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VARIATIONS IN EGG WHITE AND EGG YOLK COMPONENTS OF VIRUS AND RICKETTSIAL VACCINES

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INTRODUCTION

THE occurrence of untoward reactions following immunization procedures with virus and rickettsial vaccines has been well documented. Evidence for the role of hypersensitivity factors in many of these instances has been included in a comprehensive review of the subject by Untracht and Ratner.¹ The fact that many species of viruses and rickettsiae require culturing in the developing chick embryo, its amniotic and allantoic fluid, or its yolk sac may result in the incorporation of inseparable egg proteins during vaccine production.²⁻³ This has usually been a source of concern to those considering the administration of such vaccines to known egg-allergic patients.⁴⁻⁵ However, wide degrees of variation in tolerance to these vaccines have been exhibited by egg-sensitive persons, and an unpredictable reactive clinical response to immunization is often noted. Questions arising from such observations have led to this study, which is concerned with a qualitative antigenic analysis of common virus and rickettsial vaccines for chicken egg white and egg yolk components.

MATERIALS AND METHODS

Precipitin Tests.—The antigenic reactivity of virus and rickettsial vaccines for egg components was studied through their participating role in the formation of in vitro antigen-antibody precipitates. An attempt was made to resolve each preparation into constituent egg white and egg yolk fractions

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by employing the vaccine as a test antigen in precipitin tests with rabbit antisera to chicken egg white and to egg yolk in gel media. Patterns of reaction in agar-filled Petri dishes, according to the double diffusion technique of Ouchterlony,⁶ were thus noted for each of the following commercially available preparations:

(1) *Virus vaccines*: Polyvalent influenza virus (Eli Lilly, Lederle, Merck Sharp & Dohme, Parke Davis, Pitman-Moore), mumps (Lederle), yellow fever (National Drug), smallpox (Lederle), rabies (Squibb), lymphogranuloma venereum skin test antigen (Lederle), all of chick embryo origin, and rabies (Eli Lilly) prepared in the duck embryo.

(2) *Rickettsial vaccines of chick embryo origin*: Rocky Mountain spotted fever (Lederle) and typhus, epidemic type (Lederle).

Each band of precipitate formed within the agar was verified as a true antigen-antibody reaction by the development of coalescence with one similar band resulting from the adjacent reactions of egg white-anti-egg white or egg yolk-anti-egg yolk systems. Further identification of the antigenic component of the immune precipitate was attempted by searching for similar reactions of identity with some egg white fractions (for example, crystallized ovalbumin, lysozyme, ferric complex of conalbumin, and reference antisera) or by noting a characteristic location for the precipitin band in agar according to the immunologic patterns described by Kaminski.⁷

Antiserum to Egg White.—Rabbits were immunized with whole egg white carefully aspirated through a hole placed in the shell of a chicken egg. Each animal received a total of 12 ml. of egg white given in twelve equally divided doses of saline-diluted material over a three-week period. The first four injections were given intravenously, the next four by alternating intraperitoneal and intravenous routes, and the last four subcutaneously. Antiserum was prepared from whole blood obtained by cardiac puncture seven days following the last injection. Individual antisera, subsequently pooled, were demonstrated to contain antibody components to seven to ten distinct egg white fractions, of which one or two could also be detected in egg yolk. There is an obvious disparity in the number of egg white fractions determined by different techniques, such as detection by antigen-antibody reactions and the employment of physicochemical methods responsible for the isolation of ovalbumin, conalbumin, ovomucoid, lysozyme, globulin, ovomucin, and avidin. These differences may possibly be explained by the demonstration, with immunologic techniques, of the presence of an unidentified antigenic impurity in ovalbumin,⁹ the heterogeneity of ovomucoid,¹⁰ the multiplicity of albumin and globulin fractions, the inconstant immunochemical behavior of lysozyme in gels, and the lack of antigenicity of avidin.⁷

Antiserum to Egg Yolk.—Egg yolk was prepared by separating the intact yolk from the white of a chicken egg, carefully rolling the intact yolk on filter paper to remove adsorbent white particles, washing the intact yolk

in running water for one hour, and carefully dissecting away a small portion of the yolk sac to allow aspiration of the yolk through a needle and syringe. Rabbits were immunized with a total dose of 12 ml. of egg yolk given in twelve equally divided doses of saline-diluted material over a three-week period. One milliliter was given on each of four days of every week. All injections were given intravenously except those on the first day of the second and third weeks, which were given intraperitoneally. Individual antisera, subsequently pooled, were demonstrated to contain antibody components to seven or eight distinct constituents of egg yolk, of which at least one or two could be detected in egg white. Egg yolk was also prepared by freezing the whole chicken egg and thawing carefully removed pieces of the frozen intact yolk which could be easily separated from the frozen white without contamination. Precipitin tests with both types of yolk preparations and the rabbit antisera to egg yolk exhibited identical patterns. Chemical methods have demonstrated that egg yolk proteins consist of six fractions (lipovitellin, livetin, lipovitellenin, phosvitin, vitellin, vitellenin).⁹ The immunochemical properties of egg yolk constituents have not been characterized, however, and the antigen-antibody precipitate bands noted in gel could not be identified.

RESULTS

The results of precipitin tests recorded for each virus and rickettsial vaccine with antisera to egg white and to egg yolk as the diffusing reactants in agar gel are shown in Table I.

TABLE I

VACCINES	NUMBER OF PRECIPITIN BANDS	
	ANTISERUM TO EGG WHITE	ANTISERUM TO EGG YOLK
Mumps	1	1
Rabies (duck)	4	3
Yellow fever	5	3
Rabies	7	6
Small pox	8	7
Influenza polyvalent		
Brand A	5	6
Brand B	0	0
Brand C	7	4
Brand D	0	0
Brand E	5	4
Lymphogranuloma venereum	2	1
Rocky Mountain spotted fever	3	3
Typhus (epidemic)	1	4

The patterns of these precipitate bands representing qualitatively distinct antigen-antibody systems is diagrammatically represented in Fig. 1. These demonstrated a marked variation in the egg white and egg yolk component reactions for each vaccine.

COMMENT

In the immunization of rabbits with either egg white or egg yolk, it was found impossible to obtain an antiserum that did not possess at least one or

two common antibody components. Extreme care was taken in separatory techniques in order to avoid criticism directed to an impure, white-contaminated yolk antigen. The precipitin patterns noted in agar gel would thus tend to confirm the suspicion of a common sensitizing antigen to egg white and egg yolk reported on the basis of Schultz-Dale techniques^{2b, 11} and studies

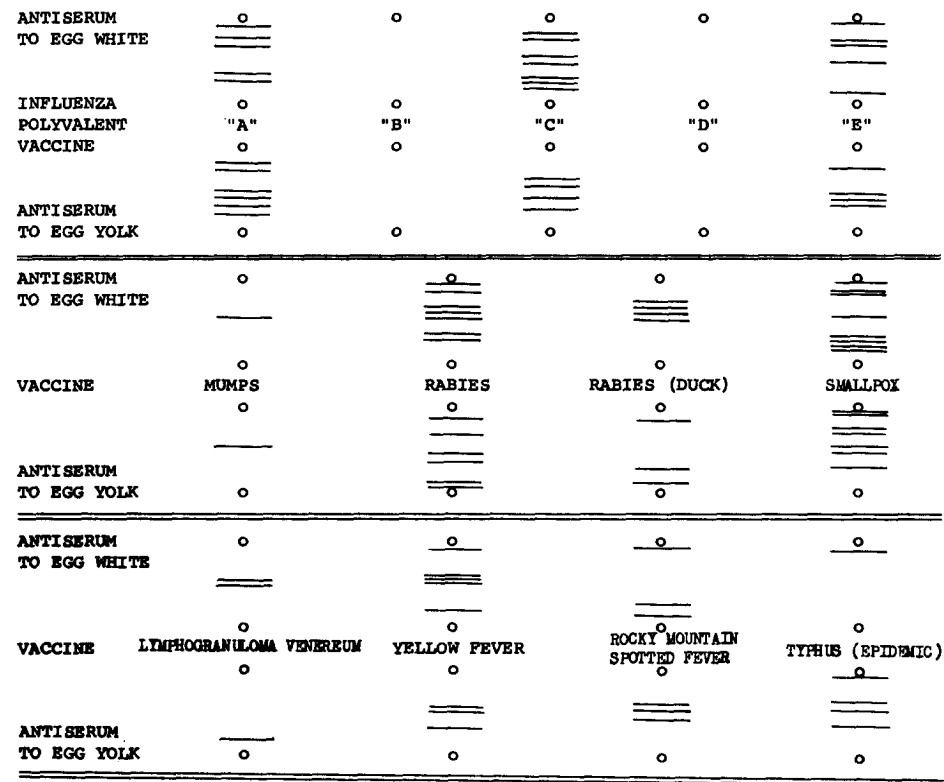


Fig. 1.—Diagrammatic representation of results of precipitin tests by the double diffusion technique (Ouchterlony) utilizing virus and rickettsial vaccines of egg embryo origin and antisera to egg white and to egg yolk as reactants. Each transverse line represents a distinct band of antigen-antibody precipitate placed in its relative position noted within the agar.

employing passive transfer of serum reagins.¹¹ Onoc¹² was able to isolate ovomucoid from egg yolk, while Marshall and Deutsch,¹³ utilizing immunologic techniques, reported the presence of conalbumin and a trace of ovalbumin in yolk. Because of our inability to obtain a chemically pure recrystallized ovalbumin fraction, it was impossible to identify absolutely this antigen, common to egg white and egg yolk, by immunologic reactions of identity. However, the precipitin band patterns in gel suggested characteristics for ovalbumin and/or conalbumin.

The detection of antigenic fractions of chicken egg white and egg yolk in rabies vaccine of duck embryo origin emphasizes the need for caution in attempting to delineate clinical allergy to virus vaccines on the basis of species

origin of the egg culture. Such cross-reactivity for many components of egg whites of other fowl with those of chicken has been recorded in serologic studies.¹⁴

Neither components of either egg white or egg yolk could be detected by specific antisera in two lots of polyvalent influenza virus vaccine. When apprised of this finding, the manufacturers were unable to offer any reasonable explanation. One did suggest, however, that the presence of an incorporated phosphate carrier might possibly be a factor, inasmuch as protein might be adsorbed to the carrier with a resultant decrease in its reactivity.

The marked variation in content of antigenic egg white and egg yolk fractions within the virus and rickettsial vaccines studied is evident when their precipitin reaction patterns are compared with common antisera. This qualitative variation existed in different lots of influenza virus vaccines, even when presumably prepared by similar methods of egg inoculation, harvesting, concentration, and purification. Reasons for this are not apparent. No evident correlation was noted between any particular egg white or egg yolk component of the vaccine and that portion of the egg (allantoic fluid, whole embryo, yolk sac) utilized in the culture of the virus or rickettsial microorganism in question.

In evaluating hypersensitivity reactions to virus and rickettsial vaccines, consideration must be given to the biologic effects and properties of the inoculated microorganisms themselves.¹ Another important factor might well be a quantitative one relating the amount of constituent egg proteins of a vaccine to the degree of clinical sensitivity of an egg-allergic recipient. This has not been a subject of study in this report. However, it has been noted that qualitative differences may exist in the reaginic antibodies of egg-sensitive man.¹⁵ Therefore, the results of this study would suggest that one important determinant of allergic reactivity or any vaccine may be the variable presence of that particular protein fraction of egg white and/or egg yolk for which the recipient possesses clinical sensitivity.

SUMMARY

1. Rabbit antisera to chicken egg white and to egg yolk demonstrated common antibody constituents by double diffusion precipitin studies in agar gel with the Ouchterlony technique. Because of impurities in the egg white fractions, the antigen common to egg white and egg yolk could not be definitely identified as ovalbumin and/or conalbumin.

2. These antisera were employed in similar precipitin test procedures to detect qualitative egg white and egg yolk components of some virus and rickettsial vaccines of chick and duck embryo origin.

3. A wide variation in the number and types of component egg white and egg yolk fractions was found to exist among the vaccines.

4. It is suggested that one determinant of allergic reactivity for a vaccine requiring chick embryo culture in its preparation might be the variable

presence of that particular protein fraction of egg white and/or yolk for which the recipient may possess clinical sensitivity.

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