A rabbit model for herpes simplex virus (HSV) stromal keratitis, produced by intrastromal injection of live virus, was used to evaluate the effects of tunicamycin and 2-deoxy-D-glucose therapy. In vivo and in vitro evidence suggests that HSV strains that produce stromal disease secrete relatively large amounts of highly antigenic glycoproteins. Also, various studies have shown that tunicamycin and 2-deoxy-D-glucose inhibit the production of complete HSV-specific glycoproteins. Thus, these drugs might be capable of mitigating the clinical manifestations of HSV stromal keratitis by reducing the antigenic load. However, when topical therapy with tunicamycin and/or 2-deoxy-D-glucose was begun in rabbit eyes, the day after intrastromal inoculation of live RE strain HSV and several days before the appearance of stromal disease, no difference in the clinical course of herpetic ocular disease was seen between the experimental (treated) and control (untreated) groups. Invest Ophthalmol Vis Sci 25:219–221, 1984

Although ocular epithelial herpetic disease does not normally produce permanent damage to the eye, stromal disease often results in irreversible scarring of the cornea, and is therefore a far more serious form of herpes simplex virus ocular infection. Wander et al have shown that experimental ocular herpetic disease produced by different strains of herpesvirus type 1 varies in a consistent and reproducible pattern. Some of these strains produce no epithelial disease or minimal conjunctivitis, some produce minimal epithelial disease, and others produce necrotizing stromal disease. In addition, these variations seem to be relatively independent of the size of the inoculum. Thus, each virus strain appears to have its own biologic properties.

Because the pathogenesis of necrotizing stromal keratitis appears to involve a destructive immunologic reaction, many investigators have tried to compare the immune systems of those who get this disease and those who do not. None of these studies has been particularly useful. Some evidence suggests that the HSV strains that produce stromal disease produce relatively large amounts of highly antigenic glycoproteins and that the strains that produce epithelial disease, produce relatively few glycoproteins in relatively small amounts. In various in vitro and in vivo studies, tunicamycin and 2-deoxy-D-glucose were found to inhibit the production of herpes simplex specific glycoproteins, and it appears likely that the presence or absence of stromal disease may be determined by the host reaction to the virus-secreted glycoproteins. The present study was designed to evaluate the effect of tunicamycin and 2-deoxy-D-glucose, either separately or in combination, on stromal keratitis in rabbits.

Materials and Methods. Virus: RE strain HSV was grown in RK-13 cells. A suspension of infected cells was used to produce the model of stromal keratitis in the rabbit eye.

Rabbits: New Zealand white rabbits weighing 2–3 kg were given intramuscular injections of chlorpromazine (Thorazine 25 mg/kg) one hr prior to inoculation of the cornea. Corneas were anesthetized by the topical application of proparacaine HCl (Ophthaine 0.5%) and injected intrastromally with approximately 0.02 ml of the RE strain of herpesvirus suspension. The injection was given with a 27-gauge needle attached to a tuberculin syringe. Both eyes of all animals were injected with the virus.

Drug Therapy: The topically treated rabbits were divided into five groups of 10 rabbits each. Beginning 1 day after infection, group 1 received two drops per eye of a 0.005% aqueous solution of tunicamycin (0.05 mg/ml) three times a day; group 2 was given two drops per eye of a 0.1% saline solution of 2-deoxy-D-glucose (1 mg/ml) three times a day; and group 3 was given two drops of each of the same tunicamycin and 2-deoxy-D-glucose solutions three times a day (total of four drops at each application). Beginning three days after infection, group 4 was given two drops of the tunicamycin solution three times a day. Group 5 was treated on the same schedule with topical normal saline and served as controls. These doses were selected after we found that solutions containing 5 mg/ml tunicamycin and 2 mg/ml 2-deoxy-D-glucose were toxic and were, in effect, the highest usable doses.

Biomicroscopy: Biomicroscopic observations were made on each eye at regular intervals in a coded manner. A slit-lamp biomicroscope with white illumination was used to evaluate the corneal stroma and anterior chamber structures. Fluorescein was placed on the cornea and observations were made with the cobalt-blue filter to quantitate epithelial ulceration.

Slit-lamp Observation Grading: Observations were made on a masked basis. Findings were graded on a scale of 0–4, corresponding with increasing pathology. Stromal disease was scored as follows: (0) clear and thin stroma; (1) detectable corneal edema, iris details clearly visible; (2) gross corneal edema with stromal swelling, iris details still distinct; (3) pupillary border no longer distinctly visible, some cellular infiltration.
Table 1. Severity of stromal disease

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>9</th>
<th>11</th>
<th>15</th>
<th>16</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tunicamycin 0.05 mg/ml tid, started 1 day after infection</td>
<td>0.09</td>
<td>0.49</td>
<td>1.38</td>
<td>1.54</td>
<td>1.75</td>
<td>1.14</td>
<td>1.08</td>
<td>0.83</td>
</tr>
<tr>
<td>2-deoxy-D-glucose 1 mg/ml tid, started 1 day after infection</td>
<td>0.18</td>
<td>0.81</td>
<td>1.25</td>
<td>1.64</td>
<td>2.25</td>
<td>1.31</td>
<td>1.44</td>
<td>1.16</td>
</tr>
<tr>
<td>Tunicamycin and 2-deoxy-D-glucose tid, started 1 day after infection</td>
<td>0.13</td>
<td>0.72</td>
<td>1.26</td>
<td>1.50</td>
<td>2.13</td>
<td>1.42</td>
<td>1.29</td>
<td>1.05</td>
</tr>
<tr>
<td>Tunicamycin 0.05 mg/ml tid, started 3 days after infection</td>
<td>0.23</td>
<td>0.85</td>
<td>1.08</td>
<td>1.45</td>
<td>2.10</td>
<td>1.58</td>
<td>1.05</td>
<td>0.86</td>
</tr>
<tr>
<td>Untreated controls</td>
<td>0.18</td>
<td>0.90</td>
<td>1.20</td>
<td>1.70</td>
<td>1.85</td>
<td>1.18</td>
<td>1.10</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Values are means of 20 eyes.

possibly present; and (4) an opaque cornea, anterior chamber structures not visible.

Results. Neither tunicamycin nor 2-deoxy-D-glucose (alone or in combination) suppressed ocular herpetic disease significantly (Table 1), regardless of administration schedule. Throughout the study period, almost no difference was noted in the treated groups compared with the control group. The study was terminated on the 18th day, when the epithelium was healed completely and stromal disease had become minimal in almost all of the animals.

Discussion. Tunicamycin: Tunicamycin, a potent inhibitor of protein glycosylation produced by Streptomyces lysosuperificus was discovered by Takatsuki, Arima, and Tamura. Although the drug was first noticed for its activity against Gram-positive bacteria, its ability to inhibit the replication of fungi, yeast, and viruses was discovered almost simultaneously. The composition and lipophilic nature of tunicamycin, coupled with its known biologic activity, have prompted in vitro investigations of its effects on the lipid-linked pathway for protein glycosylation. In experiments using microsomes from calf liver or chicken embryo, tunicamycin blocked the synthesis of N-acetyl glucosaminyl pyrophosphorylpolyisoprenol. From in vitro studies, it is evident that tunicamycin blocks only the first step in the lipid-linked pathway of eukaryotes, the synthesis of N-acetyl glucosaminyl pyrophosphoryldolichol from UDP-N-acetylgalcosamine and dolichophosphate. Subsequent steps in the pathway and the final transfer of an oligosaccharide to protein are not affected.

Studies of various substrates such as yeast, virus-infected cells, cultured fibroblasts, cultured ovary cells, etc, have shown that tunicamycin prevents the glycosylation of proteins containing N-glycosidically-linked carbohydrate. Since tunicamycin is not metabolized in vivo or in vitro, it appears unlikely that the inhibition of glycosylation observed in these investigations was caused by the incorporation of this drug into the growing oligosaccharide chains.

Unlike sugar analogues or the aminosugars, tunicamycin does not interfere with either sugar or nucleotide metabolism. More importantly, it appears not to interfere with the synthesis of the antigenic protein precursors or their fragments, even though they are not glycosylated.

2-Deoxy-D-glucose: It has been shown that 2-deoxy-D-glucose interferes with the in vivo and in vitro glycosylation of extracellular glycoproteins. In most studies, the inhibition of glycosylation was determined by comparing the incorporation of isotopically labeled sugars into particular glycoproteins in the presence and absence of the inhibitor. It is not clear whether 2-deoxy-D-glucose completely blocks glycosylation or merely causes premature termination of the growing oligosaccharide chain after it is incorporated into the chain as an analogue of glucose or mannose.

Since 2-deoxy-D-glucose is an analogue of glucose, it might also affect the synthesis of macromolecules through its metabolites or by interfering with normal sugar metabolism. Although 2-deoxy-D-glucose, in very large doses, can inhibit sugar metabolism and reduce the energy available for protein synthesis, it seems unlikely that this could be operative in the in vivo situation we observed.

The effect of 2-deoxy-D-glucose on protein secretion is very complex. The data obtained with the use of this sugar analogue do not provide definitive evidence for the hypothesis that the carbohydrate moieties of glycoproteins are required for their secretion. Similarly, there exists a certain amount of confusion concerning the effect of 2-deoxy-D-glucose (as well as other inhibitors of glycosylation) on the assembly and maturation of infective virus particles in cultured cells. It is now clear that this confusion arises from the fact that the effects of deoxy sugars depend on the virus strain and cell type employed.
since 2-deoxy-D-glucose can be incorporated into viral glycoproteins, results obtained with this compound may not be comparable to those obtained using inhibitors such as tunicamycin.

In this report, neither tunicamycin nor 2-deoxy-D-glucose proved effective in the treatment of stromal keratitis in rabbit corneas. One possible explanation is that glycoprotein production is not the sole determinant of stromal disease. Some evidence suggests a multiple antigenic etiology. Results of an in vitro study showed that in the presence of tunicamycin, antigenically-active, lower molecular-weight polypeptides were found that were antigenically and structurally related to the glycosylated proteins. In another study, a class of glucosamine-containing heterosaccharides (MW less than 3000) not present in DG-free HSV-infected cells was accumulated in the presence of 2-deoxy-D-glucose. These lower molecular-weight proteins and/or heterosaccharides also may contribute to the antigenic stimulus for the production of stromal disease, thereby negating the effect of glycoprotein inhibition. Finally, it is not known certainly that tunicamycin and 2-deoxy-D-glucose can inhibit glycoprotein production when applied topically in the kind of dosage schedule used here. Further work, possibly involving recombinant HSV strains with well-characte...