2/10/64	2/19/64	3/4/64	5/20/64
<u>Monkey #1</u> Immunization Dose, mg. (S ₄) Reaction* Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mg./kg.) Reaction	0.10 N.R.	40,000 No Zone	0.25 N.R.	40,000 No Zone	0.25 N.R.	80,000 No Zone	20,000 No Zone
<u>Monkey #2</u> Immunization Dose, mg. (S ₄) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	20,000 Positive	0.25 N.R.	80,000 No Zone	< 10,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #2

0.2% formalin at 37°C. for 2 weeks

Date

	12/12/63	1/9/64	1/16/64	2/10/64	2/19/64	3/14/64	5/20/64
<u>Monkey # 69</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	320,000 Positive	< 10,000 No Zone
	0.10 I	< 10,000 No Zone	0.25 N.R.	40,000 Positive	0.25 N.R.	640,000 Positive	40,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #2

0.2% formalin at 37°C. for 2 weeks

	<u>Date</u>						
	12/12/63	1/9/64	1/16/64	2/10/64	2/19/64	3/4/64	5/20/64
<u>Monkey # 71</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	80,000 Positive	< 10,000 No Zone
<u>Monkey # 72</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	80,000 Positive	0.25 N.R.	320,000 Positive	80,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #2

0.2% formalin at 37°C. for 2 weeks

Date

	12/12/63	1/9/64	1/16/64	2/10/64	2/19/64	3/4/64	5/20/64
<u>Monkey #73</u> Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	80,000 Positive	0.25 N.R.	320,000 Positive	20,000 No Zone
	0.10 I	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	20,000 No Zone	< 10,000 No Zone
<u>Monkey #74</u> Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #2

0.2% formalin at 37°C. for 2 weeks

Date

	12/12/63	1/9/64	1/16/64	2/10/64	2/19/64	3/4/64	5/20/64
<u>Monkey #75</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	80,000 No Zone	< 10,000 No Zone
	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	10,000 No Zone	0.25 N.R.	20,000 No Zone	< 10,000 No Zone

Monkey #76

Immunization
 Dose, mg. (SQ)
 Reaction
 Antibody Titer
 Hemagglutination
 Gel-Diffusion
 Intravenous Challenge
 Dose (mcg./kg.)
 Reaction

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #2

0.2% formalin at 37°C. for 2 weeks

Date

	12/12/63	1/9/64	1/16/64	2/10/64	2/19/64	3/4/64	5/20/64
<u>Monkey #77</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	10,000 No Zone	0.25 N.R.	80,000 Positive	0.25 N.R.	320,000 Positive	160,000 No Zone
<u>Monkey #78</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	160,000 Positive	0.25 N.R.	1,280,000 220	0.25 N.R.	2,560,000 240	640,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #1C

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

Date

	1/14/64	2/10/64	2/17/64	3/17/64	3/24/64	4/7/64	6/24/64
<u>Monkey # 152</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	160,000 No Zone	0.25 N.R.	1,280,000 145	0.25 N.R.	640,000 Positive	160,000 No Zone
<u>Monkey # 153</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	40,000 Positive	0.25 N.R.	640,000 235	0.25 N.R.	1,280,000 870	1,280,000 Positives**

* I, Illness (anemia, diarrhea)
 X, Death
 N.R. No Reaction

** Quantitative titer to be determined

Sample #10

0.2% formalin + 0.15% glycine at 25° C. for 2 weeks

Date

	1/14/64	2/10/64	2/17/64	3/17/64	3/24/64	4/7/64	6/24/64
<u>Monkey # 154</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	160,000 No Zone	0.25 N.R.	160,000 No Zone	160,000 No Zone
	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	320,000 Positive	0.25 N.R.	320,000 Positive	320,000 Positive
<u>Monkey # 155</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	320,000 Positive	0.25 N.R.	320,000 Positive	320,000 Positive

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #10

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

Date

	1/14/64	2/10/64	2/17/64	3/17/64	3/24/64	4/7/64	6/24/64
Monkey # 154 Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	160,000 No Zone	0.25 N.R.	160,000 No Zone	160,000 No Zone
	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	320,000 Positive	0.25 N.R.	320,000 Positive	320,000 Positive

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #10

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

Date

	1/14/64	2/10/64	2/17/64	3/17/64	3/24/64	4/7/64	6/24/64
<u>Monkey # 156</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	10,000 No Zone	0.25 N.R.	40,000 Positive	0.25 N.R.	640,000 Positive	80,000 No Zone
	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	< 10,000 Positive	0.25 N.R.	20,000 Positive	10,000 No Zone
<u>Monkey # 157</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	< 10,000 Positive	0.25 N.R.	20,000 Positive	10,000 No Zone
	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	< 10,000 Positive	0.25 N.R.	20,000 Positive	10,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #10

0.2% formalin + 0.15% glycine at 25°C. for 2 weeks

Date

	1/14/64	2/12/64	2/17/64	3/17/64	3/24/64	3/24/64	4/7/64	6/24/64
<u>Monkey # 158</u> Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	10,000 Positive	0.25 N.R.	160,000 Positive	80,000 No Zone	
	0.10 N.R.	160,000 No Zone	0.25 N.R.	640,000 175	0.25 N.R.	1,280,000 145	640,000 Positive**	
<u>Monkey # 159</u> Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	160,000 No Zone	0.25 N.R.	640,000 175	0.25 N.R.	1,280,000 145	640,000 Positive**	
	0.10 N.R.	160,000 No Zone	0.25 N.R.	640,000 175	0.25 N.R.	1,280,000 145	640,000 Positive**	

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

** Quantitative titer to be determined

Sample #10

0.2% formalin + 0.15% glycine at 25° C. for 2 weeks

Date

	1/14/64	2/14/64	2/17/64	3/17/64	3/24/64	4/7/64	6/24/64
<u>Monkey # 160</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	20,000 Positive	0.25 N.R.	80,000 240	0.25 N.R.	160,000 455	160,000 Positive
<u>Monkey # 161</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	40,000 No Zone	0.25 N.R.	160,000 Positive	0.25 N.R.	640,000 480	1,280,000 Positive**

* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

** Quantitative titer to be determined

Sample #10

0.2% formalin + 0.15% glycine at 25° C. for 2 weeks

Date

	1/14/64	2/14/64	2/17/64	3/17/64	3/24/64	4/7/64	6/24/64
<u>Monkey # 162</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	2,560,000 400	0.25 N.R.	5,120,000 1200	0.25 N.R.	1,280,000 1290	2,560,000 Positive**
	0.10 N.R.	10,000 No Zone	0.25 N.R.	80,000 275	0.25 N.R.	80,000 380	80,000 No Zone
<u>Monkey # 163</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.		0.25 N.R.		0.25 N.R.		

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

** Quantitative titer to be determined

Sample #12

0.3% hexamethylenetetramine at 37°C. for 3 weeks

Date

	12/13/63	1/10/64	1/17/64	2/10/64	2/20/64	3/5/64	5/20/64
<u>Monkey #81</u> Immunization Dose, mg. (sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	40,000 Positive	0.25 N.R.	160,000 Positive	80,000 No Zone
	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	160,000 190	0.25 N.R.	320,000 220	320,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #12

0.3% hexamethylenetetramine at 37° C. for 3 weeks

Date

	12/13/63	1/10/64	1/17/64	2/10/64	2/20/64	3/5/64	5/20/64
<u>Monkey #83</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer HemaGlutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	<10,000 No Zone	0.25 N.R.	40,000 No Zone	0.25 N.R.	150,000 Positive	80,000 No Zone
	0.10 I	<10,000 No Zone	0.25 N.R.	40,000 Positive	0.25 N.R.	40,000 Positive	20,000 No Zone
<u>Monkey #84</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer HemaGlutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #12

0.3% hexamethylenetetramine at 37°C. for 3 weeks

Date

	12/13/63	1/10/64	1/17/64	2/10/64	2/20/64	3/5/64	5/20/64
<u>Monkey # 85</u> Immunization Dose, mg. (SQ) Reactor * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	20,000 H: Zone	0.25 N.R.	40,000 Positive	0.25 N.R.	50,000 Positive	160,000 Positive
	0.10 N.R.	<10,000 No Zone	0.25 N.R.	640,000 100	0.25 N.R.	5,120,000 315	1,280,000 Positive

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #12

0.3% hexamethylenetetramine at 37°C. for 3 weeks

Date

	12/13/53	1/10/64	2/17/64	2/10/64	2/20/64	3/5/64	5/20/64
<u>Monkey # 87</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	20,000 Positive	0.25 N.R.	160,000 210	0.25 N.R.	5-120,000 330	320,000 No Zone
<u>Monkey # 88</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	40,000 No Zone	0.25 N.R.	80,000 No Zone	40,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #12

0.3% hexamethylenetetramine at 37° C. for 3 weeks

Date

	12/13/63	1/10/64	1/17/64	2/10/64	2/20/64	3/5/64	5/20/64
<u>Monkey # 92</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 Positive	0.25 N.R.	40,000 Positive	0.25 N.R.	40,000 Positive	160,000 No Zone
	0.10 I	< 10,000 No Zone	0.25 N.R.	---	0.25 N.R.	80,000 No Zone	40,000 No Zone
<u>Monkey # 93</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

* I, Illness (emesis, diarrhea)
 A, Death
 N.R. No Reaction

Date

	1/15/54	2/14/54	2/18/54	2/19/54	3/25/54	4/8/54	6/25/64
<p><u>Monkey # 156</u></p> <p>Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>	0.10 I	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	40,000 Positive	80,000 No Zone
<p><u>Monkey # 157</u></p> <p>Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>	0.10 I	< 10,000 No Zone	0.25 N.R.	40,000 Positive	0.25 N.R.	80,000 Positive	160,000 No Zone

* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

Sample #14

0.2% beta-propiolactone at 37° C. for 2 hours

Date

	1/15/64	2/14/64	2/18/64	3/18/64	3/25/64	4/8/64	6/25/64
<u>Monkey # 168</u>	0.10 I	< 10,000 No Zone	0.25 N.R.	40,000 Positive	0.25 N.R.	80,000 Positive	80,000 No Zone
Immunization Dose, mg. (Sq) Reaction * Antibody Titer HemaGlutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							
<u>Monkey # 169</u>	0.10 I	80,000 No Zone	0.25 N.R.	320,000 No Zone	0.25 N.R.	320,000 Positive	1,280,000 No Zone
Immunization Dose, mg. (Sq) Reaction Antibody Titer HemaGlutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

Date

	1/15/64	2/14/64	2/18/64	3/18/64	3/25/64	4/8/64	6/24/64
<p><u>Monkey #170</u></p> <p>Immunization Dose, mg. (Sq) Reaction *</p> <p>Antibody Titer HemaGlutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>	0.10 I	10,000 No Zone	0.25 N.R.	10,000 No Zone	0.25 N.R.	10,000 No Zone	20,000 No Zone
	0.10 N.R.	2,560,000 330	0.25 N.R.	1,280,000 500	0.25 N.R.	1,280,000 1260	2,560,000 Positive**
<p><u>Monkey #171</u></p> <p>Immunization Dose, mg. (Sq) Reaction</p> <p>Antibody Titer HemaGlutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>							

* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

** Quantitative titer to be determined

Sample #14

0.2% beta-propiolactone at 37°C. for 2 hours

Date

	1/15/64	2/14/64	2/18/64	3/18/64	3/23/64	4/8/64	6/25/64
<u>Monkey # 172</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	160,000 No Zone	0.25 N.R.	40,000 No Zone	0.25 N.R.	160,000 Positive	160,000 No Zone
	0.10 I	< 10,000 No Zone	0.25 N.R.	40,000 Positive	0.25 N.R.	320,000 Positive	320,000 No Zone
<u>Monkey # 173</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #14

0.2% beta-propiolactone at 37°C. for 2 hours

Date

	1/15/64	2/14/64	2/18/64	3/18/64	3/25/64	4/8/64	6/25/64
<u>Monkey # 174</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	40,000 No Zone	0.25 N.R.	40,000 No Zone	40,000 No Zone
<u>Monkey # 175</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	40,000 No Zone	0.25 N.R.	80,000 No Zone	0.25 N.R.	160,000 No Zone	40,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #14

0.2% beta-propiolactone at 37°C. for 2 hours

Date

	2/15/64	2/14/64	2/18/64	3/18/64	3/25/64	4/8/64	6/25/64
<u>Monkey # 176</u> Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	< 10,000 No Zone
	0.10 I	< 10,000 No Zone	0.25 N.R.	320,000 No Zone	0.25 N.R.	320,000 Positive	320,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

0.2% diethylpyrocarbonate at 5°C. for 1 week

Date

	1/5/64	1/31/64	2/10/64	3/10/64	3/16/64	3/30/64	6/16/64
<u>Monkey #96</u> Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	160,000 No Zone	20,000 No Zone
	0.10 I	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	160,000 No Zone	40,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #16

0.2% diethylpyrocarbonate at 5° C. for 1 week

Date

	1/6/64	1/31/64	2/10/64	3/10/64	3/16/64	3/30/64	6/16/64
<u>Monkey # 98</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	10,000 No Zone	0.25 H.R.	10,000 No Zone	0.25 N.R.	80,000 No Zone	40,000 No Zone
<u>Monkey # 99</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 X						

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #16

0.2% diethylpyrocarbonate at 5°C. for 1 week

Date

	1/6/64	1/31/64	2/10/64	3/10/64	3/16/64	3/30/64	6/16/64
<u>Monkey # 100</u> Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	40,000 No Zone	< 10,000 No Zone
	0.10 I	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	40,000 No Zone	20,000 No Zone
<u>Monkey # 101</u> Immunization Dose, mg. (Sq) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	40,000 No Zone	20,000 No Zone
	0.10 I	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	40,000 No Zone	20,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #16

0.2% diethylhydrocarbonate at 5°C. for 1 week

Date

	1/6/64	1/31/64	2/10/64	3/10/64	3/16/64	3/30/64	6/16/64	
<u>Monkey # 102</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	80,000 Positive	0.25 N.R.	320,000 Positive	0.25 N.R.	2,560,000 245	80,000 No Zone	
<u>Monkey # 103</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	320,000 Positive	40,000 No Zone	

* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

sample #10

0.2% diethylpyrocarbonate at 5°C. for 1 week

Date

	1/5/64	1/31/64	2/10/64	3/10/64	3/16/64	3/30/64	6/16/64
<u>Monkey #104</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	320,000 200	0.25 N.R.	640,000 Positive	0.25 N.R.	2,560,000 130	160,000 No Zone
<u>Monkey #105</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 X				

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #16

0.2% diethylpyrocarbonate at 5°C. for 1 week

Date

	1/6/64	1/31/64	2/10/64	3/10/64	3/16/64	3/30/64	6/16/64
<u>Monkey # 106</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 X						
<u>Monkey # 107</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	<10,000 No Zone	0.25 N.R.	160,000 No Zone	10,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

0.2% ethylene oxide at 5 C. for 2 hours

Date

	1/20/64	2/14/64	2/24/64	3/19/64	3/30/64	4/13/64	6/30/64
<u>Monkey # 180</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	<10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	40,000 Positive	20,000 No Zone
<u>Monkey # 181</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	<10,000 No Zone	0.25 N.R.	80,000 Positive	0.25 N.R.	80,000 Positive	80,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #18

0.2% ethylene oxide at 5°C. for 2 hours

Date

	1/20/64	2/15/64	2/24/64	3/19/64	3/30/64	4/13/64	6/30/64
<u>Monkey # 182</u> Immunization Dose, mg. (kg.) Reaction* Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	10,000 No Zone	0.25 N.R.	20,000 Positive	80,000 Positive
	0.10 N.R.	10,000 Positive	0.25 N.R.	40,000 Positive	0.25 N.R.	40,000 Positive	160,000 Positive
<u>Monkey #183</u> Immunization Dose, mg. (kg.) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.						

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

0.2% ethylene oxide at 5°C. for 2 hours

Date

	1/20/64	2/14/64	2/24/64	3/19/64	3/30/64	4/13/64	6/30/64
<u>Monkey # 184</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer HemaGlutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	40,000 Positive	0.25 N.R.	40,000 Positive	80,000 No Zone
<u>Monkey # 185</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer HemaGlutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	X				

* I, Illness (emesis, diarrhea)

X, Death

N.R. No Reaction

Sample #18

0.2% ethylene oxide at 5 °C. for 2 hours

Date

	1/20/64	2/14/64	2/24/64	3/19/64	3/30/64	4/13/64	6/30/64
<u>Monkey # 186</u> Immunization Dose, mg. (Sq) Reaction * Antibody Titer HemaGlutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 Positive	0.25 N.R.	40,000 No Zone	0.25 N.R.	20,000 Positive	640,000 Positive
<u>Monkey # 187</u> Immunization Dose, mg. (Sq) Reaction Antibody Titer HemaGlutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	40,000 No Zone	0.25 N.R.	40,000 Positive	20,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #18

0.2% ethylene oxide at 5°C. for 2 hours

Date

	1/20/64	2/14/64	2/24/64	3/19/64	3/30/64	4/13/64	6/30/64
<p><u>Monkey # 188</u></p> <p>Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>	0.10 I	80,000 Positive	0.25 N.R.	160,000 Positive	0.25 N.R.	160,000 80	320,000 No Zone
<p><u>Monkey # 189</u></p> <p>Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>	0.10 I	40,000 Positive	0.25 N.R.	40,000 No Zone	0.25 N.R.	80,000 Positive	20,000 No Zone

* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

Sample #18

0.2% ethylene oxide at 5°C. for 2 hours

Date

	1/20/64	2/14/64	2/24/64	3/19/64	3/30/64	4/13/64	6/30/64
<p><u>Monkey # 190</u></p> <p>Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>	0.10 I	10,000 No Zone	0.25 N.R.	40,000 No Zone	0.25 N.R.	20,000 No Zone	40,000 No Zone
<p><u>Monkey # 191</u></p> <p>Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>	0.10 I	X 10,000 No Zone					

* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

Sample #22

Exposure to methyl bromide vapor for 24 hours

Date

	1/21/54	2/14/54	2/25/54	3/20/54	3/31/54	4/14/54	7/1/54
<u>Monkey # 194</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	40,000 Positive	20,000 No Zone
	0.10 I	< 10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	< 10,000 No Zone	< 10,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #22

Exposure to methyl bromide vapor for 24 hours

Date

	1/21/64	2/14/64	2/25/64	3/20/64	3/31/64	4/14/64	7/1/64
<u>Monkey # 196</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer HemaGlutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	320,000 Positive	0.25 N.R.	160,000 Positive	0.25 N.R.	160,000 Positive	40,000 No Zone
	0.10 N.R.	10,000 No Zone	0.25 N.R.	160,000 Positive	0.25 N.R.	320,000 Positive	
<u>Monkey # 197</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer HemaGlutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Exposure to methyl bromide vapor for 24 hours

Date

	1/21/64	2/14/64	2/25/64	3/20/64	3/31/64	4/10/64	7/1/64
<p><u>Monkey # 200</u></p> <p>Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (m 3./kg.) Reaction</p>	0.10 I	< 10,000 No Zone	0.25 N.R.	< 10,000 No Zone	0.25 N.R.	X	
<p><u>Monkey # 201</u></p> <p>Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>	0.10 N.R.	160,000 No Zone	0.25 N.R.	160,000 No Zone	0.25 N.R.	160,000 Positive	80,000 No Zone

* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

Sample #22

Exposure to methyl bromide vapor for 24 hours

Date

	1/21/64	2/14/64	2/25/64	3/20/64	3/31/64	4/14/64	7/1/64
<u>Monkey # 202</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	160,000 70	80,000 No Zone
<u>Monkey # 203</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	10,000 No Zone	0.25 N.R.	20,000 No Zone	< 10,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #22

Exposure to methyl bromide vapor for 24 hours

Date

	1/21/54	2/14/54	2/25/54	3/30/54	3/31/54	4/14/54	7/1/54
<p><u>Monkey # 204</u></p> <p>Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>	0.10 N.R.	50,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	20,000 No Zone	10,000 No Zone
<p><u>Monkey # 205</u></p> <p>Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>	0.10 I	< 10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	20,000 No Zone	< 10,000 No Zone

* I, Illness (emesis, diarrhea)
X, Death
N.R., No Reaction

Sample #25

1.0% sodium gluconate at 37°C. for 4 weeks

Date

	1/13/64	2/10/64	2/17/64	3/16/64	3/23/64	4/5/64	6/23/64
<u>Monkey # 138</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 [~] N.R.	40,000 Positive	0.25 N.R.	1,280,000 235	2,560,000 Positive**
<u>Monkey #139</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	10,000 No Zone	0.25 N.R.	20,000 Positive	0.25 N.R.	640,000 125	160,000 No Zone

* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

** quantitative titer to be determined

1.0% sodium gluconate at 37°C. for 4 weeks

Date

	1/13/64	2/10/64	2/17/64	3/16/64	3/23/64	4/6/64	6/23/64
<u>Monkey # 140</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	80,000 No Zone	0.25 N.R.	640,000 No Zone	40,000 No Zone
	0.10 I	< 10,000 No Zone	0.25 N.R.	20,000 No Zone	0.25 N.R.	80,000 No Zone	80,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #25

1.0% sodium gluconate at 37° C. for 4 weeks

Date

	1/13/64	2/10/64	2/17/64	3/16/64	3/23/64	4/6/64	6/23/64
<u>Monkey #142</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	<10,000 No Zone	0.25 N.R.	160,000 Positive	0.25 N.R.	640,000 190	640,000 Positive
	0.10 N.R.	40,000 No Zone	0.25 N.R.	1,280,000 Positive	0.25 N.R.	640,000 160	320,000 No Zone
<u>Monkey #143</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

1.0% sodium gluconate at 37°C. for 4 weeks

Date

	1/13/64	2/10/64	2/17/64	3/16/64	3/23/64	4/6/64	6/23/64
<u>Monkey # 144</u> Immunization Dose, mg. (S ₂) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	80,000 No Zone	0.25 N.R.	640,000 160	640,000 No Zone
	0.10 I	< 10,000 No Zone	0.25 N.R.	160,000 No Zone	0.25 N.R.	1,280,000 Positive	40,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #25

1.0% sodium gluconate at 37°C. for 4 weeks

Date

	1/13/64	2/10/64	2/17/64	3/16/64	3/23/64	4/5/64	6/23/64
<u>Monkey # 146</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	80,000 Positive	0.25 N.R.	1,280,000 190	640,000 Positive
	0.10 I	< 10,000 No Zone	0.25 N.R.	40,000 No Zone	0.25 N.R.	320,000 No Zone	160,000 No Zone
<u>Monkey # 147</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	40,000 No Zone	0.25 N.R.	320,000 No Zone	160,000 No Zone
	0.10 I	< 10,000 No Zone	0.25 N.R.	40,000 No Zone	0.25 N.R.	320,000 No Zone	160,000 No Zone

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Exposure to ultraviolet light for 1 hour

Date

	1/8/64	1/31/64	2/12/64	3/12/64	3/18/64	4/1/64	6/18/64
<u>Monkey # 124</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	320,000 Positive	0.25 N.R.	2,550,000 Positive	0.25 N.R.	540,000 Positive	2,550,000 Positive
	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	40,000 Positive	0.25 N.R.	80,000 Positive	150,000 Positive
<u>Monkey # 125</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #26

Exposure to ultraviolet light for 1 hour

Date

	1/8/64	1/31/64	2/12/64	3/12/64	3/18/64	4/1/64	6/18/64
<u>Monkey # 126</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	160,000 Positive	0.25 N.R.	80,000 Positive	320,000 Positive
	0.10 I	10,000 No Zone	0.25 N.R.	320,000 Positive	0.25 N.R.	80,000 Positive	20,000 No Zone
<u>Monkey # 127</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Date

	1/8/64	1/31/64	2/12/64	3/12/64	3/18/64	4/1/64	6/18/64
<u>Monkey # 128</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	20,000 Positive	0.25 N.R.	320,000 Positive	0.25 N.R.	320,000 No Zone	320,000 No Zone
	0.10 I	< 10,000 No Zone	0.25 N.R.	160,000 Positive	0.25 N.R.	80,000 Positive	40,000 No Zone
<u>Monkey # 129</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction							

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #26

Exposure to ultraviolet light for 1 hour

Date

	1/8/64	1/31/64	2/12/64	3/12/64	3/18/64	4/1/64	6/18/64
<u>Monkey #132</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	80,000 No Zone	0.25 N.R.	80,000 Positive	< 10,000 No Zone
	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	320,000 Positive	0.25 N.R.	320,000 275	320,000 Positive
<u>Monkey #133</u> Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	320,000 Positive	0.25 N.R.	320,000 275	320,000 Positive
	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	320,000 Positive	0.25 N.R.	320,000 275	320,000 Positive

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Exposure to ultraviolet light for 1 hour

Date

	1/8/64	1/11/64	2/12/64	3/12/64	3/18/64	4/1/64	6/18/64
Monkey # 134 Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	20,000 No Zone	0.25 N.R.	80,000 Positive	0.25 N.R.	40,000 Positive	40,000 No Zone
	0.10 N.R.	< 10,000 No Zone	0.25 N.R.	40,000 Positive	0.25 N.R.	160,000 Positive	160,000 Positive
Monkey # 135 Immunization Dose, mg. (SQ) Reaction Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.						

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #28

Photodynamic action of methylene blue - 1 hour

Date

	1/7/64	1/31/64	2/11/64	3/11/64	3/17/64	3/31/64	6/17/64
<u>Monkey # 110</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	10,000 No Zone	0.25 N.R.	160,000 Positive	0.25 N.R.	1,280,000 No Zone	160,000 Positive
	0.10 I	< 10,000 No Zone	0.25 N.R.	80,000 Positive	0.25 N.R.	320,000 125	320,000 Positive

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Photodynamic action of methylene blue - 1 hour

Date

	1/7/64	1/31/64	2/11/64	3/11/64	3/17/64	3/31/64	6/17/64
<u>Monkey # 112</u> Immunization Dose, mg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 I	< 10,000 No Zone	0.25 N.R.	320,000 Positive	0.25 N.R.	320,000 165	80,000 Positive
	0.10 I	80,000 Positive	0.25 N.R.	160,000 Positive	0.25 N.R.	640,000 150	320,000 Positive

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Sample #28

Photodynamic action of methylene blue - 1 hour

Date

	1/7/64	1/31/54	2/11/54	3/11/54	3/17/54	3/31/64	6/17/64
<u>Monkey # 114</u>							
Immunization							
Dose, mg. (SQ)	0.10		0.25	1,280,000	0.25	1,280,000	160,000
Reaction *	N.R.		N.R.	Positive	N.R.	115	Positive
Antibody Titer							
Hemagglutination		50,000					
Gel-Diffusion		Positive					
Intravenous Challenge							
Dose (mcg./kg.)							
Reaction							
<u>Monkey # 115</u>							
Immunization							
Dose, mg. (SQ)	0.10		0.25	80,000	0.25	640,000	320,000
Reaction	I		N.R.	Positive	N.R.	115	Positive
Antibody Titer							
Hemagglutination		10,000					
Gel-Diffusion		No Zone					
Intravenous Challenge							
Dose (mcg./kg.)							
Reaction							

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Photodynamic action of methylene blue - 1 hour

Date

	1/7/54	1/31/54	2/11/54	3/11/54	3/17/54	3/31/54	6/17/64
<p><u>Monkey # 116</u></p> <p>Immunization Dose, mcg. (SQ) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>	0.10 I	10,000 Positive	0.25 N.R.	320,000 Positive	0.25 N.R.	1.280,000 Positive	320,000 No Zone
	0.10 I	80,000 200	0.25 N.R.	640,000 Positive	0.25 N.R.	640,000 140	640,000 Positive

* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction

Sample #28

Photodynamic action of methylene blue - 1 hour

Date

	1/7/54	1/31/54	2/11/54	3/11/54	3/17/54	3/31/54	6/17/64
<u>Monkey # 11E</u> Immunization Dose, mg. (Sq) Reaction * Antibody Titer Hemagglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction	0.10 N.R.	10,000 Positive	0.25 N.R.	1,280,000 Positive	0.25 N.R.	150,000 500	640,000 Positive
	0.10 I	50,000 No Zone	0.25 N.R.	150,000 Positive	0.25 N.R.	20,000 Positive	320,000 Positive

* I, Illness (emesis, diarrhea)
 X, Death
 N.R. No Reaction

Photodynamic action of methylene blue - 1 hour

Date

	1/7/64	1/31/64	2/11/64	3/11/64	3/17/64	3/31/64	6/17/64
<p><u>Monkey # 120</u></p> <p>Immunization Dose, mg. (SQ) Reaction *</p> <p>Antibody Titer HemaGglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>	0.10 X						
	0.10 I	40,000 No Zone	0.25 N.R.	160,000 Positive	0.25 N.R.	80,000 No Zone	160,000 No Zone
<p><u>Monkey # 121</u></p> <p>Immunization Dose, mg. (SQ) Reaction</p> <p>Antibody Titer HemaGglutination Gel-Diffusion Intravenous Challenge Dose (mcg./kg.) Reaction</p>							

* I, Illness (emesis, diarrhea)
X, Death
N.R. No Reaction