Presentation 14 – Phillip Pittman

USAMRIID

Studies on the Health Effects of Multiple Vaccines: Completed and Ongoing

Research Advisory Committee on Gulf War Illness Meeting
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Lafayette Building
811 Vermont Street, NW Rm 819
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Hyper Immunization

- Repeated vaccination with a variety of antigens has become common practice for immunization against a variety of pathogens.
- Common reactions have included acute local and/or systemic reactions and rare hypersensitivity reactions
- Otherwise, few other adverse events have been clearly linked to vaccination.
- Experimental animals injected with large doses of antigens may produce delayed adverse effects, such as, amyloid deposition, arteritis, etc., but similar reactions have not been observed in humans

Hyper Immunization

- Studies have been done to assess the long-term medical risk of repeated injections with multiple antigens at Fort Detrick for many years.
- In the 1950s Fort Detrick had a group of workers who had received repeated injections with multiple antigens of bacterial, rickettsial and viral origins.

What are the Fort Detrick Vaccine Safety Studies

- 1958 - Peeler RN, Cluff LE, Trever RW. Hyper-immunization of man. *Bulletin of the Johns Hopkins Hospital* 1958;103:183-98.
- 1965 - Peeler RN, Kadull PJ, Cluff LE. Intensive immunization of man: Evaluation of possible adverse consequences. *Annals of Internal Medicine* 1965;63:44-57.
- 1974 - White CS III, Adler WH, McGann VG. Repeated immunization: Possible adverse effects: Reevaluation of human subjects at 25 years. *Annals of Internal Medicine* 1974;81:594-600.

Study 1: The Peeler Study 1956-7: M & M

- **99 Caucasian males**
- **Ages 28-65 years**
- **Duration of immunization 8-12 years (1944--1956)**
- **Total amount of antigen 35.8 ml -- 74.4 ml**
- **All subjects received the followed antigens:**
  - botulism, tularemia, Rocky Mountain spotted fever, Q fever, typhus, plague, psittacosis, and the viral encephalitides. In addition,
  - brucellosis = 34; smallpox = 95; anthrax = 28; etc.
- **93 had complete medical history and physical examination**
- **Hospital and outpatient records were reviewed for each subject for the period.**

Peeler 1: Results

- **Clinical Evaluations**
  - Men NOT ill as a group!
  - Occupational illness
    - tularemia 1
    - brucellosis 1
    - Q fever 1
    - febrile illness of undetermined origin (URIs) 9
  - Physical findings
    - hepatomegaly 7
      - 2 tularemia & brucellosis
      - 5 ? Etiology
    - macroglossia 1

Peeler 1: Conclusion

- No clinical abnormality found
- Two clinical laboratory deviations noted
  - abnormal PEP pattern (~23%)
  - lymphocytosis (~25%)
- No demographically matched control group

Study 2 : 5-year follow-up 1962: M & M

- **76/99 Caucasian males**
- **Ages 33-70 years (mean age 46.3)**
- **Duration of immunization 12-18 years (1944--1962); mean 13.3 years**
- **Total volume of antigen 42 ml -- 101 ml (mean 21 ml)**
- **All subjects received the followed antigens:**
  - botulism, tularemia, Rocky Mountain spotted fever, Q fever, typhus, plague, psittacosis, and the viral encephalitides. In addition,
  - brucellosis = 34; smallpox = 70; RVF 66, Diphtheria 20, influenza 54, anthrax = 72; etc.
- **76 had complete medical history and physical examination**
- **Hospital and outpatient records were reviewed for each subject for the period.**

Study 2 : 5-year follow-up 1962: M & M

- **Additional clinical laboratory tests added compared to 1956: BUN, SGOT, SGPT, Urea clearance, Fasting glucose, U/A, VDRL, serum hexosamines, Zinc turbidity test for gamma globulin level, RF, etc**
- **Gingival (7) & renal punch biopsies (3)were performed; 4 died of intercurrent and unrelated illnesses.**
- **Controls for electrophoretic data and hexosamine determinations were 102 serial serum specimens from healthy blood donors at the Johns Hopkins Hospital Blood Bank. Same age group but not matched by other demographics.**

Study 2 : 5-year follow-up 1962: Results

- **Clinical Laboratory Findings**
  - Hematologic
    - HCT -- normal in all men.
    - Leukopenia 4
    - Leukocytosis11
    - Monocytosis 0 (3 subjects had monocytosis in 1956 --not seen in 1962)
    - Lymphocytosis
      - 1956 27% had > 40%
      - 1962 31.6%
    - Eosinophilia (> 3%) 17 in 1956; 23 in 1962
  - Renal Function
    - Proteinuria
  - Liver Function
    - Alkaline Phosphatase slightly elevated in 3 men

Study 2 : 5-year follow-up 1962: Results

- **Clinical Laboratory Findings**
  - Serum Electrophoresis --
    - No quantitative abnormalities of the various protein fractions in 1958 report or in 1962.
    - Same qualitative abnormality described in 23% in 1956 now in 34%
  - Serum Hexosamines-- mean hexosamine value elevated for test group
- **Pathological Studies**
  - 4 deaths between 1956 -1962
    - MI 3
    - carcinoma of colon 1
    - sections of liver, spleen and kidneys were examined after staining and showed no evidence of amyloid deposition or other abnormality
  - Gum biopsies (7 of the most suggestive laboratory abnormalities). Percutaneous renal punch biopsy on 3 men demonstrating persistent proteinuria. All of these sections were normal for hematoxylin and eosin and thioflavin-T.

Study 2 : 5-year follow-up 1962: Conclusion

- **"Follow-up examinations of these intensively immunized men failed to demonstrate any evidence of illness attributable to the immunizations."**
- **"There is no indication that intensive immunization interfered with the ability to produce adequate antibody titers after antigenic challenge."**
- **Several clinical laboratory abnormalities were noted but of no clinical significance**
- **No proper control group**

Study 3 : 25-year follow-up 1971: M & M

- **77/99 Caucasian males**
- **Ages 43-79 years (mean age 55)**
- **Number of immunogens = 21**
- **Total volume of antigen 52 ml -- 134 ml (mean 97 ml); mean skin tests = 55**
- **Control group was 26 age-matched, long-term, civilian, male employees from Fort Detrick who had never received special immunizations or been exposed to laboratory infections.**

Study 3 : 25-year follow-up 1971: Results

- **Laboratory Evaluations**
  - Serums concentrations of IgG, IgA, IgM, or C3 were similar for both groups.
  - Mean lymphoproliferative response to phytohemagglutinin was not significantly different for the immunized subject group and age-matched control group
  - In 1971, 15.5 years after their selection for study, 11/99 immunized persons had died, a mortality rate in agreement with the 10.76 deaths predicted by actuarial data.
    - ASCVD 4
    - Cancer 3 (oat-cell ca of lung, colon adenocarcinoma, brain tumor)
    - COPD 2
    - 2 died suddenly without postmortem examination
      - IDDM 1
      - LBBB & PVCs on old EKGs
    - Tissue sections obtained from 4 postmortem examinations and one biopsy showed no evidence of amyloidosis

Study 3 : 25-year follow-up 1971: Discussion

- **Evaluations in 1962 suggested that laboratory abnormalities might be transient because there was no continuing abnormality in some individuals and seven men who had not received an immunization within the preceding 2 years had no antigammaglobulin factors.**
- **Hexosamine elevations noted in all 3 studies—the significance of this finding is not known. The test is no longer done.**
- **Other unexplained differences: ESR, Serum iron and copper levels; serum albumin, alpha-2 globulin and beta globulin values and PTT. The significance of findings for the alpha and beta globulins is less impressive because most values for the immunized subjects fall within the 95% confidence limits of the control mean.**

Study 3 : 25-year follow-up 1971: Conclusion

- **"These data and the accompanying evaluation of an intensively immunized population provide evidence that no obvious adverse effects result from repeated immunization."**
- **There are some laboratory mean values that are different but the means often were within the normal range and do not support a clinical illness.**
- **There were no disease or clinical symptom complex found related to multiple immunization in either studies over a 25 year period.**

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Long-term health effects of repeated exposure to multiple vaccines☆

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Long-term health effects?

- The health of 155 former workers in a US military research program who had received multiple vaccines and 265 matched community controls was assessed.
- The vast majority of the study group were recruited and enrolled during a biannual alumni meeting in 1996 at Fort Detrick, MD.
- Controls were recruited from among age, race, gender matched community controls within Frederick county.

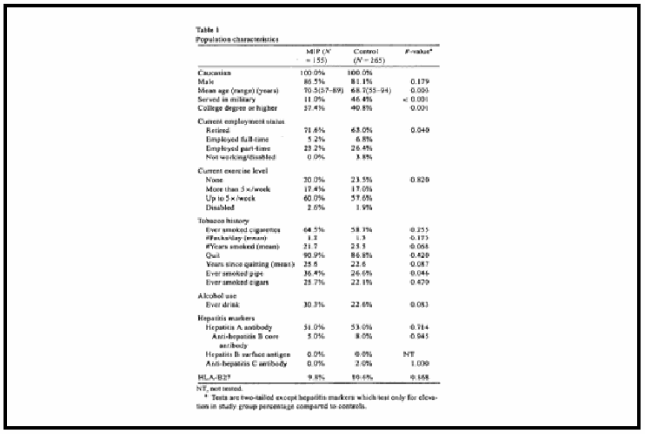


Table 1
Population characteristics

	MIP (N = 155)	Control (N = 265)	P-value*
Caucasian	100.0%	100.0%	
Male	86.5%	84.1%	0.479
Mean age (range) (years)	70.5(57–89)	68.7(55–94)	0.006
Served in military	11.0%	46.8%	< 0.001
College degree or higher	37.4%	40.8%	0.001
Current employment status			
Retired	71.6%	65.0%	0.049
Employed full-time	5.2%	6.8%	
Employed part-time	23.2%	26.4%	
Not working/disabled	0.0%	1.9%	
Current exercise level			
None	30.0%	23.5%	0.820
More than 3 x/week	17.4%	17.0%	
Up to 3 x/week	60.0%	57.6%	
Disabled	2.6%	1.9%	
Tobacco history			
Ever smoked cigarettes	64.5%	58.1%	0.253
#Packs/day (mean)	1.3	1.3	0.173
#Years smoked (mean)	21.7	23.5	0.068
Quit	90.9%	86.8%	0.420
Years since quitting (mean)	25.6	22.6	0.087
Ever smoked pipe	36.4%	26.6%	0.046
Ever smoked cigars	25.7%	22.1%	0.479
Alcohol use			
Ever drink	80.3%	22.6%	0.053
Hepatitis markers			
Hepatitis A antibody	51.0%	53.0%	0.714
Anti-hepatitis B core antibody	5.0%	8.0%	0.045
Hepatitis B surface antigen	0.0%	0.0%	NT
Anti-hepatitis C antibody	0.0%	2.0%	1.000
HLA-B27	9.8%	10.6%	0.168

NT, not tested.
* Tests are two-tailed except hepatitis markers which test only for elevations in study group percentage compared to controls.

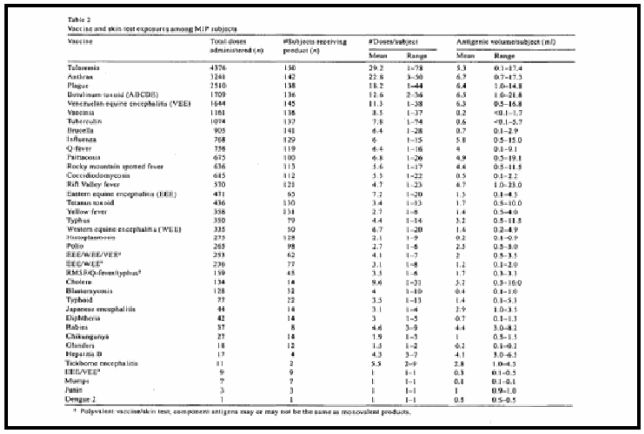
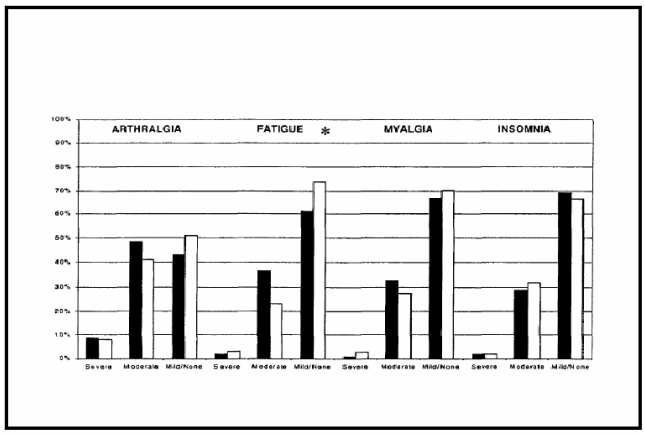
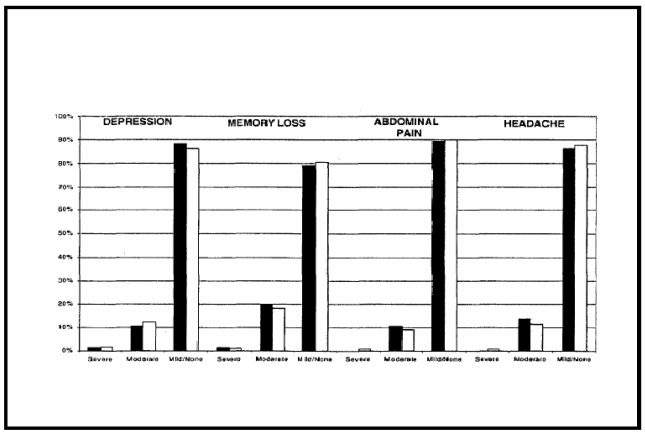


Table 2
Vaccine and skin test exposures among MIP subjects

Vaccine	Total doses administered (n)	#Subjects receiving product (n)	#Doses/subject		Antigenic volume/subject (ml)	
			Mean	Range	Mean	Range
Tularemia	4376	150	29.2	1–78	5.3	0.1–17.4
Anthrax	1284	142	22.8	3–30	6.7	0.7–17.3
Plague	2510	138	18.2	1–44	6.4	1.0–14.8
Botulinum toxoid (ABCDE)	1709	136	12.6	2–36	6.3	1.0–21.8
Venezuelan equine encephalitis (VEE)	1644	145	11.3	1–28	6.3	0.5–16.8
Vaccinia	1161	138	8.5	1–37	0.2	<0.1–0.7
Tuberculin	1074	137	7.9	1–74	0.6	<0.1–5.7
Brucella	905	141	6.4	1–28	0.7	0.1–2.9
Influenza	768	129	6	1–15	5.8	0.5–15.0
Q-fever	756	119	6.4	1–16	4	0.1–9.1
Pertussis	675	100	6.8	1–26	6.9	0.5–19.1
Rocky mountain spotted fever	636	115	5.6	1–17	4.6	0.5–11.5
Coccidiodomycosis	645	112	5.5	1–22	0.5	0.1–2.1
Rift Valley fever	530	123	4.7	1–23	4.7	1.0–23.0
Eastern equine encephalitis (EEE)	471	95	7.2	1–20	1.5	0.1–4.5
Tetanus toxoid	436	130	3.4	1–33	1.7	0.5–10.0
Yellow fever	358	134	2.7	1–8	1.4	0.5–4.0
Typhus	350	79	4.4	1–14	3.2	0.5–11.5
Western equine encephalitis (WEE)	335	50	6.7	1–20	1.6	0.2–4.9
Hemophilus	273	129	2.1	1–9	0.2	0.1–0.9
Polio	269	98	2.7	1–8	2.3	0.5–8.0
EEE/WEE/VEE*	253	62	4.1	1–7	2	0.5–3.5
EEE/WEE*	236	77	3.1	1–8	1.2	0.1–2.0
RMSF/Q-fever/typhus*	159	45	3.5	1–8	1.7	0.3–3.1
Cholera	134	14	9.6	1–21	3.2	0.3–10.0
Blastomycosis	128	32	4	1–10	0.4	0.1–1.0
Typhoid	77	22	3.5	1–13	1.4	0.1–5.1
Japanese encephalitis	44	14	3.1	1–4	2.9	1.0–3.5
Diphtheria	42	14	3	1–5	0.7	0.1–1.1
Rabies	37	8	4.6	3–8	4.4	3.0–8.2
Chikungunya	27	14	1.9	1–5	1	0.5–1.5
Cholera	18	12	1.5	1–2	0.2	0.1–0.2
Hepatitis B	17	4	4.3	3–7	4.1	3.0–6.2
Tickborne encephalitis	11	2	5.5	2–9	2.8	1.0–4.5
EEE/VEE*	9	9	1	1–1	0.3	0.1–0.5
Mumps	7	7	1	1–1	0.1	0.1–0.1
Junin	3	3	1	1–1	1	0.9–1.0
Dengue 2	1	1	1	1–1	0.5	0.5–0.5

* Polyvalent vaccine/skin test; component antigens may or may not be the same as monovalent products.





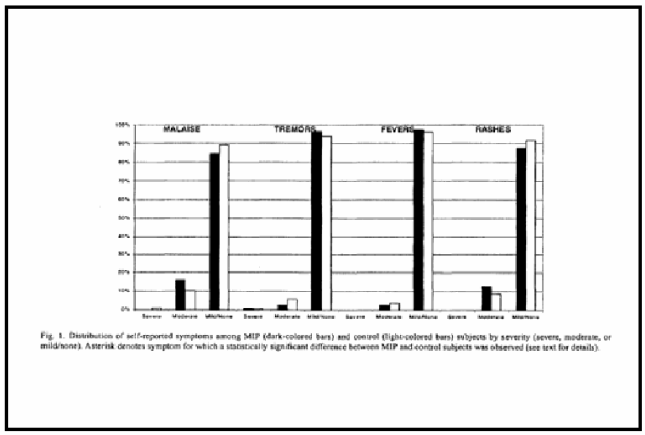


Fig. 1. Distribution of self-reported symptoms among MIP (dark-colored bars) and control (light-colored bars) subjects by severity (severe, moderate, or mild/none). Asterisk denotes symptom for which a statistically significant difference between MIP and control subjects was observed (see text for details).

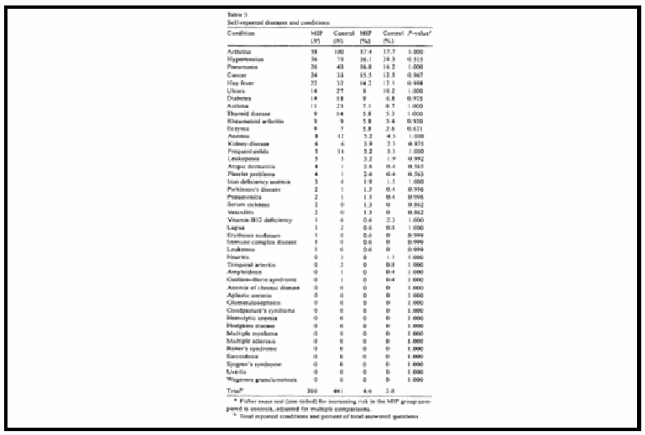


Table 3
Self-reported diseases and conditions

Condition	MIP (N)	Control (N)	MIP (%)	Control (%)	P-value*
Arthritis	98	100	37.4	37.7	1.000
Hypertension	96	79	36.1	29.8	0.313
Pneumonia	26	45	16.8	16.2	1.000
Cancer	24	35	15.5	12.8	0.867
Hay fever	33	33	14.2	12.1	0.908
Ulcers	14	27	9	10.2	1.000
Diabetes	14	18	9	6.8	0.703
Asthma	11	23	7.1	8.7	1.000
Thyroid disease	9	14	5.8	5.3	1.000
Rheumatoid arthritis	9	9	5.8	3.4	0.503
Eczema	9	7	5.8	2.6	0.621
Anemia	8	12	5.2	4.5	1.000
Kidney disease	6	6	3.9	2.3	0.575
Frequent colds	5	14	3.2	5.3	1.000
Leukopenia	5	1	3.2	1.9	0.992
Atopic dermatitis	4	1	2.6	0.4	0.363
Fibrocystic problems	4	1	2.6	0.4	0.363
Iron deficiency anemia	3	4	1.9	1.5	1.000
Parkinson's disease	2	1	1.3	0.4	0.996
Phlebitis/clots	2	1	1.3	0.4	0.996
Serum sickness	2	0	1.3	0	0.992
Vasculitis	2	0	1.3	0	0.992
Vitamin B12 deficiency	1	6	0.6	2.3	1.000
Lupus	1	2	0.6	0.8	1.000
Erythema nodosum	1	0	0.6	0	0.999
Immune complex disease	1	0	0.6	0	0.999
Leukemia	1	0	0.6	0	0.999
Neuritis	0	1	0	0.4	1.000
Temporal arteritis	0	2	0	0.8	1.000
Amyloidosis	0	1	0	0.4	1.000
Guillain-Barre syndrome	0	1	0	0.4	1.000
Anemia of chronic disease	0	0	0	0	1.000
Aplastic anemia	0	0	0	0	1.000
Glomerulonephritis	0	0	0	0	1.000
Goodpasture's syndrome	0	0	0	0	1.000
Hemolytic anemia	0	0	0	0	1.000
Hodgkins disease	0	0	0	0	1.000
Multiple myeloma	0	0	0	0	1.000
Multiple sclerosis	0	0	0	0	1.000
Reiter's syndrome	0	0	0	0	1.000
Sarcoidosis	0	0	0	0	1.000
Sjogren's syndrome	0	0	0	0	1.000
Uveitis	0	0	0	0	1.000
Wegeners granulomatosis	0	0	0	0	1.000
Total†	300	481	4.6	3.8	

* Fisher exact test (one-tailed) for increasing risk in the MIP group compared to controls, adjusted for multiple comparisons.
† Total reported conditions and percent of total answered questions.

Table 4
Protein and immunoglobulin measures

Test	MIP			Control			*P*-value[a]	
	n	Out of reference range, low (%)	Out of reference range, high (%)	*n*	Out of reference range, low (%)	Out of reference range, high (%)	Out of range, low	Out of range, high
Albumin (%)	153	10.5	20.3	265	10.6	12.5	0.997	0.238
Albumin (g/dl)	153	3.3	1.3	265	2.6	0.0	0.993	0.851
Total protein (g/dl)	153	0.7	0.7	265	0.8	1.5	1.000	1.000
Alpha-1 globulin (%)	153	1.3	2.0	265	0.8	1.5	0.994	1.000
Alpha-1 globulin (g/dl)	153	0.0	1.3	265	0.0	1.1	1.000	1.000
Alpha-2 globulin (%)	153	6.5	1.3	265	0.0	1.9	0.001	1.000
Alpha-2 globulin (g/dl)	153	0.7	0.0	265	0.0	1.5	0.970	1.000
Beta globulin (%)	153	0.0	2.0	265	0.0	1.9	1.000	1.000
Beta globulin (g/dl)	153	0.0	2.6	265	0.0	2.6	1.000	1.000
Gamma globulin (%)	153	2.0	1.3	265	1.1	2.3	0.981	1.000
Gamma globulin (g/dl)	153	1.3	3.9	265	0.4	6.4	0.927	1.000
C-4 (mg/dl)	153	0.0	31.4	265	0.0	22.3	1.000	NT[b]
Copper (ug/dl)	153	3.9	1.3	265	1.9	3.4	0.725	1.000
C-reactive protein (mg/dl)	153	0.0	2.2	265	0.0	10.9	NT	1.000
IgM (mg/dl)	153	4.6	9.8	265	5.3	5.3	1.000	0.553
IgG (mg/dl)	153	1.3	9.2	265	1.9	12.1	1.000	1.000
IgA (mg/dl)	153	3.3	9.2	265	2.3	10.9	0.975	1.000
Total IgG (mg/dl)	150	0.7	3.3	261	0.0	10.3	0.959	1.000
IgG1 (mg/dl)	152	0.7	2.0	265	0.8	4.2	1.000	1.000
IgG2 (mg/dl)	152	0.0	13.2	264	0.0	14.8	1.000	1.000
IgG3 (mg/dl)	152	0.7	1.3	264	1.1	7.2	1.000	1.000
IgG4 (mg/dl)	152	4.6	0.0	261	0.0	0.0	0.005	1.000

[a] One-tailed upper tested for MIP > control group, adjusted for multiple comparisons.
[b] Not tested.

Table 5
Rheumatological assays

Test	MIP		Control		*P*-value[a]
	n	%	*n*	%	
Anti-thyroglobulin antibody (1:20 or greater)	3	2.00	6	2.30	1.000
Anti-thyroid microsomal antibody (1:100 or greater)	7	4.60	16	6.00	1.000
Hep-2 ANA titer (1:40 or greater)	80	52.30	147	55.50	1.000
ANA fluorescence pattern					
None detected	73	47.70	118	44.50	
Centromere	0	0.00	2	0.80	0.555[b]
Nucleolar	1	0.60	2	0.80	
Speckled	79	51.60	139	52.40	
Speckled nucleolar	0	0.00	4	1.50	
Mouse kidney/stomach ANA titer (1:40 or greater)	19	12.40	26	9.80	0.975
Fluorescence pattern					
None detected	134	87.60	239	90.20	0.616[b]
Nucleolar	1	0.60	1	0.40	
Speckled	18	11.80	25	9.40	
Quantitative RF (Iu/ml)	17	11.10	27	10.20	1.000

[a] One-tailed upper tested for MIP > control group, adjusted for multiple comparisons.
[b] Fisher exact test for trend (unadjusted for multiple comparisons).

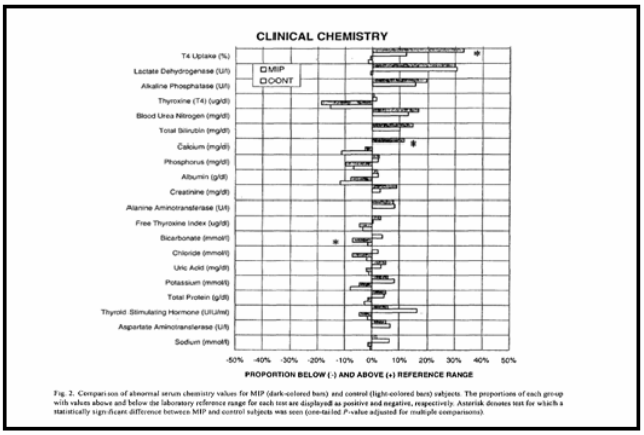


Fig. 2. Comparison of abnormal serum chemistry values for MIP (dark-colored bars) and control (light-colored bars) subjects. The proportions of each group with values above and below the laboratory reference range for each test are displayed as positive and negative, respectively. Asterisk denotes test for which a statistically significant difference between MIP and control subjects was seen (one-tailed *P*-value adjusted for multiple comparisons).

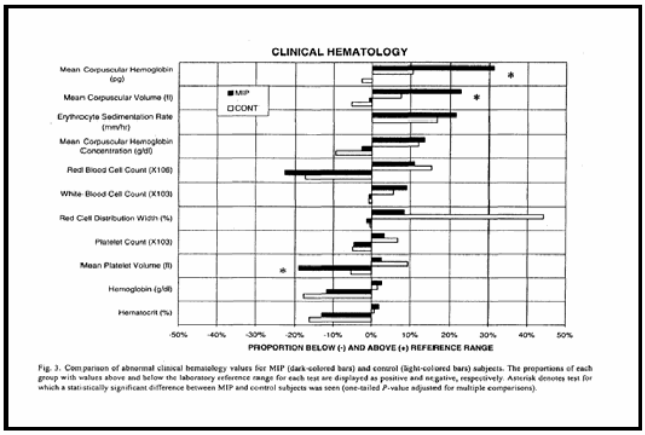


Fig. 3. Comparison of abnormal clinical hematology values for MIP (dark-colored bars) and control (light-colored bars) subjects. The proportions of each group with values above and below the laboratory reference range for each test are displayed as positive and negative, respectively. Asterisk denotes test for which a statistically significant difference between MIP and control subjects was seen (one-tailed *P*-value adjusted for multiple comparisons).

Table 6
Monoclonal paraproteins

Volunteer	Finding	Sub-class	Chain
Study-8V97	Monoclonal spike	IgM	lambda
Study-6B89	Monoclonal spike	IgG	lambda
Study-9H18	Monoclonal spike	Unk[a]	Unk[a]
Study-3N01	Monoclonal spike	IgM	kappa
Study-8P02	Monoclonal spike	IgM	kappa
Study-4S19	Paraprotein	Unk	kappa
Study-7E15	Monoclonal spike	IgG	kappa
Study-1M64	Monoclonal spike	IgG	kappa
Study-5W67	Monoclonal spike	IgA	lambda
Study-8R54	Paraprotein	IgG	kappa
Study-1U44	Paraprotein	IgA	kappa
Study-5S26	Monoclonal spike	IgG	lambda
Study-5D45	Paraprotein	IgA	kappa
Study-2Q41	Paraprotein	IgG	lambda
Study-3C28	Monoclonal spike	IgA	lambda
Study-7Y83	Paraprotein	IgG	kappa
Study-7K87	Paraprotein	Unk[a]	kappa
Study-8L76	Monoclonal spike	IgM	kappa
Study-0A47	Monoclonal spike	IgG	kappa
Control-3V34	Paraprotein	IgM	kappa
Control-0N06	Paraprotein	IgA	lambda
Control-1G06	Monoclonal spike	IgG	kappa
Control-3J71	Monoclonal spike	IgM	kappa
Control-4X66	Paraprotein	Unk[a]	lambda
Control-0Z41	Monoclonal spike	IgG	lambda
Control-0W41	Paraprotein	IgM	lambda
Control-2T41	Monoclonal spike	IgM	kappa
Control-4C44	Monoclonal spike	IgG	lambda
Control-6L20	Monoclonal spike	IgG	lambda
Control-5W15	Monoclonal spike	IgG	lambda
Control-9C83	Monoclonal spike	IgM	lambda

[a] Unk, unknown/indeterminate.

MONOCLONAL GAMMOPATHY

	Positive	Negative	
	n (%)	n (%)	TOTAL
GROUP			
Study	16 (10.3)	139 (89.7)	155
Control	12 (4.5)	253 (95.5)	265
TOTAL	28	392	420

P-value by Fisher's exact test (1-tailed) = 0.0196

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Research Paper

Detection of antibodies to squalene III. Naturally occurring antibodies to squalene in humans and mice☆

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Table 2
Age, sex, AVA doses, and antibodies to SQE among the USA/MRBD alumni

ID no.	Age	Sex	AVA # doses	SQE IgG	SQE IgM
1	59	M	5	0	0
2	66	M	33	0	0
3	63	F	6	0	0
4	62	F	31	Pos	Pos
5	68	M	Unknown	0	Pos
6	71	M	6	0	0
7	69	F	6	0	Pos
8	59	M	17	0	Pos
9	72	M	25	0	0
10	70	M	Unknown	Pos	0
11	66	M	47	Pos	Pos
12	65	M	16	0	0
13	79	M	27	0	Pos
14	70	M	0	0	0
15	Unknown	M	43	0	0
16	60	M	24	0	0
17	74	M	39	0	Pos
18	59	M	14	0	0
19	65	M	40	0	Pos
20	75	M	28	0	0
21	68	M	20	0	Pos
22	69	M	15	0	0
23	64	F	8	0	Pos
24	72	M	32	0	Pos
25	70	M	31	0	Pos
26	61	F	8	0	0
27	71	M	30	0	0
28	58	M	26	0	0
29	Unknown	M	35	0	0
30	70	M	36	0	0
31	76	M	36	0	0
32	78	M	33	0	0
33	67	M	27	0	0
34	71	M	29	0	0
35	59	M	Unknown	0	0
36	64	M	14	0	0
37	71	M	45	0	Pos
38	Unknown	M	9	0	Pos
39	70	M	24	0	0
40	72	M	20	0	Pos

Table 3
Appearance of antibodies to SQE in mouse serum as function of age

Age (months)	BALB/c % positive (# positive/total) [95% confidence interval]		B10.Br % positive (# positive/total) [95% confidence interval]		C57BL/6 % positive (# positive/total) [95% confidence interval]	
	IgG	IgM	IgG	IgM	IgG	IgM
2[a]	0 (0/49) [0.0–5.9]	0 (0/60) [0.0–4.9]	0 (0/25) [0.0–11.3]	0 (0/25) [0.0–11.3]	0 (0/25) [0.0–11.3]	0 (0/25) [0.0–11.3]
10	11 (2/18) [1.4–34.7]	32 (6/19) [12.6–56.6]	0 (0/18) [0–15.3]	10.5 (2/19) [1.3–33.1]	12 (2/17) [1.5–36.4]	30 (6/20) [11.9–54.3]
16	10 (2/19) [1.3–33.1]	37 (7/19) [16.3–61.6]	10 (2/20) [1.2–31.7]	0 (0/20) [0.0–13.9]	74 (14/19) [48.8–90.9]	90 (18/20) [68.3–98.8]
17	0 (0/19) [0.0–14.6]	63 (12/19) [38.4–83.7]	5 (1/20) [0.1–24.9]	5 (1/20) [0.1–24.9]	65 (13/20) [40.8–84.6]	80 (16/20) [56.3–94.3]
18	N.D.	39 (7/18) [17.3–64.3]	85 (17/20) [62.1–96.8]	5 (1/20) [0.1–24.9]	100 (17/17) [83.8–100]	89 (16/18) [65.3–98.6]
19	6 (1/17) [0.1–28.7]	65 (11/17) [38.3–85.8]	60 (12/20) [36.1–80.9]	25 (5/20) [8.7–49.1]	100 (18/18) [84.7–100]	72 (13/18) [46.5–90.3]
21	10 (1/10) [0.3–44.5]	62 (8/13) [31.6–86.1]	45 (9/20) [23.1–68.5]	55 (11/20) [31.5–76.9]	94 (16/17) [71.3–99.9]	62 (10/16) [35.4–84.8]
24	17 (1/6) [0.4–64.1]	86 (6/7) [42.1–99.6]	0 (0/17) [0.0–16.2]	50 (9/18) [26.0–74.0]	46 (6/13) [19.2–74.9]	58 (7/12) [27.7–84.8]
At any time point	35 (7/20)	85 (17/20)	95 (19/25)	65 (13/20)	100 (20/20)	100 (20/20)

Mice were bled at the time intervals indicated and the sera were assayed for antibodies to SQE. Serum was scored as positive for IgG or IgM antibodies to SQE if the absorbance was >3 times the baseline at both the 1:50 and 1:100 dilutions. Baseline absorbances ranged from 0.1 to 0.18 in different assays.
[a] Sera from 2 month old mice were from different animals than the retired breeders used for the remaining time points.

Further study to determine if there are there long-term adverse effects of AVA?

Hypothesis: The frequency of death, chronic disease, laboratory abnormalities, and/or degradation of quality of life in individuals who received Anthrax Vaccine, Adsorbed (AVA, BioPort Corporation) plus other vaccines administered in the Special Immunizations Program (SIP) and/or Special Procedures Program (SPP) at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Maryland, is not greater than that observed in individuals in SIP/SPP who received other vaccines but not AVA.

Further study to determine if there are there long-term adverse effects of AVA?

Objective: **To determine whether AVA accounts for differences in the frequency of death, chronic diseases, laboratory abnormalities, and degradation in quality of life in individuals in a population that is receiving or has received multiple vaccines over time.**

Further study to determine if there are there long-term adverse effects of AVA?

Objective: **This retrospective, single-site study will enroll current and former SIP/SPP volunteers (those who are currently enrolled in the SIP and those who were previously enrolled). Table 6 profiles the characteristics of the SIP/SPP participants from which the study subjects will be drawn. The maximum number of eligible SIP/SPP volunteers is 3421. Of those, 2102 have been exposed to AVA and other vaccines, whereas 1319 have been exposed to other vaccines but not to AVA.**

Further study to determine if there are there long-term adverse effects of AVA?

- **Primary outcome measures are:**
  - Death (from all causes)
  - Chronic diseases (latency)
  - Degradation of quality of life (as determined by SF-36 questionnaire)
  - Abnormal laboratory results of blood tests/assays and salivary cortisol test

Further study to determine if there are there long-term adverse effects of AVA?

- **The measure of AVA exposure is whether or not the subject received the AVA vaccine. The measures of concomitant SIP/SPP exposure are the following:**
  - Years in SIP/SPP
  - Number of non-AVA doses received
  - Volume of non-AVA doses received
  - Number of different non-AVA antigen exposures

Further study to determine if there are there long-term adverse effects of AVA?

- Potential secondary analyses to be conducted, as data permit, include:
  - Additional measures of AVA exposure.
  - Number of doses (0 is maximum)
  - Change in number of doses (1-6 or 7+ doses)
  - Volume of AVA (0 is maximum)
  - Length of time between 1st and last dose
- Non-AVA vaccine variables:
  - Live-agent vaccine/exposure
  - Vaccine administered
  - Monovalent vs. polyvalent
  - Volume
  - Adjuvant exposure
- Demographic variables:
  - Gender
  - Age in terms of receipt of 1st and last dose of each vaccine and as interview
  - Race
  - Ethnicity
- Other exposure variables:
  - Length of time since leaving SIP/SPP
  - Pre-existing conditions
  - Tobacco use
  - Alcohol use
  - Occupational exposure
  - Family medical history
- Other outcome variables:
  - Death (specific causes)
  - Reproductive/fertility disorders
  - Mean/median values for the results of blood tests and assays and salivary cortisol test

Further study to determine if there are there long-term adverse effects of AVA?

- Study Progress
  - LONG-TERM SAFETY STUDY (Ongoing)
    - Enrolled 1124
    - SF36 1124
    - CATI 958
    - Blood draws 616
  - Enrollment closes 27 April 2005

Project Whitecoat Program

An Assessment of Health Status among Medical Research Volunteers Who Served in the Project Whitecoat Program at Fort Detrick, Maryland. Military Medicine. 170, 3:183, 2005.

COL Phillip R. Pittman, Sarah L. Norris, Kevin M. coonan, Kelly T. McKee.

Project Whitecoat Program

- Between 1954 and 1973, more than 2000 men entering military service as conscientious objectors participated in Project Whitecoat as medical research volunteers for the Army's biological warfare defense program.
- Project Whitecoat was the title given to the Army research program "to use human volunteers in medical studies to evaluate the effect of certain biological pathogens upon humans in an effort to determine the vulnerability to attack with biological agents.
- The objectives of the studies involved were to develop medical defenses against biological warfare and included techniques for rapid diagnosis, improved therapeutic and prophylactic agents, and development of vaccines against biological weapons and endemic disease threats.

Project Whitecoat Program

- The program evolved after a series of meetings in 1954-1955 between representatives of the Army Surgeon General and the Seventh Day Adventist Church.
- With the background of the Church's philosophy and practice of medical service and encouragement of noncombatancy and its longstanding cooperation with the military in health and medical practice, Project Whitecoat became an accepted and respected vehicle by which conscientious objectors could serve the nation.
- From its inception in 1954 to its termination in 1973, approximately 2,300 individuals participated in this program, more than 90% of whom were Seventh Day Adventists.

Project Whitecoat Program

- **The group participated in more than 135 clinical research studies involving exposure to live agents, receipt of investigational vaccines, and studies of metabolic and psychological effects of environmental-and infection-induced stress.**
- **This study was designed to assess the long-term effects on health of these men resulting from their involvement in this vital program.**

METHODS

- Volunteers recruited from Whitecoat Alumni Association in 1998
- Questionnaire survey; returned by mail
  - 522 respondents
- Records of study participation abstracted from USAMRIID archives

EXPOSURES

- 358 volunteers "Exposed" (received study product) to:
  - Investigational vaccines: 197
  - Disease-causing agents: 211
  - Antibiotics/other therapeutic agents: 46
- 164 "Controls" (did not receive study product)

EXPOSURES (CONT)

- Participated in 1 study: 303
- Participated in 2 studies: 75
- Participated in 3 studies: 17
- Participated in 4 studies: 1

VACCINE EXPOSURES

- VEE: 73
- Tularemia: 45
- Yellow Fever: 31
- EEE: 29
- WEE: 28
- Plague: 13
- Q-fever: 11
- Rift Valley fever: 8
- Anthrax: 7
- Chikungunya: 6
- Adenovirus: 4

DISEASE AGENT EXPOSURES

- *Coxiella burnetii* (Q-fever): 58
- Sandfly fever: 30
- Staphylococcal enterotoxin B (SEB): 20
- *Francisella tularensis* (tularemia): 11
- Venezuelan equine encephalitis (VEE): 7
- *Pseudomonas* endotoxin: 2

NON-AGENT EXPOSURES

- Tetracyclines: 25
- Amino Acids: 15
- Chloramphenicol: 4
- Tyrosine: 4

DEMOGRAPHICS

		STUDY (N=358)	CONTROL (N=164)	p-value *
Race				.
	White	90.8%	91.5%	0.832
	Black	3.6%	4.3%	
	Other	5.3%	4.3%	
Male		100.0%	100.0%	NT
Mean Age (range) (yrs)		58.4 (47-76)	58.5 (46-79)	0.822
Mean Time Spent at Ft Detrick (range) (yrs)		1.5 (<1-5)	1.5 (<1-3)	0.636
Served in Military		100.0%	100.0%	NT
College Degree or Higher		55.9%	63.4%	0.126
Current Employment Status				
	Retired	11.7%	15.2%	0.145
	Employed Full-time	51.1%	40.9%	
	Employed Part-time	31.0%	38.4%	
	Not Working b/c disability	5.6%	5.5%	
*Tests are 2-tailed				
NT= Not tested				

HEALTH AND BEHAVIORAL CHARACTERISTICS

		STUDY (N=358)	CONTROL (N=164)	p-value *
Current Health Status				
	Excellent	39.4%	47.6%	0.375
	Good	46.6%	41.5%	
	Fair	10.6%	9.1%	
	Poor	2.8%	1.8%	
Current Exercise Level				
	None	25.4%	20.1%	0.579
	more than 5X/wk	10.9%	10.4%	
	up to 5 X/wk	60.1%	65.9%	
	Disabled	3.1%	3.0%	
Tobacco History				
	Ever Smoked Cigarettes	14.8%	15.2%	0.896
	#Packs/Day (mean)	1.0	1.5	0.285
	# Yrs Smoked (mean)	10.0	11.8	0.500
	Quit (among those ever smoked)	96.2%	80.0%	0.031
	Yrs Since Quitting (mean)	23.0	16.5	0.084
	Ever Smoked Pipe	7.5%	8.5%	0.726
	Ever Smoked Cigars	7.8%	7.3%	1.000
	Ever Dip Snuff/Use Chewing Tobacco	1.4%	0.6%	0.670
Alcohol Use				
	Ever Drink	15.1%	19.5%	0.206
*Tests are 2-tailed				

REPRODUCTIVE OUTCOMES

TABLE 3
REPRODUCTIVE OUTCOMES

		STUDY (N=352)	CONTROL (N=161)	p-value ^
Number of Children [mean][range]		2.0 (0-7)	2.0 (0-6)	0.502
Had Any Children		313 (21.4%)	111 (26.0%)	0.611
Had Children with Birth Defects or Mental Retardation		25 (7.0%)	15 (9.1%)	0.381
Number of Children [N=1,161]				
	"Normal/Healthy"	698 (95.6%)	306 (95.0%)	0.510
	With Birth/Congenital Defects	21 (2.3%)	11 (3.4%)	
	With Mental Retardation	8 (1.1%)	2 (0.6%)	

^Tests are 2-tailed

MEDICAL CONDITIONS (FREQUENT)

TABLE 4
SELF-REPORTED DISEASES AND CONDITIONS

CONDITION	STUDY N	STUDY %	CONTROL N	CONTROL %	p-value*
Hypertension	78	21.8%	41	25.0%	1.000
Arthritis	55	15.4%	25	15.2%	1.000
Hay Fever	55	15.4%	26	15.9%	1.000
Pneumonia	43	12.0%	24	14.6%	1.000
Cancer	26	7.3%	17	10.4%	1.000
Asthma	25	7.0%	4	2.4%	0.165
Diabetes	25	7.0%	17	10.4%	1.000
Ulcers	23	6.4%	7	4.3%	0.938
FrequentColds	20	5.6%	8	4.9%	0.998
Eczyma	13	3.6%	5	3.0%	1.000

Whitecoat Project

- Asthma reported more frequently among tularemia vaccine recipients than controls (13.3% vs 2.4%, p=0.049)
- Asthma reported more frequently in group exposed to non-agents than controls (13.0% vs 2.4%, p=0.050)
- No definite association

DEATHS & DISABILITIES

- Small number and incomplete knowledge of total N makes statistical assessment infeasible at this time.
- No link found

CONCLUSIONS

- "Exposed" and "unexposed" groups similar in terms of demographics, education, current employment status, and behavioral risk factors
- No differences between "exposed" and "unexposed" with regard to self-reported general health status and self-reported exercise activity

CONCLUSIONS (CONT)

- No differences between "exposed" and "unexposed" volunteers with regard to reproductive outcomes
- No significant differences between "exposed" and "unexposed" subjects with regard to self-reported symptoms

CONCLUSIONS (CONT)

- No significant differences between "exposed" and "unexposed" subjects with regard to self-reported diseases or medical conditions
- No differences between individuals participating in one and those participating in two or more studies with regard to any outcome measured (general health, exercise level, children, symptoms, or medical conditions)

Does receipt of multiple vaccines increase risk for adverse health effects?

- Available evidence does not suggest there are any disease or disease complex that result from repeated injections with multiple antigens.
- We are investing whether the finding of monoclonal immune globulin represents an association or an epiphenomenum.

Are antibodies to squalene related to receipt of anthrax vaccine or related to any disease, symptom or symptom complex?

- We found no such association with anthrax vaccine or to any disease, symptom or symptom complex.
- Squalene antibodies prevalence was related to increasing age.

CONCLUSION

- Vaccines, including multiple vaccine antigen injections, appear to have a safe long-term health outcome.