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Passive Cutaneous Anaphylaxis with Antigen-Antibody Complexes and Additional Antigen.* (24073)

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The effect of antigen-antibody precipitates on tissues was described as a type of foreign body reaction by Opie(1). Recent investigations have demonstrated a variety of inflammatory and "immune" reactions associated with administration of antigen-antibody complexes(2-5). The continued potential for such combined antibody to react *in vivo* with additional antigen has not been demonstrated. In the present study the ability of antibody in washed specific precipitate to sensitize tissues to a subsequent injection of antigen is demonstrated. Mixtures of antigen with antibody ranging from great antigen excess to antibody excess, as described below, were also effective. The amounts of combined antibody which sensitize approximate the minimum amounts required for sensitization by uncombined antibody.

Methods. Guinea pig antisera to 4 times recrystallized egg albumin were analyzed for antibody nitrogen by the Markham modification of the micro-Kjeldahl procedure(6). Appropriate dilutions were made of serum stored at 4°C and lacking complement activity. To aliquots of the antibody solutions varying amounts of antigen were added, as indicated in the Table. Since these solutions were made to contain minute quantities of antibody, precipitation was not ordinarily visible. The presence of antigen excess was confirmed by capillary precipitin test. Specific precipitate was prepared in quadruplicate by adding a slight excess of egg albumin to a pool of guinea pig antisera containing 146 μg AbN/ml. The precipitates were washed 3 times in cold saline, and duplicate precipitates were analyzed for nitrogen content. Tests of the first supernate confirmed the presence of excess antigen. For injection, aliquots of precipitate were vigorously agitated and suspended in saline. Albino male guinea pigs weighing 250 $\pm$ 50 g were injected intracutaneously in the flank with 0.1 ml of antigen-antibody solution, a suspension of washed specific precipitate or other materials to be tested. Animals were challenged to determine specific reactivity of

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TABLE I. Passive Cutaneous Anaphylactic Reactions in Guinea Pig Skin with 0.1 ml of Antigen-Antibody Complexes Followed by Antigen Intravenously.

	Ratio AbN/EaN at skin site								
	Antigen excess					"Equivalence"	Antibody excess		
μg AbN at skin site	1/60	1/43	1/6–1/4	1.66	4	± 7	30	150	No antigen
.2						+++ ++			
.15	+++ +++	++++ ++ +	++++ +++ +++ ++	++++ +++	++++ +++		++++ +++ +++	++++ +++ +++	++++ ++++
.075		0							++++
.03		+ + 0	++++ ++++ 0						++++
.02						++			
.01		0 0	+++ ++ +	++ + 0					+++ ++ ++ +

Reactions in the "equivalence" zone were induced with thrice washed specific precipitates prepared in slight antigen excess, followed, as in all other instances, by the inj. of antigen intrav.

the injected skin sites for passive cutaneous anaphylaxis(7) at either 2 or 18 hours after intracutaneous injection. One mg egg albumin in 1 ml saline and 0.5 ml of a 0.5% solution of T-1824 (Evans Blue Dye) were injected together intravenously. With the amounts of antibody used, reactions were generally more marked at 18 hours, and most of the results were obtained using this interval between sensitization and challenge. Furthermore, non-specific dye leakage due to trauma or other factors does not occur at this interval. Skin sites were examined for extravasation of dye 15 minutes after challenge. The area of dye leakage was measured after the animals were sacrificed, bled out and the skin reflected. Reactions were graded one to 4 plus, according to the size and intensity of the blue spot as previously indicated(8).

Results. Intracutaneous injection of 0.1 ml of a suspension of washed specific precipitate containing 0.2 μg AbN sensitized the skin of 2 guinea pigs so that intravenous injection of 1 mg egg albumin and blue dye after 18 hours elicited distinct reactions of passive cutaneous anaphylaxis. When as little as 0.02 μg AbN was present in the sensitizing precipitate, a positive reaction was also elicited. The injection of dye alone produced no dye localization at the site of injection of the precipitate. Varying quantities and proportions of antibody mixed with antigen were injected intracutaneously, one site in each guinea pig. The results of subsequent challenge with intravenous antigen and dye are indicated in the Table. With 0.15 μg AbN and excess antigen, 2 skin sites showed erythematous reactions 18 hours after sensitization and prior to challenge. Following challenge, these sites gave passive cutaneous anaphylactic reactions. Comparable sites in control animals did not cause dye localization when dye alone or antibody and dye were injected 2 or 18 hours after sensitization. A skin irritating effect has been noted for antigen-antibody mixtures in larger amounts than those used here(4).

In the Table it is apparent that antigen-antibody complexes in the range of antigen excess sensitize the guinea pig tissue to passive cutaneous anaphylaxis. This occurred regularly with 0.15 μg AbN in the injected mixtures containing approximately 400 times the amount of antigen present at "equivalence." In a few instances, it was found that 0.03 μg AbN and 0.01 μg AbN were also capable of sensitization. Complete titrations of excess antigen were not carried out at these levels of antibody nitrogen. In the region of moderate antigen excess the reactions elicited by subsequent antigen were comparable to

those caused by complexes in antibody excess, and those with antibody alone as the sensitizing agent.

A preliminary test with a polysaccharide antigen (Streptococcus C) and rabbit antibody indicates that the phenomenon of sensitization by antigen-antibody complexes may be accomplished with this system as well.

Discussion. The specific combination of antigen with antibody has been described by physico-chemical laws(6). The results of this combination are evident *in vitro* in the precipitin test and its derivative reactions, and *in vivo* in specific immune or allergic phenomena(6). In the present study the ability of specific precipitates and of antigen-antibody mixtures in great antigen excess to sensitize tissues to subsequent anaphylaxis suggests that optimal biological reactivity of antibody persists, despite apparent neutralization of antibody in *in vitro* tests. As little as 0.01 and 0.03 μg AbN, amounts approximating that required for threshold sensitivity, remain capable of sensitization despite the initial presence of excess amounts of antigen. When amounts of this order are utilized, it is improbable that multiple antigen-antibody systems account for the biological reactivity noted. In addition, the skin irritating activity of the complex(4) is considerably diminished so that control injections with dye alone do not result in dye localization. Apparently the antibody continues to react with antigen until extreme antigen excess is present, an indication of greater combining ratios of antigen with antibody than is ordinarily appreciated from *in vitro* tests.

Alternatively, the dissociation *in vivo* of antigen from antibody with conservation of the reactive properties of the antibody may be postulated. The small amount of antibody used suggests that antibody may be reutilized almost quantitatively in some processes of immunity and allergy. The present data do not permit the exclusion of either alternative.

Summary. Small amounts of antibody and excess antigen sensitize guinea pig skin so that challenge with intravenous antigen and dye elicits passive cutaneous anaphylaxis. The significance of the biological reactivity of antigen-antibody complexes is briefly discussed.

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Sendai Virus Antibody in Acute Respiratory Infections and Infectious Mononucleosis. (24074)

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Japanese investigators have recently studied a virus, variously referred to as newborn pneumonitis virus, hemagglutinating virus of mice, hemagglutinating virus of Japan, and Sendai virus, isolated from cases of human respiratory infection(1), from laboratory mice(2), and swine(3). First reports of virus isolations and of serologic evidence indicating human infection with this virus were limited to Ja-

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