

Investigation of the mechanisms by which "enhancing" antiserum prevents induction of delayed hypersensitivity to protein antigens in mice

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Enhancing antibody (referred to here as "contrasensitizer"), produced in hyperimmunized mice and injected daily into normal mice during the first 5 days of their exposure to purified protein antigens in incomplete Freund adjuvant, prevents these mice from developing delayed (tuberculin-type) hypersensitivity by specifically blocking antigen-host cell interactions in the earliest stages of sensitization. An antigen inoculum is permanently inactivated by such a series of contrasensitizer injections; but treated mice are not made immunologically unresponsive, for they can respond normally to a second inoculation not neutralized by a second course of contrasensitizer injections. The inhibitory antibody, apparently a 7S gamma-1 globulin and not a precipitin, cannot prevent activation of an anamnestic response; but its administration can be delayed as much as 4 days after original antigen inoculation and still prevent primary induction of delayed hypersensitivity. Much less effectively, it also suppresses induction of Arthus hypersensitivity. In terms of retained inhibitory activity, it is estimated to have a half-life of only 10 to 12 hours.

Passive immunological "enhancement"¹ or "facilitation"² is antigenically specific impairment of an animal's homograft rejection system by humoral antibodies to the homograft administered during the animal's initial experiences with it. Currently, enhancing antibody is believed to prevent or delay development of delayed hypersensitivity to the homograft, perhaps by interfering with interaction between graft antigens and host immunocytes. Proving this idea has been difficult because graft rejection is complex and has numerous stages at which enhancing serum might act.

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Experiments dealing with enhancement can be simplified by the use of purified, nonviable proteins rather than grafts. To date results from such experiments confirm that enhancing antisera do specifically suppress the induction of delayed hypersensitivity.^{3, 4} More such experiments, primarily on the effects of contrasensitization, are described here.* They indicate that enhancing antibody (i.e., "contrasensitizer") prevents induction of delayed hypersensitivity by inactivating the inoculum of antigen. It does not create any form of immunologic unresponsiveness nor will it inhibit activation of an anamnestic immunologic response.

MATERIALS AND METHODS

Mice

In most experiments we used CF-1 females (Carworth Farms, Portage, Mich.) but for adoptive transfer experiments we used CAF₁ females (Cumberland View Farms, Clinton, Tenn.). All experimental groups consisted of 10 mice each. The animals were maintained on Rockland Farms Mouse Diet and tap water.

Antigens

Human serum albumin (Pentex, Inc., crystallized, or Nutritional Biochemicals Corp., "4X crystalline"), bovine serum albumin (Pentex, Inc., crystallized), and chicken egg albumin (Nutritional Biochemicals Corp., "5X crystalline") served as antigens. They are designated here, respectively, as HSA, BSA, and OVA.

Inoculations and skin testings

Mice were sensitized by subcutaneous injection in opposite inguinal regions on Days 0 and 7 of an experiment with 0.1 ml. volumes of water-in-oil (w/o) emulsion containing 0.25 mg. of antigen.⁵ Boosting injections were the same as sensitizing injections, but they were delivered into axillary rather than inguinal areas. The mice were tested for hypersensitivity on Days 21, 35, and 49 by intradermal injection of 0.02 ml. of 0.1 per cent antigen in physiologic phosphate buffer on alternating shaved flanks. Arthus reactions were read at 3 hours and delayed hypersensitivity reactions at 24 hours. Our techniques and criteria are detailed elsewhere⁵⁻⁷ but can be reiterated briefly.

Each skin-test site was observed and palpated at the time of reading, and the width and thickness of the inflammation, if present, were measured with sliding metric calipers. Because none of the 3 antigens used causes inflammation exceeding 2 mm. in diameter in unsensitized mice, we currently designate a reaction as positive that is ≥ 4 mm. Since our mice usually fall sharply into non-

*The terms "enhancement" and "facilitation" were devised originally to describe the fates of tumor grafts as determined by a host's active or passively acquired immunologic response to them. Hence, the terms, when applied directly to immunologic responses themselves, mean the opposite of what they say (i.e., antibody which enhances a homograft means antibody which inhibits homograft rejection). We believe that this confusion, as well as attendant discursive clumsiness, can be avoided by use of the term "contrasensitizer."

sensitive (no reaction) or sensitive (reactions of 10 ± 3 mm. mean diameter and 4.0 ± 1.0 mm. mean thickness of folded skin), we report our results here in this fashion.

Contrasensitizer

The inhibitor of sensitization whose actions we describe is a particular class of antibody which we have purified in only very small quantities.⁸ Hence, contrasensitizer refers in this study to whole mouse antiserum rich in antigen-specific inhibitory activity and not to the purified inhibitory antibody itself. Such antiserum was prepared by injecting mice, which have good Arthus and delayed hypersensitivity reactions, intraperitoneally on 2 succeeding days with 0.1 and 1.0 mg. quantities of antigen, respectively, in 0.2 ml. volumes of physiologic phosphate buffer and then bleeding them 6 days after the second injection (timing is critical).⁸ Antiserum was separated from the pooled bloods by centrifugation after one hour of clot retraction at 37° C., stored frozen at -29° C., and usually diluted to 20 per cent with physiologic phosphate buffer for actual use. The diluted antiserum, prepared in a volume sufficing for a given experiment, was also stored frozen, being thawed each day for use and then refrozen.* Mice being treated with this substance received daily intraperitoneal injections of 0.1 ml. of the 20 per cent concentration, unless otherwise indicated.

Surgical removal of antigen inocula

In 2 experiments mice were injected for sensitization intradermally rather than subcutaneously so that their inoculation sites could be cut out at appropriate intervals. Each mouse received 0.02 ml. of w/o emulsion containing 0.1 mg. of antigen deposited with a No. 26 gauge needle over the xiphoid process. To excise the inoculum, a mouse was anesthetized with Nembutal, the inoculum and accompanying skin was lifted and cut away with small curved scissors, and the resulting incision was closed with No. 6-0 silk sutures.

Adoptive transfers

Donor mice were killed by hyperextension of the neck, and their spleens were removed and pooled in Hanks solution kept at room temperature. A collection of 10 spleens was then forced with a pestle through a square of fine-mesh stainless steel screen into 2 ml. of Hank's solution in an evaporating dish. The disintegrated product was drawn into a 10 ml. syringe through a No. 22 gauge needle and was diluted to a total volume of 10 ml. with more Hanks solution directly in the syringe, and 1 ml. of this suspension was injected intraperitoneally into each recipient mouse. Thus, each mouse received slightly less than the equivalent of one donor spleen. By the procedures used over 90 per cent of the transferred nucleated cells were viable by the eosin exclusion test.

*We have compared the activities of contrasensitizer frozen and thawed repeatedly with the same frozen in several small batches to be thawed only once at the time of use and have found that repeated freeze-thaw cycles have no practical adverse effect on contrasensitizer activity.⁸

Table I. *Effect of contrasensitizer on induction of overt hypersensitivities and of capacities for anamnestic response*

Group No.	Treatment	Per cent positive			
		5 weeks		7.5 weeks*	
		A†	D†	A†	D†
1	w/o alone	0	0	10	0
2	HSA in w/o	90	40	100	80
3	HSA in w/o + con-S	20	0	90	10
4	HSA in w/o + con-S, resection Day 3	40	0	100	10
5	HSA in w/o + con-S, resection Day 7	30	0	100	50
6	HSA in w/o + con-S, resection Day 11	70	0	100	0

On Day 0 mice were inoculated with 0.02 ml. volumes of w/o emulsion intradermally. In this, Groups 2 to 6 received 0.1 mg. quantities of HSA. "Con-S" indicates a series of 12 daily intraperitoneal injections of 0.2 ml. volumes of 20 per cent contrasensitizer beginning on Day 0. Inoculation sites were removed surgically from mice of Groups 4, 5, and 6 on Days 3, 7, and 11, respectively. Each group consisted of 10 mice.

*One week following boosting of mice in Groups 2 through 6.

† A = Arthus hypersensitivity; D = delayed hypersensitivity.

EXPERIMENTS AND RESULTS

Inhibition of the induction of Arthus and delayed hypersensitivities

Contrasensitizer delays development of Arthus hypersensitivity and prevents induction of delayed hypersensitivity as illustrated in Fig. 1 and Table I. In the experiment with results depicted in Fig. 1, mice immunized once with OVA and receiving 0.1 ml. volumes of undiluted contrasensitizer daily for the first 5 days of the experiment lagged 2 weeks behind similarly immunized but untreated mice in evolving Arthus hypersensitivity, but they all did become hypersensitive. Among the same treated mice, some eventually also developed delayed hypersensitivity, but most did not. The apparent lack of delayed hypersensitivity in these animals was not a spurious result of too insensitive a test for hypersensitivity, for, as is also shown in Fig. 1, they evinced no subliminal sensitization corresponding to a capacity to respond anamnestic to a boosting immunization.

Results from the experiment summarized in Table I are similar. Mice in this experiment were sensitized with HSA rather than OVA, sensitization was somewhat weaker than in the preceding experiment, and a continuous series of 12 rather than 5 daily treatments with 20 per cent contrasensitizer was given. The effects of the last two variables, weaker sensitization and a more prolonged series of injections of contrasensitizer, are evident in the comparatively greater suppression by contrasensitizer of Arthus sensitization than in the experiment shown in Fig. 1. But in their anamnestic responses to boosting or lack thereof, the test mice confirmed that Arthus sensitization is only suppressed, whereas induction of delayed hypersensitivity is prevented.

Surgical removal of inoculation sites

This experiment had two other purposes: to learn whether the residual depot of antigen in w/o emulsion sensitized mice by acting after contrasensitizer treatments had ceased and to find the approximate minimal time for mouse exposure to antigen in w/o emulsion to sensitize.

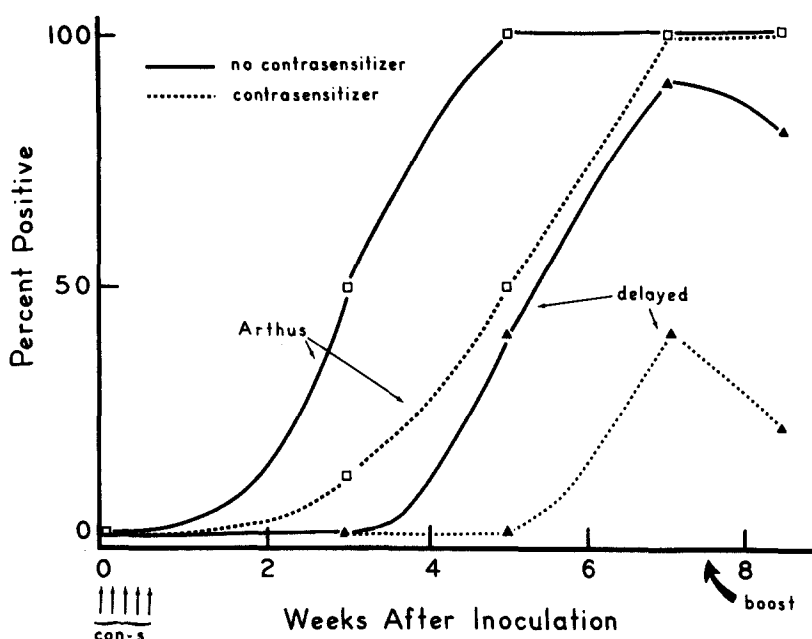


Fig. 1

Relative effects of contrasensitizer injections ("con-S") on the induction of Arthus and delayed hypersensitivities in mice injected subcutaneously on Day 0 with 0.25 mg. of OVA in w/o emulsion. Note the lack of anamnestic response to boosting in the contrasensitizer-treated mice, indicating that even this subliminal degree of sensitization has been prevented.

Mice of Groups 2 through 6 were injected intradermally with HSA in w/o emulsion, and later most of these groups had their deposits of inoculum removed surgically at different intervals as shown in Table I. Subsequent skin tests revealed no difference in the development of delayed hypersensitivity between Groups 3 and 6, the former retaining but the latter losing inoculation sites. Therefore, contrasensitizer might either make these sites nonstimulatory or the mice unresponsive to them. The same conclusion can be reached regarding Arthus sensitization, for were the inoculation sites active following cessation of contrasensitizer injections, then mice in Group 3 should have developed better Arthus hypersensitivity than mice in groups from which inoculation sites were removed; actually, the opposite result was observed. A minimal time for antigen exposure leading to development of delayed hypersensitivity could not be determined from the experiment because contrasensitizer prevented this development (see also Fig. 2). Less than 3 days of exposure to antigen is required for Arthus hypersensitivity, since mice losing their deposits of antigen at this time developed as good an anamnestic Arthus response as those with sites resected later or not at all.

Effects of contrasensitizer on induction of capacity for anamnestic responsiveness

Some of the results in Table I suggest that contrasensitizer must exert most of its effects against induction of delayed hypersensitivity early in an animal's

Table II. *Effect of contrasensitizer on later immunologic responsiveness*

Group No.	Treatment	Per cent positive			
		5 weeks		8 weeks*	
		A†	D†	A†	D†
1	w/o alone	0	0	0	0
2	HSA in w/o	100	80	100	100
3	HSA in w/o + con-S	60	0	100	100
4	HSA in w/o + con-S, resection Day 11	10	0	100	80
5	HSA in w/o, resection Day 11	90	20	100	100
6	HSA in w/o, resection Day 7	90	20	100	100
7	HSA in w/o, resection Day 3	40	0	100	100

Experimental conditions for this experiment were the same as those for the experiment described in Table I.

*Three weeks after boosting.

† A = Arthus hypersensitivity; D = delayed hypersensitivity.

Table III. *Quantitative effect of contrasensitizer on development of capacities for anamnestic response*

Group No.	Treatment	Per cent positive			
		5 weeks		7 weeks*	
		A†	D†	A†	D†
1	w/o alone	0	0	0	0
2	HSA in w/o	80	40	100	80
3	HSA in w/o + con-S at 20%	78	0	100	100
4	OVA in w/o	80	30	90	70
5	OVA in w/o + con-S at 4%	10	0	50	0

Immunization consisted of a single subcutaneous injection of 0.25 mg. of antigen in 0.1 ml. of w/o emulsion on Day 0. A continuous series of 12 daily intraperitoneal injections of 0.1 ml. of contrasensitizer was given to mice of Groups 3 and 5 beginning on Day 0. Twenty per cent contrasensitizer to HSA was used for Group 3 and 4 per cent contrasensitizer to OVA was used for Group 5.

*One week after boosting on Day 42.

† A = Arthus hypersensitivity; D = delayed hypersensitivity.

exposure to antigen in w/o emulsion and that once this critical period has passed, its further administration may be superfluous. Is this because contrasensitizer-injected mice have developed an unresponsiveness to residual antigen in the injection site, or has this inhibitor somehow neutralized the sensitizing capacity of the originally injected material? Were unresponsiveness the answer, then subsequent exposure to antigen (i.e., boosting) should fail to sensitize. The two experiments described previously support this explanation, but not conclusively, because their mice were skin tested only one week after boosting. Although they showed no anamnestic response, they may not have been tolerant and could have developed delayed hypersensitivity if given enough time. This prospect was tested in another experiment of the same type with results summarized in Table II.

Test mice in this experiment were inoculated once intradermally with HSA in w/o emulsion. Mice of Group 3 received 12 consecutive daily injections of 20 per cent contrasensitizer; mice of Group 4 were treated similarly, but their

Table IV. *Immunocompetence of spleen cells transferred adoptively from inoculated, contrasensitizer-treated donors to normal "boosted" recipients*

Group No.	Treatment	Per cent positive			
		1 week*		5 weeks*	
		A†	D†	A†	D†
1	HSA in w/o emulsion	100	100	80	100
2	HSA in w/o emulsion + con-S	50	10	100	100
3	Recipients of Group 2 cells	0	0	100	70
4	Recipients of Group 1 cells	100	40	100	100
5	Boosting control	0	0	100	80

Twenty mice in Groups 1 and 2 were inoculated subcutaneously on Day 0 with 0.25 mg. of HSA in 0.1 ml. w/o emulsion, and mice of Group 2 received a continuous series of 12 daily intraperitoneal injections of 0.1 ml. of 20 per cent contrasensitizer beginning on Day 0. Half of each group were used on Day 24 as donors for Groups 3 and 4, as indicated; Group 5 mice remained untreated until Day 31 when they and all other groups were inoculated subcutaneously ("boosted") with 0.25 mg. of HSA in 0.1 ml. w/o emulsion.

*Time after "boosting."

† A = Arthus hypersensitivity; D = delayed hypersensitivity.

Table V. *Inability of contrasensitizer to block adoptively transferred anamnestic response*

Group No.	Treatment	Per cent positive					
		3 weeks*		5 weeks*		7 weeks*	
		A†	D†	A†	D†	A†	D†
1	Cells	0	0	80	0	90	30
2	Cells + con-S	0	0	10	0	100	0
3	Cells + HSA in w/o	100	100	100	100	100	100
4	HSA in w/o	100	100	100	100	100	100
5	Cells + HSA in w/o + con-S	100	100	100	100	100	100
6	HSA in w/o + con-S	0	0	10	0	100	10
7	Con-S	0	0	0	0	0	0
8	w/o alone	0	0	0	0	11	0

Mice of Groups 1, 2, 3, and 5 received adoptive transfer of spleen cells (one spleen equivalent per mouse) from previously sensitized donor mice on Day 0; mice of Groups 2, 5, 6, and 7 received a continuous series of 12 daily intraperitoneal injections of 0.1 ml. of 20 per cent contrasensitizer beginning on Day 0; mice of Groups 3, 4, 5, and 6 were inoculated subcutaneously on Days 0 and 7 with 0.1 ml. volumes of w/o containing 0.25 mg. quantities of HSA.

*Time after first inoculation with HSA in w/o. There were 10 mice per group, except in Group 8 which had 9.

† A = Arthus hypersensitivity; D = delayed hypersensitivity.

inoculation sites were removed surgically on Day 11; mice of Groups 5, 6, and 7 had their inoculation sites removed on Days 11, 7, and 3, respectively, but they were not treated with contrasensitizer. These various groups were skin tested at 5 weeks, boosted subcutaneously with HSA in w/o emulsion, and skin tested again 3 weeks after the boosting.

As indicated in Table II by results from the 5 week skin tests, mice of Group 3 failed to develop delayed hypersensitivity initially. On the other hand, results from their second series of skin tests 3 weeks after boosting showed that all of these mice could respond normally, but not anamnesticly, to a fresh

exposure to antigen in w/o emulsion if not again treated with contrasensitizer. Hence, contrasensitizer prevents sensitization but does not induce immunologic unresponsiveness.

Evidence for neutralization of the inoculum

Is this because antigen injected into mice in w/o emulsion is stimulatory for a few days and its stimulatory effect is blanketed during this critical period by contrasensitizer, or is the antigenicity of the original inoculum blocked permanently? A comparison of the 5 week skin-test results for Groups 2 and 5 in Table II favors the latter idea, for left undisturbed (Group 2) the depot of antigen in w/o emulsion continues beyond 11 days (Group 5) to stimulate mice antigenically, especially for inducing delayed hypersensitivity (nearly maximal Arthus hypersensitivity already has been induced by the seventh day; see also Groups 6 and 7). Hence, the effect of contrasensitizer as seen in Group 3 closely resembles the surgical removal of the depot of antigen as done in Group 5. Therefore, the results from Table II taken collectively indicate that contrasensitizer durably neutralizes the antigenic stimulus of the original inoculum but has no other lasting effect.

Quality of contrasensitizer related to its capacity to inhibit

Originally we reported that contrasensitizer could block induction of overt delayed hypersensitivity but not of capacity for anamnestic response,⁶ but the previously described experiments show it to block both. This discrepancy is quantitative, for the degree of inhibition effected by contrasensitizer depends on its quantity and quality as balanced against the strength and length of antigenic stimulation. An example of this is shown in Table III in which an anti-

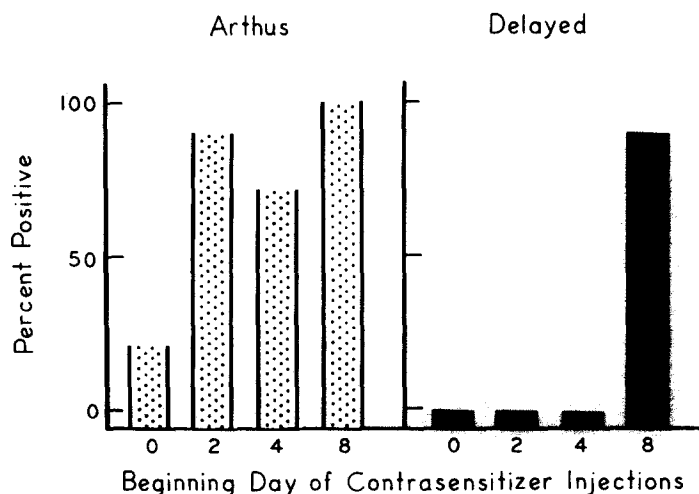


Fig. 2

Effects of delaying the first of a series of contrasensitizer injections on the development of Arthus and delayed hypersensitivities for 0, 2, 4, and 8 days following inoculation. Skin tests were performed 5 weeks after inoculation.

serum containing little contrasensitizer blocked overt development of delayed hypersensitivity to HSA but not anamnestic responsiveness; whereas in the same experiment another obviously more inhibitory antiserum, though being used at fivefold lower concentration than the weak antiserum, blocked both overt and anamnestic evolution of delayed hypersensitivity and greatly suppressed or perhaps even prevented the development of Arthus hypersensitivity to OVA. The inhibitory quality of an antiserum depends on a number of factors, such as allergic status of donors, induction regimen, and bleeding time.⁸

Effects of contrasensitizer as detected by adoptive transfer

The ability of contrasensitizer to block systemic sensitization of a mouse can be demonstrated as well by adoptive transfer experiments as by test of originally vaccinated mice: Spleen immunocytes taken from mice immunized without contrasensitizer transfer capacity for anamnestic response for both Arthus and delayed hypersensitivity to normal mice (Group 4, Table IV), whereas immunocytes obtained from similar donors treated with contrasensitizer do not (Group 3).

Location of activated immunocytes

That homogenized spleens contained the "immunocytes" transferred leads to an interesting sidelight. By this test of adoptive transfer, which would detect only immunocytes in the spleen, contrasensitizer was effective against Arthus hypersensitivity as well as against delayed hypersensitivity. Hence, this inhibitor must have prevented the development of any significant number of Arthus antibody-producing cells in donor mice spleens, which in turn indicates that in contrasensitizer-treated mice all or nearly all of the usually considerable Arthus antibodies which do appear are made outside of the spleen. Obviously, in mice not treated with this inhibitor, there are many cells within the spleen which either make or are able to make Arthus antibodies (Groups 1 and 4). A corollary to this is that anamnestic production of Arthus antibodies in contrasensitizer-inhibited animals must occur outside of the spleen. This may be true also of delayed hypersensitivity.

Lack of effect by contrasensitizer against adoptively transferred anamnesis

Although various data from the preceding experiments suggest that contrasensitizer functions by blocking allergic stimulation, either this blockade is incomplete or the effects of the inhibitor are more subtle than a physical walling-off of antigen, for contrasensitizer does not prevent activation of an anamnestic response. For instance when contrasensitizer was used in mice which received isogeneic sensitized spleen cells together with primary inoculation of HSA in w/o emulsion (Group 5, Table V), it was unable to prevent development of either Arthus or delayed hypersensitivities while being fully effective against primary sensitization (Group 6). The results from this experiment also provide incidental but clear evidence that contrasensitizer interferes only with the earliest events in sensitization and not with either intermediate stages, antibody production, or reaction between the sensitized animal and challenging

antigen used in a skin test. This incidental conclusion is confirmed by other experiments, such as the following.

Temporal aspects of contrasensitization

Several groups of mice were immunized once with HSA in w/o emulsion. Each of these groups, except the positive control mice, then received a series of 12 daily consecutive injections of contrasensitizer beginning at 0, 2, 4, or 8 days after immunization. Later, they were skin tested as usual to determine which groups had developed hypersensitivities. Results from the 5 week tests, shown in Fig. 2, show that injections of contrasensitizer can be delayed up to 4 days after immunization and still prevent development of delayed hypersensitivity. Aside from corroborating that the serum inhibitor prevents only the earliest stages of sensitization, these findings indicate that several days (at least 4) pass before development of delayed hypersensitivity reaches the stage of anamnestic cell production, whereas this stage is reached in less than 2 days for Arthus sensitization.

Fig. 2 also provides the practical information that administering contrasensitizer for more than 4 or 5 days following immunization should be superfluous; it indicated that our hitherto employed series of 12 consecutive injections should be replaceable with a more economical regimen of 5 for a single inoculation or two series of 5 for two separate inoculations. This was confirmed by experiments such as are summarized in Tables VI and VII.

According to results from the first experiment of Table VI, two interrupted series of 5 injections, begun at the time of each of two immunizations, exerted the same degree of inhibition on the development of delayed hypersensitivity

Table VI. Effectiveness of 5 day series of contrasensitizer injections for inhibiting sensitization to BSA

Group No.	Treatment	Per cent positive					
		3 weeks		5 weeks		7 weeks	
		A*	D*	A*	D*	A*	D*
1	w/o alone	0	0	0	0	14	0
2	BSA in w/o	100	100	100	100	100	100
3	BSA in w/o + con-S on Days 0-11	30	0	90	0	100	90
4	BSA in w/o + con-S on Days 0-4 and 7-11	70	10	100	10	100	60
5	BSA in w/o + con-S on Days 0-4 only	90	50	100	80	100	100
1	w/o alone	0	0	0	0	12	0
2	BSA in w/o	90	50	100	50	100	100
3	BSA in w/o + con-S on Days 0-4 only	70	20	100	70	80	60
4	BSA in w/o + con-S on Days 0-11	0	0	60	0	70	20

Except for both Groups 1 in these two similar experiments, each group consisted of 10 mice. In both experiments, mice were inoculated subcutaneously on Days 0 and 7 with 0.25 mg. quantities of BSA in 0.1 ml. w/o emulsion and were treated with daily series of intraperitoneal injections of 0.1 ml. of 20 per cent contrasensitizer to BSA on the days indicated.

*A = Arthus hypersensitivity; D = delayed hypersensitivity.

to BSA following these two immunizations as one continuous series of 12 injections starting at the time of the first immunization. But one series of 5 injections following only the first of two immunizations exhibited only marginal inhibition. This latter point is corroborated by a separate experiment also shown in Table VI.

Table VII summarizes similar findings for the HSA system, indicating that inhibition is readily evident even if immunizations are on Days 0 and 14 instead of 0 and 7, providing that matching series of contrasensitizer injections are given. The results in Fig. 2 indicated that between 5 and 8 days' uninhibited exposure to antigen is sufficient to impel the development of delayed hypersensitivity in mice beyond susceptibility to contrasensitizer. The interval between the last of the first series of contrasensitizer injections and the day of the second immunization (and first of the second series of contrasensitizing injections) in Group 2 (Table VII) was 10 days. Together, these data show that the first series "neutralized" the first immunization and that the second series could do the same for the second immunization because no anamnestic responsiveness had been effected by the initial immunization.

For effect, according to all of the preceding experiments, contrasensitizer must be present during the first few days of immunization. Some slight interference with sensitization was evident in the mice of Group 5 (Table VI), so that only traces of contrasensitizer from the first series of injections must have persisted in the second period of immunization. Matching this degree of inhibition with data on minimum concentrations of contrasensitizer known effective against sensitization,⁶ we estimate that the half-life of contrasensitizer may be between 10 and 12 hours.

DISCUSSION

Our experiments with contrasensitization, purified protein antigens, and mice have revealed some new information on how delayed hypersensitivity is in-

Table VII. *Effectiveness of 5 day series of contrasensitizer injections for inhibiting sensitization to HSA*

Group No.	Treatment	Per cent positive					
		3 weeks		5 weeks		7 weeks	
		A*	D*	A*	D*	A*	D*
1	HSA in w/o on Days 0 and 14	100	80	90	80	100	70
2	HSA in w/o on Days 0 and 14 + con-S on Days 0-4 and 14-18	70	10	70	10	90	30
3	HSA in w/o on Days 0 and 7	100	100	100	100	100	100
4	HSA in w/o on Days 0 and 7 + con-S on Days 0-11	50	10	30	0	90	20

Mice in this experiment were inoculated subcutaneously with 0.25 mg. quantities of HSA in 0.1 ml. volumes of w/o emulsion as indicated, and then were injected intraperitoneally with 0.1 ml. volumes of 20 per cent contrasensitizer, also as indicated.

*A = Arthus hypersensitivity; D = delayed hypersensitivity.

duced and how immunological enhancement can delay rejection of homografts and interfere with immunity to some tumors. Our original observation that certain humoral antibodies specifically can suppress induction of delayed hypersensitivity⁶ is reconfirmed here and extended to some analyses of how these antibodies inhibit sensitization.

The extent to which hypersensitization is suppressed depends upon a balance between quantity and quality of contrasensitizer applied and the strength and persistence of antigen exposure (Fig. 1, Tables I and III). It ranges with decreasing contrasensitization from completely prohibiting development of delayed hypersensitivity (including capacity for anamnestic recall; Tables III and IV) and strongly suppressing to occasionally preventing induction of Arthus hypersensitivity, through preventing most and impairing some development of delayed hypersensitivity and only moderately slowing Arthus sensitization, to only deferring most delayed hypersensitization and not affecting development of Arthus hypersensitivity at all.

That contrasensitizer works against the inoculum of antigen and not against the experimental animal is indicated by many of our results. Its effect is antigen specific,⁴ and it itself is an antibody probably of 7S gamma-1 class.⁸ One can reproduce this type of contrasensitization by surgically removing the antigenic inoculum some time within the first few days after its injection (Tables I and II). An inoculum counteracted by an appropriate series of contrasensitizer injections remains inactivated indefinitely after injections of the inhibiting antibody have ceased (Fig. 1), but the experimental animal retains normal immunologic responsiveness: If it is inoculated again but not once again treated with contrasensitizer, it develops delayed hypersensitivity in a normal (but not anamnestic) manner (Tables I to III). Immunocytes transferred to normal mice adoptively from mice immunized and also treated with contrasensitizer do not sensitize their recipients either actively or anamnesticly (Table IV); hence, contrasensitizer must have interfered with the earliest stages of sensitization preceding even the development of immunocytes with anamnestic responsiveness, i.e., with interaction between antigen and appropriate host cells.

We have more evidence of the same kind and of similar implication from timing experiments. One of these (Fig. 2) indicates that contrasensitizer injections will suppress the induction of delayed hypersensitivity in mice only if they are begun within 4 or less days after immunization. Results from this experiment, along with those of various others reported here, demonstrate that under our experimental conditions mice require more than 4 days, but no more than 8, to begin making delayed hypersensitivity memory cells but less than 2 days to do the same for Arthus hypersensitivity. These are not absolute values since they undoubtedly depend on conditions of inoculation, but the sequence of Arthus sensitization preceding delayed hypersensitivity is probably consistent and agrees with what we have observed before.⁷

As a result of this experiment we were able to modify our regimen of contrasensitizer injections to use a series of 5 in daily sequence to counteract each inoculation of antigen (Tables VI and VII). Thus, one such series permanently prevented one inoculation from inducing delayed hypersensitivity, and a second

series could be given to do the same against a similar inoculation given one or 2 weeks later. But contrasensitizer, like enhancing antibody in the enhancement phenomena,⁹ was not effective against anamnestic stimulation, that is, against inoculation of a sensitized mouse (Fig. 2) or of a recipient of sensitized isogeneic spleen cells (Table V).

The last point is interesting in suggesting that either blockade of an inoculum of antigen is not complete enough to prevent escape of the much lower quantities of antigen needed for triggering an anamnestic rather than primary response¹⁰ or that interaction between anamnastically responsive cells and antigen is different from primary interaction between antigen and mouse cells and can take place despite the presence of contrasensitizer. The facts that contrasensitizer must be a special class of immunoglobulin,⁸ that precipitins of apparently higher avidity than contrasensitizer and 7S gamma-2 type do not have a contrasensitizing effect,⁴ and that contrasensitizer is effective when given as late as 4 days after inoculation when antigen already has been distributed widely throughout the body would seem to favor the second explanation. Perhaps contrasensitizer interferes with antigen processing and modification by macrophages or with the RNA-antigen complex resulting from such processing. Since both of these steps are postulated as necessary to primary immunologic induction, but once anamnestic responsiveness has developed antigen can stimulate memory cells directly,¹¹ contrasensitizer would be effective against primary but not secondary antigenic stimulation as we have observed to be so. Probably contrasensitizer reacts with antigen before it is much degraded, since, as a humoral antibody with somewhat different determinant specificity than the cellular antibody whose formation it blocks,^{1, 12} it must exert its effect while these different determinants remain on the same molecule.

Data from one of the experiments testing different regimens of contrasensitization (Table VI) together with interpolation from previously published quantitative information⁶ indicate that the half-life of this immunoglobulin is 10 to 12 hours. This is an inordinately short half-life, especially for a mouse 7S gamma-1 globulin¹³⁻¹⁵ such as we suspect this to be.⁸ Maybe in addition to being catabolized this antibody is adsorbed rapidly to mouse tissues and its chances of inactivating antigen are decreased proportionately. Indeed, this ready adsorbability might be a characteristic of contrasensitizer essential for its inhibitory effects.

Our interest here has been in the effect of contrasensitizer on prevention of delayed hypersensitivity, but obviously it also suppresses Arthus sensitization. One sidelight of our adoptive transfer experiments related to Arthus sensitization was a finding, which we do not understand, that, although contrasensitizer failed to prevent systemic sensitization of treated mice, it did prevent any Arthus-sensitized cells from appearing in their spleens (Table IV).

In immunological enhancement of tumors, mice injected with nonliving suspensions of certain kinds of tumors later fail to develop immunity against living tumor cells of the same antigenic kind; thus, tumor growth is enhanced.¹⁶ This phenomenon is due in part to humoral enhancing antibodies which interfere with the delayed hypersensitivity responsible for tumor immunity; tumor

growth can be enhanced by passively transferred antibody.^{9, 16} The same kind of interference by humoral antibodies is seen also in other complex delayed hypersensitivity phenomena, such as debilitated homograft rejection^{1, 9, 16} and passive "immunity" to certain experimental autoimmune diseases.^{17, 18} Although passive enhancement has eluded ready explanation because of its complexities,^{1, 10} most evidence favors the proposition that humoral antibodies enhance by interfering with the initial development of delayed hypersensitivity.^{9, 10, 19} By proving directly that this can happen, our simplified experiments with delayed hypersensitivity* to purified protein antigens support this idea. They begin also to offer low-variable information on the nature and mode of action of enhancing antibody, or specific contrasensitizer.

Our results provide an exception to the generalization that primary induction of delayed hypersensitivity to protein antigens is less easily suppressed by injections of antiserum than such induction of humoral antibody responses.¹⁹ This may be because we have been working with a tuberculin-type rather than Jones-Mote delayed hypersensitivity. Furthermore, our results indicate that only a particular class of humoral antibody inhibits induction of delayed hypersensitivity.^{8, 21, 22} This antibody differs from the class usually used to inhibit humoral antibody formation, whose activity seems to be related more to affinity than to type of immunoglobulin.²³ The importance of this latter point is supported by the recent publication of Axelrad and Rowley.³

*Most previous simplified experiments on enhancement have dealt with humoral antibody suppression of humoral antibody formation^{10, 19, 20} which may not be the same mechanistically as humoral antibody suppression of delayed hypersensitivity.

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