Intensive Immunization of Man

Evaluation of Possible Adverse Consequences

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SINCE 1902, when Zenoni (1) first described the occurrence of amyloidosis in horses intensively immunized to diphtheria toxin, there have been numerous reports of animal experiments in which amyloidosis, as well as arteritis and other manifestations of hypersensitivity, have been induced by the injection of many different antigenic agents (2-21). That such adverse reactions to immunization have not been observed in man is doubtlessly related to the relatively trivial doses of antigen to which the human being is usually exposed. The increasing list of antigens recommended for the routine immunization of human beings plus the likelihood that the number of such agents will continue to grow indefinitely as new vaccines are developed has raised the question as to whether repeated immunization produces adverse effects in man. Besides the pathological changes observed in animals, it might be speculated that intensive immunization may interfere with the recipient's ability to respond to immunologic challenge.

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In 1958 we reported the results of clinical and laboratory investigations done in 1956 to 1957 upon a group of skilled laborers and laboratory workers who had undergone intensive immunization to a variety of bacterial, rickettsial, and viral agents for an extended period of time (22). The 99 men studied ranged in age from 28 to 65 years. Over a period of 8 to 13 years, they had received an average total of over 50 ml of various antigens as well as numerous bacterial and fungal skin tests. They had been observed closely in one clinic for evidence of illness. Investigations of these men revealed several interesting findings. Most important, there was no clinical illness that could be attributed to intensive immunization. Several laboratory aberrations were encountered. Approximately 25 % of the men had an unexplained peripheral lymphocytosis. Nearly 40 % had some abnormality of one or more tests of liver function not explained by history or physical examination. Twenty-three percent had a peculiar abnormality of serum protein electrophoretic pattern characterized by alterations in mobility of the alpha-2 and beta globulin fractions. It was noted that similar abnormalities had been described previously in patients intensively immunized with diphtheria toxin (23), in familial amyloidosis (24-26), and in the nephrotic syndrome of disseminated lupus erythematosus and the Kimmelstiel-Wilson syndrome (27-30).

The purpose of this report is to record the results of further studies of these same men during the ensuing 5-year period and to report additional data adding to the evaluation of possible chronic adverse consequences of intensive immunization.

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TABLE 1. Duration of Immunization

Range, yrs.	12 to 18
Mean, yrs.	15.3
Distribution (no. of subjects), yrs.	
12	2
13	4
14	14
15	20
16	26
17	7
18	3
Total	76

MATERIAL AND METHODS

Seventy-six of the original 99 men studied in 1956 and 1957 were available for study in 1962. Currently, their average age is 46.3 years with a range of 33 to 70 years. By virtue of their employment in laboratories handling a number of virulent infectious agents, they have been immunized against a variety of infectious diseases, including botulism, tularemia, brucellosis, anthrax, diphtheria, Rocky Mountain spotted fever, Q fever, plague, typhus, psittacosis, Rift Valley fever, poliomyelitis, tetanus, smallpox, yellow fever, influenza, and Eastern, Western, and Venezuelan equine encephalitis. The duration of the immunization period now ranges from 12 to 18 years with a mean of 15.3 years (Table 1). The antigens used for immunization varied considerably in type, so that a complete listing is impractical. They consisted of killed and attenuated live bacterial vaccines, purified bacterial fractions, toxoids, and chicken egg prepared virus vaccines (31-37). Because of the varying compositions of the vaccines, doses are recorded in volumes, and no attempt is made to quantitate the amount of protein administered. Total vaccine volumes administered now range from 42.25 to 101.5 ml with an average of 73.51 ml per subject. Table 2 compares these doses with those that had been given at the time of the original study in 1956 to 1957. The average subject has received 21 ml additional antigen in the current 5-year interval. All subjects were immunized with the antigens of botulism, tularemia, Rocky Mountain spotted fever, Q fever, typhus, plague, psittacosis, smallpox, and the viral encephalitides. Of the other antigens, yellow fever was given to 70 subjects, anthrax to 72, Rift Valley fever to 66, tetanus to 63, influenza to 54, poliomyelitis to 37, brucellosis to 34, diphtheria to 20, cholera to 1 and typhoid-paratyphoid to 1. The usual pattern of administration of such antigens was followed with an initial immunizing series and "booster" injections every 6 to 12 months.

The men received additional antigen in the form of periodic skin testing with the antigens of tuberculosis (PPD), diphtheria (Schick and Maloney), brucellosis (brucellergin), tularemia (Foshay), histoplasmosis, coccidioidomycosis, blastomycosis, and glanders. The average subject had received 20 such tests at the time of the 1956 to 1957 study and an additional 10 by 1962, with a range of from 9 to 44 tests per subject.

TABLE 2. Antigen Dose

	1956	1962
Range, ml	35.75 to 74.40	43.25 to 101.50
Mean, ml	52.85	73.51
Distribution		
ml	*no. of subjects*	
35 to 40	6	0
41 to 45	20	1
46 to 50	21	1
51 to 55	15	1
56 to 60	19	3
61 to 65	12	14
66 to 70	5	11
71 to 75	1	12
76 to 80	0	14
81 to 85	0	7
86 to 90	0	9
91 to 95	0	2
96 to 100	0	0
101 to 105	0	1
Total	99	76

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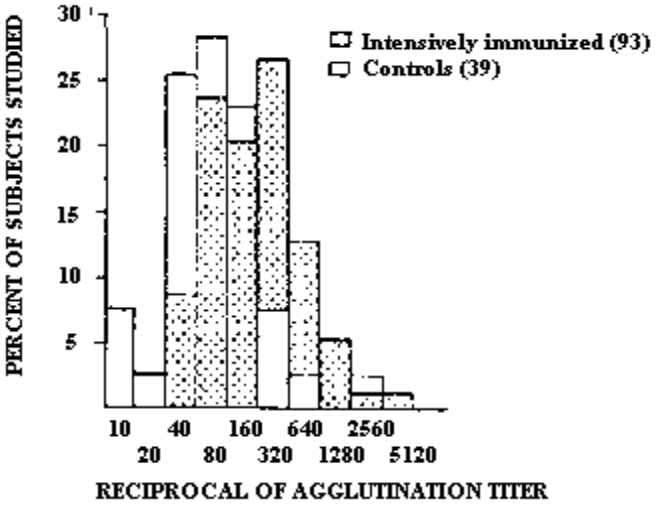
FIGURE 1. Tularemia agglutination titers after immunization with Foshay antigen. The highest titer achieved during immunization of intensively immunized subjects (*cross hatched bars*) are compared to those achieved in a group of controls immunized to tularemia alone (*open bars*).



Serologic titers to tularemia (agglutination) were performed frequently on all subjects. Titers to brucellosis (agglutination), Rocky Mountain spotted fever (CF), Q fever (CF), typhus (CF), and psittacosis (CF), were performed less frequently and are not analyzed for that reason.

All 76 of the men studied had a complete medical history and physical examination. Outpatient and immunization records of each patient were reviewed. Reliable long-term records were available on all subjects, as they were carefully followed up in one clinic throughout the immunization period, and their awareness of the infectious environment led them to seek medical attention for any symptoms suggesting possible occupational disease. The following laboratory examinations were performed on all the men: electrocardiogram, chest x-ray, urinalysis, hematocrit reading (Wintrobe), hemoglobin, total and differential leukocyte counts, blood urea nitrogen (BUN), 2-hour phenolsulphthalein excretion (PSP), urea clearance, fasting blood sugar, serum cephalin-cholesterol flocculation (CF), serum thymol turbidity (TT), serum glutamate-oxalacetate transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), bromsulphalein (BSP) retention 45 minutes after injection of 5 mg/kg of the dye, and serologic test for syphilis (V.D.R.L.). Serum hexosamines were determined by the method of Boas (38).

Serum electrophoresis was performed by the method previously described (22). Total serum protein was determined by the Biuret method in order to quantitate the albumin, alpha-1, alpha-2, beta, and gamma globulin fractions obtained by electrophoresis. The protein values thus obtained were used for statistical analysis.

The sera of 64 men were examined by the zinc turbidity test for gamma globulin level. Screening for rheumatoid factor was performed by the latex fixation test in 64 and the Rose test in 74. The sera of 64 patients were examined for ability to agglutinate human group O Rh_o red cells coated with an incomplete anti-D antibody. Four sera were analyzed in the analytical ultracentrifuge.

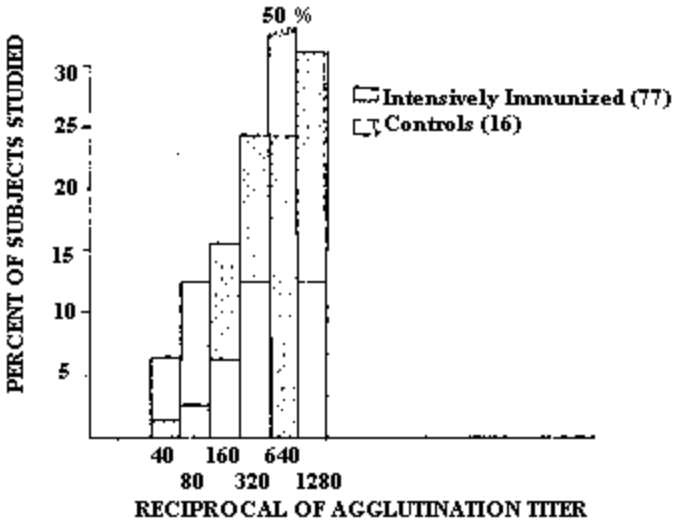


FIGURE 2. Tularemia agglutination titers after immunization with attenuated live tularemia vaccine. The highest titers achieved after a single injection of attenuated live tularemia vaccine in intensively immunized individuals (*cross hatched bars*) are compared to titers achieved in a group of controls immunized with live tularemia vaccine only (*open bars*).

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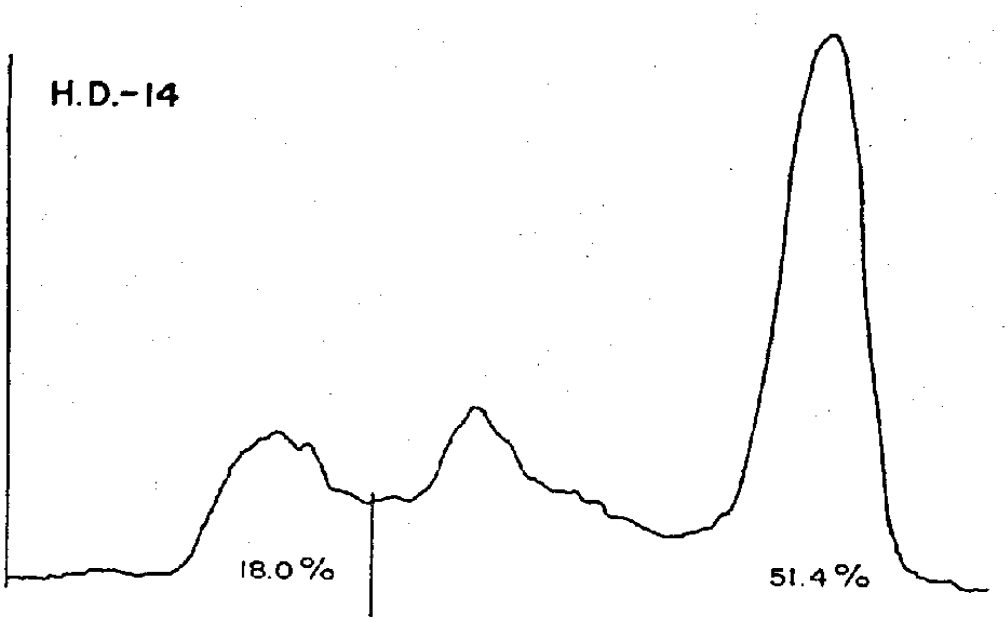

FIGURE 3. Abnormal serum electrophoretic pattern observed in 46% of intensively immunized men, showing poor separation of the alpha-2 and beta globulin fractions. Reprinted by permission of the editors from the *Bull. Hopkins Hosp*. 103: 195, 1958.

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As controls for the electrophoretic data and hexosamine determinations, 102 serial serum specimens were drawn from healthy blood donors at the Johns Hopkins Hospital Blood Bank. The control sera were obtained from male donors in the same age range as the test group. It will be apparent that this series of controls is not an ideal selection, but we were unable to obtain a group of controls of similar environmental experience who had not been immunized, either for these studies or as controls for the clinical laboratory data.

Gingival biopsies were performed by an experienced dental surgeon and were stained with hematoxylin and eosin. Material obtained by percutaneous renal punch biopsy was stained with hematoxylin and eosin and with thioflavin-T (39) for detection of amyloid. Tissue specimens of postmortem examinations performed elsewhere on three men dying of intercurrent and unrelated illnesses were received imbedded in paraffin. Sections of liver, kidney and spleen were stained with hematoxylin and eosin and thioflavin-T before microscopic examination From the beginning of the program until mid-1960, the Foshay antigen was used exclusively. Since 1960, an attenuated live vaccine has been used. Figure 1 illustrates the highest tularemia titer obtained on each of 93 of the original 99 subjects during the period when Foshay antigen was administered. These titers were compared to those of 39 controls who had been immunized to few, if any, antigens other than tularemia. Figure 2 makes the same comparison between 77 subjects and 16 controls, recording the highest titer obtained after immunization with attenuated live tularemia organisms. All men with a history of suspected or proven tularemia were omitted from both comparisons.

The intensively immunized group of men achieved titer responses after immunization which, if anything, are slightly higher than the controls. Although the size of the control groups is not adequate to reach any statistical conclusion, particularly in the attenuated vaccine comparison, it is clear that intensive immunization did not interfere with the ability of these men to respond to immunologic challenge.

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CLINICAL ILLNESS

Illnesses recorded in reviewing medical histories fell into two groups: unrelated illnesses and those that might be considered as occupational (Table 3). Of the occupation-connected infections, there were possibly 17. Three subjects had precipitous tularemia titer rises not associated with symptoms or with recent tularemia immunization. These rises were interpreted as representing subclinical tularemia. Two had proven symptomatic tularemia. One was suspected of having brucellosis. Clinical Q fever occurred once, as did an unidentified meningoencephalitis There were nine instances of fever of undetermined origin which may or may not have been related to the occupational exposure.

Of the nonoccupational illnesses, none occurred with greater frequency than would be

TABLE 3. History of Clinical Illness Among Subjects

A. Occupational	*no.*
Asymptomatic tularemia	3
Symptomatic tularemia	2
Suspected brucellosis	1
Q fever	1
Undiagnosed fevers	9
Meningoencephalitis	1
Total	17

B. Nonoccupational

1. Infectious	*no.*
Sinusitis	6
Pneumonia	3
Primary syphilis	1
Malaria	1
Paratyphoid	1
Lung abscess	1
Chronic bronchitis	1
Osteomyelitis	1

2. Cardiovascular	*no.*
Hypertension	9
Peripheral arterial disease	2
Coronary insufficiency	2
Rheumatic heart disease	2
Congestive failure (arteriosclerotic)	1
Postural hypotension	1
Coronary occlusion	1

3. Urinary Tract	
Stones	5
Infections	4
Glomerulonephritis	1
Prostatism	1
Renal cyst	1
Orthostatic albuminuria	1

4. Musculoskeletal	
Arthritis: Unclassified	3
Gout	3
Rheumatoid	1
Myositis	1
Bursitis	1

5. Gastrointestinal	
Peptic ulcer	7
Cholelithiasis	2
Colitis	1

6. Allergic	
Drug reactions	5
Rhinitis	4
Asthma	1
Urticaria	1

7. Hepatic	
Hepatitis	4
Alcoholism	1

8. Endocrine	
Colloid goiter	1
Diabetes	1

9. Miscellaneous	
Meniere's disease	1

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expected in a population of this age and sex. The significance of these illnesses lies in their possible relation to the abnormalities obtained on laboratory investigation. For this reason, in the following analysis of the various laboratory tests, subjects with a history of any antecedent illness that might influence a given test were excluded from the analysis.

No clinical illness was observed that might be attributed to intensive immunization.

TABLE 4. Lymphocyte Counts

Total WBC	Subjects	
	1956	1962
%	*no.*	
36-40*	25	22
41-44	17	14
45-50	4	6
51-55	2	2
56-60	3	2
61-65	1	0
Total abnormal	27	24

* Normal values.

PHYSICAL EXAMINATION

Abnormalities discovered on physical examination may also be classified into two groups; those that may have been and those that probably were not related to intensive immunization. In the latter category, hypertension was the most frequent finding. Nineteen of the 76 men examined had blood pressure recordings in excess of 140/90 mm Hg. Abnormal blood pressure recordings varied from mild to moderately severe. Three subjects had elevations in the range of 140/100 mm Hg: six were in the 150 to 155/90 to 105 mm Hg range. Two were recorded at 160/100, two at 180/80 to 90, and one each at 190/100 and 200/100 mm Hg.

Hepatomegaly was encountered in a total of 14 subjects. Two of these, with enlargements ranging from 1 to 3 fingerbreadths below the right costal margin, were not explained by history of alcoholism, hepatitis, or other illness in the past. Hepatomegaly occurred in one subject with each of the following diagnoses: congestive heart failure, history of tularemia, excessive alcohol intake, and in an obese diabetic.

Unexplained splenomegaly, perforated nasal septum, unexplained cardiomegaly, and macular degeneration occurred in one subject each. Two men were thought to have macroglossia, hypertrophy of the gums, and frequent ventricular premature contractions as isolated findings. Two men having historical evidence of acute rheumatic fever in the past demonstrated murmurs compatible with rheumatic heart disease. Two men were considered arteriosclerotic in excess of the expected degree for their ages.

CLINICAL LABORATORY EVALUATION

Electrocardiogram: Electrocardiographic abnormalities were found in 17 men, with the following interpretations: low voltage (2 patients) frequent ventricular premature contractions (3 patients), right bundle branch block (1 patient), incomplete right bundle branch block (1 patient), prolonged intraventricular conduction time (1 patient), left ventricular hypertrophy (1 patient), nonspecific ST-T wave abnormalities (7 patients), and old posterior myocardial infarction (1 patient). Of these, three subjects with ST-T wave abnormalities, one with right bundle branch block, one with incomplete right bundle branch block, and one with premature contractions were significantly hypertensive. The infarction had been diagnosed clinically 1 year before this evaluation. The other abnormalities were not explained by other historical or physical abnormalities.

Chest x-ray: In tabulating chest x-ray findings, calcified Ghon complexes were disregarded, since they occurred so frequently. Unexplained hilar calcifications were seen in one subject. Emphysematous changes

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occurred in two. Multiple parenchymal calcifications were seen in three. One man, recovering from pneumonia at the time of the examination, had a resolving infiltration. One subject who had had a huge pulmonary abscess due to bacteroides in 1957 showed marked pleural reaction. On one film, there was un-explained blunting of the costophrenic angles.

TABLE 5. Renal Function Tests

Proteinuria		
Degree	Subjects	
	1956	1962
	no.	*no.*
Trace	11	6
1	3	2
2	1	0
3	0	0
4	0	0
Total	15	8

Phenolsulphthalein Excretion		
2-hr excretion	Subjects	
	1956	1962
%	*no.*	*no.*
11 to 15	1	0
16 to 20	0	0
21 to 25	0	0
26 to 30	0	0
31 to 35	0	4
36 to 40	1	5
41 to 45	0	4
46 to 50	7	4
51 to 55	7	3
56 to 60	5	8
Total	21	28

Urea Clearance (1962 only)		Blood Urea Nitrogen	
Normal	Subjects	Test Results	Subjects
%	*no.*	*mg/ 100 ml*	*no.*
		Normal*	69
40 to 49	2	20	2
50 to 59	5	21	0
60 to 69	3	22	1
70 to 79	3	23	0
80 to 89	15	24	2
90 to 99	16		
Over 100	30		
Total	74	Total	74

*Normal BUN, 7 to 20 mg/100 ml.

Hematologic: Hematocrit readings were normal in all of the men. Four had total leukocyte counts in the 4,000 to 4,900/mm^3 range, and 11 had an unexplained leukocytosis of 10,100 to 15,900/mm^3. Monocytosis that occurred in three subjects in the 1956 study was not seen in 1962.

Lymphocytosis continued to occur in high incidence (Table 4). Using somewhat more stringent criteria than those applied in the 1956 study, 27% had peripheral lymphocytosis (in excess of 40%) in 1956 and 31.6% currently. Of those studied on both occasions, 37 (37.4%) have had lymphocytosis at some time but only 6 (8.1%) on both occasions. Of those demonstrating lymphocytosis, the following clinical illnesses had occurred in the remote past: arthritis (7 patients), primary syphilis (1 patient), hay fever (7 patients), Q fever (1 patient), hepatitis (4 patients), serologic asymptomatic tularemia (4 patients), chronic sinusitis (4 patients), urticaria (1 patient), acute rheumatic fever (2 patients), fever of undetermined etiology (3 patients), pulmonary granuloma (1 patient), X-ray evidence of healed histoplasmosis (1 patient), and diabetes (1 patient). Except for the allergies and diabetes, all of these illnesses were so remote in time as to make it unlikely that they would have an effect upon current lymphocyte counts. Peripheral eosinophilia (in excess of 3%) was found in 17 men in 1956 and in 23 men in 1962 (30%). Forty-two have demonstrated eosinophilia on one or both occasions. Of these, 5 had a history of hay fever, 1 of asthma, 11 of chronic sinusitis, and 2 of drug allergy. Twenty-five gave no history of allergic disorder.

Renal Function: Significant urinary findings were confined on both occasions to the detection of proteinuria. Excluding men with a history of hypertension, vascular disease, urinary infection, glomerulonephritis, diabetes, and gout, 15 had trace to 2+ proteinuria in 1956, and 8 had trace or 1+ in

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1962. Six have shown proteinuria on both occasions (Table 5).

Two-hour excretion of PSP was decreased (below 60%) in 28 men studied in 1962 (Table 5). Decreased PSP excretion in 21 men in 1956 was discounted as having little significance. The persistence of these abnormalities, however, both in individual subjects and in the group as a whole suggests that these findings may carry more significance than we had first allowed them. Of those men with decreased PSP excretion on either study, there was an incidence of 37% in those with no history of illness involving the urinary system. Of the 25 men with decreased PSP excretion in the 1962 study, 13 were decreased in 1956, 11 were normal in 1956, and 1 was not studied previously.

Urea clearance determinations were not performed in 1956. In 1962, 8 of the 76 men showed depression of this function, with values ranging from 43.2 to 62.9% of normal (Table 5). Of these, one had gout, one was hypertensive, and one was diabetic. The remaining five were clinically well.

There were three minimally elevated blood urea nitrogens (BUN) in 1962 (22, 24 and 24 mg/100 ml). Of these, one was hypertensive. In 1956, one man with a history of nephrolithiasis had an elevated BUN (21.2 mg/100 ml). In 1962, his BUN was normal (Table 5).

Of the 76 men studied in 1962, 39 (51%) have had some significant and unexplained indication of abnormal renal function on one or both occasions; 8 only in 1956, 17 only in 1962, and 14 on both occasions. Half had abnormalities of more than one test of renal function.

Liver Function: Alkaline phosphatase values were slightly elevated in three men.

Omitting subjects with a history of possible liver disease, 24 had cephalin flocculation elevations in 1962 (Table 6). Of these, 9 were among the 13 found abnormal in.1956, and most were abnormal in 1962 than 1956.

TABLE 6. Hepatic Function Tests

	Subjects	
	1956	1962
Cephalin-cholesterol flocculation, degree(48 hr.)		
	no.	*no.*
1+*	17	22
2+	13	12
3+	0	10
4+	0	2
Total	30	46
Thymol Turbidity, units		
5.1 to 5.9	4	1
6.0 to 6.9	0	2
7.0 to 7.9	1	1
8.0 to 8.9	0	5
9.0 to 9.9	1	1
10.0 to 10.9	1	2
11.0 to 11.9	0	1
12.0 to 12.9	0	0
13.0 to 13.9	0	1
Total	7	14
Serum Bilirubin, mg/100 ml		
1.01 to 1.09	0	1
1.10 to 1.19	4	1
1.20 to 1.29	6	0
1.30 to 1.39	0	1
1.40 to 1.49	1	0
1.50 to 1.59	0	2
2.05	0	1
Total	11	6
Bromsulphalein Retention (45 min), %		
5 to 6	2	12
7 to 8	3	4
9 to 10	2	2
11 to 12	0	0
13 to 14	0	1
15 to 16	0	2
17 to 18	0	1
Total	7	22

* Not abnormal.

Fourteen thymol turbidity determinations were abnormal in 1962 as compared to seven in 1956 (Table 6). Three were abnormal on both occasions. Elevations range from minimal (5.6 units) to moderate (13.6 units). There were six bilirubin elevations in 1962 as compared to 11 in 1956 (Table 6). Two were abnormal on both occasions.

Abnormal BSP retention was recorded 22 times in 1962 and 7 times in 1956 (Table 6). Of the six abnormal in 1956, four were worse in 1962 and two were lost to follow-up. Overall, 35% demonstrated some abnormality of

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TABLE 7. Serum Hexosamine Determinations

	1958		1962	
	Test	Control	Test	Control
Range	50 to 179.75	82.50 to 139.00	83.50 to 186.50	59.25 to 127.00
Mean	114.47	101.68	119.75	93.86
sp	16.70	12.37	16.67	13.14
t	5.65		11.16	
Distribution	Total subjects			
51 to 60	0	0	0	1
61 to 70	0	0	0	3
71 to 80	0	0	0	13
81 to 90	1	10	4	27
91 to 100	14	40	8	28
101 to 110	27	33	16	21
111 to 120	26	10	24	7
121 to 130	22	4	28	2
131 to 140	8	2	7	0
141 to 150	1	0	11	0
151 to 160	0	0	1	0
161 to 170	0	0	0	0
171 to 180	1	0	0	0
181 to 190	0	0	1	0
Total	88	48	75	102

liver function in 1956 as compared to 57% in 1962. Over half of these had abnormalities of more than one test of liver function.

Serum Electrophoresis: Serum electrophoretic analysis was performed in 1956 on the original 99 men, in 1958 on 84 men, and on the 76 remaining in 1962. Statistical analysis on neither occasion has shown quantitative abnormalities of the various protein fractions when the serum of the intensively immunized men was compared to the controls. Serial records revealed no consistent change with increasing years of immunization. The qualitative abnormality described in 23% of the men studied in 1956 was found again in 34% of the 84 studied in 1958 (Figure 2). Nineteen men with this change in 1958 had not shown it in 1956. Of the original 23 men with the abnormality in 1956, 4 were lost to follow-up, 7 were unchanged, 8 were less abnormal, and 4 had returned to normal. By 1958, 42 of the original 99 subjects had this abnormality at some time and 15 on both occasions. In 1962, 19 of the 76 men showed the abnormality in electrophoretic pattern. A total of 46 have now demonstrated it on one or more of the three occasions. Twelve men have shown the abnormality on each occasion studied, 9 on two of three occasions, and 25 on only one occasion.

Serum Hexosamines: Serum hexosamine determinations were made on men studied in 1958 and 1962. Comparisons between the intensively immunized and control groups are shown in Table 7. On each occasion, the mean hexosamine value for the test group was significantly elevated when compared to controls, as demonstrated by the "t" value.

Antigammaglobulin Factor: Sixty-four sera from subjects demonstrating the most striking abnormalities on other laboratory tests were examined with the zinc turbidity test for gamma globulin level, with the latex fixation test, and for their ability to

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agglutinate human group O, Rh_o red cells coated with an incomplete anti-D antibody. All 76 sera were examined with the sheep cell agglutination test (Rose test).

Ten of 64 men (16%) had gamma globulin levels above normal by the zinc turbidity test. Twelve (18.8%) showed agglutination of anti-D coated cells. Twenty-two (29%) had positive latex fixation tests. In general, the degree of latex agglutination was significantly weaker and titers lower than those seen in sera of rheumatoid arthritis patients. Rose tests were negative in all subjects. There was considerable overlap between the positive latex and the positive hemagglutination tests. Three subjects had positive latex, zinc turbidity, and anti-D agglutination tests. Sera from four men were examined in the analytical ultracentrifuge (2). These were selected because of their abnormalities in the other gamma globulin tests. In each of the four, there was elevation of the 7S peak without increase in the 19S globulins. No 22S or intermediate complexes were identifiable in either serum.

Further studies of antigammaglobulin factors on these men are in progress and will be reported in a later communication.

Serologic Tests for Syphilis: Venereal Disease Research Laboratory (V.D.R.L.) tests were performed on the 76 men examined in 1962. All were negative except one, a patient with a well-documented history of a primary syphilitic lesion.

Pathological Studies: Since the 1956 study, four men have died of illnesses unrelated to the immunization program. Three have died of acute coronary occlusions and the fourth of carcinoma of the colon. Postmortem examinations have been performed elsewhere on three of these. We have obtained tissue specimens on these three. Sections of liver, spleen, and kidney were examined after staining with hematoxylin and eosin and with thioflavin-T, but no evidence of amyloid deposition or other abnormality was found.

Gum biopsies were obtained on seven of the subjects with the most suggestive laboratory abnormalities. Percutaneous renal punch biopsy was performed on three men demonstrating persistent proteinuria. All of these sections have been examined after staining with hematoxylin, eosin and thioflavin-T, and are normal.

DISCUSSION

It is of prime significance that long-term 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.

The various clinical laboratory abnormalities are difficult to interpret, particularly because of the unavailability of an adequate control group with which to compare them, as indicated before. The incidence of lymphocytosis in the immunized persons was in excess of that expected in a healthy population. Lymphocytosis may occur in various viral infections, allergic states, blood dyscrasias, tumors, and metabolic diseases, none of which exist, at least with any considerable frequency, in the group studied. The significance of this finding is not clear, but its persistence adds to the impression that it may be explained by the intensive immunization.

It is our impression that the abnormalities of renal and liver function were more frequent than expected in the general population. Sporadic and unexplained abnormalities of renal function occur with some frequency in any "normal" population. This is particularly true of proteinuria, which may occur in normal persons at times of physical and emotional

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stress (40-45). Hepatic functional abnormalities have also been shown to occur in significant incidences in normal persons (46). The relationship of these abnormalities to intensive immunization can only be speculated upon at present.

The serum protein electrophoretic abnormality, characterized by alteration in the alpha-2 and beta globulins, was a consistent finding in several of the intensively immunized men over the 5 years of observation. The similarity of this abnormality to those abnormalities detected in hyperimmune states (23), amyloidosis (24-26, 29, 30), and the nephrotic syndrome (27-30) may suggest that it was related to presumed immunological disorders. These serum protein alterations, however, were not similar to those occurring in the sera of experimental animals in which amyloidosis is produced by feeding casein or by other methods (16, 47, 48). In such animals, the principle changes are limited to immunoglobulins and to albumin. Gamma globulin tends to rise initially and then fall as amyloid deposition begins in the liver. Albumin falls progressively only after renal amyloid deposition is severe enough to allow protein leakage from the glomeruli. Elevations of beta globulin also occur with some regularity in experimental amyloidosis; elevations of the alpha globulins occur less frequently. Alterations in the electrophoretic mobility of alpha-2 and beta globulins have not been described under experimental conditions.

Hexosamines occur in high concentration in deposits of amyloid, and elevated serum hexosamines develop in animals with experimental amyloidosis and in some patients with systemic amyloidosis (48, 49). Serum hexosamines were not performed initially in this study. The abnormalities observed subsequently, however, may suggest that amyloid deposition was induced by the intensive immunization. Nevertheless, the failure to find amyloid on biopsy of gingivae and kidney does not support this interpretation. The positive latex fixation tests and other tests of antigammaglobulin activity have been reported previously in animals experimentally injected with bacteria such as streptococci (50) and coliform bacilli (51), as well as in animals injected with bacterial antigens (52). Positive latex fixation tests have been reported in patients with subacute bacterial endocarditis (53), disappearing during convalescence, when *human* globulin is used for sensitization of the latex particles. Rose tests, using erythrocytes sensitized with rabbit globulin, are usually negative in such patients and in experimental animals which are given various antigen injections. In this regard, it is interesting that Rose tests were negative, whereas latex fixation tests and agglutination of erythrocytes coated with human globulin were positive in some 43% of these intensively immunized persons. There were seven individuals who did not receive any immunizations during the 2 years immediately before the study in 1962 after previously being intensively immunized. In none of these can antigammaglobulin factors be detected by any of the tests used. These findings indicate that antigammaglobulin antibodies develop frequently in intensively immunized individuals but may disappear as antigen injections are discontinued. It is of interest, however, that subjects with antigamma-globulin activity did not receive greater quantities of antigen than those not developing positive latex fixation tests. The implications of antigammaglobulin antibody in immunized persons are not known and may represent merely an interesting by-product of the introduction of foreign antigen.

There was no histological evidence of amyloid deposition or other adverse effect of

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immunization in the individuals who had renal and gingival biopsies. It is difficult to justify a program of extensive organ biopsy in such a population. Nevertheless, complete autopsies of a few individuals in the study dying of natural causes failed to reveal morphologic abnormalities attributable to immunization. In this regard, it should be pointed out that in the experimental animal, amyloidosis and other histologically demonstrable abnormalities result only after the administration of antigen in far greater proportionate quantities than employed in human beings. For this reason, it would be surprising, if not frightening, if morphological changes were found in these men.

At present, the most that can be said of the abnormalities observed in these intensively immunized human beings is that they seem to occur with an incidence greater than expected in a normal population. The abnormalities described are not only persistent upon repeated study but are increasing in incidence with continued immunization. Whether they represent the prodromata of anatomical changes to follow or are simply interesting temporary laboratory changes of no prognostic significance will be answered only with continued observation.

We have recently begun studies on one man, not included in the original group, who had undergone an identical immunization program for a comparable length of time. During this period of employment in the infectious environment, while undergoing immunization, he was never noted to develop positive serologic titers to any of the antigens used. He left the environment in 1958 and has since begun to have severe recurrent lower respiratory infections. Studies have demonstrated progressive hypogammaglobulinemia. The significance of this finding in relation to his intensive immunization is not clear. The results of investigations of this problem will be reported at a later date.

SUMMARY

The results of a continuing program of clinical and laboratory investigations on a group of adult men who have been intensively immunized against numerous infectious agents over a prolonged period of time have been presented. No evidence of clinical illness was found which might be attributed to immunization. Several laboratory abnormalities were encountered which may have resulted from intensive immunization. These abnormalities include: [1] an abnormal serum protein electrophoretic pattern, [2] elevated serum hexosamines, [3] a high incidence of lymphocytosis, [4] unexplained abnormalities of renal function, [5] liver function abnormalities, and [6] a high incidence of serum antigammaglobulin activity. Although there is no conclusive evidence that these abnormalities represent deleterious effects of intensive immunization, this possibility is presented and discussed.

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SUMMARIO IN INTERLINGUA

Un gruppo de 76 travaliatores de un laboratorio occupate con le investigation de un varietate de pathogene organismos bacterial, rickettsial, e virusal esseva re-evalutate con respecto al possibilitate de adverse reactiones evocate per le numerose immunisationes contra numerose agentes infectiose que illes ha recipite in le curso de un continue periodo de multe annos.

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Esseva trovate nulle evidentia de morbo clinic que pote esser attribuite al programma de immunisation. Esseva constatate, del altere latere, plure anormalitates laboratorial, incluse (1) un anormal configuration electrophoretic del proteinas seral, manifeste in mobilitate anormal del fractiones alpha-2 e globulina beta, (2) elevate concentraciones seral de hexosaminas, (3) un alte incidentia de lymphocytose, (4) inexplicate anormalitates del functionamento renal, (5) anormalitates del functionamento hepatic, e (6) un alte incidentia de activitate seral anti globulina gamma.

Le anormal configuration electrophoretic del proteinas seral es simile a illos describite in amyloidosis clinic, in le syndome nephrotic, e post intense immunisation contra toxina de diphtheria. Elevate concentraciones seral del hexosaminas ha essite describite como occurrente in animales experimental que disveloppava amyloidosis e in certe patientes con amyloidosis. Le activitate anti globulina gamma suggere un comparation con anormalitates describite in patientes con endocarditis bacterial e in animales subjicite al injection de bacterios integre e de fractiones bacterial.

Un restringite numero de examines histologic esseva effectuate, in intensemente immunisate subjectos human, in specimens de examine necroptic, de percutanee biopsia renal a punciamento, e biopsia gingival. Omnes esseva normal.

Ben que le presente studio presenta nulle evidentia conclusive de adverse resultatos de prolongate e intense immunisation, le possibilitate de tal resultatos es presente e analysate.

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