HYPER-IMMUNIZATION OF MAN[1]

RICHARD N. PEELER, LEIGHTON E. CLUFF AND ROBERT W. TREVER

Department of Medicine, The Johns Hopkins University School of Medicine and Hospital

Received for publication June 20, 1958

Repeated immunization of man with antigenic materials from a variety of pathogenic microorganisms has become an accepted measure to prevent infection. Such immunization can produce hypersensitivity to microbial antigens, and reinoculation of antigen or vaccine into the hypersensitive person may elicit acute local and systemic reactions of varying severity. Few other untoward effects from *repeated* immunization of man, however, have been clearly defined.

Intensive immunization of experimental animals has been shown (1–20) to produce delayed adverse consequences, i.e. amyloid deposition, arteritis, etc., but similar observations have not been conclusively reported in the human being, possibly related to the fact that far greater quantities of antigen are given to the experimental animal than would ordinarily be given to man.

We have had an opportunity to study a number of men who have been immunized prophylactically with a variety of bacterial, rickettsial, and viral antigens because of their employment in laboratories investigating pathogenic microorganisms. These men had received immunizations far in excess of that usually administered. To evaluate the effect of hyper-immunization in man these subjects were studied, and the results of clinical and laboratory examination are presented.

MATERIAL AND METHODS

A group of 99 Caucasian males between the ages of 28 and 65 years (Table I-A) was studied.

Diseases against which the personnel were immunized included botulism, brucellosis, tularemia, anthrax, diphtheria, Rocky Mountain spotted fever, Q fever, plague, typhus, psittacosis, smallpox, Eastern, Western, and Venezuelan equine encephalitis. The antigens used for immunization varied considerably in type, method of preparation, and concentration, so that a complete listing here is not practical. They have, however, been described elsewhere (21–27)[2], and consisted of killed bacterial vaccines, purified bacterial fractions, toxoids, and chicken egg prepared virus vaccines. The duration of the immunization period varied from 8 to 13 years during the time from 1944 to 1956 (Table I-B). The total amount of all antigens administered to the men (Table I-C) ranged from 35.8 to 74.4 ml., and varied in

[1] Supported by contract DA-18-064-404-CML-100 with the Army Chemical Corps, Fort Detrick, Maryland.

[2] Rocky Mountain spotted fever, Q fever, and Typhus vaccines, Lederle Laboratories, New York, N. Y. Plague vaccine, Cutter Laboratories, Berkeley, Cal. Smallpox vaccine, Parke, Davis and Co., Detroit, Mich.

TABLE I

Basic Data

A. Age

Range: 28 to 65 years
Mean: 40.1 years
Distribution:

age (yrs.)	(no. subjects)
28–30	4
31–35	27
36–40	26
41–45	20
46–50	9
51–55	7
56–60	3
61–65	3
Total	99

B. Duration of Immunization

Range: 8 to 13 years
Mean: 10.4 years
Distribution:

time (yrs.)	no. subjects
8	5
9	21
10	17
11	43
12	9
13	4
Total	99

C. Antigen Dose

Range: 35. to 74. ml.
Mean: 52.8 ml.
Distribution:

ml. antigen	no. subjects
35–40	6
41–45	20
46–50	21
51–55	15
56–60	19
61–65	12
66–70	5
71–75	1
Total	99

D. Skin Tests

Range: 9 to 44 per subject
Mean: 20.1 per subject
Distribution:

tests per subject	no. subjects
6–10	5
11–15	25
16–20	22
21–25	26
26–30	16
31–35	4
36–40	0
41–45	1
Total	99

E. Immunization Reactions

Range: 0 to 16 per subject
Mean: 2.8 per subject
Distribution:

reactions per subject	no. subjects
0	18
1–5	68
6–10	10
11–15	2
16	1
Total	99

each case with the duration of the immunization period. All subjects received immunization with antigens of botulism, tularemia, Rocky Mountain spotted fever, Q fever, typhus, plague, psittacosis, and the viral encephalitides. Thirty-seven were immunized against brucellosis, 95 against smallpox, 25 (all of whom had initially positive Schick tests) against

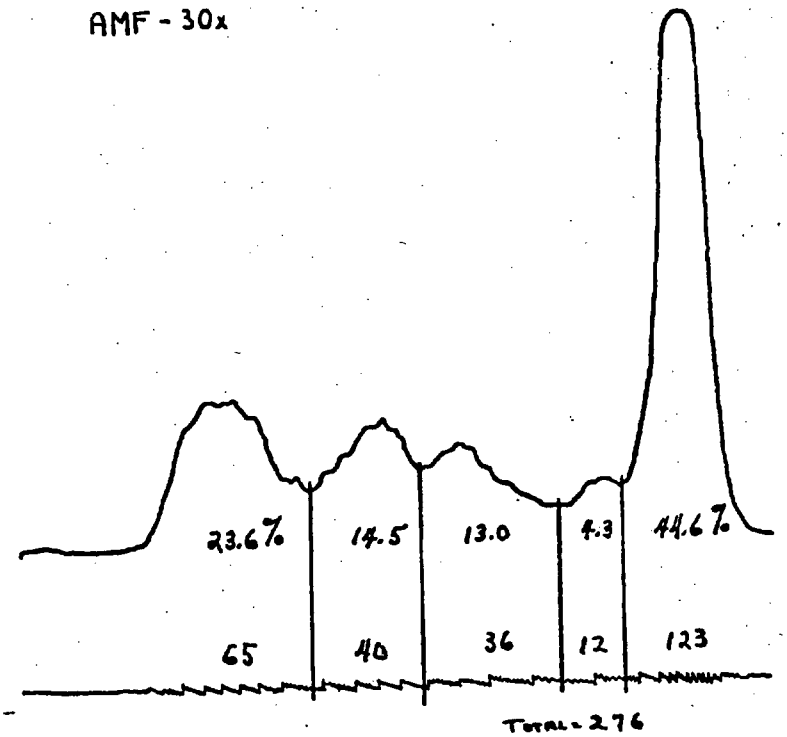

FIG. 1. Normal serum electrophoretic pattern demonstrating method of determining per cent of each protein fraction from electrophoretic curve integrated according to the area under each peak. "276" is representative of the area under the curve. Each protein fraction is expressed in per cent of total protein.

diphtheria, and 28 against anthrax. With each antigen, an initial series of immunizing injections was given, followed by "booster" injections every 6 to 12 months. In addition, subjects underwent frequent skin testing with the antigens of tuberculosis (Purified Protein Derivative), diphtheria (Schick and Maloney), brucellosis (Brucellergin), tularemia (Foshay), histoplasmosis, blastomycosis, coccidioidomycosis and glanders (28).[3] The average subject received about 20 such skin tests during the period of immunization (Table I-D). Reactions were frequent, ranging from none to 16 per subject (Table I-E). Serological titers to tularemia (agglutination) were performed frequently on all subjects. Titers to brucellosis (agglutination), Rocky Mountain spotted fever (complement fixation) (CF), Q fever (CF), typhus (CF), and psittacosis (CF) were performed less frequently and were not analyzed for this reason.

Ninety-three of the 99 men were evaluated by complete medical history and physical examination. In addition, each person's out-patient and hospital record for the period of

[3] Tularemia—1:1000 dilution of Foshay vaccine; Purified Protein Derivative Tuberculin, and Brucellergin, Sharpe and Dohme, Philadelphia, Pa., Histoplasmin and Blastomycin, Parke, Davis and Co., Detroit, Mich., Coccidioidin and Diphtheria toxin, Cutter Laboratories, Berkeley, Cal.

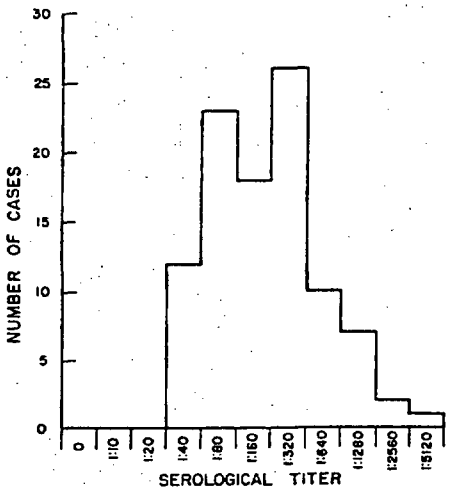

FIG. 2. Distribution curve of highest tularemia agglutinin titer obtained in the hyperimmunized persons.

immunization was reviewed. Since the subjects were followed in one clinic, reliable records of all illnesses occurring during the period of immunization were obtained.

Eighty-nine of the 99 men were evaluated by the following clinical laboratory tests: electrocardiogram, chest x-ray, hematocrit (Wintrobe), total and differential leukocyte counts, blood urea nitrogen (BUN), 2-hour phenolsulphthalein excretion (PSP), blood cholesterol, serum alkaline phosphatase activity (Bodansky units), total serum protein (TSP), serum albumin-globulin ratio, serum cephalin-cholesterol flocculation, serum thymol turbidity, and bromsulphalein (BSP) retention 45 minutes after intravenous injection of 5 mg. per kg. of the dye.

Serum for paper electrophoresis was obtained by centrifugation of venous blood after clotting and stored at 4°C. Duplicate specimens of 0.01 ml. of each serum were subjected to electrophoresis for 16 hours at 10 ma. in a Spinco Durrum-type electrophoresis cell (29) on 3 by 32 cm. Whatmann No. 3 filter paper strips, using 0.075 M veronal-veronal buffer at pH 8.6. After electrophoresis, the protein was coagulated and fixed to the paper by heating in an oven at 120°C. for 30 minutes. The protein was stained by immersing the paper strip for 8 hours in a solution containing 0.01 per cent bromphenol blue and 3.1 per cent zinc sulfate. The strips were then washed twice for 6 minutes in 5 per cent acetic acid, and fixed for 6 minutes in a 0.9 per cent solution of sodium acetate in 5 per cent acetic acid. After drying for 20 minutes at 120°C., the strips were exposed for 2 minutes to ammonia vapor. The stained strips were scanned photo-electrically in a Spinco model R Analytrol[4] for direct writing of the electrophoretic curve and automatic integration to determine the area representative of each of the 5 major protein components. Figure 1 shows a normal electrophoretic pattern with intersects drawn at the lowest point between each protein fraction. The area of the curve between the intersects was used in calculating the per cent of each protein constituent.

The total nitrogen of each serum sample was measured in duplicate by the micro-Kjeldahl method. Total *serum* nitrogen rather than total *serum protein nitrogen* was estimated. This

[4] Beckman Instruments Inc., Belmont, Cal.

choice was made because of the relative simplicity of total serum nitrogen determinations and all subjects had normal serum non-protein nitrogen levels. The fraction of total serum nitrogen represented by non-protein substances was negligible. Quantitative determination of albumin and alpha-1, alpha-2, beta, and gamma globulin expressed in mg. nitrogen per ml. of serum, was calculated from the fraction per cent obtained from electrophoresis and the total serum nitrogen. The nitrogen value for each protein component was used for statistical analysis.

TABLE II

History of Illness

A. *Probably Occupational*	
Tularemia, symptomatic	1
"Chronic brucellosis"	1
Q fever	1
Undefined fevers	9
B. *Not Occupational*	
Chronic sinusitis	10
Renal disease	10
Calculi	4
Acute pyelitis	4
Glomerulonephritis	1
Renal cyst	1
Allergy (hay fever, asthma, drug reactions)	9
Duodenal ulcer	7
Hepatitis	5
Arthritis	5
Hypertension	4
Coronary occlusion and/or angina pectoris	4
Albuminuria (orthostatic)	3
Diabetes mellitus	2
Acute rheumatic fever	2
Lung abscess	1
Osteomyelitis	1
Gout (?)	1
Cushing's syndrome (probable)	1
Colloid goitre	1
Leriche syndrome (occlusive arterial disease)	1

TABLE III

Differential Leukocyte Count

1. Lymphocytosis (> 36 per cent)

per cent	no. subjects
36–40	25
41–44	16
45–50	2
51–55	2
56–60	3
61–65	1
Total	49

For comparative analysis, the sera of 44 normal medical students, house-officers, nurses, and laboratory personnel, ages 18 to 50 years, were studied by electrophoresis in the same way as the sera from the hyper-immunized subjects. The control group selected was, in many aspects, not an adequate control for the experimental group. We were not able, however, to assemble a group of identical age, sex, and occupational exposure who had not received intensive immunization.

RESULTS

Serological Titers: Figure 2 shows a distribution curve of the highest tularemia titer obtained on each immunized subject during the period of study. No one failed to develop a rise in titer during his series of injections. When the serologic titers of the group were compared with those of persons of comparable age and duration of immunization who were immunized against tularemia only, there appeared to be no difference in the distribution of titers obtained in the two groups. This suggests that administration of multiple immunizing agents probably did not interfere with development of an adequate antibody response to this antigen.

Clinical Evaluation: As a group the men were not ill. Of the 93 patients queried, 55 had some illness in the past which might possibly alter one or more of the laboratory tests used in the study. These illnesses probably appeared no more frequently than might be expected in other individuals of comparable age, sex, and employment. There were a few instances of occupational infection. One patient had recovered from pulmonary tularemia, one may have had brucellosis, and another man had recovered from Q fever. Nine patients had short febrile illnesses of undetermined etiology without evidence of occupational disease and were diagnosed as having upper respiratory in-

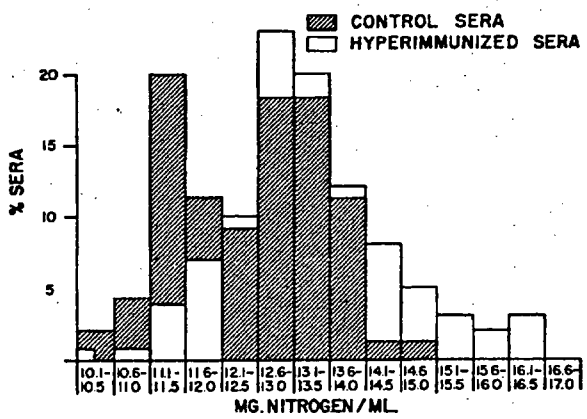

FIG. 3. Distribution of total serum nitrogen levels in 99 hyper-immunized men (99 sera) and 44 normal persons (44 sera), determined by electrophoresis.

fection. No illness was detected by anamnesis which might have been directly attributable to immunization. It is impossible, of course, to eliminate the possibility that some of these men had sub-clinical occupational illnesses. There were 5 instances in which an unexpected rise in tularemia titer occurred, and these were diagnosed as "serological tularemia". There were no similar rises in the serological titers for other diseases to which these men were exposed. One can assume, therefore, that the occurrence of such sub-clinical infection was probably uncommon.

The occurrence of hypertension and valvular murmurs among the immunized group was no different than that expected in any other population. Hepatomegaly was probably accounted for in 2 patients by a recent history of tularemia and brucellosis, respectively. In 5 other subjects there was no ready

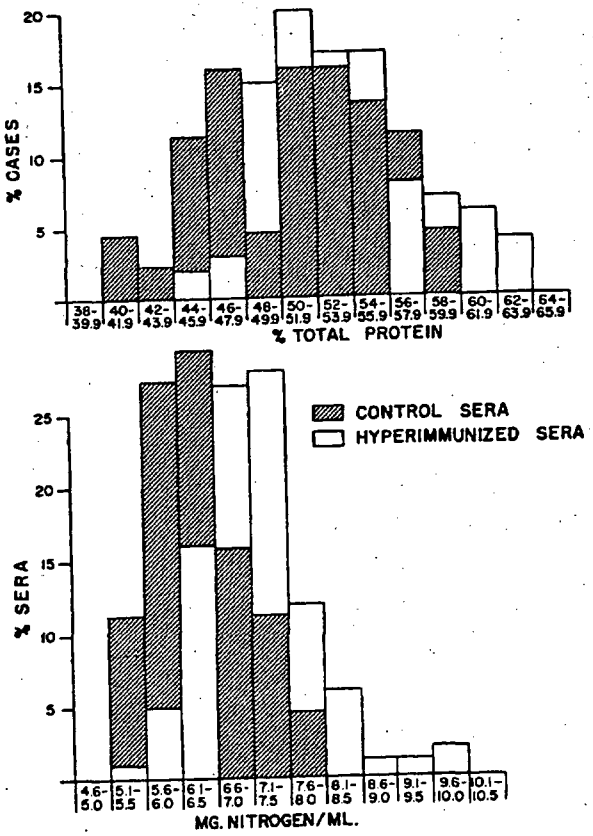

FIG. 4. Distribution of serum albumin levels, expressed as per cent of total protein and as mg. nitrogen, in 99 hyper-immunized men and 44 normal persons, determined by electrophoresis.

explanation for enlargement of the liver. Alcoholism was not diagnosed in any of the individuals. Macroglossia was detected in one patient.

Clinical Laboratory Evaluation: The hematocrit was determined in 91 subjects and the only abnormality was polycythemia in a patient with probable Cushing's syndrome. Twenty of the men examined had leukocytosis (10–16,000 per ml.), and 2 had leukopenia (4–4900 per ml.). Three subjects had a blood monocyte count in excess of 10 per cent. Seventeen patients had eosinophilia. In three of these there was a history of an allergic disorder such as hay fever. Forty-nine men had lymphocytosis (Table III). Of these, 7 had a history of an allergic illness, 4 of arthritis, three of non-specific febrile illnesses in the remote past, 3 of serological tularemia, one of clinical tularemia, and one each of Q fever, paratyphoid fever, and long standing myalgia of undetermined etiology. With the exception of one instance of serologic tularemia, the allergic

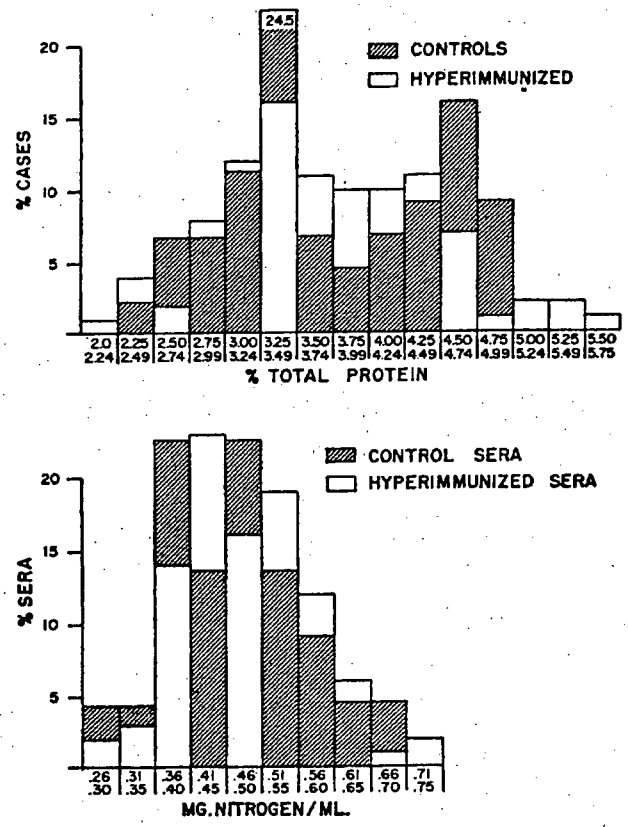

FIG. 5. Distribution of serum alpha-1 globulin levels, expressed as per cent of total protein and as mg. nitrogen, in 99 hyper-immunized men and 44 normal persons, determined by electrophoresis.

disorders and the chronic myalgia, all of these illnesses had occurred many months prior to the laboratory evaluation. It is unlikely that such remote illnesses could have had an effect upon the lymphocyte count at the time of the study.

Chest x-ray showed a few abnormalities among 89 men examined, including punctate calcification in 2, pleural thickening in one and hilar adenopathy in another. These abnormalities seemed to bear no relationship to immunization.

Proteinuria of varying degree was detected in about one-third of the patients but in only 2 subjects was this greater than one-plus, and probably could be explained by the presence of hypertension, history of glomerulonephritis, arteriosclerotic disease, or may have been accounted for on a functional basis. There was no reduction in phenolsulphthalein excretion which could not be readily explained.

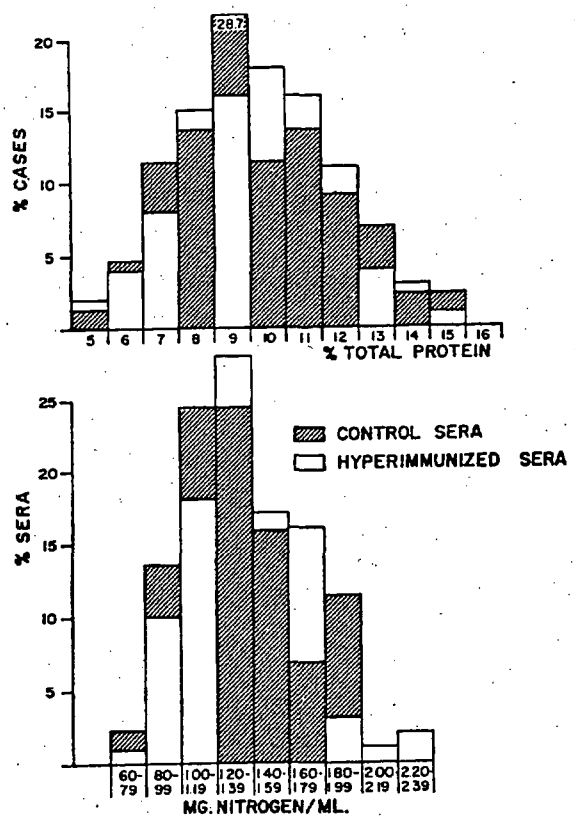

FIG. 6. Distribution of serum alpha-2 globulin levels, expressed as per cent of total protein and mg. nitrogen, in 99 hyper-immunized men and 44 normal persons, determined by electrophoresis.

In no patient was the blood cholesterol or alkaline phosphatase level significantly abnormal. Twenty-six men had a positive serum cephalin-flocculation test (1 or 2 plus) which was unexplained. In 7 persons the serum thymol turbidity was above 5 units (5–11) for an unexplained reason, and, 7 patients had a slightly impaired bromsulfalein excretion (5–10 per cent). The total serum proteins (grams per cent) and albumin-globulin ratios were not particularly abnormal in any individual.

In all, 53 subjects were found to have one or more abnormalities of liver function test. Excluded from this number are 25 persons with a past history of either allergy, arthritis, non-specific fever, tularemia, possible brucellosis, rheumatic heart disease, Q fever, probable Cushing's syndrome, osteomyelitis, or undiagnosed myalgia, all of whom had at least one abnormality of these laboratory tests.

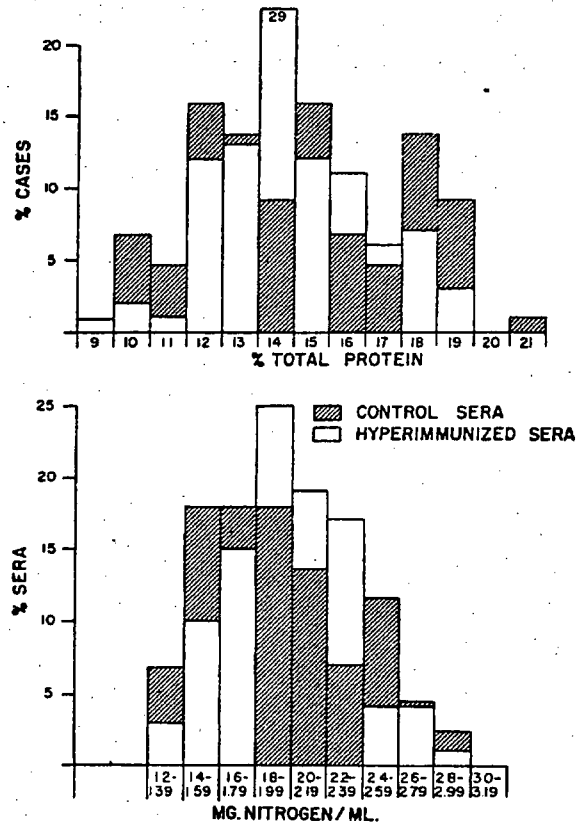

FIG. 7. Distribution of serum beta globulin levels, expressed as per cent of total protein and mg. nitrogen, in 99 hyper-immunized men and 44 normal persons, determined by electrophoresis.

An abnormal electrocardiogram seen in 4 patients was compatible with a history of coronary occlusion, rheumatic heart disease or hypertension. One unexplained right bundle branch block was found.

Serum Electrophoretic Studies: The mean total serum nitrogen and albumin nitrogen for the immunized group, estimated by electrophoresis, was significantly higher than that of the control group. There was no significant difference between the means of any of the other protein fractions in the test and control groups. Distribution curves for the various protein fraction values, calculated as per cent of the total and mg. nitrogen, are plotted in Figures 3–7. A tabular analysis is presented in Table IV. Gamma, alpha-1, alpha-2, and beta globulins were not significantly different in the immunized and control groups. The distribution curves of albumin and total serum nitrogen in the immunized group, however, were skewed toward the high levels. Eight subjects had total serum nitrogen values significantly higher than normal which could not be explained by any underlying disease. Similarly, 4 subjects had elevated total serum albumin. These 2 abnormalities occurred concurrently in 2 subjects.

There was one type of distinct qualitative abnormality of electrophoretic pattern which occurred frequently in the immunized subjects. Twenty-three of the 99 patients had serum electrophoretic patterns with a marked disturbance in the alpha-2 globulin region with poor separation of the alpha-2 and beta globulin fractions. This abnormality is illustrated in Figure 9. Although this pattern occurred in the hyper-immunized group with a frequency of 23 per

TABLE IV

Statistical Analysis of Serum Electrophoretic Fractions

Fraction	Group	mg. Nitrogen/ml. serum				Per cent, total protein			
		Range	Mean	±2 SD	SD	Range	Mean	±2 SD	SD
Gamma Globulin	Control	1.6–3.4	2.5	±1.05	0.52	14.1–26.1	20.2	±3.72	3.36
	Test	1.4–3.9	2.4	±0.98	0.49	11.2–27.0	17.7	±5.12	2.56
Beta Globulin	Control	1.2–2.8	1.9	±0.84	0.42	10.1–19.9	15.2	±5.64	2.82
	Test	1.1–3.0	2.0	±0.69	0.34	10.5–19.9	14.9	±4.54	2.27
Alpha-2 Globulin	Control	0.6–1.9	1.3	±0.64	0.32	5.3–15.8	10.1	±4.54	2.27
	Test	0.7–2.3	1.4	±0.59	0.30	5.5–15.6	10.2	±4.04	2.02
Alpha-1 Globulin	Control	0.27–0.68	0.47	±0.20	0.10	2.4–4.9	3.8	±1.49	0.75
	Test	0.28–0.73	0.49	±0.17	0.09	2.1–5.6	3.7	±1.45	0.73
Albumin	Control	5.3–7.9	6.3	±1.27	0.64	40.4–59.1	50.9	±9.90	4.85
	Test	5.4–9.9	7.1	±1.65	0.83	45.3–63.7	53.6	±13.2	6.61
Tot. Serum N	Control	10.3–14.6	12.5	±2.06	1.03	—	—	—	—
	Test	10.2–16.5	13.3	±2.34	1.17	—	—	—	—

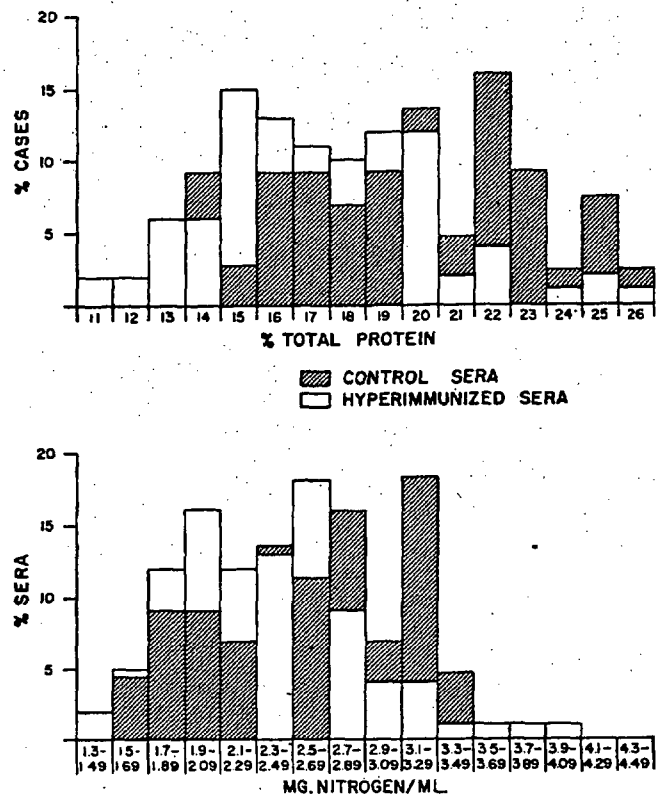

FIG. 8. Distribution of serum gamma globulin levels, expressed as per cent of total protein and mg. nitrogen, in 99 hyper-immunized men and 44 normal persons, determined by electrophoresis.

cent, it was not seen at all in the control series. Of the 23 patients with this electrophoretic abnormality, 19 were available for clinical study. The ages, duration of immunizations, number of skin tests, and frequency of immunization reactions in this group were identical to those of the immunized group as a whole as well as to the 76 subjects with normal electrophoretic patterns. There seemed to be no relation between the occurrence of this abnormality and the amount of any one antigen administered. When one compares the incidence of the various unexplained laboratory abnormalities in this group with those in the remainder of the immunized men, several differences are apparent. In the group with abnormal electrophoretic patterns, the incidence of hepatomegaly, lymphocytosis, proteinuria, and positive serum cephalin-flocculation are considerably higher. Of these 23 individuals, 3 gave histories of probable occupational infection (tularemia, brucellosis, fever of unknown origin) 7 others had arthritis, hay fever, osteomyelitis, or rheumatic heart disease.

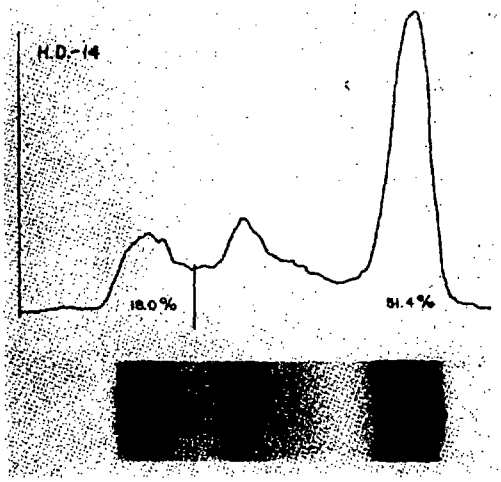

FIG. 9. Abnormal serum electrophoretic pattern observed in 23 per cent of hyper-immunized men, showing poor separation of alpha-2 and beta globulin.

DISCUSSION

In this study of hyper-immunized men, we have found no instance of illness which might be attributed to immunization; nor was there any suggestion of interference with serological response to antigenic stimulus.

The electrophoretic abnormality appears to be the most striking deviation from normal found in these intensively immunized persons. This was characterized by poor separation of the alpha-2 and beta globulin. Similar abnormalities of electrophoretic pattern have been reported to occur in several situations, including the nephrotic syndrome (29–32, 37, 38), disseminated lupus erythematosus (33), and amyloidosis (34–36). Mackay and Volweiler (37) have reported a patient with supposed Kimmelstiel-Wilson syndrome who also had poor separation of alpha-2 and beta globulin on serum electrophoresis. Kuhns (38) has shown that 9 to 19 days following a "booster" injection of diphtheria toxoid, several patients with high "skin-sensitizing" antibody titers demonstrated this same peculiar merging of the alpha-2 and beta globulin. He also found this pattern in one patient with eosinophilia following a penicillin injection and in another with pollen hay fever.

In our series of 44 normal persons the abnormality of electrophoretic pattern was not observed. We did not see a similar abnormality in the patterns of an additional 100 normal persons (medical students) kindly lent to us by Dr. Gerald Klatskin.[5] In reviewing 1000 serum electrophoretic patterns done consecutively on hospitalized patients (Dr. Gerald Klatskin), we found 6 patterns which showed some merging of alpha-2 and beta globulins. These

[5] Yale University School of Medicine, New Haven Hospital, New Haven, Conn.

patients had the following diagnoses: multiple myeloma, probable demyelinizing disease of the brain-stem, pneumococcal pneumonia with septicemia, rheumatoid arthritis, and hypertension, exfoliative dermatitis, and cholelithiasis, diverticulosis, and osteoarthritis.

In view of the low incidence of the serum electrophoretic abnormality in normal subjects (0 per cent) and in hospitalized patients (0.6 per cent), and in view of the high incidence of this abnormality in the hyper-immunized men it seems reasonable to conclude that the protein abnormality may be related to the intensive immunization.

The high incidence of lymphocytosis among the hyper-immunized men (54 per cent), and particularly in those persons with abnormal serum electrophoretic patterns (68 per cent), cannot be explained by any of the clinical observations. The possibility that the immunizations might be responsible for this finding appears likely. Intense antigenic challenge may cause lymphatic hyperplasia and could explain the leukocyte abnormality, but the significance of lymphocytosis in this circumstance is not clear.

Some abnormality of liver "function" tests was present by laboratory examination in over half (59.5 per cent) of the 89 men studied. Only the abnormalities of serum cephalin-flocculation, total serum nitrogen and serum albumin nitrogen seem of possible significance. The occurrence of similar abnormalities among normal subjects, however, makes it impossible to interpret these findings in the immunized patients.

Two findings in the hyper-immunized patients, are not explained on any common basis other than the intense immunization which they received. These include: (1) a peculiar abnormality of the electrophoretic pattern and (2) a striking incidence of lymphocytosis. Most important, however, remains the fact that the subjects in the present study are clinically well and show no evidence of interference with immunological response. These 2 abnormalities could not be correlated with the dosage of any one antigen, number of skin tests received, or occurrence and severity of reaction to the various injections.

The importance of the serum electrophoretic pattern abnormality associated with immunization cannot be readily interpreted. It seems, however, that this protein change could be indicative of an untoward effect of hyperimmunization. The possibility that amyloid deposition might be induced by intense immunization is suggested by the observations in experimental animals, wherein amyloidosis occurs following hyper-immunization (1–19). Over an 8 to 13 year period, however, there is as yet no conclusive evidence that amyloidosis or other organic disease has been induced by repeated inoculation of large doses of antigen in the men studied here.

SUMMARY

The results of clinical and laboratory studies of 99 adult men who were intensively immunized have been presented. Although the group was, on the

whole, well, two findings could probably be attributed to hyper-immunization. These abnormalities include: (1) an abnormal serum electrophoretic pattern and (2) a high incidence of lymphocytosis. Although this is not conclusive evidence of deleterious influence of hyper-immunization in man, the possibility that intensive antigenic stimulation might be associated with untoward effects is considered.

REFERENCES

1. Zenoni, C.: Toxische Amyloidosis bei der antidiphtherischen Immunization des Pferdes. Zbl. allg. P., 1902, **13**: 334.
2. Pease, H. D. and Pease, R. M.: Liver necrosis and venous thrombosis in horses actively immunized with diptheria and tetanus toxins and streptococci and their products. J. *Infect. Dis.*, 1906, **3**: 619.
3. Markus, H.: Over amyloide Degeneratie van de Lever bij ned Paard. Tijdschr. v. Veeartsenijk. Maanbl., 1904–5, **32**: 99.
4. Schonkewich, J.: De la degenerescence amyloide chez les chevaux soumis aux injections repetees de bacillus pesteux. Arch. sc. biol., 1907, **12**: 190.
5. Arndt, H. and Doerken, E.: Uber die Amyloidose der Serumpferde. Arch. wiss. prakt. Tierheilk., 1931, **63**: 1.
6. Fresch, A.: Uber eigenthumliche Produkte myotischer Keratitis mit der Reaction des Amyloids. Sitz. ber. math.-physik. Kl. Akad. Wiss. München., 1877, **76**: 109.
7. Bouchard, R. and Charrin, P.: Degenerescence amyloide experimentale. Compt. rend. Soc. biol., 1888, **50**: 688.
8. Czerney, A.: Zur Kentniss der glycogenen und amyloiden Entartung. Arch. f. exper. Path. u. Pharmakol., 1893, **31**: 190.
9. Bailey, C. H.: The production of amyloid disease and chronic nephritis in rabbits by repeated intravenous injections of living colon bacilli. J. Exper. Med., 1916, **23**: 773.
10. Smetana, H.: Experimental study of amyloid formation. Bull. Johns Hopkins Hosp., 1925, **37**: 383.
11. Jaffe, R. H.: Amyloidosis produced by injection of proteins. Arch. Path., 1926, **1**: 25.
12. Grayzel, H. G. et al.: Experimental studies on amyloidosis. Proc. Soc. Exper. Biol. & Med., 1930, **28**: 172.
13. Ibid.: Amyloidosis; Experimental studies. Arch. Path., 1934, **17**: 50.
14. Dick, G. F. and Leiter, L.: Experimental chronic diffuse amyloid glomerulonephritis in rabbits, produced by hemolytic streptococci. Tr. A. Am. Physicians, 1935, **50**: 135.
15. Ibid.: Experimental amyloidosis and hyperglobulinemia. Tr. A. Am. Physicians, 1937, **52**: 246.
16. Letterer, E.: New aspects of experimental amyloidosis. J. Path. & Bact., 1949, **61**: 496.
17. Dick, G. F. and Leiter, L.: Some factors in the development, localization, and resorption of experimental amyloidosis in the rabbit. Am. J. Path., 1941, **17**: 741.
18. Richter, G. W.: The resorption of amyloid under experimental conditions. Am. J. Path., 1954, **30**: 239.
19. Reiman, H. A. and Eklund, C. M.: Long-continued vaccine therapy as a cause of amyloidosis. Am. J. M. Sc., 1935, **190**: 88.
20. Germuth, F. G.: A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. J. Exper. Med., 1953, **97**: 257.
21. Nigg, C., Hottle, G. A., Coriell, L. L., Rosenwald, A. S. and Beveridge, G. W.:

patients had the following diagnoses: multiple myeloma, probable demyelinizing disease of the brain-stem, pneumococcal pneumonia with septicemia, rheumatoid arthritis, and hypertension, exfoliative dermatitis, and cholelithiasis, diverticulosis, and osteoarthritis.

In view of the low incidence of the serum electrophoretic abnormality in normal subjects (0 per cent) and in hospitalized patients (0.6 per cent), and in view of the high incidence of this abnormality in the hyper-immunized men it seems reasonable to conclude that the protein abnormality may be related to the intensive immunization.

The high incidence of lymphocytosis among the hyper-immunized men (54 per cent), and particularly in those persons with abnormal serum electrophoretic patterns (68 per cent), cannot be explained by any of the clinical observations. The possibility that the immunizations might be responsible for this finding appears likely. Intense antigenic challenge may cause lymphatic hyperplasia and could explain the leukocyte abnormality, but the significance of lymphocytosis in this circumstance is not clear.

Some abnormality of liver "function" tests was present by laboratory examination in over half (59.5 per cent) of the 89 men studied. Only the abnormalities of serum cephalin-flocculation, total serum nitrogen and serum albumin nitrogen seem of possible significance. The occurrence of similar abnormalities among normal subjects, however, makes it impossible to interpret these findings in the immunized patients.

Two findings in the hyper-immunized patients, are not explained on any common basis other than the intense immunization which they received. These include: (1) a peculiar abnormality of the electrophoretic pattern and (2) a striking incidence of lymphocytosis. Most important, however, remains the fact that the subjects in the present study are clinically well and show no evidence of interference with immunological response. These 2 abnormalities could not be correlated with the dosage of any one antigen, number of skin tests received, or occurrence and severity of reaction to the various injections.

The importance of the serum electrophoretic pattern abnormality associated with immunization cannot be readily interpreted. It seems, however, that this protein change could be indicative of an untoward effect of hyperimmunization. The possibility that amyloid deposition might be induced by intense immunization is suggested by the observations in experimental animals, wherein amyloidosis occurs following hyper-immunization (1–19). Over an 8 to 13 year period, however, there is as yet no conclusive evidence that amyloidosis or other organic disease has been induced by repeated inoculation of large doses of antigen in the men studied here.

SUMMARY

The results of clinical and laboratory studies of 99 adult men who were intensively immunized have been presented. Although the group was, on the

whole, well, two findings could probably be attributed to hyper-immunization. These abnormalities include: (1) an abnormal serum electrophoretic pattern and (2) a high incidence of lymphocytosis. Although this is not conclusive evidence of deleterious influence of hyper-immunization in man, the possibility that intensive antigenic stimulation might be associated with untoward effects is considered.

REFERENCES

1. Zenoni, C.: Toxische Amyloidosis bei der antidiphtherischen Immunization des Pferdes. Zbl. allg. P., 1902, **13**: 334.
2. Pease, H. D. and Pease, R. M.: Liver necrosis and venous thrombosis in horses actively immunized with diptheria and tetanus toxins and streptococci and their products. J. Infect. Dis., 1906, **3**: 619.
3. Markus, H.: Over amyloide Degeneratie van de Lever bij ned Paard. Tijdschr. v. Veeartsenijk. Maanbl., 1904–5, **32**: 99.
4. Schonkewich, J.: De la degenerescence amyloide chez les chevaux soumis aux injections repetees de bacillus pesteux. Arch. sc. biol., 1907, **12**: 190.
5. Arndt, H. and Doerken, E.: Uber die Amyloidose der Serumpferde. Arch. wiss. prakt. Tierheilk., 1931, **63**: 1.
6. Fresch, A.: Uber eigenthumliche Produkte myotischer Keratitis mit der Reaction des Amyloids. Sitz. ber. math.-physik. Kl. Akad. Wiss. München., 1877, **76**: 109.
7. Bouchard, R. and Charrin, P.: Degenerescence amyloide experimentale. Compt. rend. Soc. biol., 1888, **50**: 688.
8. Czerney, A.: Zur Kentniss der glycogenen und amyloiden Entartung. Arch. f. exper. Path. u. Pharmakol., 1893, **31**: 190.
9. Bailey, C. H.: The production of amyloid disease and chronic nephritis in rabbits by repeated intravenous injections of living colon bacilli. J. Exper. Med., 1916, **23**: 773.
10. Smetana, H.: Experimental study of amyloid formation. Bull. Johns Hopkins Hosp., 1925, **37**: 383.
11. Jaffe, R. H.: Amyloidosis produced by injection of proteins. Arch. Path., 1926, **1**: 25.
12. Grayzel, H. G. et al.: Experimental studies on amyloidosis. Proc. Soc. Exper. Biol. & Med., 1930, **28**: 172.
13. Ibid.: Amyloidosis; Experimental studies. Arch. Path., 1934, **17**: 50.
14. Dick, G. F. and Leiter, L.: Experimental chronic diffuse amyloid glomerulonephritis in rabbits, produced by hemolytic streptococci. Tr. A. Am. Physicians, 1935, **50**: 135.
15. Ibid.: Experimental amyloidosis and hyperglobulinemia. Tr. A. Am. Physicians, 1937, **52**: 246.
16. Letterer, E.: New aspects of experimental amyloidosis. J. Path. & Bact., 1949, **61**: 496.
17. Dick, G. F. and Leiter, L.: Some factors in the development, localization, and resorption of experimental amyloidosis in the rabbit. Am. J. Path., 1941, **17**: 741.
18. Richter, G. W.: The resorption of amyloid under experimental conditions. Am. J. Path., 1954, **30**: 239.
19. Reiman, H. A. and Eklund, C. M.: Long-continued vaccine therapy as a cause of amyloidosis. Am. J. M. Sc., 1935, **190**: 88.
20. Germuth, F. G.: A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. J. Exper. Med., 1953, **97**: 257.
21. Nigg, C., Hottle, G. A., Coriell, L. L., Rosenwald, A. S. and Beveridge, G. W.:

patients had the following diagnoses: multiple myeloma, probable demyelinizing disease of the brain-stem, pneumococcal pneumonia with septicemia, rheumatoid arthritis, and hypertension, exfoliative dermatitis, and cholelithiasis, diverticulosis, and osteoarthritis.

In view of the low incidence of the serum electrophoretic abnormality in normal subjects (0 per cent) and in hospitalized patients (0.6 per cent), and in view of the high incidence of this abnormality in the hyper-immunized men it seems reasonable to conclude that the protein abnormality may be related to the intensive immunization.

The high incidence of lymphocytosis among the hyper-immunized men (54 per cent), and particularly in those persons with abnormal serum electrophoretic patterns (68 per cent), cannot be explained by any of the clinical observations. The possibility that the immunizations might be responsible for this finding appears likely. Intense antigenic challenge may cause lymphatic hyperplasia and could explain the leukocyte abnormality, but the significance of lymphocytosis in this circumstance is not clear.

Some abnormality of liver "function" tests was present by laboratory examination in over half (59.5 per cent) of the 89 men studied. Only the abnormalities of serum cephalin-flocculation, total serum nitrogen and serum albumin nitrogen seem of possible significance. The occurrence of similar abnormalities among normal subjects, however, makes it impossible to interpret these findings in the immunized patients.

Two findings in the hyper-immunized patients, are not explained on any common basis other than the intense immunization which they received. These include: (1) a peculiar abnormality of the electrophoretic pattern and (2) a striking incidence of lymphocytosis. Most important, however, remains the fact that the subjects in the present study are clinically well and show no evidence of interference with immunological response. These 2 abnormalities could not be correlated with the dosage of any one antigen, number of skin tests received, or occurrence and severity of reaction to the various injections.

The importance of the serum electrophoretic pattern abnormality associated with immunization cannot be readily interpreted. It seems, however, that this protein change could be indicative of an untoward effect of hyperimmunization. The possibility that amyloid deposition might be induced by intense immunization is suggested by the observations in experimental animals, wherein amyloidosis occurs following hyper-immunization (1–19). Over an 8 to 13 year period, however, there is as yet no conclusive evidence that amyloidosis or other organic disease has been induced by repeated inoculation of large doses of antigen in the men studied here.

SUMMARY

The results of clinical and laboratory studies of 99 adult men who were intensively immunized have been presented. Although the group was, on the

whole, well, two findings could probably be attributed to hyper-immunization. These abnormalities include: (1) an abnormal serum electrophoretic pattern and (2) a high incidence of lymphocytosis. Although this is not conclusive evidence of deleterious influence of hyper-immunization in man, the possibility that intensive antigenic stimulation might be associated with untoward effects is considered.

REFERENCES

1. Zenoni, C.: Toxische Amyloidosis bei der antidiphtherischen Immunization des Pferdes. Zbl. allg. P., 1902, **13**: 334.
2. Pease, H. D. and Pease, R. M.: Liver necrosis and venous thrombosis in horses actively immunized with diptheria and tetanus toxins and streptococci and their products. J. Infect. Dis., 1906, **3**: 619.
3. Markus, H.: Over amyloide Degeneratie van de Lever bij ned Paard. Tijdschr. v. Veeartsenijk. Maanbl., 1904–5, **32**: 99.
4. Schonkewich, J.: De la degenerescence amyloide chez les chevaux soumis aux injections repetees de bacillus pesteux. Arch. sc. biol., 1907, **12**: 190.
5. Arndt, H. and Doerken, E.: Über die Amyloidose der Serumpferde. Arch. wiss. prakt. Tierheilk., 1931, **63**: 1.
6. Fresch, A.: Uber eigenthumliche Produkte myotischer Keratitis mit der Reaction des Amyloids. Sitz. ber. math.-physik. Kl. Akad. Wiss. München., 1877, **76**: 109.
7. Bouchard, R. and Charrin, P.: Degenerescence amyloide experimentale. Compt. rend. Soc. biol., 1888, **50**: 688.
8. Czerney, A.: Zur Kentniss der glycogenen und amyloiden Entartung. Arch. f. exper. Path. u. Pharmakol., 1893, **31**: 190.
9. Bailey, C. H.: The production of amyloid disease and chronic nephritis in rabbits by repeated intravenous injections of living colon bacilli. J. Exper. Med., 1916, **23**: 773.
10. Smetana, H.: Experimental study of amyloid formation. Bull. Johns Hopkins Hosp., 1925, **37**: 383.
11. Jaffe, R. H.: Amyloidosis produced by injection of proteins. Arch. Path., 1926, **1**: 25.
12. Grayzel, H. G. et al.: Experimental studies on amyloidosis. Proc. Soc. Exper. Biol. & Med., 1930, **28**: 172.
13. Ibid.: Amyloidosis; Experimental studies. Arch. Path., 1934, **17**: 50.
14. Dick, G. F. and Leiter, L.: Experimental chronic diffuse amyloid glomerulonephritis in rabbits, produced by hemolytic streptococci. Tr. A. Am. Physicians, 1935, **50**: 135.
15. Ibid.: Experimental amyloidosis and hyperglobulinemia. Tr. A. Am. Physicians, 1937, **52**: 246.
16. Letterer, E.: New aspects of experimental amyloidosis. J. Path. & Bact., 1949, **61**: 496.
17. Dick, G. F. and Leiter, L.: Some factors in the development, localization, and resorption of experimental amyloidosis in the rabbit. Am. J. Path., 1941, **17**: 741.
18. Richter, G. W.: The resorption of amyloid under experimental conditions. Am. J. Path., 1954, **30**: 239.
19. Reiman, H. A. and Eklund, C. M.: Long-continued vaccine therapy as a cause of amyloidosis. Am. J. M. Sc., 1935, **190**: 88.
20. Germuth, F. G.: A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. J. Exper. Med., 1953, **97**: 257.
21. Nigg, C., Hottle, G. A., Coriell, L. L, Rosenwald, A. S. and Beveridge, G. W.:

patients had the following diagnoses: multiple myeloma, probable demyelinizing disease of the brain-stem, pneumococcal pneumonia with septicemia, rheumatoid arthritis, and hypertension, exfoliative dermatitis, and cholelithiasis, diverticulosis, and osteoarthritis.

In view of the low incidence of the serum electrophoretic abnormality in normal subjects (0 per cent) and in hospitalized patients (0.6 per cent), and in view of the high incidence of this abnormality in the hyper-immunized men it seems reasonable to conclude that the protein abnormality may be related to the intensive immunization.

The high incidence of lymphocytosis among the hyper-immunized men (54 per cent), and particularly in those persons with abnormal serum electrophoretic patterns (68 per cent), cannot be explained by any of the clinical observations. The possibility that the immunizations might be responsible for this finding appears likely. Intense antigenic challenge may cause lymphatic hyperplasia and could explain the leukocyte abnormality, but the significance of lymphocytosis in this circumstance is not clear.

Some abnormality of liver "function" tests was present by laboratory examination in over half (59.5 per cent) of the 89 men studied. Only the abnormalities of serum cephalin-flocculation, total serum nitrogen and serum albumin nitrogen seem of possible significance. The occurrence of similar abnormalities among normal subjects, however, makes it impossible to interpret these findings in the immunized patients.

Two findings in the hyper-immunized patients, are not explained on any common basis other than the intense immunization which they received. These include: (1) a peculiar abnormality of the electrophoretic pattern and (2) a striking incidence of lymphocytosis. Most important, however, remains the fact that the subjects in the present study are clinically well and show no evidence of interference with immunological response. These 2 abnormalities could not be correlated with the dosage of any one antigen, number of skin tests received, or occurrence and severity of reaction to the various injections.

The importance of the serum electrophoretic pattern abnormality associated with immunization cannot be readily interpreted. It seems, however, that this protein change could be indicative of an untoward effect of hyperimmunization. The possibility that amyloid deposition might be induced by intense immunization is suggested by the observations in experimental animals, wherein amyloidosis occurs following hyper-immunization (1–19). Over an 8 to 13 year period, however, there is as yet no conclusive evidence that amyloidosis or other organic disease has been induced by repeated inoculation of large doses of antigen in the men studied here.

SUMMARY

The results of clinical and laboratory studies of 99 adult men who were intensively immunized have been presented. Although the group was, on the

whole, well, two findings could probably be attributed to hyper-immunization. These abnormalities include: (1) an abnormal serum electrophoretic pattern and (2) a high incidence of lymphocytosis. Although this is not conclusive evidence of deleterious influence of hyper-immunization in man, the possibility that intensive antigenic stimulation might be associated with untoward effects is considered.

REFERENCES

1. Zenoni, C.: Toxische Amyloidosis bei der antidiphtherischen Immunization des Pferdes. Zbl. allg. P., 1902, **13**: 334.
2. Pease, H. D. and Pease, R. M.: Liver necrosis and venous thrombosis in horses actively immunized with diptheria and tetanus toxins and streptococci and their products. J. Infect. Dis., 1906, **3**: 619.
3. Markus, H.: Over amyloide Degeneratie van de Lever bij ned Paard. Tijdschr. v. Veeartsenijk. Maanbl., 1904–5, **32**: 99.
4. Schonkewich, J.: De la degenerescence amyloide chez les chevaux soumis aux injections repetees de bacillus pesteux. Arch. sc. biol., 1907, **12**: 190.
5. Arndt, H. and Doerken, E.: Uber die Amyloidose der Serumpferde. Arch. wiss. prakt. Tierheilk., 1931, **63**: 1.
6. Fresch, A.: Uber eigenthumliche Produkte myotischer Keratitis mit der Reaction des Amyloids. Sitz. ber. math.-physik. Kl. Akad. Wiss. München., 1877, **76**: 109.
7. Bouchard, R. and Charrin, P.: Degenerescence amyloide experimentale. Compt. rend. Soc. biol., 1888, **50**: 688.
8. Czerney, A.: Zur Kentniss der glycogenen und amyloiden Entartung. Arch. f. exper. Path. u. Pharmakol., 1893, **31**: 190.
9. Bailey, C. H.: The production of amyloid disease and chronic nephritis in rabbits by repeated intravenous injections of living colon bacilli. J. Exper. Med., 1916, **23**: 773.
10. Smetana, H.: Experimental study of amyloid formation. Bull. Johns Hopkins Hosp., 1925, **37**: 383.
11. Jaffe, R. H.: Amyloidosis produced by injection of proteins. Arch. Path., 1926, **1**: 25.
12. Grayzel, H. G. et al.: Experimental studies on amyloidosis. Proc. Soc. Exper. Biol. & Med., 1930, **28**: 172.
13. Ibid.: Amyloidosis; Experimental studies. Arch. Path., 1934, **17**: 50.
14. Dick, G. F. and Leiter, L.: Experimental chronic diffuse amyloid glomerulonephritis in rabbits, produced by hemolytic streptococci. Tr. A. Am. Physicians, 1935, **50**: 135.
15. Ibid.: Experimental amyloidosis and hyperglobulinemia. Tr. A. Am. Physicians, 1937, **52**: 246.
16. Letterer, E.: New aspects of experimental amyloidosis. J. Path. & Bact., 1949, **61**: 496.
17. Dick, G. F. and Leiter, L.: Some factors in the development, localization, and resorption of experimental amyloidosis in the rabbit. Am. J. Path., 1941, **17**: 741.
18. Richter, G. W.: The resorption of amyloid under experimental conditions. Am. J. Path., 1954, **30**: 239.
19. Reiman, H. A. and Eklund, C. M.: Long-continued vaccine therapy as a cause of amyloidosis. Am. J. M. Sc., 1935, **190**: 88.
20. Germuth, F. G.: A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. J. Exper. Med., 1953, **97**: 257.
21. Nigg, C., Hottle, G. A., Coriell, L. L , Rosenwald, A. S. and Beveridge, G. W.:

Studies on botulinum toxoid, types A and B. I: Production of alum precipitated toxoids. J. Immunol., 1947, **55**: 245.

22. Howe, C., Miller, E. S., Kelly, E. H., Bookwalter, H. L. and Ellingson, H. V.: Acute brucellosis among laboratory workers. New England J. Med., 1947, **236**: 741.
23. Foshay, L.: Contract DA-18-064-CML-2394. Chemical Corps Biological Laboratories, Camp Detrick, Md.
24. Wright, G. W., Green, T. W. and Kanode, R. G., Jr.: Studies on immunity in anthrax. V: Immunizing activity of alum-precipitated protective antigen. J. Immunol., 1954, **73**: 387.
25. Wells, D. M. and Havens, L. C.: Diphtheria toxoid precipitated with alum: Its preparation and advantages. Am. J. Pub. Health, 1952, **22**: 648.
26. Wagner, J. C., Meiklijohn, G., Kingsland, L. C. and Hukish, H. W.: Psittacosis vaccines prepared from chick embryo tissues. J. Immunol., 1946, **54**: 35.
27. Randall, R., Mills, J. W. and Engel, L. L.: The preparation and properties of a purified equine encephalomyelitis vaccine. J. Immunol., 1947, **55**: 41.
28. (Mallein) Wilson, G. S. and Miles, A. A.: *Principles of Bacteriology and Immunology*, Fourth edition. Williams and Wilkins, Baltimore, Md., 1955, pp. 1610–1611.
29. Williams, F. G., Jr. and Durrum, E. L.: Improved hanging-strip paper electrophoresis technique. Science, 1955, **121**: 829.
30. Hardwicke, J.: Serum and urinary protein changes in the nephrotic syndrome. Proc. Roy. Soc. Med., 1954, **47**: 832.
31. Dencks, W. P. et al.: The clinical significance of analysis of serum protein distribution by filter paper electrophoresis. Am. J. Med., 1956, **21**: 387.
32. Marrack, J. R. and Hoch, H.: Serum proteins; a review. J. Clin. Path., 1947, **2**: 16.
33. McQueen, E. G. et al.: Filter paper electrophoresis of serum proteins in clinical practice. Australas. Ann. Med., 1954, **3**: 270.
34. Block, W. D. et al.: An atypical peak in the serum of patients with familial primary systemic amyloidosis. Proc. Soc. Exper. Biol. & Med., 1955, **89**: 175.
35. Ibid.: Serum electrophoretic studies of patients with familial primary systemic amyloidosis. J. Lab. & Clin. Med., 1956, **47**: 257.
36. Rukavina, J. G. et al.: Familial primary systemic amyloidosis: a review and an experimental, genetic, and clinical study of 29 cases, Medicine, 1956, **35**: 239.
37. Mackay, I. R. and Volweiler, W.: Quantitative aspects of filter paper electrophoresis of serum. Australas. Ann. M., 1954, **3**: 263.
38. Kuhns, W. J.: Immunochemical studies of antitoxin produced in normal and allergic individuals hyper-immunized with diphtheria toxoid. J. Exper. Med., 1955, **100**: 175