Fig. 2. Sections of the surface of rabbit cornea epithelium, stained with alcian blue, and observed by transmission electron microscopy. *22,000. A. normal cornea. The layer of probable mucosubstances on the cell surface measures 10 to 20 nm. in thickness. B, cornea after instillation of chloramphenicol. The layer of probable mucosubstances on the surface increased to 50 to 120 nm. in thickness.

but the pathogenesis of this kind of infection is obscure.

A thin layer of mucosubstances has been demonstrated in normal cornea as the deepest layer of the precorneal tear film.² Physiologically, this layer of mucosubstances on the cornea may have a protective function increasing the resistance of corneal surface against external influences. After application of chloramphenicol a heavy cell coat, stained strongly with alcian blue, appeared on the corneal surface. This coat is supposed in all probability to be deposits of mucosubstances or the like. The developmental mechanism of this cell coat is obscure, but mucosubstances have the property of increasing pathogenicity of bacteria.³ The formation of the heavy cell coat of mucosubstances may, therefore, accelerate corneal infections with resistant organisms after application of chloramphenicol. The possible relationship between the deposition of mucosubstances on the corneal surface after the instillation of chloramphenicol and development of corneal infections due to antibiotic-resistant bacteria after topical application of this antibiotic warrants further investigation.

From the Department of Ophthalmology, School of Medicine, Tokushima University, Tokushima-shi 770, Japan. Submitted for publication May 30, 1975. Reprint requests: Dr. Y. Mitsui, Tokushima University, Tokushima-shi 770, Japan.

Key words: corneal surface, mucosubstance, electron microscopy, chloramphenicol, alcian blue.

REFERENCES

Immunologic protection of rabbit corneal allografts: prolonged survival of allografts pretreated with homologous antibody against transplantation antigens.

JOHN W. CHANDLER.

Homologous Dutch pigmented rabbit antibody against New Zealand white rabbit transplantation antigens has been produced. This antibody is much less cytotoxic than similarly prepared heterologous guinea pig antilymphocyte serum. The homologous antibody preparation significantly prolongs corneal allograft survival.

Burde, Waltman, and Berrios¹ reported that rabbit corneal allografts pretreated with heterologous antilymphocyte serum (ALS) had pr-
longed survival times as compared to unsoaked grafts or grafts pretreated with normal serum. Chandler and co-workers\textsuperscript{2, 3} and Binder and co-workers\textsuperscript{4} confirmed these observations. However, they demonstrated that both heterologous ALS and antilymphocyte globulin (ALG) are cytotoxic for corneal cells in the presence of complement. It was further demonstrated that succinylation of ALG produced an antibody that retained its antigen binding capabilities but failed to combine with complement (C') and was non-cytotoxic. Rabbit corneal allografts that had been pretreated with succinylated ALG had significantly longer survival times than those pre-treated with either ALS or ALG.\textsuperscript{3, 4}

The present report details the preparation, testing, and efficacy of homologous antibody against transplantation antigens for the pretreatment of rabbit corneal allografts.

\textbf{Materials and methods.} Homologous antibody against transplantation antigens was prepared by immunizing Dutch pigmented rabbits with New Zealand white (NZW) rabbit lymphocytes. Details of the preparation of antibody were identical to those used to prepare heterologous antibody.\textsuperscript{4} Briefly, three Dutch pigmented rabbits were given subcutaneous injections of $10^8$ NZW rabbit thymus cells emulsified in Freund's complete adjuvant. Three weeks later each animal received an intraperitoneal injection of $10^6$ thymus cells. The antibody-containing serum was collected and pooled two weeks later. Pre-immunized serum from the same rabbits served as normal serum for these experiments. The serum was heated to $56^\circ$ C for 30 minutes, separated into 2 ml. lots and stored at $-70^\circ$ C.

Lymphocytotoxicity titers were measured by a modification of the micromethod described by Kaliss.\textsuperscript{5}

Standard operative techniques were used for rabbit corneal transplantation.\textsuperscript{7} New Zealand white donor corneas were sacrificed and the eyes immediately enucleated. Corneas with the attached scleral rims were either placed in sterile vials containing 1 ml of normal Dutch pigmented rabbit serum or 1 ml of Dutch pigmented rabbit serum containing antibody against NZW histocompatibility antigens for 30 minutes at room temperature, or transplanted immediately as unsoaked grafts. Allografts soaked in either serum solution remained clear, thin, and did not display any distinguishing characteristics as compared to unsoaked grafts at the time of surgery or in the early postoperative period. To prepare the donor button, the donor cornea was placed endothelial-side-up on a Teflon block and cut with an 8.0 mm, disposable trephine. The grafts were sutured in place using 10 interrupted 10-0 nylon sutures. There were 22 recipients of unsoaked and 22 recipients of antibody-soaked allografts, while 25 rabbits received grafts soaked in normal rabbit serum. In addition, the three Dutch pigmented rabbits that had been immunized with NZW histocompatibility antigens received allografts that had been soaked in their antibody-containing serum. Eyes with operative complications or cloudy grafts by day 7 were excluded from the statistical analysis. Animals that were eliminated from consideration had wound dehiscences (3), infection (2), or flat anterior chamber (3). No single complication predominated in one animal group. No grafts exhibit primary endothelial cell death. The sutures were removed 14 to 16 days after surgery.

The recipient rabbits were given 40,000 units of penicillin intramuscularly at the time of surgery. They also received daily topical antibiotics and cycloplegics until the day after suture removal. The grafts were evaluated daily for 21 days and three times weekly for the next eight weeks. Diagnosis of graft rejection was based on criteria outlined by Khodadoust and Silverstein.\textsuperscript{7} The day of rejection was determined as the first day that there was definite clinical evidence of rejection of any or all layers of the cornea. The animals were sacrificed three to five days later and the diagnosis confirmed by histopathologic study. Only rejected grafts were used to compute the average day of graft rejection. Student's T-test was used to statistically analyze the results.

\textbf{Results.} Fig. 1 demonstrates that a low titer of homologous cytotoxic antibody against transplantation antigens could be detected five weeks after initial immunization. In comparison, early work with heterologous guinea pig ALS typically retained 50 per cent lymphocytotoxicity at dilution of 1:1024, and succinylated heterologous ALG had a cytotoxicity curve similar to that for the homologous preparations in the present experiments.\textsuperscript{2}

To further demonstrate that the Dutch pigmented rabbits had been adequately immunized, they each received an 8.0 mm. NZW corneal allograft. Each sensitized animal rejected the allograft in second set fashion with a mean survival time of 9.7 days as compared to 26.2 days for unimmunized recipients.

A large series of nonsensitized Dutch pigmented rabbits was given 8.0 mm. NZW allografts. Table I summarizes the results of the series. The technical failure rate ranged from 9 to 12 per cent. The mean survival times for unsoaked (26.2 days) and grafts soaked in normal serum (30.6 days) were not statistically different. In contrast, allografts soaked in homologous serum containing antibody against transplantation antigens had a prolonged mean survival time (40.8 days). This was significant when compared to either unsoaked (P < 0.01) or normal soaked (P < 0.05) allografts. While the incidence of
Fig. 1. Comparison of lymphocytotoxicity mediated by heterologous guinea pig ALS and homologous Dutch anti-NZW serum in the presence or absence of C.

Table I. Survival of NZW rabbit corneal allografts in Dutch pigmented recipients after pretreatment with homologous sera

<table>
<thead>
<tr>
<th>Treatment (No.)*</th>
<th>Average rejection Day</th>
<th>No. (%)</th>
<th>Number not rejected</th>
<th>P values (mean rejection day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (20)</td>
<td>26.2</td>
<td>16 (80)</td>
<td>4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Homologous Ab+ (20)</td>
<td>40.8</td>
<td>13 (65)</td>
<td>7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Normal Serum (22)</td>
<td>30.6</td>
<td>16 (73)</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parentheses are total technically successful grafts.
†Group of grafts pretreated with Dutch pigmented serum containing antibody against NZW histocompatibility antigens.
‡There is no statistically significant difference in the incidence of graft rejection of the three groups.

Discussion. The use of donor tissue treated with antibody against histocompatibility antigens for the protection of allograft tissue was originally described by Guttman and co-workers\(^6\) in a renal allograft model. The prolongation of the kidney allograft survival noted in this model was felt to be due to the coating of the tissue and blockage of immunologic recognition. However, Wilson, Kirkpatrick, and Talmage\(^8\) demonstrated that lymphocytes are transferred with renal grafts. Lymphocytes appear to be the most satisfactory antigens for making ALS.\(^1^\) Donors pretreated with ALS are likely to carry significantly reduced numbers of lymphocytes to the recipients, and this reduced antigenic dose may account for prolonged survival of such grafts.

Corneal allograft models are free of this problem and there are now several reports using this model with pretreatment of the donor cornea with heterologous ALS.\(^1^,^4\) These reports have clearly demonstrated that heterologous ALS or its fractions can give protection against mild im-
munologic stimuli such as corneal allografts as well as prolong graft survival.1-4

However, in considering the adoption of this technique to human corneal transplantation, several considerations make a homologous antibody preparation more desirable than a heterologous preparation. There are now available techniques for measuring the number and quantity of various antibodies against human transplantation antigens. Thus, it would be possible to repeatedly make a given antibody preparation for use in corneal transplantation. A homologous preparation is less cytotoxic and therefore carries less potential for damage to the graft. Likewise, it is not necessary to chemically modify the homologous preparation to avoid this problem.

The results of the present experiments indicate that homologous antibody against transplantation antigens is successful in prolonging rabbit corneal allograft survival. Based on these studies, it would appear that similar pretreatment of human corneal allografts might be successful in prolonging graft survival and even, perhaps, in reducing the incidence of allograft rejection.

The author acknowledges the technical assistance of Ms. Patricia Skahen and Ms. Karen Vedder.

From the Department of Ophthalmology University of Washington School of Medicine, Seattle, Wash. 98195. Supported by Grant EY-01282 from the National Eye Institute. Submitted for publication Sept. 17, 1975. Reprint requests: Dr. J. Chandler.

REFERENCES

Dissecting ocular tissue for intraocular drug studies. Robert Abel, Jr.* and Gerard L. Boyle.

This report describes a convenient reproducible ocular dissection technique which has important applications for ocular antimicrobial penetration studies. Different ocular tissues can be sectioned while frozen and then plated directly on culture medium containing the test organism; after the zones of bacterial inhibition are measured at 18 hours following incubation, the tissue specimens are weighed providing more reliable evidence regarding drug concentrations. In such a fashion, a drug can be administered topically, subconjunctivally, or systemically, and assayed from the cornea to the optic nerve at various time intervals. Analysis of antibiotic in the vitreous body, which has important application in the therapy of endophthalmitis, can be routinely performed in the experimental model.

The judicious use of antimicrobial therapy is based on controlled ocular penetration studies. Traditionally, these evaluations are limited to analyzing drug concentrations in the aqueous humor and serum. Recently, certain authors have incorporated tissue dissection, in addition to performing anterior chamber paracentesis in order to study drug uptake in the eye. The purpose of this report is to present a convenient method of ocular dissection which facilitates antibiotic analysis of separate tissues with minimal contamination. For this reason, cefazolin sodium was selected as a sample antibiotic to illustrate the information that can be provided by ocular dissection.

Methods. In a previous report, albino rabbits received subconjunctival (one eye only), intramuscular, and intravenous cefazolin sodium at various time intervals before sacrifice. The discussion will be confined to the group of 46 rabbits (two to four animals for each time period)