A TRANSIENT STAGE OF SUSPECTED DELAYED SENSITIVITY DURING THE EARLY INDUCTION PHASE OF IMMEDIATE CORNEAL SENSITIVITY*

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Normal rabbits, inoculated intracorneally with soluble protein antigens, subsequently develop antibodies which diffuse into the avascular cornea and combine with the residual antigen to form a macroscopic intracorneal ring of opacification 13 or more days after the initial inoculation. This intracorneal ring has been shown to be composed of precipitated antigen-antibody complexes and inflammatory cells (1–3). Other studies have indicated that similar zones can be produced by the simultaneous injection of antigen into one pole of the cornea and homologous antibody into the opposite pole. The zone of precipitation which forms between the two injection sites has been considered the *in vivo* counterpart of Ouchterlony's diffusion system of antigen and antibody in gels (1–4). Since the corneal reaction is dependent upon the presence of precipitating antibody, it is considered the immunological parallel of the cutaneous Arthus reaction in an avascular tissue. This reaction is therefore classified among the immediate (humoral) type of hypersensitivity.

If a sufficient quantity of antigen is introduced intracorneally, one obtains a biphasic reaction in the cornea. The initial reaction is characterized by a sudden diffuse corneal clouding and a dilatation of the limbal vessels 3 to 5 days after the introduction of the antigen. At this time, there is an absence of circulating antibodies and the animal often gives a delayed type of response to skin testing. Corneal clouding of this nature will be referred to as the primary response. In contrast, the later, antibody-dependent component will be referred to as the secondary response of this biphasic corneal reaction.

The following study is a description of the primary response of the biphasic reaction obtained by the introduction of soluble protein antigens into the

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cornea of rabbits. Evidence is presented for placing this early reaction into the category of "delayed sensitivity" as described by Dienes and others (5–10).

Materials and Methods

Animals.—2 to 3 kg albino rabbits of the New Zealand strain were utilized in this study. For the passive transfer and the passive cutaneous anaphylaxis experiments, albino guinea pigs, weighing approximately 250 gm, were used.

Antigens.—Crystalline bovine serum albumin (BSA: Mann Research Laboratories, lot C2610) was reconstituted in physiological saline to give a stock solution with a protein concentration of 63.92 mg per ml. As an alternate antigen, saline reconstituted crystalline bovine gamma globulin was used (BGG: Mann Research Laboratories, lot C1945) in a stock solution 67.90 mg per ml. All the above solutions were prepared aseptically, Seitz-filtered, and tested for sterility.

PROCEDURES AND RESULTS

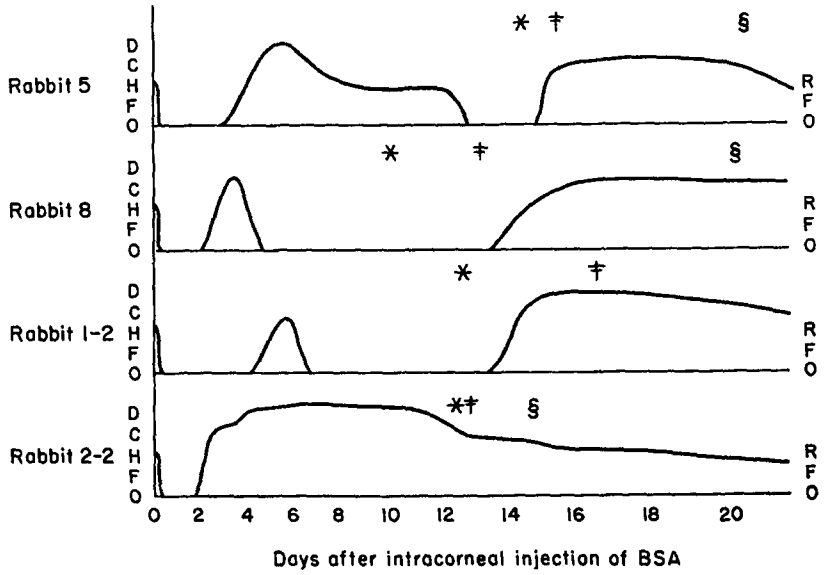
The rabbit was immobilized in a wooden holding box and several drops of ophthaine (Squibb) were instilled topically into the cul-de-sac of each eye. The eye was then proptosed and 0.06 ml of stock BSA (representing 3.8 mg) or BGG (representing 4.0 mg) was introduced intralamellarly into the cornea with a half-inch 27 gauge needle attached to a quarter milliliter tuberculin syringe. Animals were individually caged and observed at daily intervals for the succeeding 3 weeks or until sacrificed.

When approximately 4 mg of either BSA or BGG is inoculated intracorneally into one eye of a rabbit, a biphasic reaction occurs which is characterized by a diffuse clouding of the cornea 3 to 5 days after injection. This is followed by an interval in which the cornea clears and, several days later, by the development of a ring of opacification in the peripheral portion of the cornea (Text-fig. 1, Table I, Figs. 1 to 5).

As indicated in Text-fig. 1, immediately following the introduction of the antigen, an opaque bleb, representing the inoculum which has mechanically separated the corneal lamellae, can be visualized in the center of the cornea (Fig. 1). This bleb is either no longer seen or is only barely perceptible with the unaided eye 8 hours after the introduction of the antigen (Fig. 2). With the aid of the slit-lamp, however, "water clefts" may be seen at the injection site. The cornea is usually clear during the following 2 to 3 days, after which time it suddenly develops a ground glass opacity. The opacification increases, and the cornea frequently becomes so cloudy that only its surface layers can be visualized with the slit lamp (Fig. 3). This corneal response is preceded and accompanied by a dilatation of the circumlimbal blood vessels. The reaction persists for several days, then gradually clears (Fig. 4). This clearing stage is terminated by the development of a circumlimbal flare, followed in a matter of a day or so by the development of an intracorneal ring (Fig. 5). The rings so formed are dependent upon the presence of circulating antibody and persists for a variable number of days, rarely exceeding 2 weeks.

A biphasic reaction is only rarely obtained when smaller doses of antigen

are used. In our experience, it was never demonstrated when less than 1.9 mg of BSA was utilized. Moreover, 2.0 mg of BSA plus 2.0 mg of BGG mixed together and inoculated intracorneally into six rabbits failed to induce the reaction as did the intracorneal injection of 4.0 mg of autologous or homologous rabbit



TEXT-FIG. 1. Gross graphic representation of corneal reaction in rabbits 5, 8, 1-2, 2-2.
Primary reaction; left side of chart:
O, cornea clear.
F, circumlimbal flare.
H, corneal haze.
C, corneal clouding.
D, dense corneal opacification.
Secondary reaction; right side of chart:
O, cornea clear.
F, circumlimbal flare.
R, corneal ring.
* Antibody detected in aqueous of inoculated eye.
‡ Antibody detected in aqueous of uninoculated eye (interpreted as an indication of circulating antibody).
§ Neovascularization of cornea.

serum proteins (Table II). All but the latter two procedures (autologous or homologous serum proteins) did, however, induce the immediate (humoral) phase of the reaction.

Early in the course of these studies it was felt that the experimental proce-

TABLE I

Corneal Reactions in Rabbits Given 3.8 mg BSA Intracorneally, Right Eye Only

Rabbit No.	I*		II							III		IV										
	0‡	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
5	O§	O	F	C	C	H	F	F	F	O	O‖	R	R¶	R	R	R	R	R	RV	RV	RV	RV
7	O	O	O	O	F	C	C	O	O	O	O	O	O	O	O	R	R	R	R	R	R	R
8	O	F	C	H	O	O	O	O	O‖	O	F	R	R¶	R	R	R	R	R	RV	RV	RV	RV
9	O	O	F	C	D	D	C	C	H	F	F	F	FV	FV	FV	RV	RV	RV	RV	RV	V	V
1-0	O	F	D	D	D	C	H	F	O	O	F	R	R	RV	RV	RV	RV	RV	RV	RV	RV	V
1-1	O	O	H	C	C	H	H	O	O	O‖	F	F	R	R	R¶	R	R	R	R	R	R	RV
1-2	O	O	O	H	H	F	O	O	O	O	O	O‖	O	O	R	R	R	R¶	R	R	R	R
1-3	O	O	O	H	C	C	H	H	F	F	F	F	F	F	F	R	R	R	R	R	R	R
1-6	O	O	O	H	C	C	C	H	H	O	O	O	O	R	R	R	R	R	R	RV	RV	RV
1-7	O	O	F	C	H	H	C	O	O	F	R‖	R	R	R	R	R	RV¶	RV	RV	RV	RV	RV
2-0	O	O	F	F	H	H	H	H	H	O	O	F‖	R	R¶	R	R	R	R	R	RV	RV	RV
2-2	O	O	C	D	D	D	D	D	C	C	C	CR‖¶	CR	CRV	CRV	CRV	DV	DV	DV	DV	DV	CV
2-3	O	O	O	O	H	C	C	H	F	O	F	F	R	R	R	R	R	R	R	R	R	R
2-5	O	O	O	F	H	H	H	O	O	O	O	F	R	R	R	R	R	R	R	R	R	R
2-6	O	O	O	H	C	D	D	D	H‖	H	H	H	RV	RV	RV¶	RV	RV	RV	RV	V	V	V
2-7	O	O	H	H	H	H	H	H	F	R‖	R¶	R	R	R	R	R	R	R	RV	RV	RV	RV
2-8	O	O	O	H	C	H	F	F	F	O	O	O	O	O	F	R	R	R	R	R	R	R
2-9	O	F	F	C	H	H	F	O	O	O	F	R	R	R	R	R	R	R	R	R	R	RV
3-0	O	O	F	F	C	C	C	H	O	O	O	O	O	F‖	F	R	R	R	R¶	R	R	R

* Stages of corneal reaction: I, latent period; II, early clouding—corresponds to period of delayed sensitivity; III, Intermediate clearing; IV, Late clouding—corresponds to period of immediate sensitization.

‡ Numbers represent days after inoculation.

§ Reaction grade: O, cornea clear; F, circumlimbal flare; H, corneal haze; C, corneal clouding; D, dense corneal opacification; R, corneal ring; V, neovascularization.

‖ Antibody detected in the aqueous of the inoculated eye by PCA test.

¶ Antibody detected in the aqueous of the uninoculated eye by PCA test.

dures could be expedited if both corneas were utilized simultaneously to study the primary corneal reaction. In such animals, it was found that the sequence of early events was similar but that the primary response often merged imperceptibly with the secondary response (Table III). As a consequence, most of our studies have been concerned with the course of events in one eye.

Injection of Non-Allergenic Inflammatory Substances.—In order to compare the corneal clouding obtained with antigens to that obtained by non-antigenic substances, the corneas of normal rabbits were inoculated with 0.06 ml of the following substances:

1. 5 per cent gum cellulose in saline
2. Freund's incomplete adjuvant
3. 70 per cent alcohol
4. India ink

TABLE II

Corneal Reactions in Rabbits Given Various Dosages and Combinations of Antigens Intracorneally

	Primary response	Secondary response
Group A: given 1.9 mg BSA ic (0.06 ml)	1/8*	8/8
Group B: given 2.0 mg BSA + 2.0 mg BGG ic (0.06 ml)	0/6	6/6
Group C: given 4.0 mg homologous serum ic (0.06 ml)	0/4	0/4
Group D: given 4.0 mg autologous serum ic (0.06 ml)	0/4	0/4

* Numerator represents positive reactions; denominator represents total number of animals tested.

All of these substances induced an immediate inflammatory response which generally subsided by the 3rd or 4th day after inoculation. This is in direct contrast to the primary response of the reaction with antigen which commenced on the 3rd or 4th postinoculation day and persisted for several additional days.

Histological Studies.—The cellular response to the injection of either 3.8 mg BSA or 4.0 mg BGG is identical. Up to 48 hours following the introduction of antigen, only a few pseudoeosinophiles and mechanical spreading of the corneal lamellae by the injected fluid are observed in the cornea. The limbus, during this period, is infiltrated with pseudoeosinophiles and "round cells."

During the phase of the primary response, the corneal stroma is markedly edematous and heavily infiltrated with pseudoeosinophiles (Fig. 6). The limbus and iris contain accumulations of "round cells" (Fig. 7). This focal infiltration of the limbus by lymphoid-mononuclear elements is quantitatively directly proportional to the amount of antigen introduced into the cornea. Inoculation of large doses results in an extensive infiltration at the limbus, lesser doses in a diminished response (11). There is no evidence of stromal damage during this phase of the reaction. There is, however, questionable evidence of endothelial damage on slit lamp microscopy that can neither be confirmed nor denied by routine histologic preparations.

Microscopic examination of the corneas of animals inoculated with non-allergenic

TABLE III

Corneal Reactions in Rabbits given 3.8 mg BSA Intracorneally into Each Eye, Bilaterally

Rabbit No.	Eye	I*				II									IV							
		0‡	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
4-5	O.D.	O§	O	O	O	F	C	C	D	D	D	C	C	C	R	R	R	R	RV	RV	RV	RV
	O.S.	O	O	O	F	C	D	D	D	D	D	D	C	R	R	R	R	RV	RV	RV	RV	RV
4-8	O.D.	O	O	F	H	C	C	D	D	D	D	D	D	R	R	RV	RV	RV	RV	RV	RV	RV
	O.S.	O	F	C	C	C	C	C	C	C	D	D	C	C	C	RV	RV	RV	RV	RV	RV	RV
5-0	O.D.	O	F	C	C	D	D	D	C	C	C	H	H	C	R	RV	RV	RV	RV	RV	RV	RV
	O.S.	O	O	F	F	F	D	D	D	D	D	C	C	H	R	RV	RV	RV	RV	RV	RV	RV
5-2	O.D.	O	H	H	H	D	D	D	D	D	D	C	C	R	R	RV	RV	RV	RV	RV	RV	RV
	O.S.	O	C	C	D	D	D	D	D	D	D	D	R	R	RV	RV	RV	RV	RV	RV	RV	RV

* Stage of corneal reaction: I, latent period; II, early clouding (primary response)—corresponds to period of delayed sensitivity; IV, late clouding (secondary response)—corresponds to period of immediate sensitivity.

‡ Days after inoculation of BSA intracorneally.

§ Reaction grade: O, cornea clear; F, circumlimbal flare; H, corneal haze; C, corneal clouding; D, dense corneal opacification; R, corneal ring; V, neovascularization.

inflammatory substances reveals a pseudoeosinophilic infiltration and edema of the corneal stroma which is similar to that seen after intracorneal inoculation of antigenic proteins. The cellular response at the limbus, however, differentiates the two. In the non-allergenic insult, the cellular elements at the limbus are predominantly pseudoeosinophiles; lymphoid elements are not present in the large numbers commonly seen in reactions induced by antigens.

The infiltration of pseudoeosinophiles into the area of corneal edema corresponds to the period of opacification of the cornea. These cells tend to disappear from the cornea as the primary response subsides and the cornea clears before the onset of the secondary response.

During the greater part of the secondary response, particularly at its termination, the limbus is predominantly infiltered with plasma cells (Figs. 8 and 9). Months after the termination of the reaction, plasma cells are rarely seen, but lymphocytes and mononuclear cells are found in focal accumulations at the limbus.

Detection of Antibody in the Aqueous and in Ocular Tissues

The passive cutaneous anaphylaxis (PCA) test developed by Ovary (12) was utilized to detect the presence of humoral antibodies in the aqueous. In each test, specific antiserum of known titer was used as a control. Aqueous samples were drawn from 15 rabbits during various stages of the primary response of the corneal reaction and were tested for the presence of antibody; none were positive. The eyes of three rabbits were enucleated during the primary response and extracts were prepared from the reactive limbal area of the eye. Such preparations were also found to be negative for the presence of humoral antibody as determined by the PCA test.

Ten rabbits were selected from the group given 3.8 mg of BSA intracorneally and, beginning on the 5th day after injection, 0.1 ml of aqueous was removed from the inoculated and the uninoculated eyes at daily intervals. This was injected intradermally into guinea pigs to determine the presence of antibody by the PCA test. As indicated in Table I, antibody was first detected in the aqueous of the inoculated eye on the 8th day after injection and subsequently in the aqueous of the uninoculated eye. The appearance of antibody in the aqueous of the uninoculated eye indicated the presence of circulating antibody (13).

Coons' technique, utilizing fluorescent labeled reagents, was employed for the detection of antibody in frozen sections of ocular tissues prepared during various stages of the primary response of the corneal reaction to BSA. In this procedure, rhodamine-labeled BSA, prepared and kindly supplied by Dr. B. Roizman, was layered over washed tissues. After thorough washing with phosphate buffered saline (pH 7.0), the sections were counterstained with fluorescein-labeled BGG. Following another washing in buffered saline, the tissues were mounted in 10 per cent glycerol in buffered saline and examined for fluorescence. This technique was used only in those eyes which had been inoculated with BSA.

The sections thus prepared failed to reveal the presence of specific antibody. Similar sections prepared during the secondary response of the reaction demonstrated the presence of juxtanuclear specific staining in plasma cells located at the limbus and the iris tissue. This was detected 7 days after the introduction of antigen in one case and routine at 8, 9, or 10 days, confirming the more extensive studies of Witmer (14).

Cell Transfer Studies.—Several attempts were made to passively transfer a delayed type of hypersensitivity utilizing cells from previously sensitized enucleated eyes, the preauricular lymph nodes, and the buffy coat. All such tissues were removed during the primary response of the corneal reaction, 3 to 5 days after the initial injection of antigen. In order to obtain a maximum amount of tissue and to potentiate the primary response, bilateral intracorneal inoculation of antigen was performed on fifteen donor animals for each experiment.

Cell suspensions prepared from the excised limbal segment included 3 mm of the limbal area, the peripheral iris, and the ciliary body. Most of the cornea was carefully cut free in order to eliminate the possibility of introducing antigen into the recipient animal from the depot in the corneal tissues. The material to be prepared was placed into an equal volume of cold, buffered (pH 7) saline containing collagenase in a concentration of 10 per cent (weight by volume), and briefly exposed to the action of a Virtis "23" (Virtis Co., New York City) homogenizer run at full speed. The resulting brei was incubated at 37°C and slowly agitated for 15 minutes, then placed into an ice bath, and again homogenized for several minutes in order to obtain a fine particle suspension. The free collagen fibers were removed by straining the brei through several thicknesses of sterile gauze. The resulting suspension was centrifuged, washed twice with several volumes of physiologic buffered saline, and finally reconstituted in an equal volume of physiologic saline. The tissues of thirty eyes thus as routine yielded a packed cell volume of 0.7 to 1.2 cc. 50 per cent cell suspensions were inoculated intraperitoneally into each of two 250 gm albino guinea pigs, each animal receiving between 0.3 and 0.5 cc of packed cells.

Twenty four hours after injection, each guinea pig was challenged with 40 to 70 micrograms of BSA intradermally. The skin was observed 6, 10, 24, and 48 hours later and graded according to the reaction obtained. In addition, skin biopsies were obtained at 24 and 48 hours.

Of the six recipient animals inoculated with preparations of ocular cells taken from forty-five donors during the primary response of the corneal reaction (three trials), none showed a visibly positive skin reaction at any interval. The biopsy material also proved to be negative.

In addition to the utilization of ocular cells, similar attempts were made to passively transfer sensitivity using cells from the preauricular lymph nodes and the buffy coat of the same experimental donor animals.

Five to ten nodes were removed from various rabbits, and were homogenized in an equal volume of physiologic buffered (pH 7) saline. 2 ml of a 50 per cent cell suspension in saline were inoculated intraperitoneally into each of eight guinea pigs (two trials). These animals were similarly challenged with 40 to 70 micrograms of BSA intradermally.

The gross observations made at all time intervals were considered negative; however, skin biopsy material showed mild cellular infiltration indicating a positive response.

The buffy coat was skimmed from cold, centrifuged, heparinized blood. Differential cell counts revealed that in each instance, the buffy coat consisted of an unusually high percentage (70 per cent) of lymphocytes. These preparations were washed once in saline and then reconstituted as 75 per cent suspensions. 2 ml of this material were injected intraperitoneally into guinea pigs. As above, these animals were challenged with 40 to 70 micrograms of BSA intradermally 24 hours after cell transfer.

Several of these animals demonstrated positive skin reactions of a low grade. Skin biopsies showed a moderate cellular infiltration between the dermis and muscle at the site of the macroscopic reaction (Fig. 10). Consequently these too were considered positive responses.

None of the control animals were found to have either positive skin tests or positive histological preparations (Fig. 11).

TABLE IV

Skin Reactions in Rabbits Given 3.8 mg BSA Intracorneally in the Right Eye Only, Skin-Tested with Separate Injections of BSA and BGG

Rabbit No.	Days after inoculation	Results of skin test					
		6 hrs.		24 hrs.		48 hrs.	
		BSA	BGG	BSA	BGG	BSA	BGG
Primary response							
3-7	4	0	0	±	0	+ (Biopsied)	0
3-8	4	0	0	+ (Biopsied)	0		
3-9	4	0	0	+ (Biopsied)	0		
4-0	4	0	0	+	0		
Secondary response							
4-1	14	++	0	++	0		
4-2	14	+	0	++	0		
4-3	14	++	0	++	0		
4-4	14	++	0	++	0		

0, no reaction; ±, pink and slight in duration; +, redness and measurable induration, wheal at least 1 cm diameter; ++, redness and palpable induration, wheal at least 2 cm diameter.

Skin Tests for Delayed and Immediate Hypersensitivity.—At various intervals following the introduction of the test antigen (BSA) into the cornea of rabbits, both a specific (BSA) and a control (BGG) antigen were inoculated intradermally into the shaved skin on either side of the midline. Between 40 and 120 micrograms of each antigen were used in each instance, the test never being repeated on the same animal. The injection site was graded according to reactivity at 6, 24, and 48 hours after inoculation and in all cases the reaction site was biopsied for histological studies. Animals so injected during the period of the primary response often developed a mild reaction at the site of the specific (BSA) antigen. This would have been overlooked had it not been for the comparison provided by the reaction to the control skin test with BGG (Table IV).

Histologic examination of the sites of the two skin tests, however, showed a more marked difference in the reaction (Table IV and Figs. 12 to 15).

In a similar group of animals, mild Arthus reactions were produced at 14 to 28 days after intracorneal injection of antigen.

Corneal Tests for Delayed Sensitivity.—Inoculation of the cornea with the experimental antigen is frequently utilized as a definitive test for delayed sensitivity. The injection of BSA into the previously uninoculated cornea during the period of "delayed sensitivity" in the experimental eye was, therefore, used as a possible additional means of proving delayed sensitivity. This met with little success. With small doses of protein, in the range of 70 to 120 micrograms of BSA, positive responses were obtained in two out of twelve tries. On the other hand, large amounts of protein, in the range of 2 to 4 mg, routinely yielded positive responses which microscopically proved to be edema and infiltration by inflammatory cells of the corneal stroma. However, there was usually an interval of 2 to 3 days before the appearance of this positive response. The test response was thus so similar to that obtained by the intracorneal inoculation of a normal animal that it was not possible to differentiate them with any degree of certainty.

DISCUSSION

During the course of studies on the induction of corneal hypersensitivity reactions in rabbits, it was observed that concentrations of protein antigen greater than 2 mg occasionally resulted in an early clouding of the cornea. This clouding occurred approximately 3 to 5 days after the introduction of the antigen and was distinct from the expected antibody-mediated corneal ring response which ensued 9 or more days later. Subsequent studies showed that this biphasic phenomenon was reproducible, especially if the inoculum was increased to 4 mg of protein. An observation similar to this has been reported by Keckarovski who found that the introduction of undiluted, heterologous animal sera into the corneas and the anterior chambers of experimental animals induced a biphasic reaction similar to, if not identical with, that described in this paper (15). No explanation for the biphasic nature of the reaction is given in his discussion of the phenomenon.

It was first thought that this primary corneal reaction was a non-specific inflammatory response. However, failure to reproduce a similar reaction with non-allergenic reagents or with homologous or autologous proteins mediated against this supposition. The presence of a concomitant delayed skin hypersensitivity to the specific antigen in these animals suggested the possibility that the corneal reaction was a manifestation of "delayed hypersensitivity" in the sense of Dienes and others (5–10).

It is well established that a transient phase of "delayed hypersensitivity" occurs during the early induction stages in the development of immediate

hypersensitivity. This phenomenon has been called "delayed hypersensitivity" because it fulfills the criteria which define such reactions. These are: (*a*) the skin responds to minute doses of the inciting allergen with a delayed type of response, peak reactivity occurring 12 to 28 hours after intradermal challenge; (*b*) circulating antibody cannot be demonstrated either by *in vitro* or *in vivo* techniques (*i.e.*, fluorescent reagents); (*c*) reactivity is passively transferred with cells, but not with serum or cell-free products.

Many investigators believe that delayed sensitivity of this type represents a state of altered reactivity of the cells of the lymphoid-mononuclear series to the inciting antigen and, that this is an integral step in the development of circulating antibody (7, 16). There is a natural reluctance to equate delayed hypersensitivity of this nature with that obtained classically with particulate antigens, especially tubercle bacilli. This aspect of the problem is adequately and extensively dealt with in the section on delayed hypersensitivity of reference 17.

Our studies of the biphasic reaction in the cornea indicate that the primary response in the cornea is a manifestation of delayed sensitivity. This appears, at times, to be localized to the ocular tissues, inasmuch as negative skin tests have been observed during the corneal reaction. More frequently, however, a degree of generalized systemic involvement is manifested that can be detected by a delayed skin sensitivity to the specific allergen. This can be transferred passively with cells, but not with serum. During this initial reaction, circulating antibody can neither be demonstrated in the serum nor in the tissues.

In contrast, the second or later state of reactivity, often referred to as the "Wessely phenomenon" (18) has been extensively studied and shown to be dependent upon the presence of circulating antibody (1–4).

As indicated in the text of this paper, the primary corneal reaction is dose-dependent. It is not obtained unless a rather large dose of antigenic protein is inoculated into the cornea. Histologic comparison of the limbic tissues of animals that react with the limbic tissues of those that do not, shows that the rabbits receiving the larger concentrations of protein intracorneally possess a greater cellular response at the limbus. These cells consist of pseudoeosinophiles and a variety of round cells. Since the latter are particularly incriminated in the transfer of delayed sensitivity, it is felt that their presence in the reactive eye in greatly increased numbers may be the basis for this manifestation of delayed hypersensitivity in the cornea.

Several days always elapse before the primary response occurs in the inoculated eye. Generally this interval is 72 hours; however, extremes of 24 and 96 hours have been observed. Histologic sections made in such instances again reveal that the manifestation is dependent upon a cellular response at the limbus. Non-allergic inflammatory corneal reactions, on the other hand, occur without the above time lapse and result predominantly in a pseudoeosinophilic

infiltration of the corneal stroma. Little or no round cell infiltration of the limbus is observed.

Delayed type of skin reactions are recognized to be associated with at least four different immunological phenomena:

1. "Allergy of infection," the broad category including all particulate antigens which induce a delayed type of skin response. This category is synonymous with "classical delayed hypersensitivity of the tuberculin type."

2. A transient delayed type of skin sensitivity induced by soluble protein, but not carbohydrate antigens. This occurs during the early induction phases of immediate or antibody-mediated hypersensitivity and is thought by some to be a state of altered cellular reactivity to antigen by the cells involved in antibody production (7, 16).

3. Contact skin sensitivity, induced and elicited with low molecular weight–sensitizing agents (*e.g.* 2,4-dinitrophenyl protein conjugates, etc.). In this case, a delayed skin response, which is not mediated by a demonstrable antibody, can be separated from an anaphylactic sensitivity.

4. The rejection of tissue transplants or transplantation immunity.

From the foregoing, it is easy to realize that the category of delayed hypersensitivity has arbitrarily been established to cover a large group of heterogeneous phenomena having several features in common. The understanding of the mechanisms of this type of sensitivity, however, is still a matter for much speculation.

SUMMARY

1. If quantities of bovine serum albumin or bovine gamma globulin in the range of 2 to 4 mg are inoculated intracorneally into rabbits, a biphasic reaction occurs in the cornea.

2. The primary reaction, which becomes manifest approximately 3 days after inoculation and lasts several days, is characterized by a diffuse clouding of the cornea. During this period, no antibody can be demonstrated either by serological or histological techniques. The animals react with a delayed type of skin reaction and the sensitivity can be passively transferred to normal guinea pigs with the cells of pooled lymph nodes or buffy coats. The corneal reaction is therefore considered a manifestation of delayed sensitivity.

3. The secondary reaction in the cornea, usually occurring about the 14th day after inoculation is the "Wessely phenomenon." This reaction is characterized by a precipitation of immune complexes in the cornea resulting in a visible annular corneal opacity. Circulating antibody can be readily demonstrated by both serological and histological techniques and the animals demonstrate typical Arthus skin sensitivity.

4. Generally intervening between these two phases of corneal activity is a

stage in which the inoculated eye appears essentially normal. This stage is eliminated if *both* corneas are inoculated initially.

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EXPLANATION OF PLATES

PLATE 73

FIG. 1. Opacification of cornea immediately following injection of BSA. × 1.5.

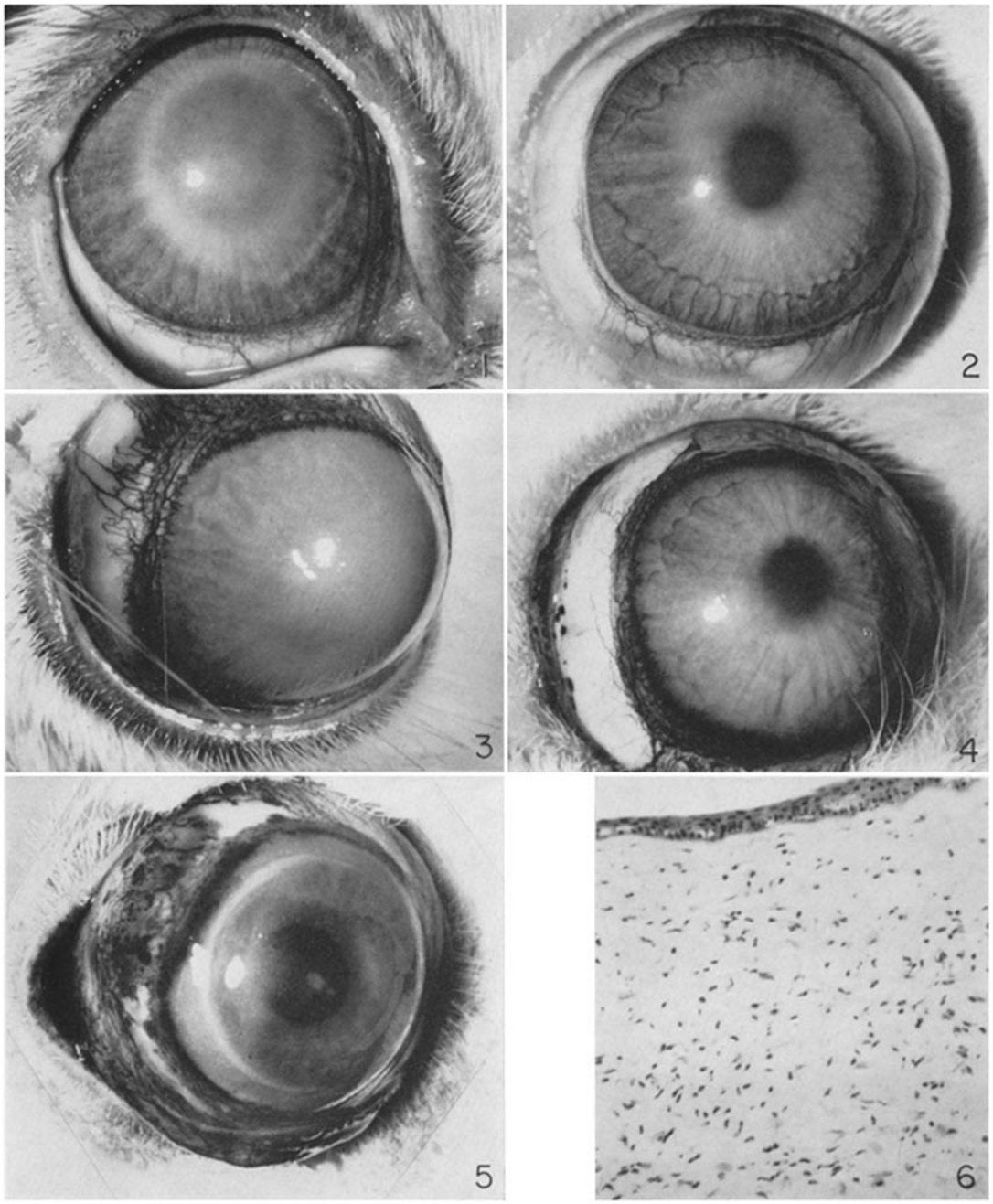
FIG. 2. Mild circumlimbal flare and iritis preceding primary response of biphasic corneal reaction. × 1.5.

FIG. 3. Primary response of biphasic corneal reaction. × 1.5.

FIG. 4. Intermediate corneal clearing. × 1.5.

FIG. 5. Secondary response of biphasic corneal reaction (Wessely ring). × 1.5.

FIG. 6. Infiltration of cornea with pseudoeosinophiles during primary response of biphasic corneal reaction. × 120.



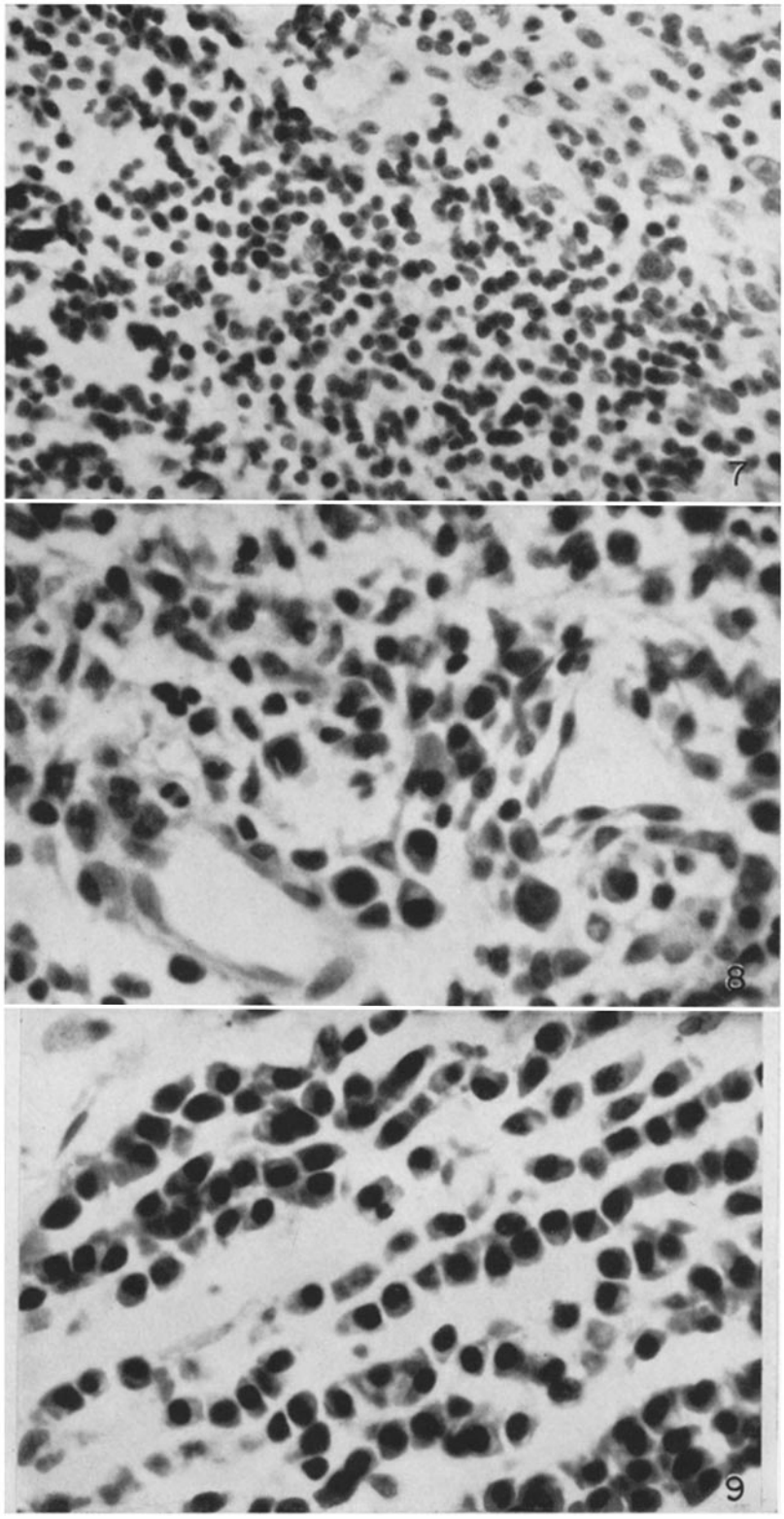
(Parks *et al.*: Corneal sensitivity)

PLATE 74

FIG. 7. Lymphocytic accumulation at limbus during primary response. × 240.

FIG. 8. Plasma cell infiltrate at limbus during secondary response. × 360.

FIG. 9. Plasma cells at limbus during secondary response. × 360.



(Parks *et al.*: Corneal sensitivity)

PLATE 75

FIG. 10. Positive delayed skin test, passive transfer with cells of buffy coat. Note lymphocytic infiltration between dermis and muscle. × 120.

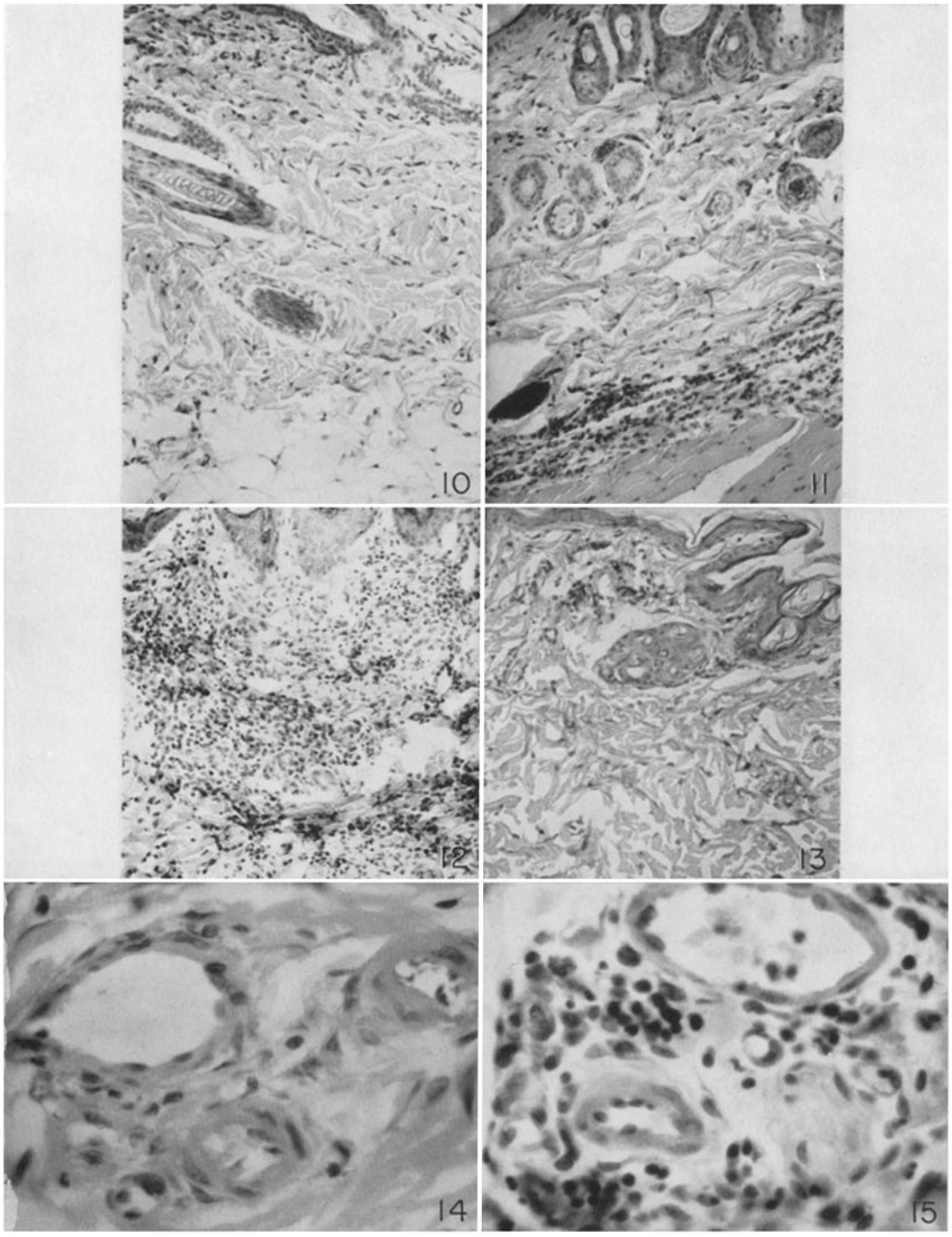
FIG. 11. Control injection site, passive transfer. × 120.

FIG. 12. Control injection site, BGG in delayed BSA-sensitive rabbit. × 120.

FIG. 13. Positive injection site, BSA in delayed BSA-sensitive rabbit. × 120.

FIG. 14. Perivascular response in control skin. × 240.

FIG. 15. Perivascular response in sensiitve skin. × 240.



(Parks *et al.*: Corneal sensitivity)