

IMMUNOLOGIC UNRESPONSIVENESS TO PROTEIN ANTIGENS INDUCED IN ADULT HYPERSENSITIVE MICE

Alfred J. Crowle, Ph.D., Denver, Colo.

EXPERIMENTATION with phenomena of immunologic unresponsiveness has become increasingly popular during recent years as a pathway toward improved understanding and control of hypersensitization and allergy, for it seems to deal directly with forces which govern initiation, maintenance, and suppression of antibody formation. Unresponsiveness usually has been induced in immunologically incompetent animals, which are so either because they are very young or because their immunocompetent cells have been injured by chemical or physical treatments. But several reports also have appeared of specific immunologic unresponsiveness elicited in normal adult nonhypersensitive animals by treatments with antigen alone.^{1, 9, 10, 18, 20, 21, 40} The present paper offers what appears to be the first evidence published that such can be achieved in adult immunocompetent animals even after they have been rendered hypersensitive.

MATERIALS AND METHODS

CF₁ strain female mice, 8 to 12 weeks old, were utilized. They were maintained on Rockland mouse pellets and water.

Two kinds of purified proteins served as antigens. One was thrice-crystallized chicken egg albumin (OVA),* and the other was crystallized bovine serum albumin (BSA).† One batch of OVA was set aside to be used only for skin tests, while several bottles of the same kind of preparation, but not necessarily of the same batch, were utilized for sensitization and desensitization. Immunodiffusion analyses¹⁵ of the skin-test batch disclosed that it contained one contaminating antigen accounting for no more than 0.01 per cent of its dry weight. Pentex BSA contained three contaminants in similar minor quantities. One batch of BSA was used for all procedures.

From the Division of Immunology of the Webb-Waring Institute for Medical Research, and the Department of Microbiology of the University of Colorado School of Medicine, 4200 East 9th Avenue, Denver 20, Colo.

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*Nutritional Biochemicals Corp., Cleveland, Ohio.

†Pentex Inc., Kankakee, Illinois.

Hypersensitization and skin-testing methods used for these experiments have been detailed previously¹⁶; they need only brief mention here. Mice were hypersensitized by injecting each twice at weekly intervals with 0.1 ml. volumes of water-in-oil (w/o) emulsion containing 0.25 mg. of antigen. Immediate or humoral, and cellular or delayed, hypersensitivities were determined by intracutaneous injection of 0.02 ml. volumes of 1 per cent antigen in physiologic phosphate buffer. Skin-test readings made at 3 hours reflect anaphylactic and Arthus types of humoral antibody hypersensitivity, and readings made at 24 hours represent delayed or cellular antibody hypersensitivity; we have shown previously for the antigen systems employed here that 3-hour reactivity can be transferred passively with serum but not with cells, and that 24-hour reactivity is transferable with lymphoid cells but not with serum.^{14, 16, 17}

Mice were desensitized by a course of seven daily intraperitoneal injections of antigen dissolved in either 0.2 or 0.4 ml. volumes of buffer, depending upon quantity of antigen used. During the course of desensitization, the presence of hypersensitivity was gauged roughly by thermistor probe rectal temperature readings performed 1 and 24 hours after each treatment.

EXPERIMENTS AND RESULTS

Pilot Experiments.—Data from several preliminary experiments were employed to devise a workable desensitizing technique. These data showed, for example, that subcutaneous desensitizing injections were impractical because of resultant extensive local allergic reactions. Rather, injections had to be intraperitoneal and given daily; if treatment were interrupted for just one day, sufficient hypersensitivity returned in most of the animals for the next desensitizing injection to kill them. Furthermore the quantities of antigen used daily had to be increased rapidly over the short period of treatment in order to avoid systemic Arthus reactions. These pilot investigations provided the basis for the following experiment.

Desensitization Experiment With OVA.—Mice were divided into three groups, two of 10 mice each and the third of 30. The first was vaccinated with injections of w/o emulsion only, and the second and third received emulsion containing OVA. Vaccinations, development of humoral and cellular hypersensitivities, and the persistent nonreactivity of unsensitized control mice over the first 6 weeks of this experiment are shown in Fig. 1.

Three weeks after the beginning of this experiment the third group of 30 sensitized mice began receiving daily desensitizing treatments as shown along the abscissa in Fig. 2; each animal in this group received, intraperitoneally, 0.1 mg. of OVA in 0.2 ml. of phosphate buffer on the first day of treatment and on succeeding days 1 mg., 10 mg., 20 mg., 20 mg., 40 mg., and 40 mg. The first five treatments were in 0.2 ml. volumes of buffer, while the last two utilized 0.4 ml. volumes. This dose schedule provided the maximum treatment which would not be fatal to most of the mice.

The most profound systemic responses to these treatments consistently oc-

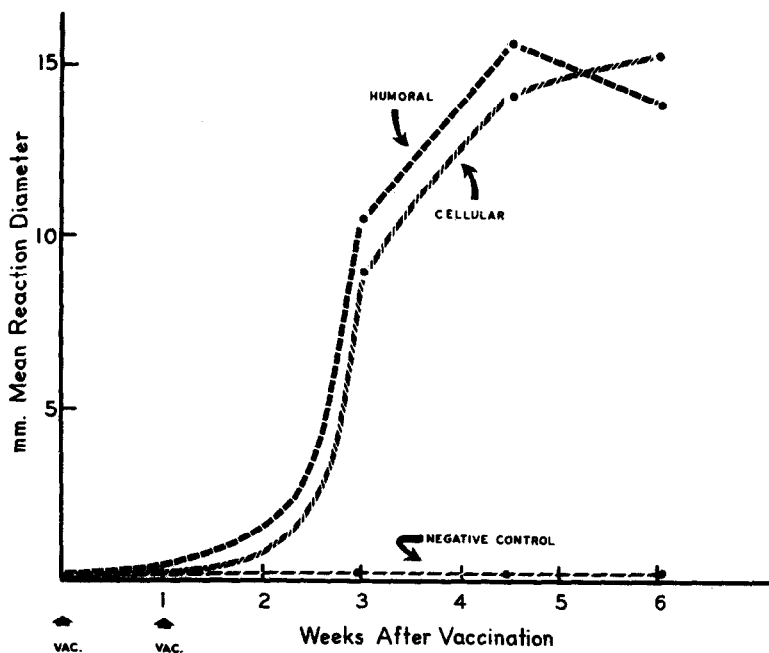


Fig. 1.—Normal development of hypersensitivity to OVA in mice vaccinated with this antigen in w/o emulsion as indicated by Vac. arrows. Edematous reaction read by diameter 3 hours after skin test was measure of humoral hypersensitivity; cellular or delayed hypersensitivity readings were of induration 24 hours after skin test. Negative control mice developed no reactions, as indicated. Curve slopes before the first data points are inferred from numerous previous experiments.¹⁶

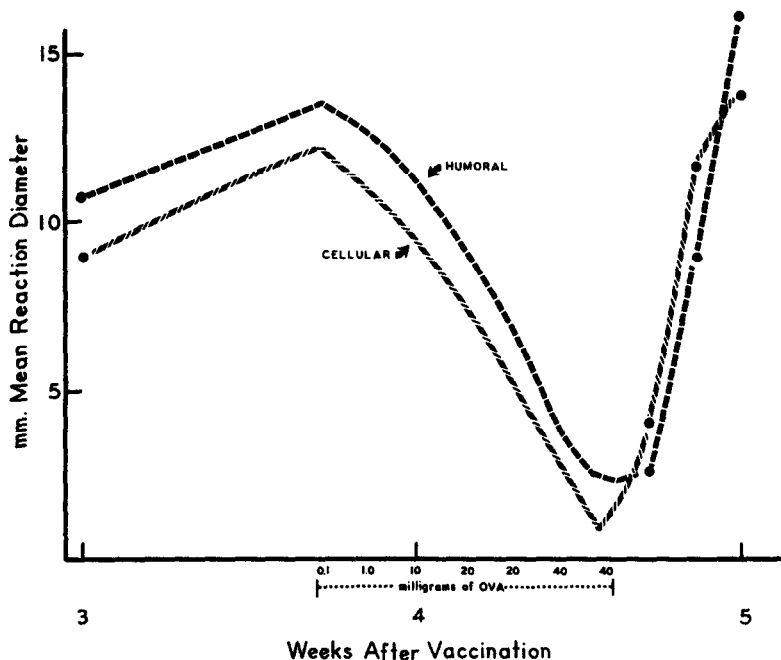


Fig. 2.—Course and effects of desensitization of allergic mice with OVA injected intraperitoneally at the daily intervals and in the quantities indicated along the abscissa. No data points are given along the curves showing drops in reactivity, because these declines are deduced from rectal temperature data rather than skin tests, which were not performed during this period of desensitization.

curred within one hour after a desensitizing injection, as determined both visually and by rectal temperature measurements; the first three treatments excited the most severe, although approximately equivalent, effects. Mean temperature readings in treated mice one hour after these first three treatments averaged 4.5° C. below normal body temperature of these mice. The last four treatments were withstood with relative impunity, and this is reflected in Fig. 2 by the two curves indicating humoral and cellular hypersensitivity reactivities, which drop to their lowest points with the last treatment.

Different groups of 5 mice selected randomly from the desensitized animals were skin-tested 24, 48, and 96 hours after the last desensitizing injection of antigen. Unsensitized control mice and untreated hypersensitive mice also were skin-tested, along with the first of these groups of 5 treated mice. The results from these tests graphed in Fig. 2 show that a significant degree of desensitization was achieved. Although not illustrated in this figure, so as to avoid a confusingly large number of curves, untreated hypersensitive mice exhibited mean skin reaction diameters of 15.7 and 14.3 mm. for immediate and delayed hypersensitivities, respectively, and unsensitized control mice showed neither type of reaction.

Return and Subsequent Loss of Hypersensitivity.—Fig. 2 also demonstrates that the desensitized mice recovered both types of hypersensitivity rapidly after their last treatment. Such recovery was complete within 96 hours, as indicated

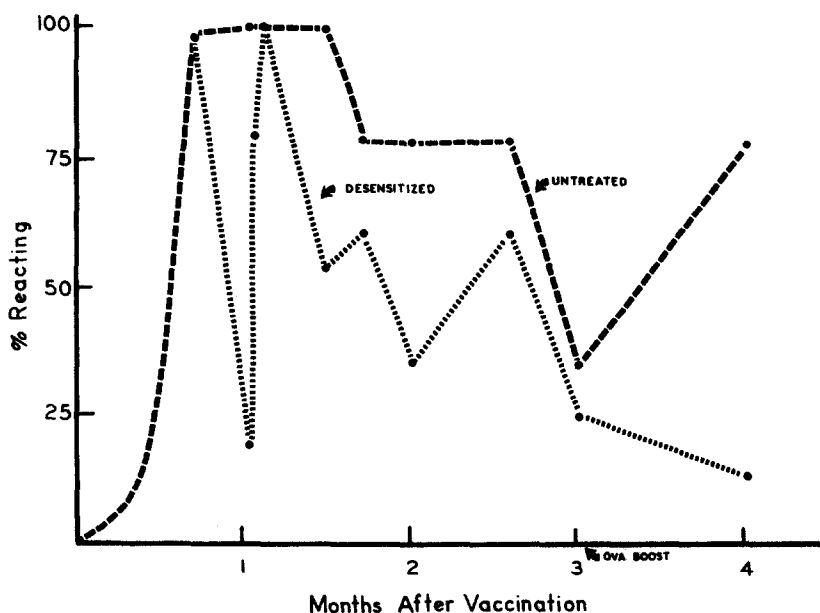


Fig. 3.—Rebound of humoral hypersensitivity to OVA and its later loss in mice desensitized with this antigen, as compared with natural waning of such allergy in untreated mice. Data are presented as per cent of mice reacting, that is, of mice developing reactions larger than 2 mm. in diameter 3 hours after skin test. Mice of both groups as well as the nonhypersensitized control group, not shown here, received a boosting injection of OVA in w/o at the time indicated along the abscissa by OVA boost.

not only by mean reaction diameter, illustrated in this figure, but also by the per cent of mice showing reactions of 2 mm. or more. From 20 per cent at 24 hours this rose through 80 per cent at 48 hours to 100 per cent within 96 hours for immediate hypersensitivity. Analogous figures for cellular hypersensitivity were, respectively, 40 per cent, 100 per cent, and 100 per cent. Thus, the length of the period of skin nonreactivity induced by this vigorous desensitizing regimen was very short.

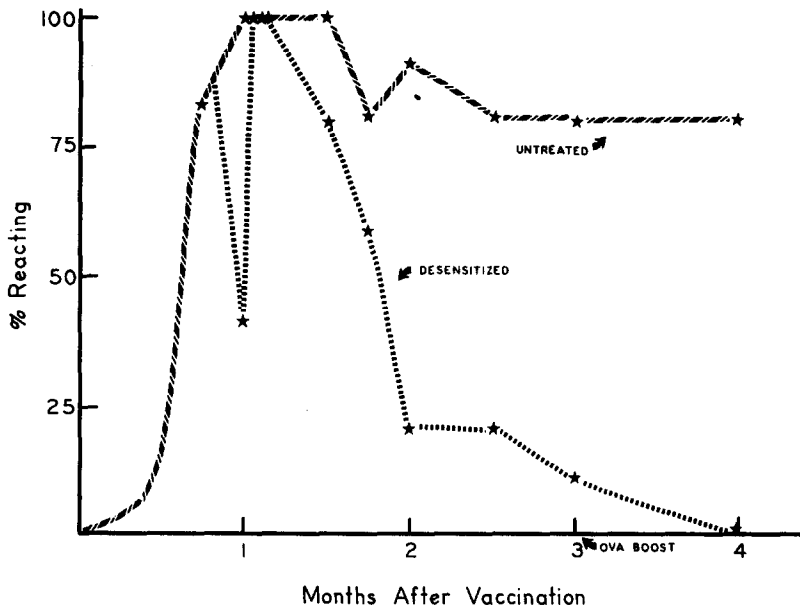


Fig. 4.—Rebound of cellular hypersensitivity to OVA and its later loss in mice desensitized with this antigen as compared with its persistence in untreated mice. Data are presented as per cent of mice developing reactions larger than 2 mm. in diameter 24 hours after skin test. An OVA boosting injection in w/o emulsion, indicated along the abscissa, failed to sensitize "desensitized" mice.

Over the following 3 months, periodic skin tests performed on alternating flanks of these mice revealed initially an unequal rate of decline of humoral hypersensitivity in treated and untreated mice as illustrated in Fig. 3. But, despite such inequality, at the end of these 3 months both groups had lost approximately equal proportions of reactors. At this time, in order to verify the difference in the way in which allergy had declined in the 2 groups, both received a single boosting injection of OVA in w/o emulsion as indicated in Fig. 3. The previously unreactive nonhypersensitized control group also was vaccinated.

This treatment obviously reactivated hypersensitivity in the undesensitized mice whose allergy had waned naturally, the proportion of reactors rising from 36 per cent to 86 per cent within a short time, but equally evident is the complete failure of desensitized mice to respond to this injection by redeveloping hypersensitivity; indeed, reactors among this group dropped from 22 to 12.5

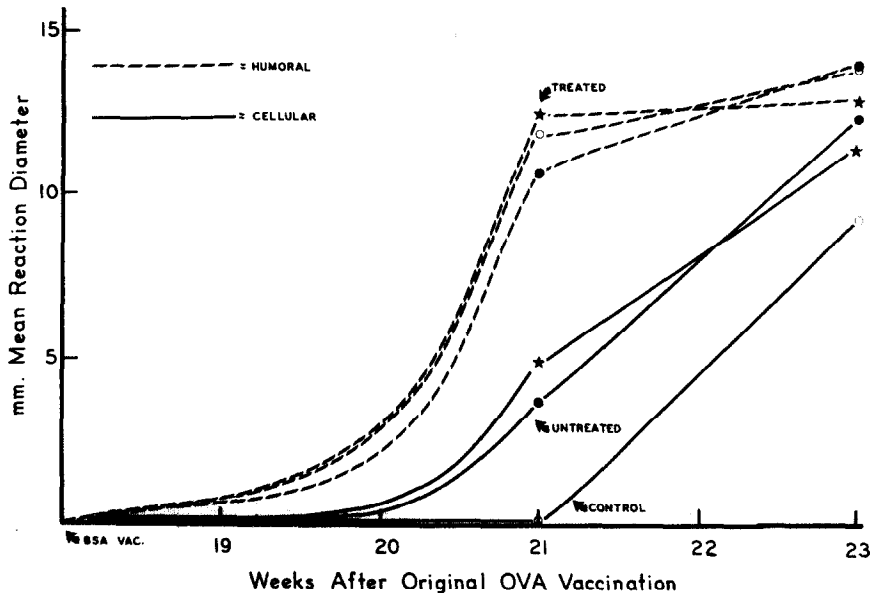


Fig. 5.—Development of humoral and cellular hypersensitivities to BSA in mice of the OVA experiment depicted in previous figures. Included in this graph are the previously nonhypersensitized control mice; these and the treated and untreated mice all received single vaccinations of BSA in w/o emulsion as indicated.

per cent. Although not illustrated in Fig. 3, this single injection of OVA in w/o sensitized all of the previously untreated control mice within the one month period shown. Thus, none of the desensitized mice could redevelop sensitivity when given a demonstrably sensitizing and resensitizing OVA injection.

Still more striking than the after-effect of desensitization on humoral hypersensitivity is that on cellular hypersensitivity; this is shown in Fig. 4. In 3 weeks most of the treated mice lost all of the allergy which they had regained temporarily after desensitization, while most of the untreated mice retained hypersensitivity. Over an additional 2 months the remaining hypersensitive, treated mice also became unresponsive, despite the OVA boosting indicated along the abscissa, while the number of sensitive mice in the untreated group remained constant.

Specificity of Unresponsiveness to OVA.—Treated unresponsive mice, untreated hypersensitive mice, and nonsensitized control mice from this experiment with OVA all were vaccinated subcutaneously in an axilla with 0.25 mg. of BSA in w/o emulsion. As illustrated in Fig. 5, each group developed humoral and cellular types of hypersensitivity to this antigen normally, showing that the observed unresponsiveness to OVA was specific. Cellular hypersensitivity appeared considerably later than humoral hypersensitivity in each group. This has been a consistent finding in all of our experiments with protein antigens in mice, both published¹⁶⁻¹⁸ and unpublished; it may bear on the current controversy over whether cellular hypersensitivity is a primitive step in hypersensitization which always precedes development of humoral hypersensitivity.

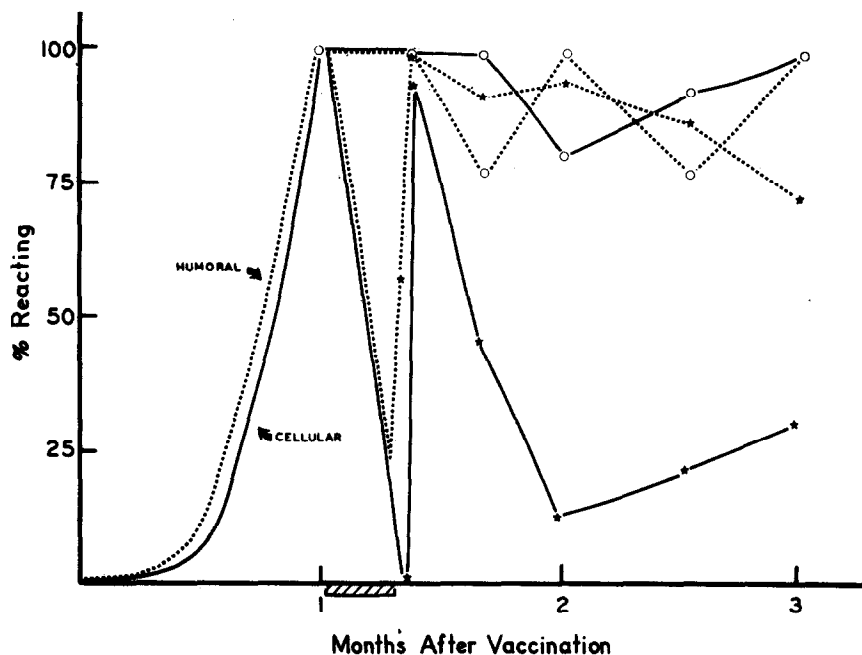


Fig. 6.—Course of humoral and cellular hypersensitivities in mice sensitized to OVA and then desensitized with this antigen over the 7-day period indicated by shading along the abscissa. This graph demonstrates that humoral hypersensitivity sometimes resists accelerated deterioration following the rebound from desensitization shown in other experiments. The desensitized group of mice is indicated by the curves connected by stars.

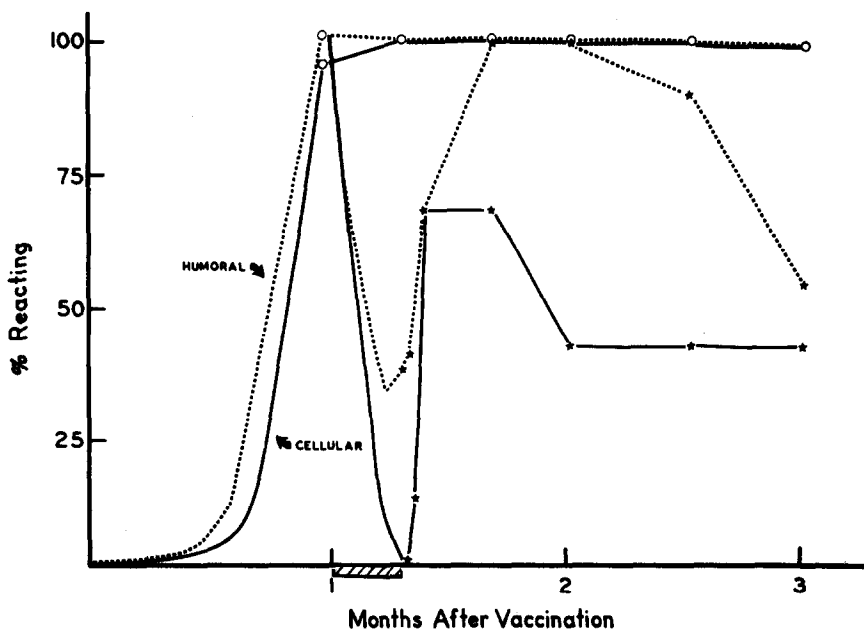


Fig. 7.—Course of humoral and cellular hypersensitivities to BSA in mice vaccinated with this antigen in w/o emulsion and then desensitized with it over the 7-day period indicated by the curve connected by stars. Note that rebound of hypersensitivity after desensitization is slow and that a significant proportion of treated mice never redeveloped delayed hypersensitivity, although they all redeveloped humoral hypersensitivity.

Distinction Between Humoral and Cellular Hypersensitivities.—The desensitization experiment described above has been repeated twice with similar results. In these confirming experiments, cellular hypersensitivity invariably declined after the post-desensitization rebound, but humoral hypersensitivity survived in one. This is illustrated by the graph in Fig. 6, which shows results from an experiment set up in the same manner and with the same temporal relationships and same numbers of mice as the original. Obviously, over a period of 3 months the decline of humoral hypersensitivity was insignificant in contrast to the marked decline of cellular hypersensitivity. Interestingly, waning of humoral hypersensitivity in untreated mice also was negligible.

Experiment With BSA.—A comparison of the development of hypersensitivities to BSA as shown in Fig. 5 with that to OVA in Fig. 1, for example, suggests that immunologic responses in mice to BSA are more sluggish than to OVA. This difference seems to carry through to recovery of hypersensitivity after desensitization. Fig. 7, which is a graph of results from an experiment in which mice were sensitized and then desensitized with BSA rather than OVA, shows this very clearly. Rebound to hypersensitivity after treatment is slower than that to OVA in the preceding experiments, and the time over which hypersensitivity subsequently declines seems to be somewhat extended. This experiment also demonstrates that the type of unresponsiveness which we have observed is not peculiar to only one kind of antigen.

In keeping with the sluggish immunologic responses of mice to BSA is the fact, not shown in Fig. 7, that the animals being treated with this antigen during desensitization developed much milder systemic reactions to it than OVA-sensitive mice did to OVA. Only the second treatment of 1.0 mg. elicited severe anaphylactic reactions. The third treatment with 10 mg. provoked modest reactions, and subsequent injections of 20 and 40 mg. of BSA caused no significant changes in systemic temperature either one or 24 hours afterward.

Also interesting, in regard to distinctions between humoral and cellular hypersensitivities and the mildness of response in mice to BSA, is the observation, shown in Fig. 7, that although each treated mouse regained humoral hypersensitivity after desensitization, some failed altogether to regain cellular hypersensitivity.

Effect of Time Between Sensitization and Desensitization.—Increasing the time interval between hypersensitization injections and desensitization treatments increases the difficulty of desensitization. When this interval was increased to 54 days instead of the usual 26 in one experiment, mortality during desensitization treatments rose from 19 per cent to 86 per cent. Although both sets of mice showed equivalent skin reactivity, the 54-day set had a more vigorous antibody-producing facility, which enabled 86 per cent of the mice to eliminate a 20 mg. dose of OVA and become anaphylactically hypersensitive once again within less than 24 hours. Those 14 per cent of mice which survived desensitization rapidly recovered hypersensitivity, but they also showed the same delayed loss of both types of hypersensitivity seen in mice first treated 26 days after vaccination.

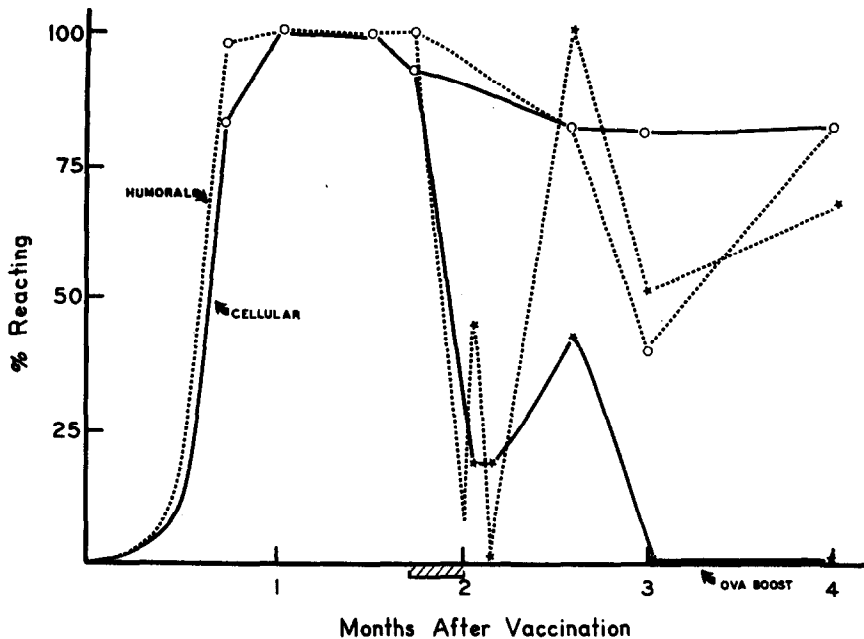


Fig. 8.—Desensitization experiment with OVA in mice treated on the fifth and seventh days of desensitization (indicated by shaded area along the abscissa) with 6-MP; treated mice are indicated by curve connected by stars. The effect of this drug was to delay rebound of humoral hypersensitivity considerably and to prevent rebound of delayed or cellular hypersensitivity altogether in more than half of the mice. Later OVA boosting, as indicated, re-elicited humoral but not cellular hypersensitivity in treated mice.

Influence of 6-MP.—Certain antimetabolites can suppress antibody formation and aid in the induction of tolerance. The effect of one of these, 6-MP, was investigated in a desensitization experiment with OVA.

Fig. 8 is a graphic summary of this experiment. Mice were given the same 7-day course of desensitizing treatments with OVA as used before, except that these were not started until 54 days after the first vaccination. On the fifth and seventh days of desensitization each of these mice, but not controls, also received 4 mg. of 6-MP in 0.4 ml. of aqueous suspending fluid injected intraperitoneally one hour after the day's desensitizing injection. As can be seen by comparing Fig. 8 with Figs. 3 & 4, 6-MP suppressed and delayed the rebound of both types of allergy. More than half of the desensitized mice never recovered cellular hypersensitivity, in contrast to 100 per cent recovery found among mice desensitized but not treated with this drug. Oddly, treated mice losing humoral hypersensitivity later redeveloped it in response to re-vaccination with OVA, but, as in previous experiments in which 6-MP was not utilized, these same mice were incapable of redeveloping delayed hypersensitivity. Incidentally, 6-MP lowered the mortality of mice being desensitized to one third that in a similar group desensitized without its aid.

Test for Humoral Blocking Factor.—The unresponsiveness described above could be attributed to development in desensitized mice of some factor interfering with normal development of skin reactions. It might be humoral or

cellular, although there seems to be no precedent for the latter possibility. The chance that a serum-blocking factor was effecting unresponsiveness is remote, according to results listed in Table I. These were obtained by collecting and pooling sera from mice paralysed to OVA at the end of an experiment, injecting this intraperitoneally in 0.36 ml. volumes into mice known to be hypersensitive to OVA, and 6 hours later skin-testing the recipients. The reactions of these mice compared with reactions which they had developed from skin tests performed a week earlier reveal no depressing effect by "anergic" serum on skin reactivity of either type.

DISCUSSION

"Immunologic unresponsiveness" is a very broad term which includes numerous varieties of immunologic indifference. Some may resemble each other mechanistically and by mode of induction; others may not. In attempts to interpret these phenomena, much importance has been attached to persistence of antigen, since it might have some suppressing effect on immunologic responses.^{19, 22, 28, 29, 36} One can scarcely doubt that the presence of antigen is needed to maintain some forms of very short-lived unresponsiveness, such as that developing during desensitization in the present experiments. In these the body is overloaded with antigen which neutralizes antibody as fast as it is formed.^{31, 32} Although as a result of this the desensitized animal fails to respond to skin tests with the same antigen, it remains immunologically fully responsive, continuing to produce antibodies which rapidly can restore skin-test reactivity when daily administration of antigen is terminated.

The possible direct role of antigen in other longer-lived forms of unresponsiveness is more difficult to visualize because so little is understood about their mechanisms. For example, repeatedly treating an allergic animal with antigen may cause it to form serum-blocking factors which interfere with development of delayed hypersensitivity skin reactions.^{4, 33, 34, 38} Presumably, in instances like these, antigen initiates "unresponsiveness" but is not so directly involved in its maintenance as in the overloading form of unresponsiveness mentioned above. This and the lag which we have noticed in our experiments between cessation of antigen treatment and development of unresponsiveness would make very attractive an explanation of this tolerance based on development of such a blocking factor, but we have not been able to detect anything of this sort in serum by passive transfer.

Antigen may have no direct role in maintaining forms of tolerance induced by injecting it in small quantities in immunologically incompetent or temporarily incapacitated animals. The often verified close correlation between longevity of tolerance and length of treatment with most nonreplicating antigens certainly suggests that persistence of tolerance depends on persistence of antigen, but the role of antigen could be faintly analogous to its function under other circumstances of maintaining hypersensitivity or immunity by the anamnestic response; its original effect may need to be recalled periodically in order to subsist for more than a short while. In these forms of unresponsiveness non-

TABLE I. IMMEDIATE AND DELAYED HYPERSENSITIVITY SKIN REACTIONS IN ONE GROUP OF ALLERGIC MICE BEFORE AND AFTER TREATMENT WITH "ANERGIC" SERUM*

HYPERSENSITIVITY	REACTIONS† BEFORE TREATMENT		REACTIONS AFTER TREATMENT	
Immediate	18	4.8	12	4.4
	12	4.3	16	5.8
	16	4.3	14	5.6
	14	4.0	13	4.3
	14	4.7	13	4.6
	10	3.0	15	4.2
	12	4.1	14	4.3
	7	3.0	12	3.8
	13	4.8	12	4.1
	15	3.8	15	5.8
Mean:	13.1	4.1	13.6	4.7
Delayed	14	7.2	18	8.6
	1	1.5	17	6.4
	16	7.6	16	6.0
	16	6.4	18	6.4
	17	8.3	18	6.8
	17	5.1	1	1.5
	18	7.8	18	8.0
	13	3.7	20	8.7
	15	5.7	18	6.2
	1	1.5	17	4.3
Mean:	12.8	5.5	16.1	6.4

*Immediate hypersensitivity reactions read at 3 hours and delayed at 24 hours after skin-testing. "Anergic" serum injected intraperitoneally 6 hours before skin-testing. The two columns of figures record, respectively, millimeters of reaction diameter and reaction thickness.

†Reactions "before treatment" were those developed from skin tests performed one week previously in the same group of animals.

reactivity is maximal during the latter part of treatment and shortly afterward; then, depending upon such factors as the nature of the antigen and immunologic intactness of the animals, it wanes slowly or rapidly. Such could be explained in two general ways, assuming that the unresponsiveness is not purely physiologic or due to production of "blocking" antibodies. The first is that antibody production is barred at its origin by small amounts of antigen or antigen derivatives,^{7, 11, 22, 35, 36, 42} and tolerance wanes as these are exhausted. The second is that all or nearly all immunocompetent cells potentially responsive to an antigen are incapacitated and that tolerance fades away only when these cells are replaced by natural biologic events, such as release from some source organ or mutation from a closely related line of cells.^{13, 19, 22, 28, 39, 42} Continuance of tolerance explained by the first idea would require a supply of antigen sufficient to maintain effective blockade of the antibody-producing apparatus, whereas duration of the second could be ensured by periodic treatments with enough antigen to kill specifically any immunocompetent cells which happen to have arisen in the interval.

Some examples have been reported of immunologic unresponsiveness in nonallergic, adult, immunocompetent animals induced by treatments with very small quantities of antigen. One of these is Felton's classic form of paralysis elicited in mice with microgram quantities of pneumococcal polysaccharide.²¹ The quantity of this antigen is small enough that the paralyzing dose for one

strain of mice is immunizing for another.⁶ A second example is found in the recent report of Battisto and Miller¹ who injected 10 to 100 meg. quantities of picryl chloride or bovine gamma globulin by mesenteric vein to induce tolerance in guinea pigs to delayed hypersensitivity to these two antigens. A third example is provided in our own recently published work.¹⁸ We found that skin-testing mice with 200 meg. of OVA within a critical short interval after these animals had been given a sensitizing injection of this antigen in w/o emulsion inhibited their development of delayed hypersensitivity to it. We also reported, and since have confirmed (unpublished work), that the same effect can be obtained with 1-chloro-2, 4-dinitrobenzene with a skin-test dose of 5 meg., a quantity of allergen which by single application is insufficient to sensitize CF¹ mice.¹⁷

These experiments which indicate that under certain conditions (e. g., early re-exposure to antigen) or with some antigens (e. g., pneumococcus polysaccharide) a long-lasting tolerance can be established with very small quantities of antigen would favor the notion that such tolerance is due to selective eradication of a clone of cells specifically responsive to the antigen. Indeed, good evidence for this regarding Felton's paralysis is provided by the work of Brooke and Karnovsky,⁶ and similar evidence for tolerance to BSA has been published recently by Sercarz and Coons.³⁶ Both reports indicate that the body's set of immunocompetent cells in tolerant animals has lost its complement of those cells specifically responsive to the tolerated antigen. The difference in ease with which tolerance is induced and maintained in these respective systems may be due less to mechanistic divergencies between them than to such factors as the relative susceptibility to catabolism of each antigen or to a disparity in the number of different antigenic determinants on each (see below).

The form of tolerance which we have reported here, and it does seem to be a specific tolerance according to the generally accepted meaning of the term^{19, 22, 29} rather than any sort of physiologic indifference,^{5, 24, 25, 27} requires injection of massive doses of antigen. At first thought this would seem to distinguish it from the forms of tolerance discussed in the preceding two paragraphs and relate it more closely to forms of tolerance induced only by injecting large quantities of antigen. But some reflection on its characteristics reveals similarities to both varieties of tolerance. Thus, large doses of antigen are required probably because already hypersensitized mice are being used; their populations of antibody-making cells are very high and can be coped with only by proportionally intense exposures to antigen.⁴¹ Yet because immunologic responsiveness (i.e., antibody production) is maintained vigorously throughout the course of desensitization, which is only a temporary antigen overloading effect, free antigen cannot persist in most of the treated mice for more than 24 hours after the last treatment. Consequently, since tolerance begins to appear several days after antigen has been eliminated from the circulation in the body, the developing unresponsiveness would appear to be due to a delayed manifestation of injury specifically incurred by the immunocompetent clone of cells responsive to the antigen employed. One might imagine this clone

to have been overstimulated into antibody production at the expense of reproduction, so that it eventually dies out.⁴²

Two of the instances mentioned above of tolerance induced in adult immunocompetent animals by very small quantities of antigen principally concerned delayed hypersensitivity; the third involved humoral antibodies to a polysaccharide. There is an engrossing common ground to these three systems having to do with the number of different antigenic determinants possibly involved in each form of tolerance, and this relationship gives satisfying meaning to one of the outstanding aspects of the observations presented in the present paper, to wit, that although the tolerance which we describe involves both humoral and cellular types of hypersensitivity, it does so unequally. Thus, unresponsiveness appears more readily and rapidly to cellular hypersensitivity than to humoral, to which it may develop slowly or not at all.

At least two explanations can be offered for this peculiarity. Assuming that both types of hypersensitivity are directed against one species of protein antigen, there still is a difference in number of antigenic determinants involved¹⁷; this point undoubtedly bears on any experiments on immunologic unresponsiveness, as vividly suggested by the recently published works of Gordon²³ and of Coe and Salvin.¹² With the cellular or delayed hypersensitivity directed toward a much broader portion of the molecule and therefore possibly only one determinant, the immune mechanism saturation needed to induce paralysis should be more readily reached for this type of hypersensitivity than for the humoral type, since the latter involves several determinants on one kind of antigen. Incomplete saturation for any one of these would lead to maintenance of reactivity to the antigen, if different cell populations react independently to these different determinants. Since polysaccharides usually have considerably fewer different determinant sites than proteins,⁴ the same sort of explanation would prevail for humoral antibody tolerance to them and the ease and permanence with which it can be induced.

The possible existence of these different cell populations^{17, 22} partly suggests the second explanation which can be offered for differences in development of unresponsiveness to humoral and cellular hypersensitivities. This is that either the total number of cells responsible for the two kinds of hypersensitivity in an animal differs or their half-lives differ or, probably, both. With such an explanation one would expect the two types of hypersensitivity to decline toward tolerance at different rates as, indeed, they do in our experiments. One also might expect to find one kind of hypersensitivity more affected by 6-MP treatments than the other, and this is what occurred in the experiment graphed in Fig. 8.

This explanation and the one preceding it are compatible with each other. From them one would predict, as we have found in practice, that unresponsiveness should become succeedingly harder to induce, especially for humoral hypersensitivity, as the time for allergy to develop is prolonged before desensitization treatments begin. Tolerance should be easiest to induce, particularly for cellular hypersensitivity, just as allergy is developing, when potentially reactive

immunocompetent cells have been stimulated to begin activity but have not yet had time to reproduce extensively, and reactions to minor antigenic determinants still remain dormant. One also would expect induction of tolerance to be aided by any treatment which interferes with reproduction of immunocompetent cells such as, indeed, is generally well known to occur, and such as we have demonstrated in our present experiments with 6-MP.

This concept also may help explain comparable observations made during study of more complicated allergic phenomena. For instance, Kies and co-workers^{26, 37} discovered that skin tests with an aqueous solution of encephalitogen given to guinea pigs a few days after they had been vaccinated with an encephalitogenic w/o emulsion suppressed development of experimental allergic encephalomyelitis in these animals, that is, suppressed development of a disease apparently caused by delayed hypersensitivity to nerve tissue.¹⁷ Another absorbing example is to be found in the work of Benjamini and co-workers.² They have reported that hypersensitivity to flea bites passed through various phases of waxing and waning if guinea pigs were exposed frequently over several weeks to flea bites. Delayed hypersensitivity disappeared before immediate.

The tolerance which we have described appears to be one of those possibly due to specific injury of an animal's immunologic system. Its relationship to specific unresponsiveness induced by Dresser in adult nonallergic mice with nonparticulate bovine gamma globulin²⁰ is uncertain. Dresser's observations may be peculiar to gamma globulins, since Mitchison³⁰ did not obtain a like effect with BSA. Or, as is suggested by Dresser's immunodiffusion analyses but not so interpreted by him, they may apply only to a particular kind of antibody or a certain antigen determinant (cf. Ref. 8); and they are not concerned with cellular hypersensitivity. The tolerance which we have reported here does not seem to be restricted to one kind of antigen, for we have shown that it can be induced to two very different ones, OVA and BSA, and experiments in progress indicate that it also will develop to bovine gamma globulin used under conditions different from those of Dresser's experiments. Allergy and anergy responses to these three proteins seem to differ only in the tempo with which their various stages succeed each other.

Our results may find their greatest theoretical utility in providing rational methods for controlling a particular clone of immunocompetent cells, regardless of which antigen is to be used. Practically, their value lies in suggesting how desensitization procedures some day can be developed for man which specifically will destroy, rather than temporarily suppress, the immunologic apparatus responsible for some undesirable hypersensitivity.

SUMMARY

A form of lasting immunologic unresponsiveness has been induced with protein antigens in adult mice already hypersensitive to these antigens. This specific tolerance appears as a delayed aftereffect of intense desensitizing treat-

ments, and it develops gradually after a rapid rebound of desensitization-suppressed hypersensitivity. This immunologic inertia is explained as possibly due to antigenic overstimulation of a clone of immunocompetent cells specifically responsive to the antigen involved, so that it abandons capabilities for its reproduction in favor of efforts to produce antibody and thus eventually is eradicated. The unresponsiveness studied cannot be explained as due either to skin test-blocking serum factors or to nonspecific immunologic or physiologic body changes.

The paralyzing effects reported in this paper influence both humoral and cellular, or delayed, types of hypersensitivity, but the latter is more strongly affected. This distinction is explained on the basis of differences in antigenic determinants involved in the two types of hypersensitivity and of the possibility that different kinds of immunocompetent cells are responsible for each. Data are presented which indicate that tolerance interfering with humoral hypersensitivity becomes progressively more difficult to induce as the time for hypersensitivity development increases.

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