group with recurrent HSV keratitis compared to the control group (Table 1). There were no significant differences in any of the other antigens tested.

An increased prevalence of HLA-A1 and HLA-B8 was not found, which has been previously reported in patients with recurrent herpes labialis infections.6

Two primary areas in which to look for an explanation of the recurrences in herpes infections are the infecting organism and the host response. A difference in the strain or virulence of HSV found in recurrent keratitis compared to nonrecurrent infections has not been demonstrated but cannot yet be excluded. However, a variable host response seems to be a more likely explanation. The increased prevalence of the HLA-B5 antigen in the recurrent HSV keratitis host provides new evidence that an abnormal immunoreactivity exists in this population. Newer HLA antigentic loci, which are only now being established, may have closer linkages with immunoreactivity sites in the cell's genes and allow further examination of the recurrent HSV keratitis population.7

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Key words: ALA tissue types, herpes simplex virus, histocompatibility antigens, recurrent herpes keratitis.

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Antinuclear antibodies (ANA) were examined in the sera of 28 patients with chronic open angle glaucoma (COAG) and 24 normal volunteers by an immunoperoxidase technique. Unlike the results of a previous report, no significant difference in titers was found between the two groups. A highly objective radioimmunoassay for DNA antibody was also employed, giving similar results. The increased incidence of ANA at low dilutions in both COAG and normal groups in this study and its absence when measured by radioimmunoassay suggest that positive ANA reactions at such low dilutions are the result of nonspecific binding. The lack of demonstrable differences from normal in ANA- or DNA-binding antibody does not support the results of earlier studies suggesting a humoral immune or autoimmune mechanism in COAG.

The reports in 1963 and 1966 of immunoglobulin and plasma cells in the trabecular meshwork of glaucomatous eyes by Becker et al.1,2 have offered an immune or autoimmune basis for this disease. More recently, this work has been refuted,3 but not before Waltman and Yarian4 reported an increased incidence of antinuclear antibody (ANA) in patients with chronic open-angle glaucoma (COAG). These authors reported that 44 percent of the patients with COAG had positive ANA reactions at a 1:5 dilution, when compared to 7.5 percent for normal volunteers. At higher serum dilutions (1:30) 18 percent of the COAG patients were still positive. Such a high proportion of positive response at the 1:5 titer was unexpected. On the assumption that such a high incidence of positive ANA reactions was due to nonspecific interactions, we repeated the study and included a highly objective radioimmunoassay for DNA-binding antibody5 to eliminate subjective description of microscopic observations.
Fig. 1. ANA detection by immunoperoxidase method. Positive (right) and negative (left) ANA results with snap-frozen mouse-liver touch prints. (Original magnification ×50.)

Materials and methods

Patients and controls. Blood was obtained from 28 patients with COAG who were diagnosed by one of the authors (G. L. S.). All patients considered to have COAG had characteristic glaucomatous visual fields and optic nerve head changes, as well as intraocular pressure consistently greater than 21 mm. Hg. Twenty-four controls consisted of employees and volunteers with no ophthalmic disease. Serum was stored at -20° C. until used.

ANA testing. Freshly obtained mouse liver was cut with a razor, and "touch prints" made on slides. The slides were snap-frozen and stored at -50° C. for up to 2 weeks before being discarded. For use, the slides were thawed, washed twice in phosphate-buffered saline (PBS), and incubated with serum diluted 1:5 or 1:30 with PBS for 30 min. The slides were washed twice in saline and incubated with horseradish peroxidase-conjugated IgG fraction goat anti-human immunoglobulins (IgA + IgG + IgM) (Cappel Laboratories, Downingtown, Pa.) diluted 1:100 in PBS containing 10 percent normal goat serum for 30 min. The slides were again washed twice and incubated with Karnovsky's reagent to develop the color. Finally, the slides were washed, dehydrated, mounted, and graded by one of the authors (N. T. F.).

DNA antibodies. The radioimmunoassay for DNA antibodies of Leon et al. was employed. Briefly, purified double-stranded radioactive DNA, obtained from E. coli grown in the presence of [115I]-iododeoxyuridine is incubated with a 1:10 dilution of serum. Bound radioactivity is determined after ammonium sulfate precipitation of antibodies.

Statistics. Chi-square values were determined from 2 × 3 contingency tables using a Hewlett-Packard 25C calculator (Hewlett-Packard Co., Palo Alto, Calif.)

Results. Positive ANA reactions were unambiguous and easy to grade (Fig. 1). The distribution of titers shown in Table I shows that there is no significant difference between the COAG patients and the normal population (χ² = 2.5519, p > 0.20).

DNA-binding antibodies are absent in most people. Most normal persons bind less than 7 percent (i.e., percent of radioactive DNA found in the precipitate), the region from 7 to 15 percent is considered a gray zone, and greater than 15 percent binding is reported as positive. It can be seen in Table II that there is no sig-
shows that there is no significant difference in frozen sections and monospecific antisera 3 are tive ANA titers in both the normal and COAG was further bolstered by the report of Waltman was in a control subject. This positive control population.9 The results presented in Table I and technique, we compared both groups by fluorescent and immunoperoxidase methods using frozen-section mouse liver immunofluorescence method.8 Other authors 1°> 1X have reported identi-

cification difference in distribution of DNA antibodies between the COAG patients and the normal volunteers (x² = 1.3514, p >0.50).

Discussion. An immunologic basis for COAG was introduced to the literature as long ago as 1962, when Becker et al.1 reported the presence of immunoglobulin in the trabecular meshwork of formalin-fixed glaucomatous globes. In an enlarged series Becker et al.2 confirmed their earlier report and noted the presence of plasma cells in the trabeculae.

The importance of humoral immunity in COAG was further bolstered by the report of Waltman and Yarian,4 who showed positive ANA reactions in 44 percent of patients with COAG when compared to only 7.5 percent of the normal controls. On the other hand in 1973, Henley et al.8 found little cell-mediated immunity to several intraocular antigens by leukocyte-migration inhibition in COAG patients.

At low dilutions, the incidence of false positive ANA titers can be high even in a young control population. The results presented in Table I show that in our study there was no statistical difference between the ANA titers of the normal and COAG groups. The increased number of positive ANA titers in both the normal and COAG groups may represent a slightly higher sensitivity when the touch-print mouse liver immunoperoxidase technique is used, as compared to the frozen-section mouse liver immunofluorescent and immunoperoxidase methods using frozen sections of mouse liver.

In order to avoid any ambiguity due to titer and technique, we compared both groups by radioimmunoassay for DNA antibodies. Table II shows that there is no significant difference in the activities between the normal and the COAG groups and that the only "positive" DNA binding was in a control subject. This positive control subject's value was 20 percent binding. The levels for patients with active lupus erythematosus range from 92 to 98 percent binding, depending on the severity of the disease.5, 7

Finally, the early reports of immunoglobulin in the trabeculum1, 2 have not been confirmed when frozen sections and monospecific antisera3 are used. Thus we have come full circle on the role of humoral immunity in COAG. The earlier report4 of increased incidence of ANA reactions in a population of COAG patients probably reflected nonspecific antibody cross-reactive at a low-dilution in a middle-aged or older population. The inability to detect DNA-binding antibodies in COAG patients by the more sensitive radioimmunoassay confirms our opinion that ANA testing at such low dilutions has led to the erroneous suggestion that ANA plays a role in the etiology or disease process of COAG.

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Indomethacin treatment before lens extraction and vitrectomy-lensectomy reduces postoperative inflammation as measured by protein determination of the ocular fluid.

Certain components of ocular inflammation have been shown to be mediated by prostaglandins and can be reduced by the use of prostaglandin synthetase inhibitors such as acetylsalicylic acid1, 2 or indomethacin.3-6 Ocular effects associated with prostaglandins include increased intraocular pressure, miosis, conjunctival hyperemia, and increased anterior chamber protein concentration.3, 4, 6

Prostaglandins have been implicated also in inflammation caused by anterior chamber paracentesis,5-7 cryotherapy,5 and postmechanical5, 6 and postchemical trauma.5 Because cataract extraction and vitrectomy in patients are accompanied by ocular inflammation in the postoperative period, it was the aim of this study to determine whether indomethacin could reduce inflammation after routine lens extraction and vitrectomy-lensectomy.

Material and methods

Lens extraction group. Ten albino rabbits were anesthetized with intravenous pentobarbital sodium. Lens extraction was performed in both eyes of each rabbit according to the following techniques. A limbal incision was made with a No. 15 Bard-Parker blade and enlarged to 180 degrees with corneoscleral scissors. The anterior capsule was ruptured, and the lens expressed through the wound. The incision was closed with running 9-0 nylon sutures, topical ophthalmic ointment (Neosporin) was applied, and the eye taped closed for 1 day.

The rabbits were divided into two groups prior to lens extraction. (1) Five rabbits (10 eyes) underwent lens extraction and received intraperitoneal indomethacin treatment according to the following regimen: 25 mg. three times at 1 day preoperatively; 25 mg. two times on the day of surgery; and 25 mg. two times at 1 day postoperatively. Medication was administered as a 1.25 percent suspension of indomethacin in 0.1M phosphate buffer, pH 8.4 (2) Five control rabbits (10 eyes) underwent lens extraction; control injection was done with normal saline intraperitoneally following the same schedule as for indomethacin injections.

Rabbits were killed 24 hr. postoperatively. Paracentesis of the aqueous humor was done with a 25-gauge needle through the cornea, withdrawing as much fluid as possible. Protein analyses were done on 0.2 ml. of each sample, which was diluted to 2.0 ml. with 0.9 percent sodium chloride. Trichloracetic acid, 1.9 ml., was added to each sample. After 5 min. the optical density was read at 420 nm. with a spectrophotometer. Protein concentrations were calculated by comparison with a standard solution.

Vitrectomy-lensectomy group. Eight rabbits (16 eyes) underwent vitrectomy-lensectomy. Of these eight animals, four were treated with indomethacin following the same regimen as described above. Four others received intraperitoneal injections with normal saline and served as controls. The animals were anesthetized by intravenously given pentobarbital sodium. Mydriasis was accomplished with topically applied 1 percent cyclopentolate hydrochloride and 10 percent phenylephrine hydrochloride. A lateral canthotomy was performed, and traction sutures of 4-0 silk were placed on both lids and the superior and inferior recti. The conjunctiva was dissected away from the sclera temporally, and a 2 mm. sclerotomy was done 2 mm. posterior to the limbus with a No. 11 blade. A purse-string suture of 5-0 polyglactin 910 (Vicryl) was placed around the sclerotomy