Experimental Study on Fixation of Allergic Reaction to the Eye.

By

Akira Urayama.

(From the Ophthalmologic Department of Tohoku University, Sendai. Director: Prof. Y. Hayashi.)

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As is well known, the idea of allergy first suggested by Pirquet in 1906 has brought about a brilliant achievement in the recent development of pathology. In the background of this development, especially in the experimental field, there were two basic types of phenomena; that is, the so-called general and local anaphylaxis, or tissue-allergy. The latter was first described in 1903 by Arthus, so is usually known as the Arthus phenomenon. Because of its comparatively permanent findings in morphology this reaction was so adequate to experimentation on animals that a number of workers made an attempt to apply it in the same manner to the other different tissues and organs as the author previously did to the skin. Consequently, up to this time there have been reported many results concerning the allergic or, as Roessle has called it, hyperergic inflammation at the site of a local injection of antigen, for instance in the kidneys, joints, lungs, pleura, liver, appendix, genitals, muscles, tendons, arterial vascular system and so on. These investigations have indeed made a great contribution toward explaining the pathogenesis of several practical diseases, but it is also true that they do not elucidate the subject in full, for there is somewhere a gap between the occurrence of natural diseases in man and such an artificial process as direct injection of antigen into localized areas of animals. It may rather be said that these experiments were still within the category of the classic example designated by Arthus. So one of the experimental approaches to be next planned was considered in pursuing the possibility that the allergic tissue reaction might be produced under more natural conditions than mentioned above, and further studies have been made on the skin, muscles, pleura, joints and some arteries by several pathologists and dermatologists, using non-antigenic physical stimuli such as heat, cold, mechanical or functional irritation, combined with an allergic mechanism.

On the other hand, in ophthalmology, besides the special kind of studies on the antigenicity of lens protein and of uveal pigment of the eye itself, there were reported, too, many studies on various ocular portions:
on the cornea, of which a typical one is noted as Wessely's phenomenon (Wessely, Poppen, Rhiem, Jirman, Ishida); on the uvea (Mazzei, Marchesani chesani, Rhiem, B.C. Seegal and D. Seegal, Brown, Rin, Moriji, Takahashi); on the conjunctiva (Rhiem, Hayashi, Funaishi); on Tenon's capsule (Isayama) and others (Spadavecchia, Nectoux). But these studies were as a rule carried out by local administration of foreign protein, for example foreign serum or egg white, into the cornea, anterior chamber, vitreous body, subconjunctival tissue, retrobulbar tissue or spaces between the sheaths of the optic nerve, Vv. vorticosae, A. carotis, or occasionally by instillation into the conjunctival sac. With regard to experimentation related to nonspecific stimulation, we do not find any other reports except the staphylococcic conjunctivitis or the traumatic uveitis in sensitive rabbits recorded by Rhiem, the inoculation of certain bacteria or toxin into the anterior chamber recorded by Brown, that of glycerin recorded by B.C. Seegal and D. Seegal, and local administration of chloroform, histamine, uric acid and bouillonpeptone etc. recorded by Spadavecchia.

The experiment here reported was carried out with the following purpose in mind: to determine whether the allergic tissue reaction can be localized upon the eyes of sensitive animals by means of local nonspecific stimulation, when it is done simultaneously with the second injection of antigen by intravenous route, and to study, if possible, the nature of ocular lesions in comparison with clinical cases.

At the present time, when the estimation of the allergy theory on clinical ophthalmic problems is not yet definitely understood and requires further critical study, it is, I believe, of great significance to bring about closer relations between experimental and clinical fields.

**Experimental.**

Methods and Materials.

The experiment was studied in the eyes of 35 rabbits, albino and a few mixed-breed, weighing from 1800 to 3000 gm., and as the antigen, sterile pig serum inactivated and occasionally cattle serum were supplied.

When the Arthus phenomenon was found to be positive after repeated subcutaneous or intracutaneous injections, commonly of 5 times, of small doses of the serum at the backskin, the following eliciting treatments were done locally on the right eye, the left eye being always untouched, of rabbits: Group I was treated with paracentesis and Group II with exposure of the corneal surface to heat of 45–50 C. thermophore or to cold of dry ice, for 5 to 15 minutes. The second injection was made immediately in every case in the marginal ear vein with a dose of 3,0 to 6,0 cc. of the same serum, and sometimes those methods were repeated. Few of the animals died
following the intravenous injection, but the surviving animals were all killed by air embolism at different stages after the clinical observation, at various interval of 1 to 40 days. As a control, some of the animals were put to the test without being previously sensitized.

Microscopic sections were made using 10 per cent formalin as a fixative, imbedding in paraffin or celloidin, and staining with hematoxylin-eosin, van Gieson’s stain and, in case of need, with other special stains.

Results.

Group I: 15 albinos were put to use, 5 being retained as controls.

Clinical Observation: A few hours after the eliciting treatments, consisting of the second injection and the paracentesis, an acute irritative symptom of the eye appeared, reaching after 12 to 24 hours the highest manifestation with hypersecretion, lacrimation, swelling of the lids, chemosis, ciliary injection, and amounts of fibrinous exudate on the iris or in the anterior chamber, occasionally with effusion of blood into it. The iris became hyperaemic, edematous, and in a large proportion of cases a transparent dark-red shadow, which appeared to be bleeding in the area of ciliary processes, was discovered in the root-zone of the iris. The fundus was most frequently obscured by the haze of the anterior chamber. After a few days these irritations of the affected eye subsided relatively rapidly; the exudate and blood in the anterior chamber were considerably absorbed, remaining only in fine deposits on the pupillary border and the anterior lens capsule, and the shadow of bleeding on the back of the iris. In a few cases the reaction was less in degree; except for the mild hyperaemic symptom and the slight exudation in the anterior chamber none of the changes occurred. The opposite eye showed in general no response, but a few symptoms of increased lacrimation, chemosis or bulbar redness appeared in some of the rabbits.

Histologic Examination: Among the animals there were in fact several minor differences, partly due to the individuality of different rabbits and partly due to the various intervals of time observed, but the histologic findings as a whole can be summarized as follows: the cornea is almost intact except for a little cellular infiltration in the superficial tissue at the limbus. In the middle stages of reaction the anterior chamber is filled with a large number of erythrocytes and fibrin containing leucocytes, particularly in the angle. The stroma of the iris becomes loose or slightly edematous, in which the smaller vessels and capillaries are extremely congested, and in most of the animals extravasation of the erythrocytes is shown. In one rabbit which was killed at the later stages perivascular infiltrate of small round cells is shown. A slight but diffuse or rarely nodulous
form of infiltrate consisting of lymphocytic, polymorphonuclear, and less of plasma cells, is found in most animals mainly in the posterior layer of the iris (Fig. 1.). In the ciliary processes appears extensive edematous swelling, with varying sizes of Greefe's follicle and massive hemorrhage (Fig. 2., 3.).

In some animals the vessels are so intensively dilated and congested as to become static in appearance, and infrequently thrombosis with positive fibrin stain is shown. The fibrinoid homogenization is, however, in no case confirmed. The cellular reaction is more marked in the pars plana than in the ciliary processes and is present near the pigment cell layer with chiefly lymphocytes, polymorphonuclear, and less of plasma cells and histiocytes (Fig. 4.). In the posterior chamber the bleeding, with wandering cells and fibrin, is found without an exception in company with the high changes of the ciliary body. In the vitreum too, adjacent to the retina, a few cells are seen apparently by migration in more than half the cases. Except for the engorgement of the retinal vessels with some of the surrounding leucocytes, appearing in a few sections, the retina as well as the lens
seems to be free from the present reaction. The choroidal vessels are in every portion considerably dilated, and filled with erythrocytes, leucocytes and blood plasma, especially near the ora serrata, with edema surrounding them. In addition, the choroid is infiltrated by the majority of the lymphocytes and polymorphonucleares with occasional mononucleares, diffuse with nodular accumulation, leading to a thickened tissue (Fig. 5.). In the sclera perivascular infiltration and vascular congestions were marked in some sections. Otherwise the optic nerve remained uninvolved. On the other hand, in the untouched eye symptom slighter than, but corresponding to those of the treated eye, such as swelling and bleeding in the ciliary processes, round cell infiltration in the pars plana, dilation and congestion of the vessels with cell infiltration in the choroid were sometimes seen.

In summary, the reaction here caused is an acute inflammatory one characterized mainly by the occurrence of a disturbance of circulation and cellular infiltration, particularly in the uveal tract. In contrast to these findings of sensitized animals, those of the control animals were remarkably less in degree, although there occurred edema in the ciliary processes, development of Greefe’s follicle, hemorrhage in the posterior chamber and engorgement in the choroid.

Group II: This group was further divided into two groups by a kind of nonspecific stimulation; namely, 13 of the animals were exposed to heat, and 7 of them to cold, with 2 to 3 rabbits in each group being retained as controls.

Clinical Observation: In a few hours after the treatments the anterior segment of the eye became irritated, while on the second day there was increased swelling of the lida, hyperaemia and chemosis of the conjunctiva with hypersekretion and pericorneal injection, and occasional hemorrhage. The cornea, in addition to the epithelial changes, showed extensive edematous parenchymatous haziness, which increased until the visibility of the fundus was poor. In the iris there appeared hyperaemia, swelling and exudation, and in the anterior chamber massive serofibrinous exudate, frequently with hyphaema. These symptoms usually reached their height from the second to the fourth day, then decreased gradually. In the meantime the capillary loops at the corneosclerotic junction, which had been exposed to the local stimuli, became markedly red, extending inward as new-growth of vessels from the limbus to the centre of the cornea. Usually after 7 to 10 days the irritated symptom of the injured eye regressed, being healed with some corneal clouding. In respect to the corneal findings of these groups, exposure of the cornea to high or to low temperature brought certain different changes, but the findings of other ocular portions were similar in both groups. Some of the untouched eyes of the animals became also slightly irritated, as in Group I. On the other 2nd,
the control animals were not alike in the degree of their changes and the absence of the exudative reaction in the iris.

**Histologic Examination:** On the cornea there appeared patches of round cells in the subconjunctival areas of the limbus, deformity and desquamation of the epithelium, edema or swelling of the fibrous bundle in the substantia propria with leucocytous infiltrate, and corneal vascularization in the anterior layer; in the anterior chamber severe serous exudation with hemorrhage and migration of polymorphonuclear leucocytes; in the stroma of the iris engorgement and edema, occasionally infiltration of round cells or extravasation; in the ciliary processes remarkable dropsical swelling, congestion of the vessels with hemorrhage, and in the pars plana cellular reaction, the same as in the former Group. In addition, it seems noteworthy that the severest reactivity, quite extensive hemorrhagic and cellular reactions, was found in the corneoscleral region in one rabbit (Fig. 6.). In the choroid slight engorgement and a few infiltration cells appeared. The changes in the untouched eye, marked with disturbance of circulation and cellular reaction mainly in the vascular tissues, were distinctly milder than those in the treated eye.

In short, the chief lesions of this Group existed in the parenchymatous edematous changes of the cornea and the exudative hemorrhagic reaction of the iris and ciliary body; in other words, in the acute inflammatory reaction of several tissues surrounding the anterior chamber. In the control animals the corneal findings were almost similar to those of the sensitized animals, but the other tissues, for instance the iris or the ciliary body, did not become so inflamed.

**Discussion.**

As a result of the above-mentioned ophthalmoscopic and microscopic examination, it was primarily recognized that as for the intensity and ex-
tent of inflammatory reaction that occurred in the ocular tissue there was a distinct difference between the sensitized and the unsensitized animals.

But when we inquire analytically into the foregoing experimental conditions there appear at least two questions which should be considered in explanation of the pathologic findings presented. The first question concerns the tissue lesion, for which the local stimulation itself is responsible. Particularly, the corneal findings due to influences of the low or high temperature may be once left out of the problem of the “allergic” histopathologic features, for even in the control animals almost similar change of the cornea were found as well. However it is also to be kept in mind that in some of the highly sensitized animals abnormally high changes were presented in the corneoscleral region.

The second question consists in the influences of foreign protein that was used as the antigen. It was Iga who gave a detailed account of this subject and who with v. Szily disputed an allergic basis of cellular infiltration in the ocular portion of the animals. Indeed it would be true that because of its rich vascular character the uvea is the site of the predilection of the toxic reaction, but in view of the relatively small dose of serum used in the formal experiment and the mildness of the changes in the control animals caused by the injection of the same dose as that of the second injection, we are obliged to hesitate in saying that the primary effect of the serum toxin is a causative factor of the experimental results.

Further, in view of the fact that certain changes in the opposite eye, which probably belong to the contralateral response recorded by Rhiem, Jahiel, Isayama, Moriji, Takahashi and others, were found exclusively in the sensitized animals and, moreover, have had a close resemblance to the histopathologic picture of the first eye, the impression gained is that the contralateral eye of the animal, if highly sensitized, would take part in the reaction on the onset of the inflammation of the first eye. The most that can be said here is that though the picture must be consequent, as Seegal stated, on the generalized hypersensitiveness, some unknown factors in connection with an action from the one eye to the other might play a rôle.

One of the standpoints to be discussed when we are going to define the allergic phenomenon has been a matter of the specific histologic picture advanced by Roessle and his school. In 1923 Gerlach described thoroughly the tissue lesion at the site of serum injection, such as circulatory changes, edema or swelling of the connective tissue, leucocytic reaction and hemorrhage in highly sensitized cases; in short, the inflammatory reaction marked by hemorrhagic exudative character. Klinge thereafter pointed out fibrinoid homogenization of the connective tissue, leucocytic-histiocytic infiltration, and in addition edematous or myxomatous swelling of the connective tissue, proliferating cellular reaction, formation
of nodules consisting of leucocytes and histiocytes, occasional occurrence of giant cells, participation of changes in the walls of vessels and formation of granuloma, etc. According to Masugi\textsuperscript{12}) the character of the tissue allergy lay in anaemic capillaritis with edematous or fibrinoid swelling of the connective tissue and swelling of the bundle of fibres, disturbance of circulation such as stasis and fibrin thrombosis in the capillaries and pre-capillaries, sometimes leading to necrosis of the tissue, and participation of arterial changes. In this way they noted especially the changes in the connective tissue although they were not prepared to consider it per se as conclusive evidence of the existence of an allergy.

My study failed to develop the fibrinoid homogenization of the connective tissue, the arterial changes and others, so I can not deny that in histopathology there is a difference between the picture presented and the so-called allergic-hyperergic one. But it is also necessary to note the special relationship of the eye structure in anatomy and the experimental proof of the fact that when antigen comes from a far distinct place via the blood stream, its morphology can possibly vary in various degrees and forms from the typical picture. But the hemorrhagic-exudative character as Gerlach called, and then the rapid and violent onset of the phenomenon in the clinical course, which is said to be one of the most characteristic signs of allergic inflammation, were surely demonstrated in our cases, too.

Recently some authors\textsuperscript{13}) are inclined to be of the opinion that the allergic nature of the phenomenon should be defined from another point of view, in other words from the angle of the mechanism of the onset instead of the resultant morphologic picture. Looking back on my experimental processes, the paracentesis, which causes an immediate drop in intraocular tension, engorgement and changed permeability of the vessels, and the changes of aqueous components etc., is one of the severest stimulations to the eye, so that if some substances such as antigenic ones simultaneously administered by way of the blood stream they would have reason to reach the eye rather than any other organ, and this fact seems to be applicable even to the following case where the local stimulation due to low or high temperature would bring engorgement or something irritative to the eye, though relatively limited to the anterior segment.

Therefore, it should be clear that my experimental treatment has led to the concentration of the antigen, the eliciting of an antigen-antibody reaction and then the fixation of reactive changes to the ocular tissue. And, as the changes obtained arose from the basis of sensitization, their essential nature should be after all attributed to the allergic mechanism, while the mechanical or physical stimuli should be regarded as a contributory factor in the development of that inflammation. In view of these results and this discussion, the possibility that a manifestation of
hypersensitiveness or allergic hyperergy can be fixed to the eye by means of the nonspecific stimulation, even if antigenic substances are not locally administered, has now been proved through the present work.

Conclusions.

The experimental work reported in this paper showed the possibility that an allergic ocular reaction can be produced not only in previously specifically sensitized eyes, but also in eyes that have been, under certain conditions, exposed to a nonspecific mechanical or physical stimulation. The experimental proof of the fact that the pathologic changes obtained have been partly owing to the antigen coming from a distant place via the blood stream suggests to us that there is a wider field of a hematogenous form for the pathogenesis of manifestation of ocular allergy.

References.