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Effect of Adjuvants on Vaccination of Human Beings Against Influenza.*

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Immunization of human subjects against influenza Types A and B with adequate vaccines prepared from infected allantoic fluids of the chick have afforded protection against the natural or experimental disease for a limited period of time. In most instances the duration of the immune state has been found to correlate roughly with the height of the serum antibody level in the vaccinated individuals.[1] The titer reaches its peak about 2 weeks after vaccination and decreases thereafter more or less rapidly until the prevaccination level is reached, usually 4-12 months after immunization. This time relationship seems to hold regardless of the kind of vaccine used.[2] Therefore, the problem of protection against influenza lies not so much in the preparation and use of vaccines producing maximal antibody response in men, but rather in the maintenance of adequate antibody levels for a prolonged period of time.

The studies of Freund and his co-workers[3] have shown that the addition of certain adjuvants (lanolin-like substances and killed tubercle bacilli in paraffin oil) increase and prolong antibody production to various antigens in rabbits and guinea pigs. Friedewald[4] applied these findings to the immunization of animals against influenza. Recently Salk[5] reported the adjuvant effect of calcium phosphate on vaccination against influenza in mice. A study was begun in this laboratory in June, 1944, using influenza vaccines combined with adjuvants, except for the tubercle bacilli, in human beings and a brief summary of the results up to the sixth month is presented below.

Centrifugally concentrated influenza virus vaccine of Types A and B prepared according to the method of Stanley,[6] formed the stock material.† One part of this stock vaccine was emulsified in one part of Falba‡ to which 4 parts of mineral oil§ were added with constant agitation. Eighty female subjects received subcutaneously 0.3 ml of this vaccine (0.45 mg protein) either in a single or divided into 2 simultaneous injections. A similar group of 80 was injected with 0.3 ml of the same stock vaccine diluted 1:4 in buffered saline (0.7 mg of protein). All individuals were bled before vaccination and 2 weeks, 1, 2, 3, and 6 months thereafter. The sera were tested for their ability to inhibit chick red cell agglutination by influenza viruses A and B,[7] using a modification of Hirst's technic. The geometric mean antibody titers (initial serum dilution) were determined for the various groups. Since there was no difference between the subjects receiving the dose in one or 2 injections of

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2 Hirst, G. K., Rickard, E. R., Whitman, L., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1942, **75**, 495.

3 Freund, J., and McDermott, K., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 548; Freund, J., and Bonanto, M. V., *J. Immunol.*, 1944, **48**, 325.

4 Friedewald, W. F., *J. Exp. Med.*, 1944, **80**, 477.

5 Salk, J. E., *Science*, 1945, **101**, 122.

6 Stanley, W. M., *J. Exp. Med.*, 1945, **81**, 193.

† The vaccine was prepared by Dr. B. Hampil of Sharp and Dohme, Inc., for the Committee on Medical Research. It contained per ml 2.5 mg each of the PR-8 and Weiss strains of influenza A and 4 mg of the Lee strain of influenza B virus.

‡ Distributed by Pfalz and Bauer, Inc., New York.

§ Atreol No. 9, of the Atlantic Refining Company, U. S. P. For further information see Halbert, S. P., Smolens, J., and Mudd, S., *J. Immunol.*, in press.

7 Hirst, G. K., *J. Exp. Med.*, 1942, **75**, 49.

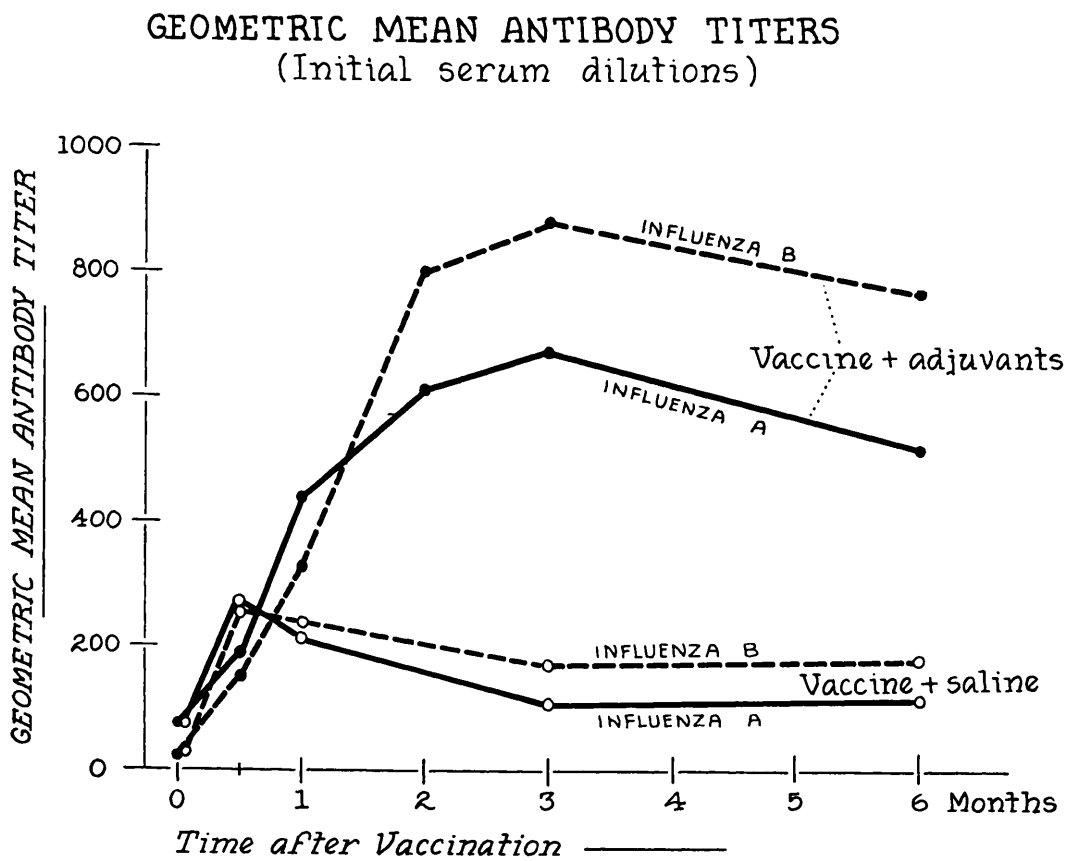

FIG. 1.
Geometric mean antibody levels over a period of 6 months following vaccination.

the Falba-mineral oil vaccine (FMV) the data of the two groups were combined. The results for the FMV and the saline vaccine (SV) series are presented in Fig. 1.

The titers in the SV series reached their peak in 2 weeks and fell gradually thereafter. In the FMV group the 2-weeks-result was inferior to that of the SV series but the titer continued to rise and reached its maximum in 2 to 3 months and only slight decreases were noted at the 6-month interval. The maxima of the FMV series were 2.5 to 3 times higher than the maxima of the SV series. By the time the FMV group reached its maximum the geometric mean antibody level was about 5 to 6 times higher than in the SV series at the corresponding time (3 months) and after 6 months the difference was still 4 to 5 fold.

Practically all individuals in the FMV series had antibody titers of more than 128 against both influenza A and B viruses and 93% and 96% respectively were still in this group at the end of 6 months. On the other hand in the SV series at this time 66% and 52% respectively had titers below this level. On the basis of experimental studies[1] one may consider individuals with titers of more than 128 as probably immune, those with lower levels as potentially susceptible. Sufficient data on this point regarding epidemics are lacking.

These results show that addition of Falba and mineral oil to influenza vaccines improves the antibody response in human beings and keeps the antibodies at a high level as compared with the results of vaccination with a slightly larger amount of antigen suspended in buffered saline. The technic of injection deserves further study and the optimal total and relative amounts of adjuvants and vaccine need to be determined. Nodules of varying

dimensions (up to 3 cm diameter) could be palpated in the subcutaneous tissues of most individuals. These decreased gradually in size and were still evident in roughly 40% of the cases 6 months after vaccination. In 2 cases abscesses were noted about 2 months after the injection, one of which had to be drained surgically. This number among 120 sites of injection is not too discouraging in the light of the early experience with alum precipitated toxoids.

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Effect of Amino Acids on Acetylcholine Synthesis.*

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In the presence of serum and spinal fluid more acetylcholine is synthesized by the frog brain *in vitro* than in the absence of these body fluids.[1,2,3] In search for the agents responsible for the greater acetylcholine synthesis the effect of amino acids was investigated.

Experimental. The effect of the substances used on the acetylcholine synthesis was studied by the method described previously.[2] Mixtures containing varying amounts of the substances, 100 mg minced fresh frog brain, 3 mg physostigmine salicylate, 4.8 mg glucose, and 3 cc Ringer's solution were shaken and incubated aerobically for 4 hours at 37°C. After incubation the amounts of acetylcholine synthesized were assayed biologically on the sensitized rectus abdominis muscle of the frog.

The amounts of acetylcholine synthesized are given in Table I. Most of the amino acids in the concentrations used (10^{-2} to 10^{-5} mol.) increased the amount of acetylcholine synthesized. The naturally occurring form of the amino acids seemed to increase the synthesis more than the naturally not occurring form.

Nachmansohn and John[4] under different experimental conditions (different enzyme preparation, different substrate, and anaerobically) found a similar increase of the amount of acetylcholine synthesized in the presence of *l* (+) glutamic acid, cysteine, alanine, phenylalanine, *l* (—) tryptophane, *l* (—) proline, and valine.

It was also found[5] that acetylcholine synthesis in the presence of serum from the working arm was 40% less than in the presence of serum from the immobilized arm. Since it is possible that during muscle work some transamination occurs[6] the effect of free ammonia on the synthesis of acetylcholine was also investigated. It was found that ammonia decreased the acetylcholine synthesis. Therefore, if ammonia accumulates during performance of muscle work it may depress the local acetylcholine synthesis, a synthesis known to occur during performance of prolonged work by cholinergic systems.[7,8] The accumulation of ammonia may thus contribute to the genesis of muscle fatigue.

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