THE "DELAYED HYPERSENSITIVITY" INDUCED BY ANTIGEN-ANTIBODY COMPLEXES*

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In recent publications, Uhr and coworkers (1, 2) have described the induction of "delayed hypersensitivity" in guinea pigs by means of injections of antigen-antibody complexes in the region of antibody excess. The intradermal route was suggested as a determinant of the degree of sensitivity attained, although injections were made into the footpads rather than into the skin. This hypersensitivity is described as developing against the antigenic moiety of the complex. This state has been described on the basis of reactivity occurring 24 hours after skin test injections, and in the absence of humoral antibodies. No other objective criteria have been applied to its delineation. However, it appears from the descriptions given that this reactivity resembles that seen in allergy of the delayed type. Good and coworkers (2 *a*) have reported similar findings in normal and agammaglobulinemic patients injected with diphtheria toxoid-horse antitoxin floccules.

These observations called to mind similar reactive states observed by a number of investigators (3–7) some years ago, in human beings and animals, following small injections of foreign sera. In those instances the "delayed" reactive state proved to be evanescent, coming on usually within several days after the initial sensitizing injections, and at various times disappearing to be succeeded by humoral antibodies and the more conventional wheal and flare or the Arthus type of skin reactivity. It seemed to us that the "delayed" responses reported by Uhr *et al.* might be further examples of these observations rather than of the more stable, persisting, delayed reactivity seen to occur during infectious processes, or following the injection of antigens along with killed tubercle bacilli (22) or a certain lipoidal component of these into normal animals (8–12), or after exposure of the skin to inducers of contact dermatitis.

The experiments to be described have demonstrated that the hypersensitivity of apparently delayed type, which occurs after injections of antigen-antibody complexes, follows equally well after injections of antigen alone, as the older

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work suggests, and as a recent paper by Salvin (13) confirms. This reactive state is very temporary and it may be succeeded by no apparent further reactivity of the animal in respect to antibodies or Arthus reactivity, or both these responses may eventuate, depending upon the intensity of the antigenic stimulus applied. Certain characteristics of this state place it in a category of its own, distinguishable from classical delayed hypersensitivity on the one hand, and from Arthus and related immediate reactivities on the other. In order to avoid a tangle of terminology, the ephemeral delayed-like skin reactions will be referred to as of Jones-Mote type, since these investigators (6) were among the earliest to describe reactions of the kind dealt with here.

Methods

Guinea pigs of random genetic background were used in these experiments. The animals varied between 400 and 600 gm. in weight; these fluctuations did not appear to influence the results.

The antigen-antibody system employed consisted of three-times crystallized egg albumin and antibody produced against it in rabbits. Complexes were produced by first determining the point of optimal flocculation by the method of Dean and Webb (14), using a constant serum dilution (1:5 or 1:15 with two different sera) as dictated by preliminary trials. Determinations of the equivalence point were made on the basis of visual observations of rapidity of flocculation as well as by titration of supernatants for excess antigen and antibody.

In the preparation for sensitizing injections, the appropriate mixture of antigen and serum was made, incubated for 1 hour at 37°C., and placed in the refrigerator for 24 hours. The precipitated complexes were then washed in the cold centrifuge, with cold saline, three times. The quantity of complex injected into animals was adjusted to contain the desired dose of antigen. This varied in different experiments, as will be described.

For injection, the complexes (or antigen alone when used) were suspended in a mixture of aquaphor and paraffin oil (saline:aquaphor:paraffin oil in the ratio of 1:1:3, or of 1:0.15:0.85. as used by Uhr *et al.* (2)), or in saline, arlacel, and paraffin oil in the same ratios to total volumes of 0.5 ml. for subcutaneous injections. For intradermal injections, the appropriate quantities of complexes or antigen were injected in a total volume of 0.2 or 0.4 ml., distributed 0.1 ml. per site in the animal.

Skin tests were carried out with egg albumin alone, either 0.5 or 1.0 mg. (The small test doses used by Uhr *et al.* did not provoke adequate reactions in animals of any of the groups to be described.) The tests were done between 5 and 7 days and between 16 and 20 days after the single antigenic stimulus; each test of course was carried out in an individual animal; *i.e.*, the 16 to 20 day tests were not repeats in those tested at 5 to 7 days. Skin tests were read after 24 and 48 hours; at the latter time animals were also bled for serological testing in some experiments.

Two serologic tests were used. The first was a modification of the Ouchterlony reaction carried out in wells in agar plates. 0.1 ml. quantities of undiluted sera were tested against 0.1 ml. of dilutions of egg albumin in order to meet the possibility that very small amounts of antibody might fail to precipitate with concentrated antigen even after the latter had diluted itself by diffusion to the limits permitted by the spacing of wells. It was found eventually that a dilution of 1:20,000 of egg albumin was suitable for weakly reactive as well as stronger sera. More reliance, however, was placed upon the second test used, the passive cutaneous anaphylactic reaction of Ovary (15). This permits the detection of even minute quantities of nonprecipitating antibodies, as little as 0.003 γ of antibody N (16). Albino guinea pigs were injected

intracutaneously on the abdominal surface with 0.1 ml. quantities of each serum to be tested, undiluted, four tests per guinea pig. Six hours later the animals were given 10 mg. of egg albumin mixed with 0.25 ml. of 1 per cent Evans blue dye, per 100 gm. body weight, *via* the saphenous vein (17). Reactions were read 1 hour later, and at intervals thereafter through 24 hours. The earliest readings were in no case improved upon by later ones.

In one experiment, corneal injections were made in an effort to obtain objective evidence of the nature of the hypersensitive state induced by the procedures used. The inoculum consisted of a solution of 20 mg. per ml. of egg albumin in saline, in a volume sufficient to cause visible clouding of the central portion of the cornea after injection. This method was shown in a previous report (10) to cause corneal damage in guinea pigs with high levels of delayed hypersensitivity induced by a stimulus consisting of egg albumin and tubercle bacillary lipopolysaccharide.

EXPERIMENTAL RESULTS

1. Antigen-Antibody Complexes in Water-Oil.—Skin test and serologic findings in animals which had received 60, 30, or 3 γ of egg albumin combined with rabbit antibody in the region of antibody excess are shown in Table I and Fig. 1. Tests were carried out between the 5th and 7th day for early responses, and between the 16th and 20th day for later responses. The dose of antigen combined with antibody and the route of injection, whether subcutaneous or intradermal, appeared to make no difference, so that all results are shown together.

In the first tests the majority of the animals developed skin reactions which were flat, erythematous, and indurated, simulating in appearance the delayed type of reaction seen in the typical tuberculin test. These reactions were higher at 24 hours, but maintained themselves quite well at 48 hours. Only one animal of nine tested showed a minor reaction indicative of circulating antibodies in the passive cutaneous anaphylactic test. Judged by the criteria available here—the appearances of the reactions, their fairly good persistence for 48 hours, and the absence of circulating antibodies—these animals would be judged to have developed hypersensitivity of the delayed type.

These early appearing skin reactions induced by complexes were larger in tests made on the 5th day after antigen injection than on the 6th or 7th day. The number of tests carried out here is too small to permit conclusions on this point, but similar results were seen in animals sensitized with antigen alone, as described below (Table II, Fig. 2).

Tests carried out between 16 and 20 days after antigen injection (in animals which had not been subjected to the first skin test), revealed a change, in that the majority of the guinea pigs now failed to show significant skin reactivity of any kind. Most of the 24 hour skin test readings were below 10 mm. in diameter, and as seen from the control skin test animal data (Table VI) reactions of this size cannot be considered significant with the test dose of antigen employed here. Five of the animals showed 24 hour reactivity above the 10 mm. level; these fell to the region of 5 mm. by 48 hours. These minor responses may

TABLE I

Skin Test Responses and Antibodies in Guinea Pigs Sensitized by Antigen-Antibody Complexes in Water-Oil Emulsion

Sensitizing dose E.A.	Early skin test Day	Skin test readings* 24 hrs.		Skin test readings* 48 hrs.		Antibody tests: Agar diffusion	Antibody tests: P.C.A.‡	Late skin test Day	Skin test readings* 24 hrs.		Skin test readings* 48 hrs.		Antibody tests: Agar diffusion	Antibody tests: P.C.A.‡
3 γ	5	20	1.0	17	1.0		?	19	0		0			?
		14	1.5	15	1.5		±		0		0			0
		19	1.0	16	1.5		0		16	1.0	6	0.2		0
		22	1.0	14	1.5		0		12	1.0	5	0.2		0
		18	1.0	4	0.3		0		13	1.0	7	0.3		0
		18	1.5	7	0.3		0		6	0.2	3	0.2		0
		22	1.5	17	1.5		0		8	0.3	3	0.2		0
		21	1.0	15	1.0		0		3	0.3	0			0
	7	13	0.5	7	0.5		0	20	6	0.5	0		0	0
		9	0.5	0					5	0.2	0		0	0
		9	0.5	0					3	0.2	0		0	0
		8	0.5	0					7	0.5	0		0	0
									11	1.0	0		0	0
									12	1.0	3	0.2	0	0
									7	0.5	5	0.2	0	?
30 γ	6	20	2.0	14	1.0			17	6	0.5	4	0.2		
		20	2.0	16	1.0				7	0.5	3	0.2		
		13	1.0	0					7	0.5	5	0.2		
		12	1.0	0					9	0.5	6	0.5		
		13	1.0	10	0.5				5	0.5	4	0.2		
		6	0.5	0					5	0.5	4	0.2		
		7	0.5	0										
		11	1.0	8	0.5									
60 γ								16	7	0.2	0			
									3	0.2	0			
									0		0			
									0		0			
									0		0			
									0		0			
									8	0.5	4	0.2		
									0		0			
									4	0.2	0			
									4	0.3	0			
									0		0			
									0		0			

* Readings of skin tests represent measured mm. diameter of reaction, and estimated mm. of thickness.

‡ Passive cutaneous anaphylaxis.

represent Arthus reactivity, but in the absence of demonstrable circulating antibody this possibility seems remote (18, 19). Sera collected at this time failed to show antibody by the agar-diffusion precipitation test in seven instances, and revealed only two very questionable positive reactions of fifteen sera tested by the passive cutaneous anaphylaxis method.

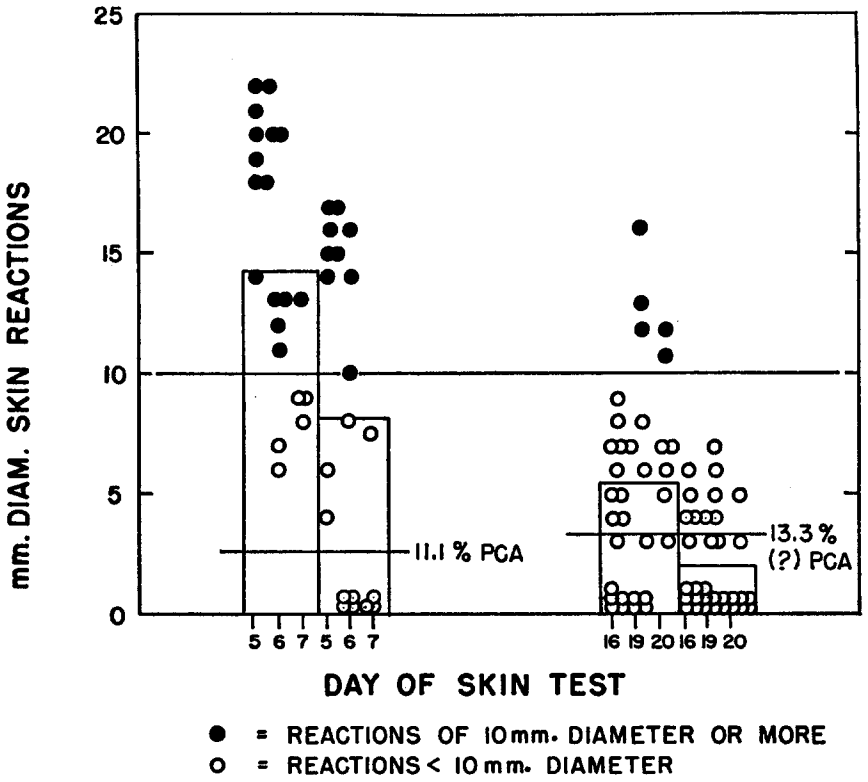

FIG. 1. Antigen-antibody complexes in water-oil (60, 30, and 3 γ antigen). Skin reactions in guinea pigs sensitized with egg albumin-antibody complexes. The height of the box in each case represents the mean diameter of all reactions shown for that period.

These results confirm the finding of Uhr and coworkers (1, 2) that guinea pigs receiving antigen-antibody complex in water-oil adjuvant develop a delayed form of skin reactivity in the first few days after injection, in the absence of circulating antibodies. However, at 2 to 3 weeks after injection virtually all reactivity of the skin has disappeared, and no significant antibody formation has occurred. The early-appearing tuberculin-like skin reactivity differs from the classical tuberculin type of hypersensitivity in the rapidity of its appearance after antigenic stimulation and in its failure to persist beyond a few

TABLE II

Skin Test Responses and Antibodies in Guinea Pigs Sensitized by Antigen Alone in Water-Oil Emulsion

Sensitizing dose E.A.	Early skin test day	Skin test readings		Antibody tests		Late skin test day	Skin test readings		Antibody tests	
		24 hrs.	48 hrs.	Agar diffusion	P.C.A.		24 hrs.	48 hrs.	Agar diffusion	P.C.A.
3 γ	6	13 1.0	10 0.5		0	19	21 2.5	7 0.5		+
		20 2.0	12 1.5		0		38 3.0	10 1.0		+
		18 1.5	20 2.0		0		9 0.3	12 0.3		+
		14 2.0	13 1.0		0		8 0.5	7 0.3		+
		20 1.5	14 1.0		?		6 0.2	5 0.2		+
		8 0.5	5 0.2		0		5 0.2	0		+
		15 1.0	11 1.0		0		4 0.2	6 0.2		+
		12 1.0	10 1.0		0					
	7	12 0.5	3 0.2			20	16 0.3	3 0.2		
		10 0.5	0				25 2.0	8 0.5	+	+
		20 0.5	6 0.3				12 1.0	5 0.2	0	+
		6 0.3	5 0.2				23 1.5	7 0.5	+	+
		4 0.2	0				30 2.0	10 0.5	0	+
		11 0.5	0				31 3.0	23 2.5	+	+
		6 0.5	5 0.2				25 3.0	15 2.0	+	+
		9 0.3	3 0.2				35 3.0	12 1.5	+	+
							23 2.0	10 0.5	+	+
30 γ	6	16 2.0	11 1.0							
		18 2.0	16 1.0							
		20 2.0	18 0.5							
		15 1.0	8 0.5							
		16 1.0	7 0.5							
		17 2.0	10 0.5							
60 γ							8 0.5	3 0.2		
							63 2.0	0		
							40 2.5	0		
							25 2.0	0		
							22 1.0	0		
							19 1.5	5 0.2		

Legend as in Table I.

days. As will be seen below, this composite hypersensitive and serologic picture can be reproduced with appropriate injections of antigen alone; it does not depend upon any special properties of antigen-antibody complexes.

2. Antigen Alone in Water-Oil Adjuvant.—The characteristics of responses to egg albumin alone are best considered in two categories: those following

injections of larger doses of antigen, from 60 to 3 γ, and those following injection of 1 γ or less.

Larger doses of antigen: The results of skin and serologic tests in animals which had been injected subcutaneously or intradermally with 3, 30, or 60 γ of egg albumin are shown in Table II and Fig. 2. The responses of these animals in the early tests resemble closely those of the complex-injected subjects tested

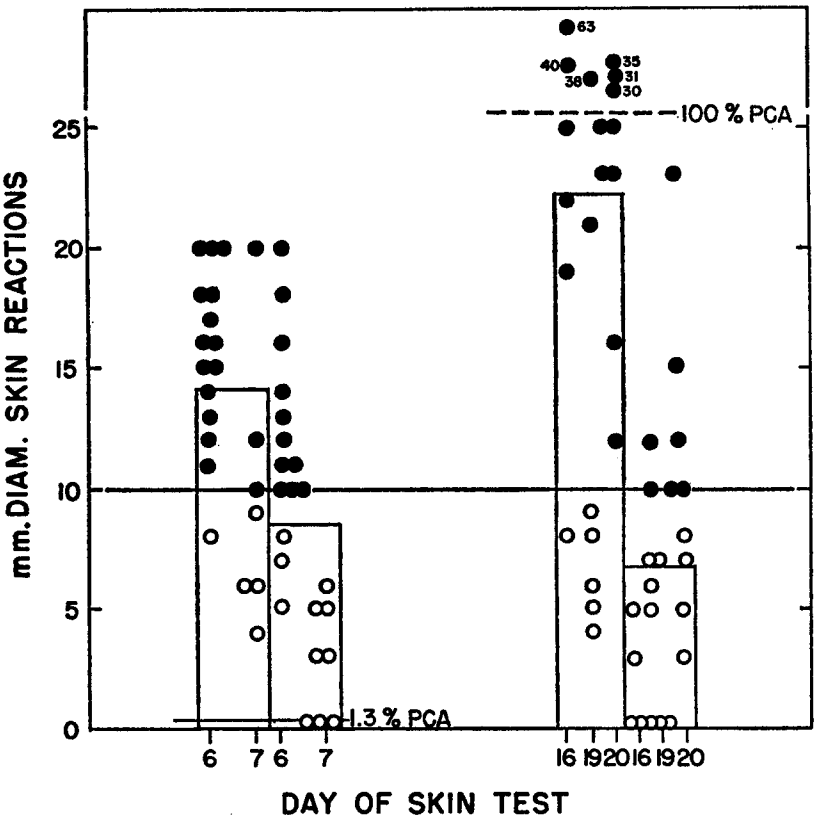

FIG. 2. Antigen in water-oil (60, 30, and 3 γ antigen).

at 5 to 7 days after the sensitizing stimulus. In the later tests, however, a different phase of reactivity developed both in the skin and in the blood. Most of the guinea pigs tested for the first time between the 16th and 20th day following antigen injection developed reactions which in appearance were characteristically of Arthus type, being marked chiefly by palpable soggy edema and moderate erythema, and a sharp decline in extent between 24 and 48 hours. Concomitantly, all of the fifteen sera revealed antibody by the passive cutaneous anaphylactic test, and six of eight tested showed lines of precipitation in the agar diffusion test.

The evidence so far presented reveals (*a*) that antigen can induce the same early-appearing skin reactivity of the Jones-Mote type in the absence of serum antibody as that which follows the injection of antigen-antibody complexes, but (*b*) that later immunologic responses to these two kinds of treatment differ. In the case of the complex-treated animals, after 2 or 3 weeks all signs of response have for the most part been lost, while the antigen-injected groups have developed circulating antibodies and Arthus reactivity. We believe that

TABLE III

Skin Test Responses and Antibodies in Guinea Pigs Sensitized by Antigen-Antibody Complexes in Arlacel-Water-Oil Emulsion

Sensitizing dose E.A.	Early skin test day	Skin test readings				Antibody tests		Late skin test day	Skin test readings				Antibody tests	
		24 hrs.		48 hrs.		Agar diffusion	P.C.A.		24 hrs.		48 hrs.		Agar diffusion	P.C.A.
3 γ	5	13	0.5	6	0.3		0	19	13	1.5	11	0.5		0
		20	1.5	14	1.0		0		19	1.5	6	0.3		0
		10	0.5	3	0.2		0		12	1.5	9	0.5		0
		19	1.5	20	1.5		0		6	0.3	0			0
		15	0.5	8	1.0		0		4	0.2	0			0
		22	1.0	11	1.0		0		8	0.5	4	0.3		0
		25	2.0	11	0.5		0		13	1.5	3	0.2		0
		13	1.5	14	1.0		0		11	1.0	0			0
	7	14	1.0	10	0.5			20	13	1.0	6	0.5	0	0
		13	0.5	6	0.3				18	1.0	0		0	0
		10	0.5	0					17	1.5	3	0.2	0	0
		10	0.5	5	0.3				15	1.5	8	0.3	0	0
		10	0.5	0					21	1.5	6	0.5	0	+
		9	0.5	3	0.2				20	1.5	8	0.3	0	±
		4	0.2	0					13	1.5	5	0.2	0	±

Legend as in Table I.

this divergence of later responses depends simply upon the magnitude of the antigenic stimulus; that the early-appearing Jones-Mote type of "delayed" skin reactivity can be induced by exposure to large or small quantities of antigen, and that a small amount of antigen such as might be liberated from antigen-antibody complexes *in vivo* suffices for this. The later appearance of circulating antibody and Arthus reactivity, however, requires a greater antigenic stimulation such as is provided by the doses of egg albumin alone used here. The experiments which follow bear out this interpretation in two ways; first, by indicating that antigen-antibody complexes can induce the "biphasic" reaction provided a more efficient adjuvant than paraffin oil in water is em-

ployed, and, on the other hand, by showing that animals receiving small quantities of antigen alone may in most instances be restricted to the early (Jones-Mote) hypersensitive response shown by those treated with complexes in water-oil.

3. Antigen-Antibody Complexes in Arlacel Adjuvant.—Several years of experience in using arlacel as the emulsifying agent in water-oil emulsions has shown us that the inclusion of this substance in a vaccinating inoculum increases the adjuvant activity of the mixture beyond that seen with aquaphor as the emulsi-

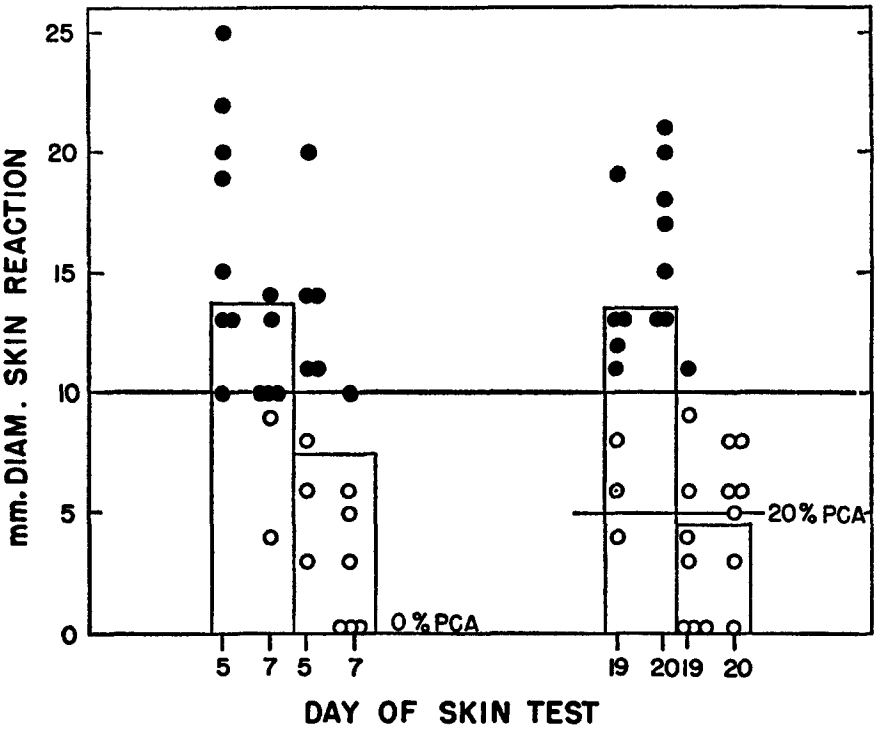

FIG. 3. Antigen-antibody complexes in arlacel.

fying agent. Animals were given, in the arlacel vehicle, 3 γ of egg albumin complexed with antibody in the region of antibody excess, and were skin-tested and bled at the usual intervals. Results are shown in Table III and Fig. 3. The delayed-appearing type of reactivity was found at 5 to 7 days after the sensitizing injection in the absence of antibodies, but on the 19th and 20th day a number of skin reactions appeared, resembling those seen in animals receiving larger doses of egg albumin alone; twelve of the fifteen guinea pigs responded with Arthus-like reactions. Several (three of fifteen) had at this time produced antibodies demonstrable by the passive cutaneous test, although none of seven of these sera reacted in the agar precipitation test.

4. Antigen in Smaller Doses.—If the degree of antigenic stimulation determines whether the immunologic response will be mono- or biphasic, then it should be possible to demonstrate that with appropriately small doses of anti-

TABLE IV

Skin Test Responses and Antibodies in Guinea Pigs Sensitized by 1 γ of Antigen Alone in Water-Oil

Early skin test day	Skin test readings		Antibody tests		Late skin test day	Skin test readings		Antibody tests	
	24 hrs.	48 hrs.	Agar diffusion	P.C.A.		24 hrs.	48 hrs.	Agar diffusion	P.C.A.
5	11 0.5	11 0.5	0	0	20	5 0.5	0	0	0
	12 0.3	7 0.3	0	0		7 0.5	0	0	0
	11 0.5	14 0.5	0	0		3 0.2	0	0	0
	12 0.5	14 0.5	0	0		15 1.0	7 0.3	0	0
	14 0.3	9 0.3	0	0		10 0.5	5 0.2	0	0
	15 0.5	9 0.5	0	0		9 0.5	3 0.2	0	0
	6 0.5	7 0.3	0	0		6 0.2	0	0	0
	11 0.3	4 0.2	0	0		11 1.0	10 0.3	0	0
6	22 2.0	19 1.0	0			19 0.3	18 0.3	0	+
	22 1.5	23 0.3	0	0		14 0.5	4 0.3	0	+
	14 1.5	14 0.5	0	0		7 0.5	0	0	+
	23 2.0	23 0.5	0	+		0	0	0	+
	22 2.0	23 0.5	0	0		0	7 0.3	0	+
	31 2.0	23 0.5	0			0	0	0	+
	27 1.0	11 0.3	0	0		0	3 0.2	0	
	17 1.0	21 0.3	0	+		14 1.5	9 0.5		0
	9 0.5	6 0.5		0		6 0.2	2 0.2		0
	20 1.0	11 1.0		0		4 0.2	5 0.2		±
	6 0.5	6 0.5		0		3 0.2	3 0.2		±
	11 0.5	21 1.5		0		4 0.2	0		±
	24 1.5	24 2.0		0					
	20 2.0	15 1.0		0					
	16 1.5	10 1.0		+					
	22 2.0	23 1.5		0					

Legend as in Table I.

gen alone, the monophasic responsiveness shown by animals receiving antigen-antibody complexes in aquaphor-water-oil is duplicable.

Egg albumin in water-oil emulsion was injected in doses of 1 γ intradermally, and skin and serologic tests were carried out at 5 or 6 and at 19 days following the injection. The results of this experiment are shown in Table IV and Fig. 4. Once more, the early test results are similar to those seen following inoculations of antigen-antibody complexes or of larger doses of antigen alone. Again, as was seen also in one case in the complex-treated animals (Table I), only occa-

sional instances of serologic reactivity were found by the passive cutaneous anaphylactic test here, in three of twenty-two sera tested.

In the 19 day tests the skin responses for the most part are negative; indeed, in their distribution they resemble those seen with antigen-antibody complexes in water-oil (Table I and Fig. 1) rather than those appearing in animals receiv-

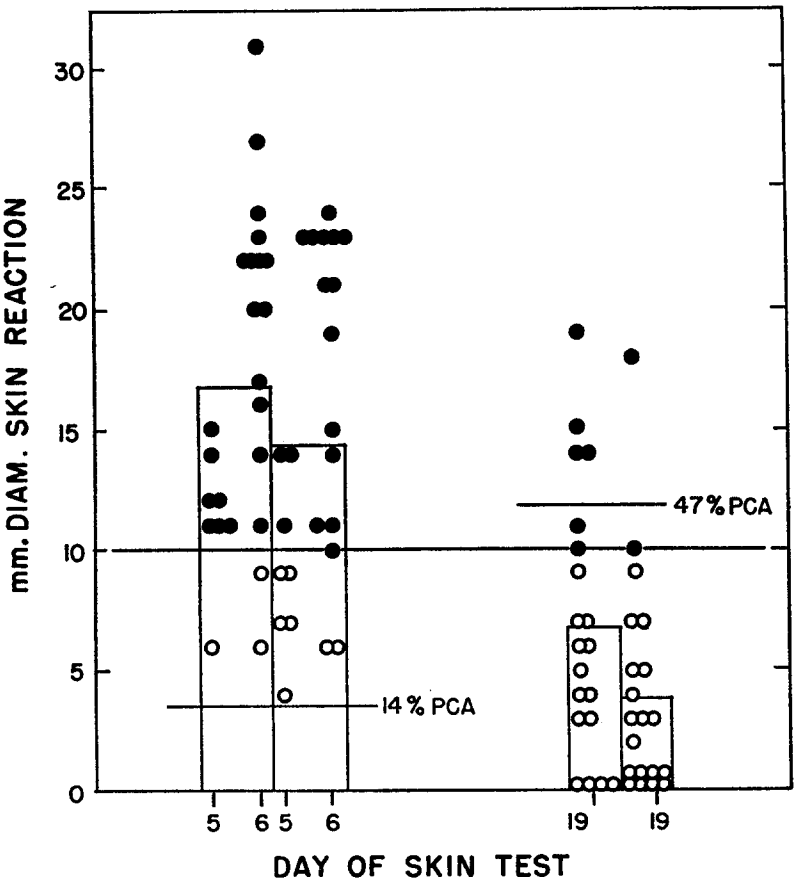

FIG. 4. Antigen in water-oil (1 γ).

ing either complexes in arlacel or larger doses of antigen alone. At this level of antigen dosage, more than half the animals failed to show the serologic aspect of the biphasic response shown by animals receiving larger doses of antigen alone, ten of nineteen sera tested showing no antibody in the passive cutaneous anaphylaxis test. It is interesting that none of the nine positive reactors in this test were positive in the agar diffusion test, indicating that this 1 γ level of antigenic stimulus has approached closely the meager immunologic inductive-

TABLE V

Skin Test Responses and Antibodies in Guinea Pigs Sensitized by 0.75, 0.5, 0.25, 0.10, 0.01, and 0.001 γ of Antigen in Water-Oil

Sensitizing dose E.A.	Early skin test day	Skin test readings		Antibody tests		Late skin test day	Skin test readings		Antibody tests	
		24 hrs.	48 hrs.	Agar diffusion	P.C.A.		24 hrs.	48 hrs.	Agar diffusion	P.C.A.
0.75 γ	5	8 0.3	6 0.3	0	0	19	9 0.5	4 0.2	0	0
		6 0.3	5 0.2	0	0		6 0.3	0	0	0
		12 0.5	7 0.5	0	0		7 0.5	5 0.2	0	0
		7 0.5	8 0.5	0	0		7 0.5	3 0.2	0	0
		6 0.3	5 0.2	0	?±		6 0.2	0	0	0
		7 0.3	6 0.2	0	0		6 0.3	0	0	0
		9 0.5	7 0.3	0	0		3 0.2	0	0	0
		10 0.5	2 0.2	0	0		8 0.5	0	0	0
0.50 γ	5	3 0.2	3 0.2	0	0	19	8 0.5	0	0	0
		0	0	0	0?		5 0.2	0	0	0
		0	0	0	0		5 0.2	4 0.2	0	0
		3 0.2	0	0	0		9 0.5	4 0.2	0	0
		0	4 0.2	0	0		5 0.2	3 0.2	0	±?
		4 0.2	6 0.3	0	0		5 0.2	3 0.2	0	0
		3 0.2	2 0.2	0	0		7 0.3	0	0	0
		3 0.2	0	0	0		6 0.2	0	0	0
0.25 γ	5	6 0.2	5 0.2	0	0	19	7 0.5	5 0.2	0	0
		5 0.2	2 0.2	0	0		3 0.2	2 0.2	0	0
		3 0.2	3 0.2	0	0		3 0.2	3 0.2	0	0
		6 0.3	6 0.2	0	0		6 0.5	0	0	0
		7 0.5	6 0.3	0	0		5 0.2	4 0.2	0	0
		7 0.5	5 0.2	0	0		7 0.5	3 0.2	0	0
		7 0.5	4 0.2	0	0		6 0.2	0	0	0
		6 0.5	9 0.5	0	0		3 0.2	0	0	0
0.10 γ	5	4 0.2	0		0	19	3 0.2	0		0
		4 0.2	3 0.2		0		0	0		0
		6 0.5	5 0.2		0		0	0		0
		6 0.5	5 0.2		0		0	0		0
		7 0.5	5 0.2		0		0	0		0
		5 0.5	0		0		0	0		0
		6 0.5	0		0		0	0		0
		2 0.2	2 0.2	0	0		0	0		0
		3 0.2	0	0	0		7 0.5	0	0	
		3 0.2	3 0.2	0	0		4 0.2	0	0	
		6 0.2	0	0	0		3 0.2	0	0	
		4 0.2	2 0.2	0	0		10 1.0	0	0	
		4 0.2	0	0	0		6 0.5	0	0	0
		4 0.2	3 0.2	0	0		5 0.2	0	0	±?
		2 0.2	0	0	0		8 0.5	3 0.2	0	0
							4 0.2	3 0.2	0	0

TABLE V (*Continued*)

Sensitizing dose E.A.	Early skin test day	Skin test readings 24 hrs.	Skin test readings 48 hrs.	Antibody tests Agar diffusion	Antibody tests P.C.A.	Late skin test day	Skin test readings 24 hrs.	Skin test readings 48 hrs.	Antibody tests Agar diffusion	Antibody tests P.C.A.
0.01 γ	6	6 0.5	0	0		20	3 0.2	0	0	0
		7 0.3	4 0.2	0			3 0.2	0	0	0
		5 0.2	0	0			0	0	0	0
		8 0.5	0	0			0	0	0	0
		5 0.2	0	0			0	0	0	0
		0	0	0			0	0	0	0
		6 0.2	3 0.2	0			0	0	0	0
		7 0.3	0	0						
0.001 γ		8 0.5	3 0.2	0						
		3 0.2	0	0						
		8 0.3	2 0.2	0						
		3 0.2	0	0						
		3 0.2	0	0						
		8 0.5	3 0.2	0						
		3 0.2	0	0						
		3 0.2	0	0						

Legend as in Table I.

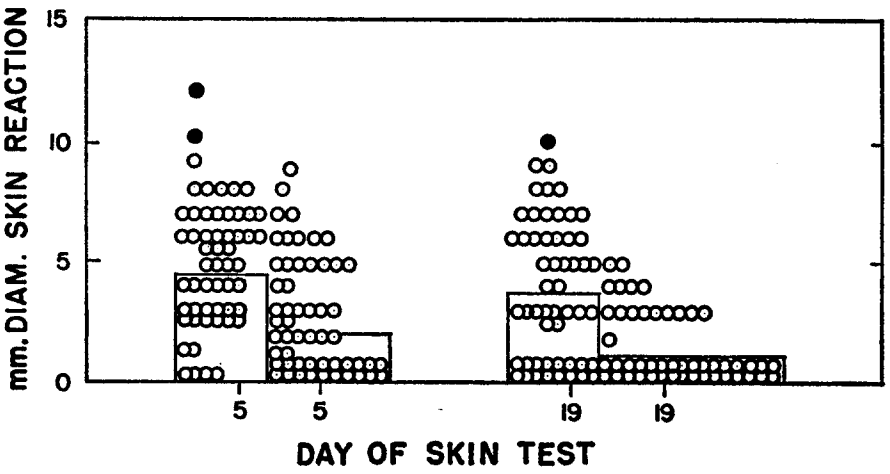

FIG. 5. Antigen in water-oil (0.75, 0.5, 0.25, 0.10, 0.01, and 0.001 γ).

ness shown by the small amounts of antigen which may become dissociated from injected complexes in the body.

In an effort to simulate the latter situation more completely, graded smaller quantities of antigen were injected into groups of animals, consisting of 0.75, 0.5, 0.25, 0.10, 0.01, or 0.001 γ of egg albumin in water-oil emulsion. The results

TABLE VI

Control Skin and Serologic Tests

Skin test reactions				Antibody tests	
24 hrs.		48 hrs.		Agar diffusion	P.C.A.
8	1.0	6	0.5	0	0
7	0.5	3	0.2	0	0
4	0.2	3	0.2	0	0
6	0.5	4	0.2	0	0
4	0.2	0		0	0
6	0.2	0		0	
8	0.5	3	0.2	0	0
6	0.2	0		0	0
4	0.2	0			
9	0.5	0			0
4	0.2	0			0
3	0.2	0			0
5	0.2	0			0
0		0		0	0
0		0		0	0
0		0		0	0
0		0		0	
0		0		0	
0		0			0
0		0			0
0		0			0
0		0			0
0		0			0
4	0.2	5	0.2	0	0
2	0.2	0		0	0
5	0.2	3	0.2	0	0
5	0.2	2	0.2	0	0
5	0.2	3	0.2	0	0
3	0.2	0		0	0
5	0.2	2	0.2	0	0
6	0.3	5	0.2	0	0
3	0.2	0			
3	0.2	0			
4	0.2	0			
5	0.2	0			
0		0			
3	0.2	0			
5	0.2	0			
0		0			
4	0.2	0			
6	0.5	0			
7	0.5	3	0.2		
4	0.2	0			

of these tests are shown in Table V and Fig. 5. Only two significant skin reactions occurred at 5 or 6 days, in animals receiving 0.75 γ of antigen. At 19 and 20 days, two of forty-three sera produced questionably positive passive cutaneous anaphylactic reactions. It is apparent that below the level of 1 γ, egg albumin usually fails to provide in guinea pigs even the minimal immunologic stimulus required for the induction of the Jones-Mote type of "delayed" hypersensitive reactivity.

Skin Test and Serologic Controls.—As a basis of reference for the various skin test and serologic results discussed, Table VI shows the ranges of skin test reactions seen at 24 and 48 hours in normal guinea pigs, and the results of a series of agar diffusion precipitation and passive cutaneous anaphylactic tests carried out with the sera of these normal animals.

DISCUSSION

The recent demonstration that the injection of antigen-antibody complexes formed in the region of antibody excess into guinea pigs results in the induction of so called "delayed hypersensitivity" (1, 2) led to the present study. It has been general opinion that true delayed hypersensitivity comes about through the presence in the tissues of infectious agents, or as the result of contact of the skin with any of a number of simple chemical substances which may sensitize to the later occurrence of contact dermatitis (20, 21). Both these examples of delayed hypersensitivity have been experimentally reproducible by means of injections into animals of allergens mixed with killed mycobacteria (22), or of antigens mixed with a lipopolysaccharide constituent of the tubercle bacillus (8–11), or with partially synthesized analogues of such a lipopolysaccharide (12).

The proposal that this kind of hypersensitivity may be induced by a protein antigen simply associated with its antibody has many implications; one is that delayed hypersensitivity should be expected to arise eventually following any antigenic stimulus provided the antigen is supplied a second time, for if antibodies are circulating as a result of the first stimulus, the second might form complexes *in vivo* to arouse in the subject a responsiveness of the delayed type. During many years of immunologic investigations one would expect that observations of this kind should have been recorded, but to our knowledge this has not been the case.

The reports of Uhr and coworkers (1, 2), on the other hand, were reminiscent of other work reported some years ago suggesting that a peculiar kind of delayed-appearing skin reactivity may occur in human beings and animals early after antigen injections, characterized by the absence of circulating antibodies and by an evanescence of the reactive state (3–6). This reactivity, which we term the "Jones-Mote" type, does not fit the concept of classical delayed hypersensitivity, largely because of its early appearance and its failure to per-

sist at a time after the sensitizing stimulus when the classical hypersensitivities of the tuberculin or of the contact types are just beginning to get under way.

The present studies show that a type of skin reactivity related in several characteristics to delayed hypersensitivity occurs within several days after injection of antigen-antibody complexes, but that this reactivity occurs as well following the injection of antigen alone. In the latter case, this transient phase of reactivity may be replaced later by typical Arthus responsiveness accompanied by circulating antibodies. Something of the same biphasic reactivity may be seen if antigen-antibody complexes are injected in a more potent adjuvant mixture containing arlacel, and, on the other hand, the biphasic reaction may be converted to the monophasic one in which only the early-appearing delayed-like skin reactivity occurs if very small amounts of antigen alone are employed for sensitization. It appears from these facts that the antigen-antibody complex may act as the provider of a minor antigenic stimulus, resulting from some small degree of dissociation of antigen from antibody *in vivo*, and that this situation may be simulated by the injection of very small quantities of antigen alone.

The characteristics of the "delayed" Jones-Mote type of skin reactivity described here include the flat, indurated nature of the reaction, its tendency to recede only moderately after 48 hours, and its occurrence in the absence of demonstrable serum antibodies. More objective tests to prove the cellular nature of this response would be desirable; in the present studies we attempted to apply the corneal reaction in a number of instances, but these were negative. The corneal test can be a good objective criterion of the presence of classical delayed hypersensitivity when it is positive. We have noted in earlier studies (8, 10) that in animals sensitized to tuberculin by BCG vaccination, or in those sensitized to tuberculoprotein or to egg albumin by the injection of these antigens along with tubercle bacillary "wax" or lipopolysaccharide, only those animals showing levels of skin reactivity approaching necrosis are apt to react positively in this test. The reason for this lies probably in the fact that without blood supply to provide secondary inflammation, evidence of injury depends upon primary damage done to the hypersensitive cells through contact with antigen, and this does not become visible unless the injury is severe. In the present instance, failure of the test does not help either to distinguish the skin reactivity described from that of the tuberculin type, or to relate it. In this connection, however, Dienes and Mallory (5) described the histologic characteristics of this type of reaction as being similar to that of the tuberculin response, with early infiltration by monocytes.

The significance of this early-appearing but ephemeral delayed-like hypersensitivity cannot now be assessed. Neither its palpable and visual characteristics nor its occurrence in the absence of circulating antibodies permits it to be classified as a reaction of Arthus type; on the other hand, although both

these facts are consonant with its classification as a reaction of delayed hypersensitivity, its fleeting appearance limited to a few days following an antigenic stimulus is not compatible with this concept.

It may be worth speculating that the Jones-Mote type of delayed reactivity described is perhaps related to the "tissue immunity" presently under discussion in respect to resistance to tumor graft transplantation. Mitchison and coworkers (23, 24) have described the occurrence in mice of an early period of tissue (lymphocytic) immunity to tumor cells following implantation. This tissue immunity is transient, and it correlates chronologically quite well with the period of reactivity seen here. In the case of the tissue experiments, the 5 to 10 day period of ability to reject transplants was followed by a period of no immunity, but during this later period circulating antibodies appeared, revealed as hemagglutinins for the erythrocytes of the animals supplying the vaccinating tissue. Again, the time of antibody formation is chronologically similar to that seen in animals receiving larger doses of antigen in the present studies. The simultaneity of the biphasic responses in both cases suggests that the "tissue immunity" which exists in appropriately vaccinated mice may be an expression of the kind of immunologic tissue responsiveness with which this work deals. Snell (25) has recently commented along these lines.

SUMMARY AND CONCLUSIONS

The "delayed hypersensitive" reactivity induced by antigen-antibody complexes has been studied from the standpoints of the role of such complexes in establishing this state, and the relationship of this state to classical delayed hypersensitivity.

It has been shown that the reactivity established by antigen-antibody complexes appears early after injection, disappears within a few days, and is characterized by several properties which make it appear similar to true delayed hypersensitivity, including its appearance, its relative persistence for 48 hours, and its occurrence in the absence of antibodies. By the same tokens, it may be distinguished from hypersensitive reactions of the immediate type. It is referred to here as reactivity of the Jones-Mote type.

Antigen alone stimulates exactly the same kind of early reactive state, but with larger doses of antigen this is later replaced by other immunologic responses including circulating antibodies and Arthus reactivity. If sufficiently small doses of antigen are employed, however, the "monophasic" reaction which follows antigen-antibody complexes consisting of the Jones-Mote type of skin responsiveness may be seen.

The dermal reactivity under discussion is unlike classical delayed hypersensitivity chiefly in its evaneescnt character; it is present only during a few days early after antigen administration.

It is suggested that this kind of reactivity, which may perhaps require a cate-

gory of its own, may be related to the "tissue immunity" to tumor transplants which has been observed in mice.

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