individuals, including normal men as well as those with respiratory diseases, is being presented elsewhere.

(2) Production of Antifibrinolysin

In previous studies,[9] it was demonstrated that bacteriological and clinical evidence of β-hemolytic streptococcal infection is not necessarily sufficient to establish an etiological diagnosis; rather, the development of specific antibody during convalescence is required. In known streptococcal infections, such as scarlet fever and epidemic sore throat, there is a significant increase in antistreptolysin antibodies in about 85 per cent. of the convalescent sera. For the purpose of the present study, patients with exudative tonsillitis or pharyngitis from whom types 3, 5, 19 or 12[10] streptococci were isolated in one or more of three cultures of the throat, and who developed antistreptolysin antibodies during convalescence, are included as *proved* instances of streptococcal infections. In all, 110 hospitalized soldiers are included in this analysis.

The distribution of the cases according to type of streptococcus and the results of the antifibrinolysin determinations are recorded in Table 1. The type 3

TABLE 1

Antifibrinolysin Response in Streptococcal Infections

Type of Group A β-Hemolytic Streptococcus	Occurrence of cases	Antifibrinolysin test		
		No. of cases positive	No. of cases negative	Per cent. positive
3	Endemic	1	9	10
5	Epidemic	15	61	20
19	Endemic	8	5	62
12	Epidemic	10	1	91

and type 19 infections occurred endemically during 1943 and 1944. In contrast, the type 5 infections were the result of a food-borne epidemic, and at least nine of the type 12 infections occurred in a single small outbreak. Presumably, the sporadic cases were produced by several strains of the given types, while the epidemic cases resulted from a single strain.

The variation in the antifibrinolysin response in these subjects was marked. Only one of the type 3 infections exhibited a significant response, whereas a rise in antibodies was demonstrated in 62 per cent. of the type 19 infections. Similarly, there was a difference between type 5 epidemic cases with only 20 per cent. positive antifibrinolysin tests and type 12 infections with 91 per cent. These marked differences in antibody formation suggested that the development of antifibrinolysin in man might be related to the fibrinolytic capacity of the infecting organism.

[9] Commission on Acute Respiratory Diseases, *Jour. Am. Med. Assn.*, 125: 1163, 1944.

[10] Type specific rabbit serums were made available through the generosity of Drs. Homer T. Swift and Rebecca C. Lancefield.

A test was devised therefore to measure the amount of fibrinolysin produced by these streptococci *in vitro*. The type 12 strains were not available for study. The average production of fibrinolysin of nine of the type 3 strains was found to be 40 units per ml of culture medium, that of the type 5 strains 90 units, and of the type 19 strains 180 units. These results suggest that the antifibrinolysin response in the subjects reported here is related to the ability of the homologous organism to produce fibrinolysin *in vitro*. It should be emphasized, however, that the amount of fibrinolysin produced is not necessarily a property of a given Lancefield type, but may be a strain characteristic. For example, some carrier strains of type 3 streptococci, isolated from the same population groups supplying the above cases, have the ability to produce large amounts of fibrinolysin.

Summary

The results of a study of the streptococcal fibrinolysin reaction and its inhibition by sera containing specific antibody are presented. It was possible to devise a quantitative antifibrinolysin test by controlling the various factors influencing the reaction. In man, the antifibrinolysin response was found to vary according to the strain of streptococcus responsible for the infection.

Commission on Acute Respiratory Diseases[11] in Collaboration with
Melvin H. Kaplan

THE IMMUNIZING EFFECT OF CALCIUM PHOSPHATE ADSORBED INFLUENZA VIRUS[1, 2]

For the purpose of enhancing the antigenic activity of certain proteins, toxins and infectious agents a variety of adjuvants have been employed. As applied to influenza virus vaccines, Friedewald[3] has described the adjuvant effect of oily substances and acid-fast bacilli when combined with formalin-inactivated virus. In view of the local reactions resulting from subcutaneous injections of such mixtures in animals, Friede-

[11] Members and professional associates of the Commission on Acute Respiratory Diseases are: John H. Dingle, Major, M.C., A.U.S., Director; Theodore J. Abernethy, Major, M.C., A.U.S.; George F. Badger, Captain, M.C., A.U.S.; Joseph W. Beard, M.D.; Norman L. Cressy, Major, M.C., A.U.S.; A. E. Feller, M.D.; Irving Gordon, M.D.; Alexander D. Langmuir, Captain, M.C., A.U.S.; Charles H. Rammelkamp, Jr., M.D.; Elias Strauss, Captain, M.C., A.U.S.; and Hugh Tatlock, 1st Lieutenant, M.C., A.U.S.

[1] From the Department of Epidemiology and the Virus Laboratory, School of Public Health, University of Michigan, Ann Arbor.

[2] These investigations were aided through the Commission on Influenza, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army.

[3] Wm. F. Friedewald, SCIENCE, 99: 453, 1944.

wald has indicated the unlikelihood that the adjuvants he employed could be safely used in humans. Bodily, Corey and Eaton,[4] in a report of experiments with alum-precipitated influenza virus, did not note any adjuvant effect. The purpose of the present report is to record the results of preliminary studies indicating the enhancement and prolongation of the immunizing effect in mice of formalinized influenza virus when absorbed on calcium phosphate[5] and to describe certain other properties of such a preparation.

Allantoic fluid from chick embryos infected with the PR8 strain[6] of influenza virus, Type A, was used as a source of virus. The virus was rendered non-infectious in 48 hours by 0.05 per cent. formalin at 4° C. To each 100 cc of the formalinized fluid was added 1.5 cc of 1 M solution of calcium chloride and 1.5 cc of 1 N solution of sodium hydroxide. (In some fluids the addition of phosphate may be needed to precipitate all the calcium chloride as calcium phosphate at pH 8.0–8.5.) After two washings with 0.05 M phosphate buffer, pH 7.5, the precipitate was resuspended in a volume of the same buffer equal to the volume of the adsorbed fluid. The quantity of virus present in the various fractions was estimated by titrating the hemagglutinating capacity.[7] To do this it was necessary to release the virus absorbed on the calcium phosphate by dissolving the precipitate in citrate solution.[5] The hemagglutinin titers[8] of the formalinized allantoic fluid and the resuspended precipitate of calcium phosphate were 2,560, while the titer of the supernatant fluid after adsorption was 16. Hemagglutinin was not detected in the wash fluids.

The relative immunizing capacities of a single subcutaneous injection of the formalinized allantoic fluid suspension of virus and the calcium phosphate adsorbed virus were tested in mice. Three groups of young adult Swiss mice were selected. The animals in one group were each given 0.5 cc of the allantoic fluid suspension of virus subcutaneously in the region of the upper back; the mice in the second group were similarly treated with the suspension of virus adsorbed on calcium phosphate; a third group was set aside as untreated controls. At intervals of 4, 8 and 14 weeks after vaccination, mice from each of the three groups were tested for immunity to intranasal infection with graded doses of mouse-adapted PR8 virus. The results are shown in Table 1. Serum was obtained, 10 weeks after vaccination, from 5 mice in each group, to determine to what extent serum antibody titers reflected the difference observed in the degree of immunity of the mice inoculated with the respective preparations. It was of interest that the antibody titers,[8] as measured by the agglutinin-inhibition reaction,[7] were 256 and 512, in the animals vaccinated with free and adsorbed virus, respectively.

TABLE 1

IMMUNITY IN MICE FOLLOWING SUBCUTANEOUS INOCULATION OF CALCIUM PHOSPHATE—ADSORBED AND UNADSORBED FORMALINIZED INFLUENZA, TYPE A (PR8)

Interval between vaccination and infection	Vaccine	Intranasal test dose (*Virus dilutions*)						
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
4 weeks	Adsorbed	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0	.....	.....	.....	.....
	Unadsorbed	4,6,7,8, ++++,++	4,5,++,+,±,0	+,+,+,0,0,0	.....	.....	.....	.....
	Controls	.....	.....	.....	.....	5,5,5,5,6, ++++	6,6,7,8,10,+	10,++++,++, ++,++,0
8 weeks	Adsorbed	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0	.....	.....	.....
	Unadsorbed	4,4,5,5,5,+	5,5,++,+,+,±	5,5,7,++,++,+	6,+++,++,+, ±,0	.....	.....	.....
	Controls	.....	.....	.....	.....	5,6,6,7,7,7	9,++++,+++, ++,+,±	+++,+++,0, 0,0,0
14 weeks	Adsorbed	+,+,+,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0	0,0,0,0,0,0	.....	.....	.....
	Unadsorbed	.....	6,6,6,7,8,8	6,6,6,+++,+,+	+++,+++,++ +,±,0	+++,++,++, 0,0,0	.....	.....
	Controls	.....	.....	.....	4,5,5,5,6,6	4,5,5,6,6, ++++	9,++++,++++, +++,++,+	+++,+++,+, +,+,0

Numerals denote day of death of individual mice.
Symbols 0 to ++++ indicate degree of pulmonary involvement in survivors autopsied on 10th day after infection.

The sites of inoculation were examined for indications of inflammatory reaction; none were seen externally. On dissection, the precipitate of calcium phosphate was found freely movable in the subcutaneous tissue, without gross evidence of inflammation. Histological examination revealed a typical foreign-body reaction with marked reticulo-endothelial response. The deposit diminished in size in the course of four months, but had not disappeared at the end of this interval.

No untoward reactions were observed in 5 human

[4] H. L. Bodily, M. Corey and M. D. Eaton, *Proc. Soc. Exp. Biol. and Med.*, 52: 165, 1943.
[5] J. E. Salk, *Proc. Soc. Exp. Biol. and Med.*, 46: 709, 1941.
[6] T. Francis, Jr., SCIENCE, 80: 457, 1934.
[7] G. K. Hirst, SCIENCE, 73: 335, 1941.
[8] J. E. Salk, *Jour. Immunol.*, 49: 87, 1944.

subjects who were given a 1 cc injection, subcutaneously, of the calcium phosphate-virus suspension containing the virus adsorbed from 1 cc of formalinized allantoic fluid containing Type A virus and 1 cc of fluid containing Type B virus. The sites of inoculation were examined over a period of 18 days. The reactions observed were similar to those seen in 5 other subjects who had received a corresponding dose of allantoic fluid suspension of both viruses. The sharp stinging pain that followed the injection of the formalinized allantoic fluid did not occur after the injection of the adsorbed material which was free of formaldehyde.

The stability of the hemagglutinating capacity of the formalin-inactivated virus adsorbed on calcium phosphate and of the original formalinized allantoic fluid suspension of virus, stored at 4° C., room-temperature and 37° C., has been tested over a period of 4 months. The results are shown in Table 2. Whether

TABLE 2

STABILITY OF HEMAGGLUTINATING CAPACITY OF CALCIUM PHOSPHATE—ADSORBED AND UNADSORBED FORMALINIZED INFLUENZA VIRUS, TYPE A (PR8), AFTER STORAGE FOR 4 MONTHS AT DIFFERENT TEMPERATURES

Temperature	Preparations	Final dilutions								
		40	80	160	320	640	1280	2560	10,240 5120	
4° C.	*Adsorbed	+	+	+	+	+	+	+	±	0
	*Unadsorbed	±	+	+	+	+	+	+	±	0
Room temperature	Adsorbed	+	+	+	+	+	+	+	±	0
	Unadsorbed	+	+	±	0	0	0	0	0	0
37° C.	Adsorbed	+	+	+	+	+	+	0	0	0
	Unadsorbed	0	0	0	0	0	0	0	0	0

* Titers after 4 months at 4° C. were the same as at the start of the experiment.
Symbols: + = complete agglutination;
± = partial or slight agglutination;
0 = no agglutination.

the greater stability of the adsorbed-virus is due to removal from prolonged contact with formaldehyde or to some protective effect of adsorption will be determined.

Further studies are in progress to determine the mechanism of the adjuvant effect and the quantitative relationship between dosage and antigenic activity of free and adsorbed virus as measured by antibody response and immunity to infection. A more detailed report of these studies in other hosts as well as mice will be made.

JONAS E. SALK

PROLONGATION OF PENICILLIN ACTIVITY BY MEANS OF ADRENALIN[1]

THE rapid absorption and excretion of penicillin following its injection by various routes is well known, and interest has been shown in methods to prolong effective blood concentration levels of this antibiotic. Thus the use of penicillin in beeswax-peanut oil mixtures[2] and the application of ice bag chilling at the site of injection[3] have been reported as procedures which prolong penicillin absorption. It occurred to

AVERAGE BLOOD CONCENTRATION LEVELS OBTAINED IN RABBITS FOLLOWING THE INJECTION OF AQUEOUS AND ADRENALIN SOLUTIONS OF CALCIUM PENICILLIN

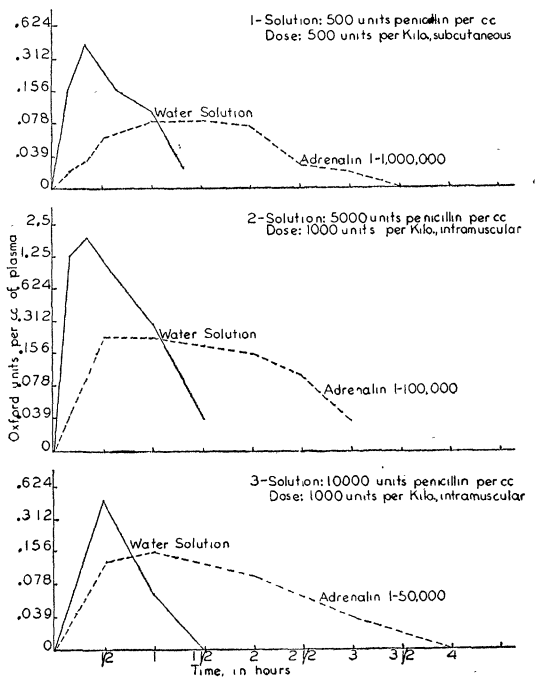

FIG. 1. (1) Each curve represents an average of values obtained from 10 determinations made on 5 rabbits. (2) Each curve represents an average of values obtained from 3 determinations made on 3 rabbits. (3) Each curve represents an average of values obtained from 5 determinations made on 5 rabbits.

the authors that adrenalin, because of its vasoconstricting properties, might afford a practical method for accomplishing this effect. The behavior of adrenalin is well established and the injection of this substance is not attended by the objectionable local tissue reactions[4] sometimes resulting from the use of vehicles such as vegetable oils which are being employed to hinder the absorption of certain therapeutic agents.

Tests were run by *in vitro* methods to determine

[1] From the Collis P. and Howard Huntington Memorial Hospital, Pasadena, and the Departments of Bacteriology and Pathology, School of Medicine, University of Southern California, Los Angeles, California. Valuable technical assistance was provided by Patrice Morrow.

[2] M. J. Romansky and G. E. Rittman, SCIENCE, 100: 196–198, 1944.

[3] M. Trumper and A. M. Hutter, SCIENCE, 100: 432–434, 1944.

[4] R. C. Page and E. J. DeBeer, *Am. Jour. Med. Sciences*, 205: 812–814, 1943.