Ocular hypersensitivity to epinephrine

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The clinical course and degree of disease severity in patients experiencing topical epinephrine hypersensitivity have been classified and correlated with circulating antiepinephrine antibody. The incidence of antiepinephrine antibody has also been studied in two control groups: (1) patients with other external ocular disease and (2) patients with no known ocular disease. Cross-reactivity between epinephrine, phenylephrine, and a triphenylmethane dye have been described.

The possibility that hypersensitization occurs to topical epinephrine has long been suspect in clinical ophthalmology.[1] Relationship between antiepinephrine antibody and clinical signs has not been described. The following studies will attempt to classify clinical stages of topical epinephrine hypersensitivity and to correlate such changes with variation in antiepinephrine antibody.

Materials and methods

Antigen preparation. Crystalline epinephrine was prepared as a 1 per cent solution in normal saline (0.15M, pH 6.5) and allowed to polymerize at 4° C. over a 6 month period. This solution appeared yellowish brown in color and was composed of polymerized epinephrine (P.E.).* (The general polymerization reaction for epinephrine has been expressed as follows:

epinephrine → adrenochrome → melanin.[3, 4, 5]

Antiserum preparation. Antisera were utilized either as whole serum or concentrated salt-precipitated globulin.[6] In general, fresh whole serum tended to give a higher antibody titer than concentrated globulin.

Immune testing procedures. A modification of the Preer capillary precipitin test[7] was performed on all antisera as follows: Glass capillary tubes approximately 4.0 cm. in length and 1.0 mm. internal diameter were sealed at one end. The tubes were filled with three successive layers (Fig. 1, *A*). Layer C contains 1 per cent P.E., B, 0.8 per cent agarose* in sodium phosphate buffer (0.7M, pH 7.4), and A, 25 per cent dilution of whole antiserum or concentrated globulin. The immune reaction occurred at the interface AB, with low-titered sera (Figs. 1, *AI*, 1, *BI*), and throughout zone B, with high-titered sera (Figs. 1, *AIII*, 1, *BIII*). Frequently, low-titered sera (especially globulin) yielded a fine turbidity at the AB interface. To control such false-positive determinations, the precipitin band was shifted to the *BC* interface by suspending P.E. in agarose (final concentration was 0.2 per cent epinephrine in 0.4 per cent agarose, C_1). After several days, a precipitin band appeared at the BC_1 interface (Figs. 1, *AII*, 1, *BII*).

Immunodiffusion[8] was performed on high-titered sera (>1/64). P.E. was precipitated as a brown precipitin band with such antisera (Fig. 2).†

Antigen control. A second polymerized antigen,

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*To enhance the rate of polymerization, one may introduce epinephrine into an alkaline buffer in the presence of heavy metal ions, e.g., cuprous sulfate.[2]

*L'Industrie Biologique Française, Gennevilliers, France.

†The possibility that polymerized epinephrine was adsorbed onto precipitated γ globulin was considered. This was ruled out by adding P.E. to several precipitin systems, e.g., BSA-rabbit antiBSA, hemocyanin-rabbit antihemocyanin, human uveal antigen–human antiuveal antigen.

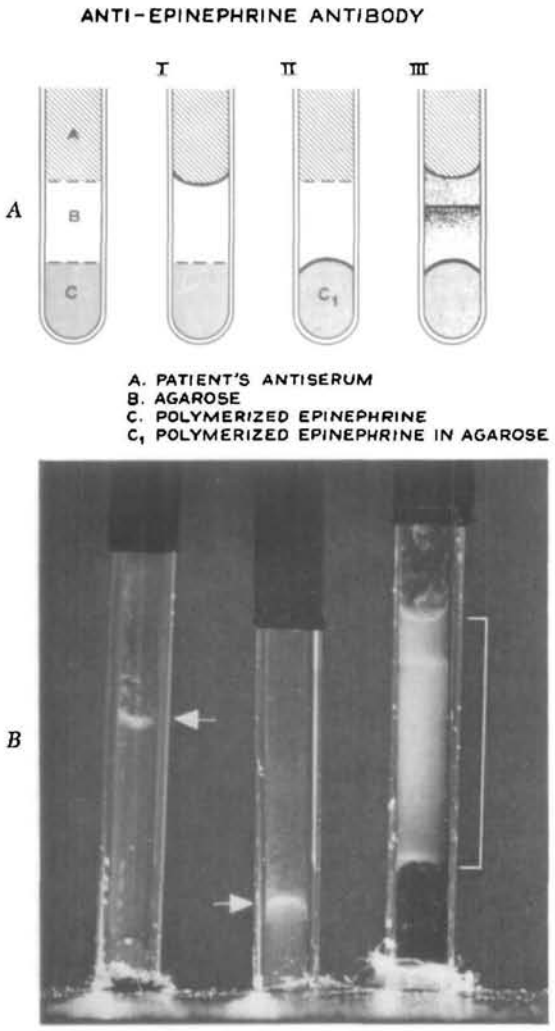

Fig. 1, *A* and *B*. Capillary diffusion precipitin test. *I*, Precipitin band at *AB* interface. *II*, Precipitin band at *BC*, interface. *III*, Heavy precipitin band throughout the agarose zone.

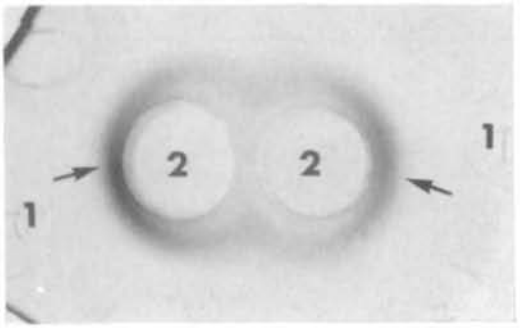

Fig. 2. Demonstration of antiepinephrine antibody by immunodiffusion. *1*, P.E.; *2*, antiserum; arrows indicate the dense brown precipitin band.

alphazurine 2G,* an epinephrine analogue, phenylephrine, and pilocarpine were evaluated for cross-reactivity with epinephrine by immunodiffusion and immunodiffusion absorption against P.E.

Selection of patients. Patients were divided into three groups. The first group was composed of individuals in whom epinephrine was considered to be the immunizing antigen responsible for the observed clinical signs. The second group of patients demonstrated other external ocular diseases which frequently overlapped with the clinical signs seen in epinephrine hypersensitivity. Diagnoses in this group included: the allergic conjunctivitises (seasonal, vernal, and nonseasonal), topical hypersensitivity to other medications (neomycin and pilocarpine), staphylococcus keratoconjunctivitis, epithelial and stromal herpetic keratitis, Sjögren's syndrome, congenital telangiectasia, and phlyctenulosis. (After re-evaluation of therapeutic histories, several patients were discovered to have used epinephrine-containing drops. However, their clinical signs were considered to be unrelated to topical epinephrine therapy.) The third group was composed of individuals with no known ocular disease.

Results

Morphologic changes accompanying epinephrine hypersensitivity. Clinical changes were limited to the anterior eye and were bilateral in each of the 9 evaluated cases. Conjunctival involvement was characterized by vascular engorgement of the limbus, as well as the bulbar and palpebral conjunctiva. Although an early follicular reaction occurred, this soon gave way to a varying degree of conjunctival chemosis. The complete conjunctival picture is best described as a low-grade anaphylactoid reaction (Fig. 3). In several cases, the conjunctival reaction was accompanied by low-grade iritis, characterized by fine flocculent lens precipitates, up to 1+ cells and flare, and an occasional fine granular KP. Fine corneal infiltrates occurred more frequently. Their usual distribution was subepithelial, adjacent to the superior or inferior limbus. Three patients demonstrated diffuse central and peripheral subepithelial infiltrates (1, 2, 3 in Table I).

The clinical course of this disease has been evaluated in 5 patients (1, 2, 3, 6, 7,

*Allied Chemical Corp.

in Table I) each of whom agreed to remain on topical epinephrine therapy. In each case, maximal disease was achieved after several months of topical medication. However, with continuing therapy, the severity of clinical signs tended to regress toward low-grade conjunctivitis, characterized by slight hyperemia and minimal chemosis.

A concomitant dimunition in antiepinephrine antibody titer was noted at this stage of minimal disease (Table I, minimal column). (As yet, no patients have demonstrated an exacerbation of clinical signs.)

Comparative occurrence of antiepinephrine antibody in immunized and nonimmunized individuals. The occurrence of antiepinephrine antibody in patients undergoing topical immunization has been found to be significantly greater than in nonimmunized patients with ocular disease or patients without known ocular disease (Table II). Five conjunctivitis patients demonstrated antibody titers of ¼ or greater. (Two had used topical drops containing epinephrine; the other 3 provided unsatisfactory therapeutic histories.)

Of the 3 antiepinephrine positive patients with no known eye disease, one demonstrated an antibody titer of 1/16. This patient died of a severe anaphylactic reaction following intravenous injection of alphazurine 2G for determination of burn depth.[9] When this dye was compared with P.E. by immunodiffusion, a reaction of identity occurred (Fig. 4), indicating cross reactivity between the two antigens.

The possibility that the described clinical signs were related to a second immunizing antigen, e.g., pilocarpine, was considered. All but one patient (Table I, 5) were receiving pilocarpine for glaucoma control. However, cross-reactivity between these patients' antisera and pilocarpine was not demonstrable.

Similarly, the possibility that phenylephrine, an epinephrine analogue and a widely utilized decongestant by the ophthalmic drug industry, may function as an immunizing antigen in the control series was considered. Although direct immuno-

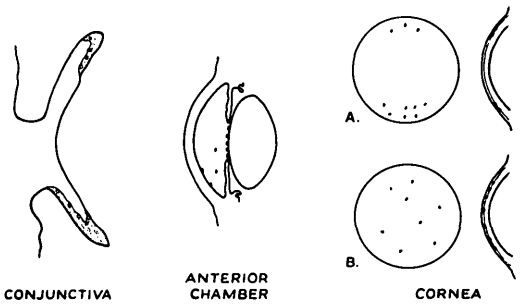

Fig. 3. Clinical changes in epinephrine immunization.

Table I. Correlation between clinical signs and antiepinephrine antibody titer

Case	Age (years)	Race	Sex		Topical epinephrine usage (months)	Conjunctiva	Iritis	Cornea	Antibody titer
1	81	White	Male	Maximum	6	++	–	++	>1/256
				Minimum	4	+	–	–	¼
2	85	White	Male	Maximum	4	++	+	++	1/64
				Minimum	¾	+	–	–	¼
3	72	White	Female	Maximum	10	++	+	++	1/64
				Minimum	1	+	–	–	⅛
4	54	White	Male		72*	+	–	+	⅛
5	69	White	Male		2	+	+	+	⅛
6	61	White	Male	Maximum	12+*	+	–	±	¼
				Minimum	4	+	–	–	¼
7	65	White	Female	Maximum	4	++	–	–	1/16
				Minimum	1	+	–	–	¼
8	79	White	Female		84*	+	+	–	⅛
9	63	White	Female		5	–	–	–	¼

*Patients had used an epinephrine solution for approximately the stated period.

Table II. Incidence of antiepinephrine antibody*

		Total patients	*Positive*	*%*
I	Antiepinephrine syndrome	9	9	100
II	Other external diseases	36	5†	14
III	No known eye disease	29	3‡	10

*Antibody titer ¼ or greater as determined by the modified capillary diffusion technique.

†Two of the five who were positive had been on an ophthalmic preparation containing epinephrine for a prolonged period.

‡The patients demonstrating antiepinephrine antibody had received intravenous alphazurine 2G for evaluation of third-degree burn.

I vs. II, χ^2 9.8, $P > 0.01$.
I vs. III, χ^2 10.6, $P > 0.01$.

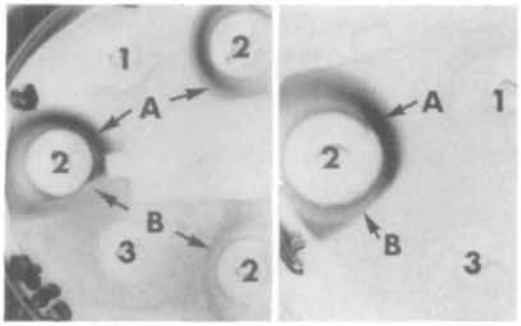

Fig. 4, *A* and *B*. Reaction of identity between P.E. and alphazurine 2G. *1*, P.E.; *2*, antiepinephrine antiserum; *3*, alphazurine 2G; *A*, P.E., antiepinephrine precipitin band (brown); *B*, alphazurine, antiepinephrine precipitin band (blue).

diffusion was negative, diffusion absorption demonstrated appreciable diminution of the precipitin band.

Discussion

Specificity of clinical signs. The epinephrine-induced clinical syndrome closely paralleled the clinical signs seen in rabbits after topical immunization to nonreplicating antigens.[10, 11] The conjunctivitis and iritis most closely resembled the changes produced by dextran 10* or sulfanilic acid, whereas the corneal changes were similar to those seen with dextran 10. Similarly, the severity of disease was well correlated with the height of precipitating antibody titer.

When compared to other human diseases, EKC (epidemic keratoconjunctivitis) and experimental inclusion conjunctivitis[12] closely mimic this syndrome. However, antibody titer infrequently correlates with the height of clinical disease in replicating antigen systems. Other topical hypersensitizing agents, e.g., atropine, pilocarpine, physostigmine, etc., usually produce a more severe reaction. Topical allergens (pollens, etc.) may produce a similar picture.

The common parameter in these models is most likely the immune reaction. The clinical signs observed during epinephrine hypersensitization would seem to represent one variant of a more general immune model. In the already mentioned animal experiments, the characteristics and course of the anterior segment disease were strongly dependent upon the size and quality of the immunizing antigen. Whether this is also true of human disease remains to be demonstrated.

The stage of resolution. The resolution of clinical signs coupled to simultaneous diminution of antibody while conjunctival epinephrine therapy is continued most likely represents a variant of desensitization. A similar observation has already been made in rabbits during conjunctival immunization to BSA, dextran, and sulfanilic acid, although the desensitized state could not be maintained. Whether this disease will recur on continued epinephrine therapy can be determined only by long-term follow-up.

The in vitro precipitin reaction. It seems evident that some polymerization of epinephrine is necessary to achieve in vitro antibody precipitation. The degree and rapidity of precipitation are dependent upon the degree of polymerization (colorimetrically determined). After adequately polymerizing the epinephrine molecule, the characteristic brown "melanin" color imparted to the precipitin band qualitatively identifies the precipitated P.E. The elliptical shape of the band is best explained by the

*Pharmacia, Inc.

rapid diffusion of small molecules in the P.E. spectrum.

The capillary diffusion technique offers a simple semiquantitative precipitin test. The relative neutrality of the agarose lattice allows for enhanced clarity of precipitin bands. False positivity at the antibody interface has been controlled by immobilizing the P.E. in agarose and subsequently shifting the precipitin reaction to the antigen interface.

Antigenic specificity of epinephrine. Cross-reactivity with alphazurine 2G has been demonstrated. (Mutual antigenic identification and antisera reactivity occurred.) No cross-reactivity was demonstrated to phenylephrine or pilocarpine, the only other molecules tested.

The universality in nature of the active antigenic group or groups of P.E. is highly speculative. Cross-reactivity to alphazurine 2G was a chance observation. Before a more exhaustive evaluation of molecular cross-reactivity can be undertaken, a better understanding of the intermediate products between adrenochrome and melanin must be available.

The occurrence of antiepinephrine antibody in ocular and nonocular control groups presents an interesting epidemiologic problem. Whether such reactions represent exposure to topical epinephrine or cross-reactions cannot be readily determined. The frequent utilization of the cross-reacting epinephrine analogue, phenylephrine, in ophthalmic decongestants and the widespread use of such compounds in the general population could explain previous exposure in these control populations.

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Discussion

Dr. Marvin Sears, New Haven, Conn. The author may be complimented on his most interesting and original study of sensitization to topically applied epinephrine. This compound is a valuable agent in our therapeutic armamentarium against glaucoma simplex. Not infrequently, sensitization to epinephrine develops and may become severe enough to require withdrawal of treatment. Therefore the proper diagnosis and management of the sensitized patient becomes an important problem. The present study describes the signs and symptoms of epinephrine sensitization and attempts to relate the cause of the disease to the serum titers of antiepinephrine antibody in the afflicted patients.

Two techniques have been used to demonstrate the presence of antibody to epinephrine. In low titered sera a capillary precipitin test was used and in high titered sera the Ouchterlony type of immunodiffusion was used. Both of these techniques are well known to immunologists and designate

specific antibody provided that certain criteria are met. There must be adequate numbers of control sera to react with the test antigen. It should be possible to modify the location and nature of the precipitin and diffusion bands by altering the relative concentrations of antibody and antigen. Further, several antigens must be employed to determine the presence of cross-reactions. Finally, the antibody should be concentrated in the γ globulin serum fraction. While the present experiments meet some of these criteria, they do not meet all of them. In particular, the appearance of unusual circular immunodiffusion rings in Ouchterlony gel and the lack of a clean cut band in the precipitin reactions except at the interface should dictate the need for more precise controls. It may well be that the low molecular weight of the antigen, epinephrine, has introduced diffusion artifacts into the system. If so, the specificity of these reactions can be further evaluated by reducing the concentration of antigen in the reaction. Similarly, if specific antibody is present, it should be possible to demonstrate activity in the γ globulin fraction of the serum either by concentration procedures alone or with the help of immunoelectrophoresis. If activity is lost with the former operation but regained with the restoration of the sera, it suggests that nonspecific protein is responsible for the reaction.

On the clinical level one may question whether or not patients with epinephrine sensitivity lose their signs and symptoms with continued administration of the drug. It is the experience of several ophthalmologists that such patients often worsen with continued administration of epinephrine so that the drug no longer can be used. In those patients who do improve with continued administration of epinephrine, it would be premature to conclude that the reduction of serum titers concurrent with decreasing symptoms represents desensitization. One must first demonstrate specificity of the antibody in question. Second, desensitization is a complex phenomenon and takes different forms in different systems. The mechanism for desensitization is not yet entirely agreed upon by immunologists. One among many questions which may be raised is whether a blocking antibody could account for the low titered serum.

In addition, no controls for the glaucoma population itself are presented. If an antibody to epinephrine is present, how do we know that there is not something special about the glaucoma group so that it makes antibodies to epinephrine as a consequence of, or concurrent with, the glaucomatous disease process? In other words, no glaucomatous nonepinephrine treated patients are included in the study.

Finally, even if we assume that antiepinephrine antibody is present, how do we know that the symptoms of the sensitivity reaction are related to it, and not to some underlying altered vascular reactivity of the tissues which is now elicited by administration of epinephrine?

In summary, I enjoyed reading this most original and provocative paper. This study represents an important beginning investigation into the nature of epinephrine sensitivity. I think it will yield useful information not only with respect to adrenergic compounds, but may also serve as a model for the detection of sensitivity to other topically applied agents.

Response by Dr. Aronson. I wish to thank Dr. Sears for his interesting comments. Of the many questions raised, I shall attempt to answer three.

The circular characteristics of the precipitin band in Ouchterlony are unusual. However, one must remember that the antigen, *Polymerized epinephrine,* is characterized by a wide spectrum of molecular sizes, so that molecular diffusion of the antigen and antibody will be unequal. (Such mixtures frequently demonstrate a circular band.)

The concept of "desensitization" (with quotation marks) presents a problem in semantics. Our definition includes the concomitance of decreasing antibody with decreasing clinical signs or symptoms in the presence of the hypersensitizing antigen. This definition probably does not meet the criteria for desensitization of most of this audience.

I must apologize to Dr. Sears for not including data for immune globulin characteristics of the antisera. One of our patients had a strong enough antiserum for such evaluation (reverse immunoelectrophoresis), and demonstrated both γM and γG globulins.

I would like to re-emphasize the preliminary characteristics of these studies. In such studies, there is no substitute for a large series of involved patients. Needless to say, we hope to expand this series.