Direction of Research on Vaccination against Influenza—New Studies with Immunologic Adjuvants*†

JONAS E. SALK, M.D., F.A.P.H.A., ANGELA M. LAURENT, AND MARY LYNCH BAILEY, M.D.

Virus Research Laboratory, Department of Bacteriology, School of Medicine University of Pittsburgh, Pittsburgh, Pa.

IN the years following the epidemic of influenza A in 1943, when vaccination reduced decisively the incidence of this disease, interest in the use of the vaccine mounted sharply at first and then declined abruptly. To explain this, in part, Figure 1 has been prepared as a summary of some of the recent experiences with vaccination against influenza. During the epidemic of influenza A in 1943, in the studies conducted by the Commission on Influenza,[1] attack rates of 7.1 per cent and 2.2 per cent respectively were observed in control and vaccinated groups. In 1945, during the epidemic of influenza B, attack rates of 11.2 per cent and 0.9 per cent respectively were recorded in unvaccinated and vaccinated populations. The latter figures represent the composite results of studies in military units under observation at the University of Michigan [2] and at Yale University.[3] In striking contrast to these findings, in which three times as many cases occurred in controls in 1943 and ten times as many in 1945, no significant difference was observed in 1947 in treated and untreated individuals in an outbreak caused by a virus of the type A group.[4-6] The absence of any effect in 1947, it is unanimously agreed, was due to the prevalence of a virus significantly different antigenically from the viruses contained in the vaccine. The type A antigens then represented in the vaccine, although highly active in the formation of homologous antibody in vaccinated human subjects, induced little or no antibody for the 1947 viruses; [4] tentatively, for convenience, strains related to the 1947 viruses have been referred to as type A-prime.

Although the principle was established firmly in 1943 and 1945 that vaccination could be effective in reducing the incidence of influenza, the 1947 experience raised questions that remain to be answered. The antigenic composition of the vaccine was broadened for the following year to include coverage of the portion of the type A spectrum heavily represented in the A-prime strains of 1947, and in the spring of 1948 it was observed [7] that a greater measure of protection was afforded by the new vaccine when compared with the earlier preparation.

Before discussing the problem of antigenic variation it is desired to draw attention to another question that will be considered in more detail later. The observation has been made repeatedly, not only in the field trials of 1943 and

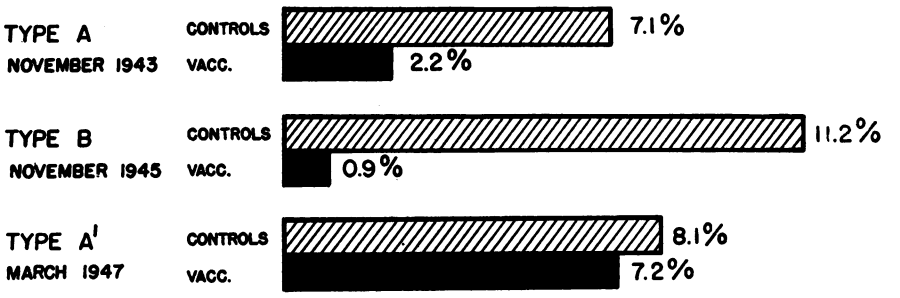
* Presented before the Epidemiology Section of the American Public Health Association at the Seventy-eighth Annual Meeting in St. Louis, Mo., October 31, 1950.

† This investigation was conducted under the auspices of the Commission on Influenza, Armed Forces Epidemiological Board, Office of the Surgeon General, United States Army, Washington, D. C.

FIGURE 1

INCIDENCE OF INFLUENZA IN CONTROL AND VACCINATED GROUPS.

FIELD STUDIES BY COMMISSION ON INFLUENZA



1945 (Figure 1) but in studies conducted with vaccines of broader antigenic coverage prepared subsequent to 1947, that not all vaccinated individuals are protected. This observation is related to one of the most prominent of the problems still to be resolved—that of enhancing the effectiveness of the procedure for prophylaxis to effect not merely a statistically significant reduction in the incidence of disease among treated individuals but to furnish distinct protection for all vaccinated persons. Under such circumstances the administration of vaccine to a sufficient proportion of a population could be expected to prevent the epidemic phenomenon.[8] In addition, of course, it would be desirable to be able to maintain the period of effective resistance for more than one season.

The importance of these problems has been recognized for a long time and their solution is a logical extension of developments to the present. However, attention to these questions has, to a great extent, been overshadowed since 1947 by problems related to the existence of diverse antigenic varieties, within the A type especially. In view of the importance of the question of antigenic variation, it is desired to comment briefly upon this aspect of the problem of influenza immunization before discussing those questions dealing with enhancement of the effectiveness of vaccines.

ANTIGENIC VARIATION

There exists among some an attitude of futility regarding the possibility of ever devising a suitable preventive against influenza. This attitude is based largely upon the interpretation of the 1947 occurrence and subsequent events[9, 10] as indicating that the virus of influenza A is constantly changing its antigenic coat of many colors and that we will always be vaccinating against the agent that prevailed the year before. Upon critical reflection this view appears to be rather shortsighted and one formulated without due consideration of all of the pertinent facts.

When one considers that but two distinct immunologic types have been identified as the cause of human disease, in almost two decades since the discovery of the viruses of swine[11] and human influenza,[12-14] it would appear that the capacity possessed by these agents for the development of major variants is limited. If the contrary were true, one

might have expected that a myriad of unrelated antigenic varieties would have developed in the course of human history, many of which might have been expected to have persisted and certainly to have been encountered in the interval since the influenza viruses were first discovered. In further support of the view that the extent is limited to which antigenic variation might occur, is the evidence that antigenic variants which develop in the course of laboratory passage differ from the parent culture in a measurable but only minor degree.[15-17] No one has ever encountered a major laboratory variant, the antigenic properties of which extend outside the presently known limits of the spectrum for the two recognized types. As for variations in pathogenicity of influenza viruses for man or laboratory animals, it appears that such changes occur without corresponding changes in antigenic character; and information is inadequate in regard to association of particular antigenic varieties and the more severe epidemics or pandemics.

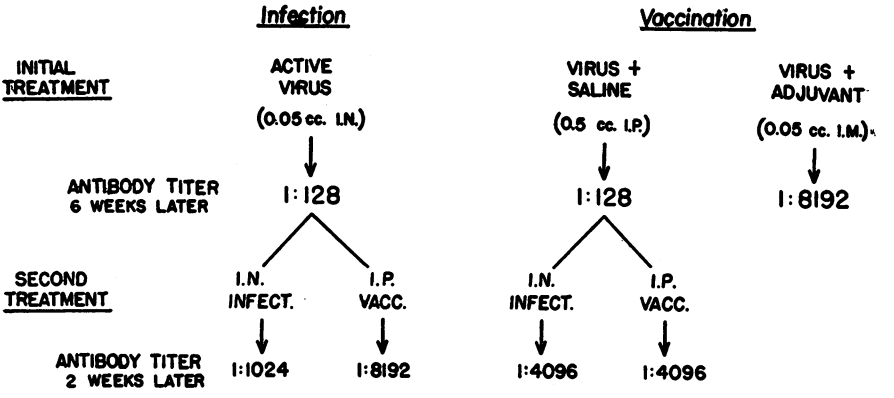
As a working hypothesis it might be suggested that there exists a finite, but as yet undetermined, number of antigenic facets characteristic of each of the two types of influenza viruses and that these are arranged in different combinations in the strains that differ from one another. To this postulate it would not be unreasonable to add that in a culture of any one strain it is probable that the virus population is more or less heterogeneous and that one particular combination of antigenic components predominates to give the "strain" its characteristic behavior or properties. A corollary of this might be that under suitable circumstances of selection, through the suppression of particles that are in the majority or through the operation of some factor favoring certain of the virus particles that are in the minority, the antigenic behavior or other characteristics of the "strain" might change. If the view is correct that a finite number of antigenic components exist, and that the strains that occur from time-to-time are restricted in their capacity to vary within definite limits characteristic of the particular type, it would seem that we have at our disposal the means for synthesizing a vaccine preparation that could be expected to be effective against future strains. From this viewpoint, future strains would consist merely of different combinations of the various antigenic components belonging to each type.

Whether or not this hypothesis is valid, will be revealed in time; however, it is quite in accord with the mechanism of the behavior of other infectious agents and provides a more hopeful outlook for the ultimate control of influenza by vaccination than does the concept expressed recently by several workers of constant change with the development of new varieties and disappearance of the old.[9, 10]

It is evident that the ultimate goal in the continued study of influenza immunization is the synthesis of an immunizing agent effective against all antigenic varieties about which we now know; and for the development of such a preparation to induce an effective lasting immunity. For achieving this objective, it will be necessary to maintain a continued search for influenza viruses in interepidemic periods as well as in well defined outbreaks, and then to match immunologically current viruses to strains included in vaccines of the broadest antigenic composition. Within limits, differences among strains determined by comparison of serum from immunized animals may not necessarily reflect differences of significance for vaccination of man. For evaluating recently isolated strains for significance of antigenic differences demonstrable in laboratory animals, serologic studies have been made recently in man, in collaboration with Colonel Adam J. Rapalski. From

FIGURE 2

COMPARISON OF ANTIBODY RESPONSE IN MICE TO INFECTION AND TO VACCINATION.



these investigations it does not seem that it will be difficult to select a group of strains for inclusion in a vaccine that will encompass the complete antigenic spectrum of the known influenza A and influenza B viruses. These studies are to be reported separately; for this reason, the remainder of this paper will be concerned with studies directed toward enhancing the immunizing effect of the vaccine.

PROCEDURES FOR ENHANCING VACCINE EFFECTIVENESS

From the very beginning of studies on influenza immunization, both in animals and in man, the desirability for achieving the highest possible levels of antibody was thoroughly appreciated. It has been assumed, and with good reason, that the elevated levels of antibody are associated with a corresponding enhancement of immunizing effect.[18-21] Before describing current studies it is desired to mention the various procedures that have been explored to raise the titer of serum antibody to the highest levels possible. The methods employed are as follows: (1) multiple inoculations,[22] (2) increasing the concentration of virus in the vaccine,[23] (3) intracutaneous inoculation,[24] (4) selection of strains of high antigenic capacity as well as broad antigenic coverage,[25] and (5) the use of adjuvants.[26] All but the last of the five procedures listed have been tried with little success beyond that which can be achieved with the vaccines and the procedures presently in use.

In regard to the use of adjuvants, it will be recalled that Friedewald [27] and the Henles [28] applied, in mice and man respectively, a method described by Freund [29] involving the emulsification of antigens with mineral oil in an effort to enhance the antigenic effectiveness of influenza virus vaccine. This approach was abandoned, however, because of the occurrence of undesirable local reactions, not only in experimental animals but in human subjects as well.

NEW STUDIES WITH IMMUNOLOGIC ADJUVANTS

The desirability of pursuing the question of the applicability of mineral oil

TABLE 1

Development and Persistence of Antibody in Monkeys Vaccinated Intramuscularly with Influenza Virus (PR 8) Combined with Adjuvant or with Saline

Interval Post Vacc.	Monkey No.	Hemagglutination-Inhibition Antibody Titers in Monkeys Inoculated with Adjuvant Vaccine *			Saline Vaccine †		
		1	2	3	4	5	6
Pre-vacc.		<8	<8	<8	<8	<8	<8
1 week		256	256	256	16	16	8
2 weeks		2,048	2,048	2,048	64	64	64
4 weeks		4,096	4,096	2,048	128	128	8
6 weeks		32,000	8,000	8,000	128	128	<8
2 mos.		65,000	16,000	16,000	128	64	<8
3 mos.		65,000	32,000	Dead	64	64	<8
4 mos.		32,000	32,000	—	64	64	<8
6 mos.		16,000	16,000	—	64	64	<8

* 1 ml. of a mixture of equal parts of PR 8 vaccine and Adjuvant — mineral oil (Bayol F) + emulsifying agent (Arlacel A).

† 1 ml. of a mixture of equal parts of PR 8 vaccine and saline.

adjuvants to the solution of the problem of an effective vaccine for man is amply clear. It seemed to us, therefore, that some way must be found to circumvent the undesirable local reactions encountered by other investigators. In the course of certain other studies [30] it was observed that no local reactions occurred using Arlacel A [31] rather than Falba [27, 28] as the emulsifying agent, and a light mineral oil called Bayol F rather than the heavy mineral oil used earlier. It now appears that the undesirable local reactions may have been due in part to the reagents employed and in part to the fact that the preparations were administered subcutaneously rather than intramuscularly as has been done in these studies. These changes in procedure have permitted an intensive reinvestigation of the possible application of mineral oil adjuvants to the immunization of man against influenza.

Before presenting a summary of some recent experimental data, it is desired to illustrate the effect upon antibody response to *infection* as compared with response to *vaccination* and the difference between vaccination with a simple antigen as compared with an antigen combined with an adjuvant. In Figure 2 it can be seen that a single intranasal inoculation of active virus is no more effective in inducing antibody formation than is vaccine given intraperitoneally. Furthermore, a solid immunity is not engendered either by the intranasally induced infection or by the intraperitoneally administered vaccine. This is indicated by the second antibody rise after the second treatment repeated six weeks later. The high level of antibody following a single antigenic stimulus with virus and adjuvant is shown for comparison with the effect of the other procedures.

STUDIES IN MONKEYS

As a preliminary to studies in man, a series of investigations was begun in monkeys at our disposal after their usefulness for studies on poliomyelitis was exhausted. The initial experiments were designed to determine whether or not undesirable reactions would result and to evaluate the degree of effectiveness of the virus-oil emulsion in stimulating the formation of antibody. It was of interest also to determine the smallest amount of virus that, when incorporated with the oil, would be effective in antibody formation. These experiments will be reported in detail elsewhere; however, it is desired to indicate the trends that have been observed.

Table 1 shows the development and

TABLE 2

Hemagglutination-Inhibition Antibody Titers in Serum of Individual Monkeys 8 Weeks After Vaccination with Diminishing Quantities of Virus Combined with a Constant Amount of Adjuvant (First Expt. 1.0 ml. I.M.)

Vaccine (1.0 ml. I.M.)	Quantity of Stock Vaccine Incorporated in Inoculum * for Each Group				
	0.50 ml.	0.15 ml.	0.05 ml.	0.015 ml.	0.005 ml.
Virus	65,000	65,000	8,000	1,024	2,048
+	16,000	16,000	4,096	1,024	1,024
Adjuvant	16,000	8,000	2,048	512	512
				Controls	
Virus	128		16	<8	
+	64		8	<8	
Saline	<8		<8	<8	

* Virus Diluted Serially (½ log steps) and then incorporated with oily adjuvant.

persistence of antibody in monkeys in the six months following inoculation of virus with adjuvant or virus and saline. The marked enhancement in antibody response to the virus-adjuvant combination is strikingly evident; as is the degree of persistence of the elevated antibody titer. The similarity of these data with those published by the Henles,[28] on their experiments in man, is quite striking. The vaccines employed in these studies were inoculated into the calf muscle and at no time was there any impairment of function nor clinically discernible evidence of inflammatory reaction. Although a total of more than seventy monkeys was involved in this and other experiments, evidence of reaction was strikingly absent, in contrast to the observations made by the Henles who encountered subcutaneous nodules in almost all of their human subjects and abscesses in two out of eighty instances. Detailed histologic studies are in progress; however, it can be reported now that no gross evidence of inflammation was evident upon sectioning muscles at intervals up to four weeks following inoculation. With inocula of 0.1 ml. used in some experiments, the site of deposition of the emulsion cannot always be found.

To determine the smallest quantity of virus that will induce a measurable antibody response, two experiments have been carried out thus far. Table 2 shows the results of one such experiment where it can be seen that the quantity of antigen inoculated was reduced to the equivalent of 0.005 ml. of vaccine and still induced a high level of antibody. In another experiment, in which 0.1 ml. of emulsion was inoculated, antibody formation was still demonstrable with a quantity of antigen equivalent to 0.000005 ml. of vaccine. In contrast to this, vaccine without adjuvant in dilutions below 0.05 ml. was no longer effective.

Since the data shown in Tables 1 and 2 represent the results of antibody titrations determined by the agglutination-inhibition reaction, it was of interest to see to what extent the high levels of antibody detected by this *in vitro* method reflect virus neutralizing activity measured *in vivo*. The close correspondence of the titers of agglutination-inhibition antibody and neutralizing antibody measured *in ovo* is shown in Table 3.

The studies in monkeys reported very briefly here are being extended. In addition, these investigations are being carried into other experimental animals where quantitative data can be obtained regarding the relationship between the high levels of antibody achieved and the degree of immunity to infection.

TABLE 3

Serum Antibody Titer Measured by Hemagglutination-Inhibition and Virus-Neutralization in ovo

Vaccine	Monkey	Antibody Titer	
		*Hemaggl.-Inhib.**	*Virus-Neutral.†*
Virus	1	65,000	200,000
+	2	32,000	80,000
Adjuvant	3	16,000	16,000
Virus	4	128	56
+	5	128	32
Saline	6	64	<5

* Highest dilution of serum inhibiting 4 units of hemagglutinin.
† Fifty per cent endpoint of neutralization vs. 1,000 EID_{50}.

TABLE 4

Experiments with Adjuvants in Progress in Human Subjects

Expt.	*Material Inoc.*	*Vol. Inoc. ml.*	*Quantity of Virus Inoc. CCA units**	*No. of Subjects*	*Reaction*
I	Virus + Adjuvant	0.1	15	12	None
	Adjuvant alone	0.1	0	12	None
II	Virus + Adjuvant	0.25	300	6	None
	Virus + Adjuvant	0.10	120	5	None
III	Virus + Adjuvant	0.25	300	46	None or minimal and transient
	Virus + Saline	0.25	300	30	

* CCA content of new standard vaccines is 500 units/ml.

STUDIES IN MAN

After observing the safety and effectiveness of the preparations employed in monkeys, a series of studies in human subjects were begun to determine the extent to which the observations made in the experimental animal also applied to man. Table 4 contains certain details of the first three experiments. In the initial trial, 0.1 ml. of a mixture of equal parts of adjuvant and vaccine, containing 500 CCA units per ml., was injected intramuscularly into one arm and a similar volume of saline plus the adjuvant was injected into the other arm. In twelve individuals there was neither subjective nor objective evidence of inflammation.

The same technique of inoculation was employed in a second experiment in which 0.1 ml. or 0.25 ml. of a mixture of virus plus adjuvant was administered in two different groups. This time the preparation of virus employed consisted of a mixture of the PR8, FM1 and Lee strains and contained approximately 2,500 CCA units per ml.* When it was observed that no untoward effects occurred, a third experiment was set up in which one group was given 0.25 ml. of the emulsion containing equal parts of the concentrated virus preparation and adjuvant, and the other group was given 0.25 ml. of an inoculum consisting of the concentrated virus preparation mixed with an equal volume of saline. In this group, again, local reactions were absent or minimal. The minimal reactions consisted of an awareness for a day or two that an injection had been received. It was less marked in the group given the virus-adjuvant mixture than the group given virus in saline. Examination two

* This highly concentrated preparation was obtained through the kindness of Dr. I. W. McLean, Jr., Parke, Davis and Company.

weeks after inoculation revealed no abnormalities.

Although the serologic tests indicate a distinctly greater antibody response in the virus-adjuvant group as compared with the virus-saline group, the results are not presented at this time because a sufficient interval has not yet elapsed for the antibody level to reach a plateau in the groups given virus plus adjuvant. The results are such that testing on a much larger scale is already under way to establish further the safety as well as the limits of effectiveness of this procedure.

CONCLUDING REMARKS

It is the purpose of this paper, in part, to review from one perspective the present status and future prospects of influenza immunization and to introduce a hopeful note, with new data, to counter the pessimism that has prevailed abroad for a long time, and in this country since 1947. It is evident that progress has been made and that this progress, which began before 1947, was not negated but rather was furthered by the experience of that year. It is evident, also, that much remains to be done before the problem of influenza immunization is brought to conclusion.

To test the hypothesis that a finite number of antigenic varieties exist, search will continue for virus strains that occur sporadically in interepidemic periods as well as for strains isolated during epidemics to determine the relationship between the new outcroppings and the viruses included in a vaccine designed to cover the known antigenic spectrum of type A and type B viruses.

Field trials which are now in progress will be continued into the future for the purpose of testing under natural conditions the protective effect of any new vaccine preparations that appear safe and more effective.

In regard to the studies with adjuvants, additional work will be required before the most effective combination of virus and adjuvant will be devised and tested on a wider scale. Nevertheless, prospects are hopeful for a preparation that will induce consistently high levels of antibody that will be maintained for much longer periods than occur with preparations in use at present.

REFERENCES

1. (a) Members of 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. A Clinical Evaluation of Vaccination against Influenza. A preliminary report. *J.A.M.A.* 124:982, 1944.
   (b) Members of 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. The 1943 Studies of Vaccination against Influenza: A series of seven papers. *Am. J. Hyg.* 42:1, 1945.
2. Francis, Thomas, Jr., Salk, Jonas E., and Brace, William M. The Protective Effect of Vaccination against Epidemic Influenza B. *J.A.M.A.* 131:275, 1946.
3. Hirst, George K., Vilches, A., Rogers, O., and Robbins, Clarence L. The Effect of Vaccination on the Incidence of Influenza B. *Am. J. Hyg.* 45:96, 1947.
4. Francis, Thomas, Jr., Salk, Jonas E., and Quilligan, J. J., Jr. Experience with Vaccination against Influenza in the Spring of 1947. A preliminary report. *A.J.P.H.* 37:1013, 1947.
5. Sigel, M. M., Shaffer, F. W., Kirber, M. W., Light, A. B., and Henle, W. Influenza A in a Vaccinated Population. *J.A.M.A.* 136:437, 1948.
6. Loosli, C. G., Schoenberger, J., and Barnett, G. Results of Vaccination against Influenza During the Epidemic of 1947. *J. Lab. & Clin. Med.* 33:789, 1948.
7. Salk, Jonas E., and Suriano, Philip C. Importance of Antigenic Composition of Influenza Virus Vaccine in Protecting against the Natural Disease. Observations during the Winter of 1947–48. *A.J.P.H.* 39: 345, 1949.
8. Salk, Jonas E. The Control of Influenza by Immunization. *Lancet* 67:18, 1947.
9. (a) Andrewes, Christopher H. Adventures Among Viruses. II. Epidemic Influenza. *New England J. Med.* 242:197, 1950.
   (b) Andrewes, Christopher H. Influenza in Perspective. *Edinburgh M. J.* 56:337, 1949.
10. Hilleman, M. R., Mason, R. P., and Rogers, N. G. Laboratory Studies on the 1950 Outbreak of Influenza. *Pub. Health Rep.* 65:771, 1950.
11. Shope, R. E. Swine Influenza. III. Filtration Experiments and Etiology. *J. Exper. Med.* 54:373, 1931.
12. Smith, W., Andrewes, C. H., and Laidlaw, P. P. Virus Obtained from Influenza Patients. *Lancet* 2:66, 1933.
13. Francis, Thomas, Jr. A New Type of Virus from Epidemic Influenza. *Science* 92:405, 1940.
14. Magill, Thomas P. A Virus from Cases of Influenza-Like Upper Respiratory Infections. *Proc. Soc. Exper. Biol. & Med.* 45:162, 1940.
15. Burnet, F. M., and Bull, D. R. Re-Examination of the Influenza Virus Strain "Melbourne Egg." *Australian J. Exper. Biol. & M. Sc.* 22:173, 1944.

16. Francis, Thomas, Jr. Apparent Serological Variation within a Strain of Influenza Virus. *Proc. Soc. Exper. Biol. & Med.* 65:143, 1947.
17. Hirst, George K. Studies on the Mechanism of Adaptation of Influenza Virus to Mice. *J. Exper. Med.* 86:357, 1947.
18. Henle, W., Henle, G., Hampil, B., Maris, E. P., and Stokes, J., Jr. Experiments on Vaccination of Human Beings against Epidemic Influenza. *J. Immunol.* 53:75, 1946.
19. Francis, Thomas, Jr., Salk, Jonas E., Pearson, Harold E., and Brown, Philip N. Protective Effect of Vaccination against Induced Influenza A. *J. Clin. Investigation* 24:536, 1945.
20. Salk, Jonas E., Pearson, Harold E., Brown, Philip N., and Francis, Thomas, Jr. Protective Effect of Vaccination against Induced Influenza B. *J. Clin. Investigation* 24:547, 1945.
21. Salk, Jonas E., Menke, W. J. Jr., and Francis, Thomas, Jr. A Clinical, Epidemiological and Immunological Evaluation of Vaccination against Epidemic Influenza. *Am. J. Hyg.* 42:57, 1945.
22. Beveridge, W. I. B. Lack of Increase in Antibody After Second Injection of Influenza Virus in Man. *Australian J. Exper. Biol. & M. Sc.* 22:301, 1944.
23. Salk, Jonas E. Reactions to Concentrated Influenza Virus Vaccines. *J. Immunol.* 58:369, 1948.
24. Bruyn, Henry B., Meiklejohn, Gordon, and Brainerd, Henry D. Influenza Vaccination: A Comparison of Antibody Response Obtained by Various Methods of Administration. *J. Immunol.* 62:1, 1949.
25. Salk, Jonas E., Laurent, Angela M., and McGinnis, Rachel C. Differences in Antigenic Capacity among Influenza Virus Strains, *Federation Proc.* 8:410, 1949.
26. Freund, Jules. Some Aspects of Active Immunization. *Ann. Rev. Microbiol.* 1:291, 1947.
27. Friedewald, W. F. Adjuvants in Immunization with Influenza Virus Vaccines. *J. Exper. Med.* 80:477, 1944.
28. Henle, W., and Henle, G. Effect of Adjuvants on Vaccination of Human Beings against Influenza. *Proc. Soc. Exper. Biol. & Med.* 59:179, 1945.
29. Freund, Jules, and McDermott, Katherine. Sensitization to Horse Serum by Means of Adjuvants. *Proc. Soc. Exper. Biol. & Med.* 49:548, 1942.
30. (a) Salk, Jonas E., Lewis, L. James, Bennett, Byron L., Youngner, J. S., and Bubash, George R. The Use of an Adjuvant to Facilitate Studies on the Immunologic Classification of Poliomyelitis Viruses. *Bact. Proc.* p. 80, 1950.
(b) Salk, Jonas E., and Youngner, J. S. Antibody in Yolk of Eggs and in Serum from Hens Inoculated with Viruses of Influenza and Poliomyelitis. *Federation Proc.* 9:390, 1950.
31. Freund, J., Thomson, J. J., Hough, H. B., Sommer, H. E., and Pisani, T. M. Antibody Formation and Sensitization with the Aid of Adjuvants. *J. Immunol.* 60:383, 1948.