SUPPRESSION OF ALLERGIC UVEITIS BY 6-MERCAPTOPURINE*

BY E. WIROSTKO, M.D., AND S. P. HALBERT, M.D.

(From the Department of Ophthalmology, College of Physicians and Surgeons, Columbia
University, New York)

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There has been increasing interest in methods of suppressing immune responses because of their great potential value in clinical medicine. Various agents are known to decrease or suppress antibody formation generally: total body Roentgen irradiation (1), saturation of the reticuloendothelial system with particulate colloidal agents such as carbon (2), protein deficiency (3), pyridoxine deprivation (4), and steroids (5), all have been shown to be effective.

Recently, compounds that act as metabolic inhibitors have been found useful (6). 6-mercaptopurine (6-MP) is such an antimetabolite. Synthesized as an analogue of the nucleic acid constituent, adenine, and the purine base, hypoxanthine, it was originally studied as an antineoplastic agent. It antagonizes purine metabolism and appears to interfere with nucleic acid synthesis (7). It has been demonstrated that 6-MP is effective in inhibiting the formation of antibody in some species of animals following stimulation with protein antigens (8). Using this drug, other investigators have prevented allergic encephalomyelitis (9), and prolonged the survival time of various tissue homografts (10, 11).

The eye is unique in several respects. In spite of having a number of avascular structures it has been repeatedly demonstrated that an experimental allergic reaction can be readily produced by a single dose of antigen injected into the vitreous body. This is followed by an acute iridocyclitis appearing within 7 to 10 days, persisting for several more days, and subsequently subsiding. In addition, the vitreous body appears to act as a depot for antigen with a resulting enhancement of antibody response (12). Furthermore, local antibody production within the eye has been well documented (19). Because of these earlier observations it was felt worthwhile to study the effects of 6-MP on experimental allergic uveitis from several points of view, with an attempt to correlate the clinical, immunological, and histopathological findings.

Materials and Methods

New Zealand albino female rabbits weighing 2 to 4 kg were used throughout the experiments. Most animals were obtained from a single dealer and were given a standard diet.

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Crystallized bovine serum albumin (Lot BX6, Pentex, Inc., Kankakee, Illinois) was used to induce the uveitis. A solution of 50 mg/ml in 0.85 per cent NaCl was filtered through a Seitz filter, tested for sterility, and frozen in aliquots at -20°C for use as needed.

After anesthetizing the rabbit with nembutal and ether, both eyes were gently massaged for a short while to achieve hypotony. Each eye was then injected with 0.15 ml of the antigen solution into the vitreous. The injection was made slowly at the region of the upper equator with a 27-gauge needle. The amount of material lost was slight, if any.

6-MP (Burroughs, Wellcome and Co., Inc., Tuckahoe, New York) at 50 mg/ml was dissolved in 0.85 per cent NaCl containing 4 ml of 10 N NaOH/100 ml. The drug was given daily by deep intramuscular injections into the hind limbs for 14 days at a dose of 5 mg/kg body weight commencing on the day that the albumin was injected intraocularly. Control animals were given similar injections intramuscularly of the same diluent, without 6-MP.

The eyes were examined in the gross, and with the slit lamp. The uveitis was classified 0 through 5 based on the following criteria:

0, normal
1, trace iris hyperemia, no other changes.
2, mild iris injection, trace flare and ciliary flush.
3, moderate iris hyperemia; moderate amount of flare with cells and precipitate over lens and cornea.
4, large amount of iris hyperemia; flare evident in the gross; fibrin and cells abundant; vitreous opacities present.
5, extreme iris hyperemia with miotic pupil, intense photophobia, and lacrimation; extensive fibrin deposition and cellular precipitates; dense vitreous opacities seen when the interior of the eye could be observed.

Eyes were observed prior to the experiment, several hours after injection, and daily throughout the experiment. Both eyes of each animal were graded, and in most cases the degree of uveitis was the same. Photographs of the eyes were taken at appropriate intervals.

Control sera and aqueous samples were obtained 1 day prior to antigen administration. Blood samples were then collected from the ear artery on the 5th, 9th, 14th, 21st, and 30th day after intraocular albumin injection. Aqueous fluid was withdrawn from the left eye only on similar days. Sera were heat-inactivated at 57°C for 30 minutes, and then absorbed with an equal volume of washed packed sheep red blood cells. The aqueous fluid was studied directly. The degree of uveitis was not usually affected by the paracenteses. Protein determinations on the aqueous and sera were done by absorption measurements at 280 and 260 nm wavelengths, and the concentration estimated according to Kalckar. (13) The ratios were typical for proteins.

The antibody titers of both fluids were determined using the hemagglutination technic of Baylén (14), as modified by Wide and Gemzell (16) for use with formalin-fixed sheep red blood cells. Determination of residual antigen in the aqueous and vitreous was done by the hemagglutination-inhibition technic. (15)

Globes were removed at various intervals, fixed with 10 per cent formalin, embedded in paraffin, sectioned in the horizontal plane, and stained with hematoxylin and eosin for histologic study. Representative eyes were selected for microscopic examination.

RESULTS

Following the injection of bovine serum albumin intraocularly, a mild, temporary, rapidly developing, inflammatory reaction was produced in both the treated and control rabbits. This reaction occurred several hours after injection, remained for 1 or 2 days, and was presumed to be due to the trauma
of injection. There appeared to be no difference in the severity of the reaction between the treated and the control group of rabbits.

During the 5th to 9th days, the eyes of the untreated group of rabbits developed a spontaneous inflammatory allergic reaction of varying degrees. This reaction reached a peak in 2 to 3 days and cleared slowly so that by the 15th day, most eyes were normal in the gross. The earlier the onset, the more severe and prolonged the inflammation tended to be. All of the twenty-seven control rabbits which were followed past the 9th day developed a uveitis of varying severity. On the other hand, during the 14 days of treatment, most of the thirty rabbits given 6-MP showed little or no ocular inflammation. Nine rabbits showed a mild uveitis, while only one rabbit developed a 3+ response. The remaining twenty showed no detectable reaction. Comparison of the clinical results in the two groups are summarized in Table I. These data represent the maximum uveitis which occurred at any time after the injection and up to 14 days. Photographs at various intervals were obtained to document the clinical course. Fig. 1 depicts the appearance of representative eyes of the two groups 12 days after intraocular antigen injection. Following cessation of therapy with 6-MP, it was of interest that eight rabbits revealed either an increased degree of previously existing uveitis or the appearance of a mild inflammation within 4 to 6 days. By the end of the 30 days all eyes were quiet. The time relationship of the clinical events in the two groups is summarized in Text-fig. 1. The days charted were chosen as being most illustrative of the course of disease.

Serum antibody titers against bovine serum albumin were determined and the results are given in Text-fig. 2. The sera of control animals showed a prompt rise to high titers within 9 days. In several rabbits, antibodies could be detected as early as the 5th day. By the 14th day titers were maximum and remained

<table>
<thead>
<tr>
<th>Maximum Degree of Uveitis (0 to 14 days)</th>
<th>Control</th>
<th>6-MP</th>
</tr>
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<tbody>
<tr>
<td>5+</td>
<td>13*</td>
<td>0</td>
</tr>
<tr>
<td>4+</td>
<td>7</td>
<td>0</td>
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<tr>
<td>0</td>
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<tr>
<td>Total</td>
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<td>30</td>
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</tbody>
</table>

* Figures represent the number of animals in each category.
elevated during the course of the experiment. Animals treated with 6-MP showed a striking suppression of antibody titer in the serum. By the 14th day of treatment, eight animals revealed no detectable serum antibodies, and the rest displayed low levels. Following cessation of the drug a slow but increasing titer of antibody appeared in the sera.

The titers in aqueous fluid generally paralleled those in the serum (see Text-fig. 3). However, the appearance of antibodies in the aqueous was not as prompt. Analysis of Text-figs. 1 to 3 reveals that there is a general correlation between the rise of serum and aqueous antibody titers, and the development of ocular inflammation in both groups.

The maximum degree of uveitis developing during the first 14 days (the period of 6-MP administration), and the antibody titers in the sera are compared in Text-fig. 4. It will be seen that treated animals showed little or no uveitis and low serum antibody titers. The control animals revealed a greater degree of inflammation and this was generally associated with high serum antibody titer levels. A titer of 1:64 in this system appeared to represent a critical level of antibody associated with severe uveitis.
Determination of residual antigen in the vitreous and aqueous revealed that bovine serum albumin could be detected in these specimens prior to the appearance of antibodies. The concentration of residual antigen was inversely proportional to the length of time after injection. Generally no antigen could be detected in either the control or treated groups after the 14th day.

There was a large variation in protein values among the aqueous samples and this could be roughly correlated with the intensity of uveitis at the time of sampling. The degree of uveitis and the aqueous protein content in the two groups of animals are compared in Table II. The aqueous samples were those obtained at either the 9th or 14th day, at a time when the uveitis was most evident. As expected, protein determinations of sera showed no apparent difference in values between the two groups.

The histologic picture of the control group was essentially that described by previous investigators (17, 18). The milder inflammatory changes were in the anterior segment of the globe (limbus, ciliary processes, and iris) while the more severe reactions extended to the choroid and vitreous. With the appearance of the spontaneous allergic reaction on the 7th day the eyes of the
control group revealed a moderate amount of mononuclear cell infiltration in the limbus, ciliary body, and iris. At the height of the reaction, in sections obtained during the 12th and 14th day, there was dense infiltration with lymphocytes, monocytes, mast cells, and large undifferentiated mononuclear cells in the ciliary body, iris, choroid, and on the optic nerve head. Scattered clumps of similar inflammatory cells were also seen in the vitreous. Sections of eyes removed on the 21st day revealed a subsiding inflammatory response. Focal nests of small mononuclear cells, essentially lymphocytes and plasma cells, persisted in the ciliary body, vitreous, and choroid. No other signs of active inflammation were present. Sections of eyes obtained on the 30th day appeared normal save for the scattered presence of nests of mononuclear cells observed previously.

Animals treated with 6-MP revealed similar inflammatory responses but they were less severe and were delayed in onset and course. One eye on the 9th day which clinically revealed beginning uveitis, displayed the typical histologic findings of a mild iridocyclitis much like that seen in the control rabbits. Large

Text-Fig. 3. Suppression of aqueous antibody titers by 6-MP. Note the striking resemblance of this graph to Text-fig. 2.
lymphocytes, monocytes, and undifferentiated mononuclear cells were evident in the ciliary processes, iris, and over the optic nerve head. The specimens of two eyes on the 14th day revealed normal anterior segments and vitreous. The only significant finding was a mild infiltration of the optic nerve head with nests of deeply stained mononuclear cells, essentially small lymphocytes, and monocytes. Scattered polymorphs and early plasma cells were occasionally seen. Sections taken on the 21st day resembled those seen on the 14th day. However,

the ciliary processes and choroid showed a scattered infiltration with small lymphocytes. Two specimens were obtained on the 30th day. One revealed a normal anterior segment but with mild infiltration of the choroid with nests of small deeply staining cells, presumably lymphocytes. The other eye displayed a mild uveitis affecting the ciliary body, choroid, and optic nerve head. Lymphocytes and monocytes predominated but plasma cells and large undifferentiated mononuclear cells were also seen. Fig. 2 illustrates some of these findings.

To determine whether 6-MP alone had any significant effect on the eye,

Text-Fig. 4. A graph relating the maximum degree of uveitis to maximum sera antibody titers during the initial 14 days of the experiment (the time of 6-MP administration). Note that rabbits which developed the more severe uveitis displayed titers of 64 or greater. Each point represents one animal.
three rabbits as another control were treated with 6-MP for 14 days. 0.15 ml of isotonic saline was injected into the left eye of each rabbit; the right eye was untouched. The gross clinical course and the histologic picture revealed no effect on normal eye structures.

### TABLE II

Comparison of Aqueous Fluid Findings in the Control and Treated Animals

Degree of uveitis is that recorded at time of paracentesis. Rabbits arranged in descending order of severity of uveitis. The samples were obtained from individual animals on different days during the course of the disease, and do not necessarily represent the maximum degree of uveitis which developed.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Degree of uveitis</th>
<th>Aqueous protein (mg/ml)</th>
<th>Antibody titer</th>
<th>Rabbit No.</th>
<th>Degree of uveitis</th>
<th>Aqueous protein (mg/ml)</th>
<th>Antibody titer</th>
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<td></td>
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### DISCUSSION

These experiments clearly demonstrated that 6-MP will inhibit the appearance of allergic uveitis following a single intraocular injection of bovine serum albumin in rabbits. These findings were evident both at the clinical and histological levels. The results appear to be due to interference with the immune response since a good correlation was demonstrated between the antibody titers and the height of uveitis in both the control, and 6-MP animals during the period of treatment.

Following cessation of drug therapy a rise in antibody titer occurred but this was not usually accompanied by an apparent or significant degree of uveitis.
It may be that an insufficient amount of residual antigen was present to initiate the allergic reaction.

The treated animals revealed a slow rise of serum antibody titer when 6-MP was discontinued, but at the termination of the experiment a significant difference in the titers of the two groups was still clearly evident. Since the antigen doses were the same, it might be expected that comparable final serum antibody titers would be seen. The most likely explanation, again, is that insufficient residual antigen was present to further stimulate antibody production.

There is no evidence in this experiment to suggest an anti-inflammatory action of 6-MP. Using steroids, Fernando (18) was able to inhibit the appearance of uveitis in a similar experimental situation. However, he also noted that steroids suppressed the initial non-specific inflammatory response associated with the trauma of injection. Animals treated with 6-MP in this experiment revealed no significant difference in the clinical picture or histologic findings of the initial reaction to the trauma of injection when compared to the control group.

The aqueous protein determinations revealed a significantly lower content in treated animals. There was a rough correlation between the degree of uveitis and the protein content in all animals studied. Inasmuch as 6-MP did not alter serum protein levels, it was felt that the lower aqueous protein values merely reflected the inhibition of the allergic uveitis, and was not due to any specific effect of the drug on the aqueous metabolism. The findings of lower protein content in the treated group served as a further check on the clinical evidence that 6-MP suppressed the allergic uveitis.

The histologic findings also bore out the clinical findings that 6-MP suppressed the inflammatory allergic response. The tissue changes that appeared during the course of therapy were less marked than those observed in the controls. During treatment many eyes showed no uveitis at all. Following cessation of therapy the microscopic findings were either normal or those of a low grade uveitis, at a time when control specimens were showing resolution. At the termination of the experiment, control eyes revealed no inflammatory exudate, while treated specimens revealed a delay in the appearance of the uveitis and also, consequently, in its healing. It is important to point out that the specific cellular response and its manner of distribution in tissues of the eye were similar in both groups.

It is interesting to speculate on the significance that these findings may have in the treatment of human uveitis. It has been presumed that ocular hypersensitivity plays an important role in the pathology of both granulomatous and non-granulomatous uveitis. In the former instance, the lesions are believed due to both the invading pathogen and the hypersensitivity that develops to products of the organism. In the latter instance no specific organisms have been incriminated, but hypersensitivity phenomena are believed to be involved.
Non-specific treatment of the human disease has been limited almost exclusively to the use of corticosteroids. These drugs owe their effect in large part to an anti-inflammatory action. However, in certain situations they suppress immune responses in large doses. On the basis of the studies reported here, an evaluation of drugs like 6-MP which primarily inhibit antibody formation might be indicated for the treatment of certain forms of uveitis.

Pyrimethamine (daraprim), a drug which is known to exert a toxoplasmacidal effect, has been used extensively in the treatment of toxoplasma uveitis. However, pyrimethamine is a folic acid antagonist, and in experimental animals this compound has been shown to suppress antibody formation. It may well be that part of the beneficial effects seen in treatment of this disease may be due to the latter effect of suppressing antibody formation. Studies to determine this action of pyrimethamine are being carried out.

**SUMMARY**

Experimental uveitis in rabbits was induced by single intraocular antigen injection. Treatment with 6-MP for 14 days suppressed the allergic inflammation and antibody response. A good correlation was demonstrated between the degree of uveitis and the antibody titer.

**BIBLIOGRAPHY**

18. Fernando, A. M., Immunological studies with 125I labeled antigen in experimental uveitis, _Arch. Ophth._, 1960, 63, 515.
EXPLANATION OF PLATES

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FIGS. 1 a to 1 d, Photographs of representative eyes.

FIG. 1 a. Untreated rabbit prior to intraocular injection.

FIG. 1 b. Same untreated rabbit 12 days after intraocular bovine serum albumin injection. Note marked (5+) inflammation.

FIG. 1 c. 6-MP-treated rabbit prior to intraocular injection.

FIG. 1 d. Same 6-MP rabbit 12 days after intraocular bovine serum albumin injection. Note absence of inflammatory response.
(Wirostko and Halbert: Suppression of allergic uveitis)
PLATE 86

Figs. 2 a to 2 f. Comparison of histological sections from untreated, and from 6-MP-treated rabbits given a single dose of bovine serum albumin intravitreally. All were stained with hematoxylin and eosin. (X 100).

Fig. 2 a. Untreated allergic uveitis involving the ciliary body. 14 days after injection.

Fig. 2 b. Ciliary body of 6-MP-treated rabbit. 14 days after antigen injection.
(Wirostko and Halbert: Suppression of allergic uveitis)
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Fig. 2 c. Untreated allergic uveitis involving the choroid. 9 days after injection. Retina (top) is also somewhat affected.

Fig. 2 d. Essentially normal choroid of 6-MP–treated rabbit. 9 days after antigen injection. Retina (top) is normal.
(Wirostko and Halbert: Suppression of allergic uveitis)
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Fig. 2 e. Untreated allergic uveitis involving the optic nerve head. 14 days after injection.

Fig. 2 f. Mild inflammatory response in the optic nerve head in a 6-MP-treated rabbit. 14 days after antigen injection.
(Wirotko and Halbert: Suppression of allergic uveitis)