

cedure and the yields were considerably increased.

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Biologic Reactivity of Antigen and Antibody in Specific Precipitate.* (24731)

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Study of effects of union of antigen with antibody upon specific functional properties of either is of significance in understanding the immune process. Recently, it has been reported that soluble complexes of antigen and antibody can cause immediate reactions which resemble systemic or local anaphylaxis (1,2). Our report(3) indicated that antigen-antibody complexes injected as mixtures in equivalence or in great antigen excess could sensitize local skin sites in guinea pigs so that subsequent challenge by intravenous injection of antigen specifically elicited local anaphylactic reactions. The present study indicates ability of washed specific precipitate, injected intravenously, to sensitize to systemic anaphylaxis when injection of antigen is given subsequently. Furthermore, washed specific precipitate, injected intravenously, acts like antigen to elicit local anaphylactic reactions at skin sites previously sensitized with antibody alone. Thus, the specific biologic reactivity of both antigen and antibody is demonstrated in the complex formed by their union.

Materials. The antigen used was 4 times recrystallized hen egg albumin. Rabbit and guinea pig antisera were analyzed for antibody nitrogen by quantitative precipitin studies(4). Specific precipitate was prepared at equivalence or slight antigen excess as determined by appropriate supernate tests. The precipitate was washed 3 times in cold saline similar to the technic used for preparation of specific precipitate for quantitative estimation of antibody. Washed specific precipitate con-

taining known amounts of antibody and antigen was then suspended with vigorous agitation in chilled saline to make suspensions containing 15 to 140 μ g AbN/ml. There was evident variation in particle size of these suspensions. Different preparations of specific precipitate varied somewhat in sensitizing capacity. It has not been determined whether these differences are due to variations between differing antisera or to small differences in ratio of antigen to antibody in the precipitates. The data are presented for ranges of concentration of antibody. The guinea pigs used for systemic or cutaneous anaphylaxis were albino males weighing 250 ± 50 g.

Results. Systemic anaphylaxis. To determine if specific precipitate could sensitize to subsequent challenge injection of antigen, the following experiment was conducted. Guinea pigs were injected intravenously with washed specific precipitate containing from 15 to 140 μ g AbN (rabbit). The recipients of these amounts of specific precipitate tolerated the injections well, although with larger amounts of specific precipitate, signs of mild anaphylactic reaction were occasionally observed. At varying intervals thereafter, 1 mg egg albumin was injected intravenously and the animals observed for manifestations of systemic anaphylaxis. Reactions were graded as follows:

0 No reaction; + Chewing, nose scratching, bristling of hair; ++ Coughing, pronounced respiratory embarrassment; +++ Loss of stability, convulsions; ++++ Death. About half of the animals which survived the respiratory component of anaphylaxis showed additional signs of prostration in varying de-

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TABLE I. Systemic Anaphylaxis in Guinea Pigs Sensitized with Washed Specific Precipitate.

μ g rabbit AbN inj. i.v. as specific precipitate	Hr between sensitization and challenge		
	5	24	48-72
15-30	0, 0, +	0, 0, 0	0, +, +
	+, +, ++	0, 0, + +, +, +	+, ++, ++ ++, ++
70-140	0, +, +	0, 0, +	++, ++++
	+, ++, +-	+, +, +	
	++, ++	+, ++, ++	
		++, ++, ++	
		++, ++++	
		++++, ++++	
		++++, ++++	

Guinea pigs inj. intrav. with suspensions of washed specific precipitate. At intervals indicated, they were challenged by intrav. inj. of 1 mg egg albumin. Degree of typical anaphylactic shock is graded as noted in text.

gree, which differed from those commonly observed in guinea pig anaphylaxis. When severe, these animals were prostrate for as long as one hour. Only severity of the respiratory component, as indicated on the scale above, is presented. Some degree of sensitization was regularly achieved with washed specific precipitate (Table I). Of the 19 guinea pigs injected with 70-140 μ g AbN as specific precipitate and challenged with egg albumin 24 hours later, 5 died with signs of typical anaphylaxis. Post mortem findings were also typical of guinea pig anaphylaxis.

The combined antibody was less effective than uncombined antibody in sensitizing to subsequent challenge. Minimum amount of antibody employed, 15 μ g AbN, if injected as uncombined antibody, would be expected to sensitize the animals so that those challenged at 24-72 hours would have severe or fatal anaphylaxis(4,6). No animals sensitized with 15 to 30 μ g AbN in the specific precipitate showed fatal reactions, although milder manifestations were observed.

Passive cutaneous anaphylaxis (PCA). The following experiment demonstrates that specific precipitate can also act as antigen to elicit PCA. Guinea pigs were sensitized by intracutaneous injection of 0.3 or 0.03 μ g AbN of guinea pig anti-ovalbumin. Eighteen hours later 1-1.5 ml of a suspension of thrice washed specific precipitate and 0.5 ml of a 0.5% solution of Evans blue dye (T-1824) were injected intravenously. The reactions of dye localization were graded as follows:

0 No reaction; + 1-5 mm; ++ 6-10 mm; +++ 11-15 mm; ++++ >15 mm dye localization. The results of challenge with specific precipitate are indicated in Table II. Amounts of antigen injected are estimated from analysis of antisera employed to make the precipitate, using an Antibody Nitrogen/Antigen Nitrogen ratio of 10:1. Also indicated in Table II are results obtained using comparable amounts of uncombined antigen. It is evident that small amounts of antigen contained in specific precipitate are able to elicit PCA reactions. Smaller amounts of uncombined antigen sufficed to elicit reactions of

TABLE II. Comparison of PCA Reactions in Sensitized Guinea Pigs Challenged with Washed Specific Precipitate or Small Amounts of Soluble Antigen.

Antigen N contained within specific precipitate (μ g)	Reactions
10	++, ++, ++++, ++++
3-5	++++, ++++, ++++, ++++, ++++, ++++, ++++
1-1.5	0, +, ++, ++++
.1	0, 0, 0
Soluble anti- gen N (μ g)	
3	++++
1	++++, ++++, ++++, ++++
.1	0, +, +, ++, ++, ++++

Guinea pigs were inj. intracut. with 0.3 μ g guinea pig AbN, one test site/animal. Eighteen hr later animals were challenged by intrav. inj. of dye and specific precipitate or dye and soluble antigen, and intensity of PCA reactions noted as indicated in text.

equal intensity, and the spot of blue dye appeared somewhat more rapidly in animals challenged with the soluble antigen. When guinea pigs were sensitized with 0.03 μ g AbN, positive reactions were not obtained following challenge with specific precipitate.

The supernate recoverable from recentrifuged suspensions of specific precipitate did not contain sufficient antigen to cause reaction at skin sites. Skin sites injected with 1/10 dilutions of normal rabbit serum did not show dye localization when animals were challenged with specific precipitate and dye, nor did injection of dye and uncombined antibody result in dye localization at test or control sites.

Discussion. Our data demonstrate that the constituents of specific precipitate, antigen and antibody, retain their ability to cause specific biologic reactions. Reactivity of antibody in antigen-antibody complexes has recently been demonstrated for passive anaphylaxis locally(3) and is now established for systemic anaphylaxis as well. It is noteworthy that the same specific precipitate, containing minute amounts of antigen, was able to elicit local anaphylaxis in passively sensitized animals.

Ability of constituents of specific precipitate to react like either antigen or antibody alone may be accounted for, in part by studies which indicate that at equivalence the available combining sites on both antigen and antibody are not fully saturated(4,5). In addition,

some dissociation of antigen-antibody complex may occur. Two factors may be related to the apparent quantitative differences between previous(3) and present studies. In this report rabbit antibody was used for local sensitization. Sensitization of guinea pig skin with complexes of rabbit antibody prepared in extreme antigen excess had not been demonstrated in contrast to results with guinea pig antibody complexes. Furthermore, the intravenously injected precipitates may fail to reach sites accessible to soluble antibody to elicit specific manifestations of anaphylaxis in the guinea pig.

Summary. The capacity of washed specific precipitate to sensitize guinea pigs so that a subsequent injection of antigen elicits systemic anaphylaxis is demonstrated. Capacity of specific precipitate to act as an antigenic challenge and elicit passive cutaneous anaphylaxis is also shown. Quantitative relationships of the amounts of antigen and antibody involved are noted.

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Influence of Archaic Reproduction Cycle on Sensitivity Response of Rat Dorsolateral Prostate to Sex Hormones.* (24732)

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Previous studies have shown that Zn⁶⁵ uptake by the rat dorsolateral prostate (DLP) reflects high natural zinc content of the gland (1). It was also demonstrated that amount of Zn⁶⁵ taken up by DLP is under hormonal

control(2), and represents a functional state of the gland(3). Recent publication conducted throughout the years 1955, '56 and '57 revealed distinct seasonal variations in capacity of DLP to concentrate Zn⁶⁵. This seasonal pattern was characterized by 2 periods of high Zn⁶⁵ uptake, namely, during February-March

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