in the glaucoma patients are significantly different from
the normal control subjects and gg patients
and different from Kurose's results in uveitis
patients.6

No circulating BM or IC antibodies were
demonstrated in any patients using human
esophagus as the antigen.

Positive ANA reactions are usually found
in patients with connective tissue or collagen
diseases. It is nonspecific, being positive primarily
in systemic lupus erythematosus and less
frequently in rheumatoid arthritis and scleroderma.
However, it is often considered indicative of
altered immune function or autoimmunity.

None of our glaucoma patients had clinical
evidence of collagen diseases, and yet 44 per
cent had positive ANA reactions indicating some
possible disturbance in immune function in these
patients. This correlates with the work of Bigger,
Palmberg, and Becker8 where lymphocytes from
glaucoma patients were noted to be abnormally
sensitive to prednisone in vitro. Our findings in
gg patients, however, are divergent. He found
that lymphocytes from gg patients were also
abnormally sensitive to prednisone, and we failed
to find an increased incidence of positive ANA
reactions in these patients.

Becker, Keates, and Coleman1 demonstrated
abnormal deposition of gamma-globulin in the
trabecular meshwork of 75 per cent of the glau-
coma eyes studied. The tissue they used was
formalin-fixed and not ideal for immunofluorescent
studies, but the glaucoma eyes were significantly
different from the control eyes. Furthermore, they
were able to demonstrate the presence of plasma
cells in these eyes and not in the control eyes.

All these findings raise the provocative questions
of glaucoma as a connective tissue disorder, or
one associated with altered immune function.

From the Department of Ophthalmology,
Washington University School of Medicine, 660
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Cycitis produced in rats by cyclophos-
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Cyclophosphamide (CY), an alkylating drug,
damages proliferating cells in bone marrow, lym-
phatic system, intestinal epithelium, skin, and
tumors. In addition, CY causes hemorrhagic cysti-
tis1 and choroid plexitis,2 probably by direct
toxic effects on these nonproliferating tissues. The
plexitis was characterized by fibrin-rich edema,
slight leukocytic infiltration, necrosis, and hemor-
rhages which were accelerated by concomitant
administration of endotoxin.3,4 Because of the
similarity in form and function between choroid
plexus and ciliary processes, we have now ex-
amined the eyes histologically and found similar
lesions.

Lewis and BN rats from Microbiological As-
ociates, Inc., and CFE rats from Carworth Farms
were maintained in groups of five in hanging
wire cages on Purina Laboratory Chow and
tap water ad libitum. CY was dissolved in saline,
25 mg. per milliliter, immediately before use. A
dose of 250 mg. per kilogram of body weight
was injected subcutaneously into young adult
rats, usually 200 to 300 grams. Endotoxins (Difo
Laboratories) were injected in the dorsal penile
vein. The rats were killed by ether anesthesia
and exsanguination. The eyes were fixed by im-
mersion in acetate-buffered 10 per cent formalin.
Both globes were bisected, embedded in paraffin
in entirety except for removal of the lens, cut,
and stained with hematoxylin-eosin.

Two days after inoculation of CY, the ciliary
body and processes were distended by eosinophile,
structureless, edema fluid in the stroma (Figs. 1
and 2). There were occasional erythrocytes,
polymorphonuclear neutrophils, or mononuclear
leukocytes. The overlying epithelium sometimes
had microcysts between the two layers, degenera-
tive changes, or attenuation. The cysts and edema
fluid contained a reticulated network of fibrin in
Fig. 1. Ciliary processes are greatly distended by eosinophilic edema fluid. There are a few polymorphonuclear neutrophils in the fluid. The iris (below) is normal. Hematoxylin-eosin, ×330.

Fig. 2. The ciliary processes on right are edematous. On the left, edema has led to formation of a fluid-filled intraepithelial cyst. The iris (below) is normal. Hematoxylin-eosin, ×330.

Phosphotungstic acid-hematoxylin stains. A few capillaries contained fibrin plugs. The other parts of the eye had no edema or other lesions, except that an intra-epithelial cyst ("Greef vesicle") of the posterior surface of the iris was seen in one rat. Cyclitis was absent five hours after inoculation, present in some animals after 24 hours, fully developed after 48 hours, and receding after eight days (five rats or more at each time).

Cyclitis was observed in more than 20 experiments involving over 200 rats. Despite the constant dose of CY, the incidence varied from 30 per cent to 100 per cent in individual experiments. It might have been more uniform if histologic sampling had been more extensive. Daily repetition of CY increased the frequency and severity. Contrariwise, cyclitis was infrequent when the usual CY dose was subdivided among five injections at two-hour intervals or when the CY dose was reduced to 125 mg. per kilogram, and no lesions were found after 100, 50, or 10 mg. per kilogram or in normal control rats, or in rats treated with a number of other drugs. The incidence did not depend on sex, strain, or route of CY administration (subcutaneous, intraperitoneal, or intravenous). Similar lesions can be produced in rabbits, cats, and dogs.

At later stages, hemorrhagic cyclitis was grossly visible in situ. The development of hemorrhages was greatly accelerated by concomitant administration of 0.5 or 1.0 mg. of endotoxin, probably due to thrombocytopenia. Although no lesions were detected five hours after the combined treatment, by 24 hours many eyes had edematous cyclitis and some eyes were hemorrhagic. At 48 hours, most eyes had cyclitis and many were hemorrhagic. The hemorrhages distended the stroma of the ciliary processes, attenuated the overlying epithelium, filled the microcysts, and occasionally leaked into anterior and posterior chambers and contiguous retina. A few eyes had hemorrhagic intra-epithelial cysts of the posterior surface of the iris (Fig. 3).

Endotoxin causes edema and leukocytic infiltration of the ciliary body in rabbits and increased permeability of the blood-aqueous barrier in rats. These facts, and the acceleration of hemorrhages in CY cyclitis by endotoxin, induced us to investigate the ocular effects of endotoxin itself in rats. Escherichia coli endotoxin 026:B6, 1 mg. dissolved in saline, was injected intravenously into 13 rats. The ciliary processes did not become edematous or hemorrhagic, but there was a small, focal infiltrate of neutrophils in one rat. Salmonella enteritidis endotoxin, 0.5 mg., produced more frequent and more severe neutrophilic infiltrates (in six out of 12 rats), but even...
Fig. 4. Polymorphonuclear leukocytes fill the stroma of the ciliary body and some of its processes. This was the most severe lesion detected after endotoxin alone. Hematoxylin-eosin, x240.

these lesions were focal (Fig. 4). In these instances, free-floating leukocytes were present in the posterior and occasionally anterior chamber. These lesions were noted 24 or 48 hours but not five hours after endotoxin injection. They were not increased by a second injection of endotoxin five hours before death.

Although these endotoxin lesions were quite different from CY cyclitis, they suggested the need to determine whether endogenous endotoxin played any role in the cyclitis produced by injection of CY alone. Seven rats were given small doses (0.2 mg.) of E. coli endotoxin on the three days preceding the CY injection (this procedure was known to produce tolerance to the effects of 1 mg. of endotoxin in rats\(^5\)). All these rats developed cyclitis equal to that in the control animals that were pretreated with saline. Thus, tolerance to endotoxin had no effect on CY cyclitis. In addition, CY was injected into germ-free CFE male rats in an isolator chamber, thus assuring the absence of endogenous endotoxin. Four rats were killed after six days and all had cyclitis and choroid plexitis. Their feces contained coagulase-negative Staphylococcus epidermidis, probably introduced during the injections. As this organism was gram-positive, it seemed justified to conclude that endogenous endotoxin was not required for the production of cyclitis and choroid plexitis by CY.

What mechanisms are involved in CY cyclitis? CY is not cytotoxic until it is activated in the liver.\(^6\) Small amounts of CY and its metabolites enter cerebrospinal fluid.\(^7\) It is presumed that the choroid plexus is injured during the process of secreting metabolites into the fluid, or possibly in the reverse process of transport from fluid to blood, or merely because it is bathed in the fluid.\(^2\) It is likely that CY and its metabolites enter aqueous humor, although we know of no studies on this problem. The marked similarity between the lesions in choroid plexus and ciliary processes suggests similar mechanisms are at work.

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From the Pathology Department, New York Medical College Center for Chronic Disease, Bird S. Coler Memorial Hospital, Roosevelt Island, New York, N. Y. 10017. Supported wholly by Grant 536C12 from the National Multiple Sclerosis Society. Submitted for publication May 28, 1974.

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