TABLE II. Effect of Aflatoxin B_1 on Organ Weights of Beef Cattle.

Ration[a] aflatoxin B_1 (ppb)	Mean organ weights (gm/100 lb body wt.)				
	Liver ± SE	Kidneys ± SE	Spleen	Heart	Adrenals
Control	605 ± 12	90 ± 3	98	193	1.5
+ 100	665 ± 18[b]	99 ±12	103	194	1.9
+ 300	610 ± 10	97 ± 3	83	189	1.8
+ 700	739 ± 54[b]	107 ± 3[c]	92	208	2.1
+1000	720 ± 51[b]	107 ± 3[c]	89	193	1.6

[a] Ten steers per group; 2 steers died on 1000 ppb group.
[b] $p = <.05$
[c] $p = <.01$

on the 59th day and one on the 137th day of the feeding trial.

Conclusions. Under simulated practical conditions, graded levels of aflatoxin fortified cottonseed meal were fed to young beef cattle. The levels of aflatoxin B_1 fed in the ration to groups of 10 steers for 133–196 days were 0, 100, 300, 700, and 1000 parts per billion (ppb). Significant growth inhibition, decreased feed efficiencies, and increased liver and kidney weights were observed in the steers fed 700 and 1000 ppb. Gross evidence of liver damage in these groups was also detected. No apparent abnormalities were seen in the groups fed 100 and 300 ppb aflatoxin.

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Received July 28, 1967. P.S.E.B.M., 1968, Vol. 127.

Specificity of Inhibition by Antiserum of the Development of Immediate and Delayed Hypersensitivities in Mice* (32653)

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Antiserum taken from hyperimmunized mice can impair development by other mice of delayed hypersensitivity to protein antigens (1). We have suggested that this effect is specific because inhibitor appeared in the sera of donor mice only upon their being heavily re-exposed to the antigen to which they were already hypersensitive. Nevertheless, such massive exposure to one antigen conceivably might elicit formation of a broadly effective inhibitor, like the α-globulin of Kamrin (2) and Mowbray (3–5), or of excess immunoglobulin which could suppress formation of the same kind of product (i.e., antibody) by feedback mechanisms specific for that class of immunoglobulin rather than for antigen (6). To examine these possibilities we set up the experiments reported here, seeking direct evidence for the antigenic specificity of this inhibition.

Materials and Methods. We used CF-1 female mice and purified protein antigens according to previously described techniques (1). Crystalline human serum albumin (Pentex, Inc.), bovine serum albumin (Pentex,

* This investigation was supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, grant AI-02689.

TABLE I. Specificity of Inhibiting Antiserum against Development in Mice of Arthus (A) and Delayed (D) Hypersensitivities.

Vaccination	Treatment	3 Weeks[a]		5 Weeks		7 Weeks	
		A	D	A	D	A	D
w/o emulsion	none	0[b]	0	0	0	30	0
HSA in w/o	none	100	40	100	90	100	100
BSA in w/o	none	100	60	100	70	100	80
HSA in w/o	antiserum to HSA	**60**	**10**	**10**	**10**	**50**	**30**
BSA in w/o	antiserum to HSA	100	60	100	100	90	50
HSA in w/o	antiserum to BSA	100	80	100	100	100	100
BSA in w/o	antiserum to BSA	**0**	**0**	80	**0**	100	**0**

[a] Skin tests performed at 3, 5, and 7 weeks after vaccination.

[b] Per cent reacting (skin edema or induration greater than 3 mm in diameter); 10 mice per group. Readings reflecting significant suppression of hypersensitivity are boldfaced.

Inc.), and chicken ovalbumin (Nutritional Biochemicals Corp.) served as antigens (HSA, BSA, and OVA, respectively). Our mice were hypersensitized by two subcutaneous injections given in opposite inguinal regions one week apart, of 0.1-ml volumes of water-in-oil (w/o) emulsion containing 0.25-mg quantities of antigen. They were skin-tested 3, 5, and 7 weeks after the first injection on alternating flanks with 0.02-ml volumes of 0.1% antigen in physiologic phosphate buffer; Arthus reactions were read 3 hours after skin-testing, and delayed hypersensitivity reactions were read at 24 hours (7).

Antisera containing inhibitor, which we refer to here for convenience as "contrasensitizer," were produced by injecting mice, previously immunized with antigen in w/o emulsion as indicated above, with a series of 0.1-, 1.0-, and 10-mg quantities of antigen dissolved in 0.2-ml volumes of buffered saline,[1] these three injections being given intraperitoneally on succeeding days. Then, these donor mice were bled out 6 days after the 10 mg injection.[2] For the first experiment, the pool of inhibitor antiserum to HSA was employed at a concentration of 61.5% and that to BSA at 25% (both had passive hemagglutination titers of 10,280). In the second experiment the pool of anti-OVA antiserum was used at 20% (passive hemagglutination titer of 81,920). This is the concentration that we usually use in our experiments, but maximum concentrations allowed by our supply of antisera were used in the HSA–BSA experiment to insure critical evaluation of specificity. The antisera were diluted at the beginning of the experiment with buffered saline and stored frozen between usages. Mice were injected with 0.1-ml volumes of these preparations every day for 12 consecutive days, beginning on the day of the first vaccination.

Absorption of the antiserum containing contrasensitizer to OVA was performed, for the second experiment, by using a quantity of OVA previously ascertained able to remove the antiserum's precipitins and to remain in slight excess as free OVA in the supernatant fluid. The effectiveness of absorption and the quantity of free OVA remaining were determined by quantitative microimmunodiffusion tests (9).

Results. The first experiment, as shown in Table I, included three control and four test groups of 10 mice. Of the control groups, one was injected with w/o emulsion without antigen, and the other two were vaccinated with HSA and BSA, respectively. Test groups were vaccinated with either HSA or BSA and treated with homologous or heterologous inhibiting antiserum.

[1] For contrasensitizer to be produced, the series of injections of 0.1, 1.0, and 10 mg of antigen must not begin until hypersensitivities are fully developed, usually from 4 to 5 weeks after initial vaccination.

[2] Contrasensitizer is produced in high titers by mice only for a short time following the course of three injections described; hence, this bleeding time is critical (8).

TABLE II. Effect of Antigen-Absorbed Antiserum against Development in Mice of Arthus (A) and Delayed (D) Hypersensitivities.

Vaccination	Treatment	3 Weeks		5 Weeks		7 Weeks	
		A	D	A	D	A	D
w/o emulsion	none	0[a]	0	0	0	70	0
OVA in w/o	none	100	100	90	100	100	100
OVA in w/o	antiserum to OVA	100	**20**	**40**	**0**	**10**	**10**
OVA in w/o	absorbed antiserum	100	**40**	90	70	80	**50**
OVA in w/o	antigen-antibody precipitate	100	100	100	100	90	90
OVA in w/o	0.01% OVA[b]	100	70	100	90	90	80

[a] Per cent reacting. Readings reflecting significant suppression of hypersensitivity are boldfaced.

[b] One-tenth milliter per day as indicated in text.

Results from this experiment, recorded as the percentage of mice reacting (Table I) indicate that mice vaccinated with HSA and treated with antiserum to this antigen or vaccinated with BSA and treated with antiserum to that antigen developed little or no delayed hypersensitivity, whereas mice vaccinated with HSA and treated with antiserum to BSA developed delayed hypersensitivity normally. HSA-vaccinated mice, which were treated with the higher concentration of antiserum, also exhibited sustained impairment of Arthus sensitization. Thus, the antiserum inhibitor or contrasensitizer has antibody-like specificity of effect.

If, as these results suggest, contrasensitizer is some kind of antibody, then absorption of antiserum containing this substance with excess antigen might remove or inactivate it. To test this possibility, we absorbed such an antiserum to chicken OVA with a moderate excess of OVA, centrifuged out the resulting heavy flocculate, and compared the absorbed antiserum (now containing 0.01% free OVA) with unabsorbed antiserum for inhibitory activity. The undissociated, washed antigen–antibody precipitate also was tested; and a control group receiving injections of 0.01% OVA in place of antiserum was included in the experiment to take into consideration possible inhibitory effects that injections of free antigen might have (10).

The results of this experiment are summarized in Table II. They demonstrate that absorption of contrasensitizer-containing antiserum diminished its inhibitory activity considerably, indeed, probably almost completely. Injections of OVA alone gave just a suggestion of interference with hypersensitization. Since the absorbed antiserum also contained free OVA, this interference, though not significant by itself, might have enhanced the otherwise only marginal contrasensitizer activity left in the absorbed antiserum. Injections of antigen–precipitin complex did not interfere with sensitization.

Discussion. Our previous report (1) that injections of appropriate antisera can interfere with the induction of delayed hypersensitivity to purified proteins in mice is confirmed here, and direct evidence for the antigenic specificity of this effect is provided. Thus, "contrasensitizer," which we call this inhibitor, appears in serum only following hyperimmunization with antigen, and its inhibitory effect is confined to sensitization by the inducing antigen. Hence, it is not a nonspecific immunosuppressive serum constituent like the α-globulin of Kamrin (2) and Mowbray (3–5). Rather, it resembles the antiserum substance(s) responsible for specific homograft and tumor enhancement phenomena (11–14) and for interference with induction of experimental allergic encephalomyelitis (15).

As we found before (1), inhibitory antisera also specifically suppressed development of Arthus hypersensitivity, though consistently to lesser degree. This might be happening because in mice immunologic responses to pro-

tein antigens develop in the anaphylaxis-Arthus-delayed hypersensitivity sequence in priority, timing of appearance, and in strength of antigenic stimulation required (8,10,16–18; cf. also 19); hence, contrasensitizer could be effective against all three types of response by similar mechanisms but in inverse proportion to their comparative priorities of development—being the most effective against the least readily induced (i.e., delayed hypersensitivity). On the other hand, a basic difference in the mechanisms for suppression of the respective hypersensitivities might account for this distinction. If the former explanation proves to be true, then immediate and delayed hypersensitivities are more closely related than generally has been supposed, a suggestion supported by recent experiences with split tolerance (10,16,20,21).

Contrasensitizer probably is a humoral antibody, because it can be inactivated or absorbed from antiserum by antigen. Although such absorption results in heavy antigen–antibody precipitation, contrasensitizer may not be a precipitin: some antisera with inhibitory activity have low precipitin titers and vice versa, and inhibitor titer in mice drops much faster with time than precipitin titer (8). Titers of antibody detected by passive hemagglutination correlate much better with titers of contrasensitizer, but not invariably. Therefore, how antiserum inhibitory activity is diminished by absorption with antigen will remain unclear pending acquisition of more information. Preliminary analyses indicate that probably contrasensitizer is an immunoglobulin of β-globulin electrophoretic mobility (8). Especially notable has been our consistent failure (8) to raise contrasensitizer in mice sensitized with dextran, an antigen which induces cellular but no humoral antibodies in mice (22).

We have no good idea, yet, of how this inhibitor suppresses development of delayed hypersensitivity. But the antigenic specificity of its effect demonstrated here may mean that it acts analogously to suppression of 19S antibody production by 7S antibody (23,24). This specificity would weigh against an interpretation of effect based on suppression by excess product (i.e., immunoglobulin), such as reported by Dubiski and Fradette (6), especially since there is no reason to believe that contrasensitizer is the "antibody" of delayed hypersensitivity in mice.

Summary. The data reported in this paper confirm our previous report that hyperimmunized mice can produce antiserum which will interfere, in other mice receiving it, with development of delayed hypersensitivity and, to a lesser extent, Arthus hypersensitivity to purified proteins. They also provide direct evidence that this inhibitory effect is specific, and that the inhibitor may be a humoral antibody. It probably is not a precipitin.

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Received August 28, 1967. P.S.E.B.M., 1968, Vol. 127.