

STUDIES IN EGG WHITE ANTIGEN CONTENT OF INFLUENZA VIRUS VACCINES

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IT IS WELL KNOWN that egg proteins are found in viral and rickettsial vaccines and that the use of such vaccines in egg-sensitive persons may result in allergic reactions. The manifestations which have been reported encompass nearly the entire spectrum of allergic phenomena.^{1, 2} The incidence of such reactions following the administration of egg-contaminated vaccines has been reported to be about 0.5 per cent of the general population.² A severe constitutional reaction from a test dose (0.02 ml.) with influenza virus vaccine has been reported.³

Stull⁴ studied the egg protein content of viral and rickettsial vaccines by passive-transfer tests with egg reagin-bearing sera. Single passive-transfer tests were performed by him with each antigenic substance and with each antiserum. Ratner and Untracht² were able to show by random direct skin testing that the vaccines prepared by red blood cell elution method contained the greatest amount of egg proteins, and elicited larger skin reactions than vaccines prepared by Sharples centrifugation or by alcohol precipitation. In a recent publication by Cohen and Mines,⁵ a marked variation in the egg white and egg yolk components of viral and rickettsial vaccines was demonstrated. These studies were performed with anti-egg rabbit sera in agar diffusion plates by the Ouchterlony technique.

The purposes of the present study are to determine, by means of the passive-transfer method in adequate trials, the amount of egg white antigen which is present in commercially available influenza virus vaccines, and to compare their egg white antigen content. The authors are aware that influenza virus vaccine contains both egg white and egg yolk antigen components. Because of the unavailability of a fully satisfactory egg yolk extract, we have confined our report to egg white as the indicator of egg antigen content of the vaccine.

METHODS AND MATERIALS

Sera obtained from two patients (S and R) which contained reagins to egg were used for the passive-transfer studies. Clinically, egg-containing foods

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caused asthmatic attacks and bouts of diarrhea in Patient S and initiated intense pruritic episodes in Patient R. Direct intracutaneous testing with egg white extract in a concentration of 0.001 mg. N per milliliter caused a marked (4+) skin reaction in both patients. Further indication of the potency of the sera was evidenced by the fact that passively sensitized skin sites flared up after ingestion of egg or fowl by the recipients. Neither serum contained reagins to penicillin.

The egg white extract was prepared by the Allergy Laboratory of The Jewish Hospital of Brooklyn. For study purposes, it was prepared with buffered saline in tenfold dilutions, the testing strengths varying from 1 mg. N per milliliter to 0.0000001 mg. N per milliliter.

The influenza virus vaccines were obtained commercially. Three basic methods are used to concentrate the virus. The red blood cell elution method entails concentration of the virus by adsorption on and elution from red blood cells of chick embryo (brands A and E). By the Sharples centrifugation method, concentration of the virus is accomplished by differential ultracentrifugation (brands B and D). With the protamine sulfate precipitation method, concentration is achieved by precipitation with protamine sulfate and separation by ultracentrifugation (brand C). The vaccines were polyvalent or monovalent and manufactured by Eli Lilly and Co., Parke, Davis and Co., Merek Sharp & Dohme, Lederle Laboratories, and Pitman-Moore Co. For testing purposes the vaccines were prepared in tenfold dilutions with buffered saline up to a dilution of 1:1,000,000.

Eight to eighteen skin sites were prepared on the arms of forty-three nonallergic recipients by the intracutaneous injection of 0.05 ml. of serum. Each recipient was instructed to maintain a diet free of egg and fowl for twenty-four hours prior to preparation of the skin and for the duration of the tests. Sites were tested three to four days after sensitization. When the same recipients were resensitized for another passive-transfer test, the opposite and previously unused arm was prepared after a rest period of one week.

Passive-transfer testing was started with high dilutions of both egg white and influenza virus vaccine. If no reaction was elicited, testing was continued successively with tenfold stronger solutions of the antigens at different sites. About 0.01 ml. of antigen was injected intracutaneously into the passively sensitized sites and into adjacent normal skin as controls. After fifteen minutes, the reactions at the test and control sites were graded according to the size of the wheal and surrounding erythema. The smallest amount of antigenic material which elicited a definite passive-transfer reaction on a sensitized site and a negative control test was considered to be the threshold level of that antigen for the particular recipient. The threshold responses for each test substance were determined on numerous subjects in order to ascertain the reaction pattern of the majority of recipients. Finally, the reaction patterns for egg white and for the five brands of vaccine were compared, and the equivalent content of egg white in the various vaccines estimated.

RESULTS

The threshold reaction for egg white was determined in twenty-eight recipients sensitized with undiluted S serum. An egg white solution containing 0.00001 mg. N per milliliter was found to be the lowest concentration which could elicit a positive passive-transfer reaction in eighteen of the twenty-eight recipients (64 per cent) sensitized with this serum (Fig. 1). Seven recipients (25 per cent) showed a threshold response at the level of 0.0001 mg. N per milliliter, while one recipient each showed threshold responses at 0.0000001 mg. N, 0.000001 mg. N, and 0.001 mg. N per milliliter, respectively.

MINIMAL AMOUNTS OF EGG WHITE REQUIRED TO ELICIT PASSIVE TRANSFER REACTIONS WITH UNDILUTED EGG- SENSITIVE SERA.

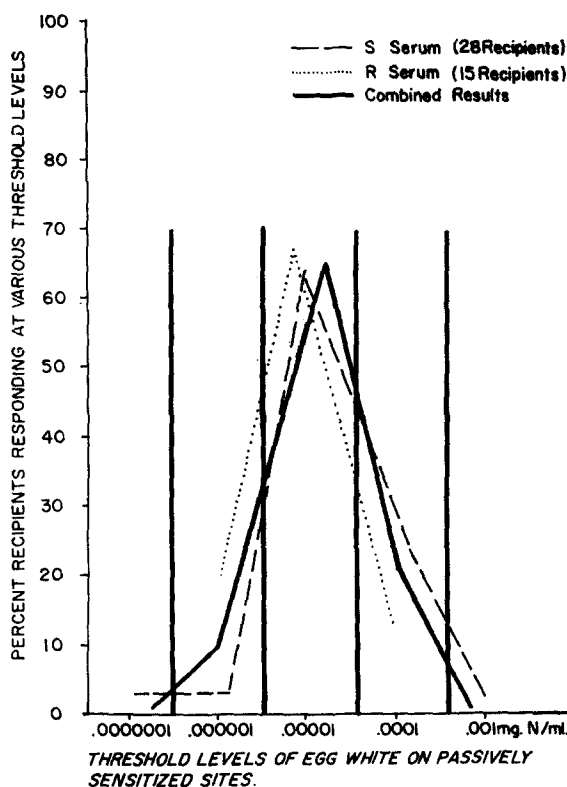


Fig. 1.

Serum R was studied in a similar manner in fifteen recipients. In ten of the fifteen subjects (66 per cent), the concentration of egg white solution required to elicit a threshold reaction was 0.00001 mg. N per milliliter (Fig. 1). Three recipients showed a threshold response at the level of 0.0000001 mg. N per milliliter. In two recipients, the threshold response was elicited with egg white solution containing 0.0001 mg. N per milliliter.

As revealed in Fig. 1, the distribution curve of threshold responses to egg white with R serum is almost identical to that obtained with S serum. It seemed reasonable, therefore, to combine the results obtained with both sera. The results thus represent the findings obtained in forty-three recipients.

Tests were then done with serial tenfold dilutions of the five brands of influenza virus vaccines on sites sensitized with undiluted sera S and R. The results are portrayed in Fig. 2. Of forty-three recipients tested with dilutions of brand A, nineteen (44 per cent) revealed a threshold response with a 1:10,000 dilution, and 20 (46 per cent) reacted with a 1:1000 dilution. Of thirty-

MINIMAL DILUTIONS OF VARIOUS BRANDS OF INFLUENZA VIRUS VACCINE REQUIRED TO ELICIT PASSIVE TRANSFER REACTIONS WITH UNDILUTED EGG-SENSITIVE SERA (R & S).

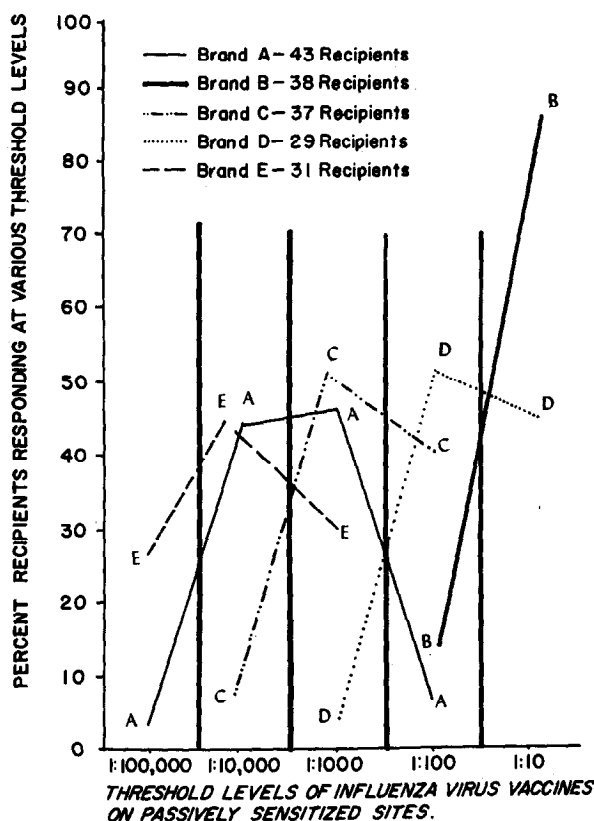


Fig. 2.

eight recipients tested with brand B, five (13 per cent) showed a threshold response to the 1:100 dilution and thirty-three (87 per cent) reacted to the 1:10 dilution. With brand C, the threshold response in nineteen of thirty-seven recipients (51 per cent) occurred with the 1:1000 dilution, and in fifteen recipients (40 per cent) with the 1:100 dilution. Among the twenty-nine recipients tested with brand D, the minimal dilution of vaccine which elicited

a passive transfer reaction was 1:100 in fifteen recipients (51 per cent) and 1:10 in thirteen recipients (45 per cent). With brand E, eight of thirty-one recipients (26 per cent) showed a threshold response at the level of 1:100,000 dilution, fourteen (45 per cent) responded minimally at the 1:10,000 level, and nine (29 per cent) responded at the 1:1000 level.

The responses of the majority of the recipients to the various brands of influenza virus vaccines formed several distinct patterns. Brands A and E, prepared by the chick red blood cell elution method, reacted mainly in the 1:10,000 to 1:1000 zone. Brands B and D, prepared by the Sharples centrifugation method, reacted predominantly in the 1:100 to 1:10 zone. Brand C, prepared by the protamine sulfate precipitation method, reacted in the 1:1000 to 1:100 zone.

By comparing the threshold reactions for egg white obtained in the majority of the recipients with those for the vaccines, it was estimated that the approximate content of egg white antigen in the vaccines is the following:

Brands B and D	—0.0001–0.001	mg. N per ml.
Brand C	—0.001 –0.01	mg. N per ml.
Brands A and E	—0.01 –0.1	mg. N per ml.

DISCUSSION

Of the various antigenic components in vaccine of chick embryo origin, egg white has been shown to be the most active.⁴ Thus, this antigen was used in these studies to indicate the approximate content of egg protein in various brands of influenza virus vaccine.

In previous reports on passive-transfer studies dealing with the content of egg protein in typhus vaccine, single passive-transfer tests were employed for each antigenic substance.⁴ In view of the fact that recipients are known to vary in their responses to passive-transfer tests, it was considered necessary to employ numerous subjects in these semiquantitative studies.

The minimal concentration of egg white extract necessary to elicit a positive passive-transfer reaction on sites sensitized with two comparable reaginic sera was determined in forty-three nonsensitive subjects. In 65 per cent of these recipients, the threshold level for egg white was 0.00001 mg. N per milliliter.

The threshold passive-transfer response to influenza virus vaccine was then determined with dilutions of five brands of vaccine in numerous subjects. Although threshold reactions ranged from 1:10 to 1:100,000 dilutions of the various brands, definite patterns of response were evident, which seemed related to the method employed in concentrating the virus during preparation of the vaccine. Brands A and E, which were concentrated by the red blood cell elution method, responded predominantly to the 1:10,000 to 1:1000 dilutions. Brands B and D, which were concentrated by the Sharples centrifugation method, responded predominantly at dilutions of 1:100 to 1:10. Brand C, concentrated by the protamine sulfate precipitation method, responded mainly at the 1:1000 and 1:100 levels. By comparing the threshold responses obtained in the majority of recipients to egg white, the approximate amount of egg white antigen in

influenza virus vaccine was estimated. Those vaccines concentrated by the Sharples centrifugation method contained the least amount of egg white antigen, the approximate equivalent of 0.001 to 0.0001 mg. N per milliliter. Those vaccines concentrated by the red blood cell elution method contained the highest concentration of egg white antigen, the approximate equivalent of 0.01 to 0.1 mg. egg white antigen N per milliliter. Vaccines, concentrated by the protamine sulfate precipitation method, contained egg white antigen to the approximate equivalent of 0.001 to 0.01 mg. N per milliliter. Thus vaccines prepared by the red blood cell elution method contained approximately ten to one thousand times more egg white antigen than vaccines prepared by the other methods.

A recent report by Cohen and Mines⁵ is of interest in relation to the studies herein described. These authors studied the egg white and egg yolk content of the same brands of influenza virus vaccine used by us. They employed the gel diffusion technique of Ouchterlony, with anti-egg white and anti-egg yolk rabbit serum. Differences in egg white antigen content in the various vaccines were demonstrated. In their studies however, no precipitin bands were found with those vaccines which were concentrated by Sharples centrifugation. On the basis of their results, one might gain the impression that this type of vaccine is devoid of egg white antigen. In the passive-transfer studies described herein, those brands of vaccines which were concentrated by Sharples centrifugation method were found to contain egg white antigen, although in lesser amounts than in the other brands. This finding demonstrates the greater sensitivity of the passive-transfer technique as compared to the gel diffusion method for this type of study.

As was previously mentioned, the Sharples centrifuged vaccines contained approximately 0.001 to 0.0001 mg. of egg white antigen N per milliliter. Thus, a prophylactic dose of this vaccine would contain about ten to one hundred times more antigen than is present in the average intracutaneous test with egg white. Although it is possible that patients with mild egg sensitivity might be able to tolerate such doses without untoward effects, it should be considered hazardous to use any of these egg-containing vaccines in an egg-sensitive individual. In view of the demonstrated fact that the influenza virus vaccines concentrated by the Sharples centrifugation method contain the least amount of egg white antigen, it would be wise to use this type of vaccine, especially in mass immunization programs when adequate allergic histories may not be obtained.

SUMMARY AND CONCLUSION

The present investigation was undertaken to determine the egg antigen content of various brands of influenza virus vaccine. Since egg white has been shown to be the most active antigen in vaccine of chick embryo origin, it was used as the indicator to estimate the amount of egg protein in the virus vaccines. Passive-transfer studies were done on forty-three normal recipients,

numerous trials being made with each dilution of egg white solution and five brands of vaccine. The approximate amount of egg white antigen in influenza virus vaccine was estimated by comparing the concentration of egg white extract needed to elicit a threshold passive-transfer reaction in the majority of recipients with the equivalent responding dilutions of the brands of vaccine.

The results indicate that influenza virus vaccines concentrated by the Sharples centrifugation method contained less egg white antigen, by a factor of ten to one thousand, than vaccines concentrated by chick red blood cell elution or the protamine sulfate precipitation methods. For this reason it is suggested that Sharples centrifuged vaccines be used for mass immunization programs. However, even those vaccines which have been found to contain the least amount of egg white antigen may not be safe to administer to egg-sensitive individuals.

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