

## OCULAR TOXICOLOGY OF MARIJUANA: AN UPDATE

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### Abstract

This review, encompassing the basic and clinical effects of marijuana and several of its constituents on the eye, primarily covers the literature since 1981. Available toxicologic data are discussed with reference to the overall effects on the eye of the drugs derived from marijuana. Both the cannabinoids and water-soluble derivatives are presented in the framework of their potential development as glaucoma medications, based on new pharmacologic and toxicologic data. The cannabinoids, as represented by cannabigerol and new prodrugs, both of which have reduced toxicologic profiles, may provide a novel approach to their clinical use. The water-soluble compounds have been shown to cause morphologic effects in the eye that correlate with the changes in intraocular pressure. The possible modes of action of these compounds are presented. Considerable further work is needed with the water-soluble compounds, now discovered in several plant sources, to identify these common chemical moieties responsible for the reduction in intraocular pressure. Concurrent toxicologic studies are also needed to establish whether the responses seen with the parent molecules are duplicated with metabolites or lower-molecular-weight, active derivatives.

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## Historical Review

This review provides an update on clinical and basic research on marijuana, in particular in relation to effects on the eye, that has occurred since a prior review in 1982.<sup>1</sup> Several extensive reviews of the various clinical and basic effects of marijuana have appeared in the recent literature,<sup>2-5</sup> and the reader interested in specific areas of cannabinoid research such as behavioral, immunologic, and numerous systemic pharmacologic effects is referred to these publications.

Marijuana has had varied uses over the past 2,000–3,000 years, ranging from a sedative, an anesthetic, an analgesic, to a multiplicity of other medical uses.<sup>6</sup> Use of marijuana for medical purposes fell into disrepute in the early 1900s both in Europe and the United States due to the highly variable potency of the preparations and because of the development of specific medications that were more potent in addressing specific symptoms. While marijuana is still used socially in some cultures as a purported stimulant, and in others associated with religious rituals,<sup>7, 8</sup> it was not until the latter part of the twentieth century that marijuana as a pleasure-inducing drug entered into the United States and Western Europe. Utilization of the material since that time has been associated with the liberalization of ethics and social behavior.

Continued research efforts since the 1960s have focused on many areas of marijuana effects.<sup>9</sup> These studies are extremely important, since, although marijuana use has been reported to be falling slightly in the overall population, it is still the most widely utilized drug after alcohol and tobacco. The distribution of the use of marijuana, however, appears to have changed somewhat over the past few years, with increased utilization by younger persons. In spite of considerable attention, and research, devoted to the various constituents of marijuana and the undoubted progress that has been made, many unanswered questions remain regarding the pharmacologic and toxicologic effects of both the parent material and the individual constituents.

## Medical Effects of Marijuana

During the past 20 years, a number of health hazards of marijuana have been documented, but many still remain ambiguous. Some of the health hazards inferred from animal studies have been difficult to identify in humans, mainly due to the facts that (1) human marijuana intake is intermittent, (2) it is used by younger people who are in better health than an elderly population would be, and (3) marijuana hazards are often difficult to separate from the concomitant use of alcohol and tobacco.

Of all the effects noted with marijuana, those that occur consistently are an increase in pulse rate, orthostatic hypotension with higher doses, conjunctival hyperemia, and euphoria.<sup>10</sup> The euphoric effects correlated temporally with the tachycardia and conjunctival hyperemia, but while all these responses correlated somewhat with the plasma concentration of  $\Delta^9$ -tetrahydrocannabinol (THC),<sup>11</sup> by no means was this relationship clear-cut.<sup>12</sup> Although the acute effects of marijuana use are

fairly well documented, the effects of chronic use are considerably less well known. Published field studies, although few in number, have shown no proven major health consequences from chronic heavy use of marijuana, but there are many drawbacks with these studies that preclude definitive conclusions being drawn. For many of the suggested detrimental effects of marijuana use (i.e., suppression of immunologic responses, chromosomal damage, effects on fetal development, and cellular metabolism, etc.), the clinical significance is unclear.<sup>4, 10</sup> The major clinical effects in humans appear to be respiratory and pulmonary toxicity, although effects on many organ systems have been noted in experimental animals treated with marijuana.<sup>13</sup>

### Effects of Cannabinoids

Marijuana contains over 400 chemicals of which 60 or so, called the cannabinoids, are unique to this plant.<sup>14</sup> The most widely occurring cannabinoid is  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), and many of the pharmacologic and toxicologic effects of the crude material marijuana have been duplicated to a large extent by this compound. The lipophilic cannabinoids vary in terms of their psychoactive potency,  $\Delta^9$ -THC being the most potent. Of the other major compounds that naturally occur in marijuana, cannabinol and cannabidiol, cannabinol has about one-tenth the potency of  $\Delta^9$ -THC<sup>12</sup> and cannabidiol far less. A variety of pharmacokinetic studies have shown that 11-hydroxy- $\Delta^9$ -THC is the only metabolite that can contribute to the overall effects of  $\Delta^9$ -THC in humans, although this may apply only after oral ingestion, since the data indicate that it is unlikely that 11-hydroxy- $\Delta^9$ -THC contributes to the pharmacologic effects of  $\Delta^9$ -THC after smoking or intravenous injection.<sup>12</sup> Hydroxylation of  $\Delta^9$ -THC in the 11 position is the major initial reaction in most species, and thus this metabolite assumes a major role in considerations of the relative effects of the parent compound and any metabolites. In fact, this metabolite (11-hydroxy- $\Delta^9$ -THC) was shown to have equipotency with  $\Delta^9$ -THC when given intravenously in humans.<sup>15</sup>

The pharmacologic effects of cannabinoids are characterized by their low toxicity<sup>13, 16</sup> and their multiplicity of effects.<sup>16</sup> The lowest effective dose of  $\Delta^9$ -THC required to induce a specific pharmacologic response is usually much lower than that required to produce toxic effects in the same species. Each of the cannabinoids tested in humans induces many side effects, yet overdosing with cannabinoids is hard to achieve, at least on an acute basis.<sup>13, 16</sup> Many of the side effects are the consequence of actions in the central nervous system; these include behavioral responses, effects on body temperature, effects on brain neurochemical systems, and neurophysiological effects, in addition to cardiovascular effects due primarily to a central effect.<sup>16, 17</sup> While the central nervous system effects of the cannabinoids are well founded, cannabinoids have been shown not to be mutagenic in the *in vitro* Ames test (whereas extracts and smoke condensates of marijuana are mutagenic).<sup>13</sup> Individual cannabinoids increase the incidence of micronuclei in bone marrow and cause

abnormal sperm in mice; thus cannabinoids might impair chromosomal segregation.<sup>13</sup> All pure cannabinoids have been shown to impair the synthesis of DNA, RNA, and protein at concentrations of about  $10^{-6}$  M when tested in tissue culture.<sup>13</sup> Effects on the immunosuppressive capacity of different species<sup>18</sup> and impaired reproductive and endocrine functions have also been noted.<sup>19</sup>

To a large degree, therefore, the effects of the cannabinoids reflect those of the parent material, marijuana, although the responses to the latter are tempered by the relative contributions of, and interaction with, other compounds such as cannabinol and cannabidiol.<sup>20</sup> These compounds can modify the absorption of  $\Delta 9$ -THC as well as modulate some of the pharmacologic and toxicologic responses either through direct competitive interaction or through the induction of responses in direct opposition to those of  $\Delta 9$ -THC. The ubiquitous effects of the cannabinoids, and marijuana, on many biological target systems have been attributed to either direct effects on certain biochemical or physiological processes or to perturbations of cell membranes. Perhaps the best explanation lies in some composite of the numerous effects;<sup>21</sup> thus most of the noted pharmacologic and toxicologic responses may arise from multiple actions. The highly lipophilic nature of the cannabinoids is strongly supportive of a role in affecting the molecular spatial organization in cell membranes that would subsequently alter enzyme reactions and interactions as well as receptor distribution. Arguments against specific cannabinoid receptors include the absence of a specific cannabinoid antagonist, and certainly there is little evidence to indicate that specific receptors exist for cannabinoids, although the degree to which cannabinoid structure induces a membrane effect is not established.<sup>21</sup>

### Ocular Effects of Cannabinoids

As indicated above, the most evident ocular sign of marijuana or  $\Delta 9$ -THC use, whether by inhalation, intravenous injection, or oral routes, is conjunctival hyperemia or redness. Ocular side effects of acute marijuana intoxication include diplopia (double vision), impairment of accommodation (ability to focus on near and distant objects), photophobia, nystagmus (involuntary eye movements), transient disturbances of vision, and blepharospasm (forced blinking).<sup>22</sup> As much as can be discerned, the ocular effects of chronic marijuana use are essentially the same as those for acute use, namely, congestion of blood vessels around the eye (both conjunctival and lid hyperemia), various alterations in ocular vision, and minor changes in tear production.<sup>23</sup>

Various cannabinoids reduce intraocular pressure in humans and several other species when given intravenously.<sup>1</sup> In addition, marijuana and  $\Delta 9$ -THC, whether taken by inhalation or orally, also decrease intraocular pressure in most normal volunteers and in the majority of volunteer glaucoma patients. One further study indicated that orthostatic hypotension occurs within 10 min after inhalation of 2%  $\Delta 9$ -THC cigarettes,<sup>24</sup> as had been noted previously even with a synthetic THC homolog.<sup>25</sup>

Although the inhalation of marijuana smoke and the ingestion of  $\Delta^9$ -THC capsules was found to reduce acutely the intraocular pressure of both normal volunteers and volunteer glaucoma patients, the overall responses varied considerably from one individual to another.<sup>1, 26</sup> Nevertheless, there was an apparent dose-response relationship in the administered cannabinoid and intraocular pressure.<sup>24, 27-32</sup> The fall in intraocular pressure was noted to be temporally dissociated from the euphoric effects.<sup>30</sup> The major problem with these and subsequent studies on humans<sup>26</sup> was to dissociate further the desirable responses of a fall in intraocular pressure from the undesirable side effects of euphoria, conjunctival hyperemia, and systemic hypotension. While the aforementioned studies focused on the acute effects of cannabinoids, only two studies examined the more chronic effects of continued use either of marijuana smoking or oral  $\Delta^9$ -THC ingestion.<sup>26</sup> Neither study added to the level of knowledge of marijuana's effect on the eye, other than those already delineated.

Several studies have been made in animals of the ocular responses to various cannabinoids. Early studies focused on  $\Delta^9$ -THC,<sup>1, 26, 33, 34</sup> but later studies expanded the scope to include many derivatives.<sup>35, 36</sup> Comparisons of a number of cannabinoids given intravenously indicated that various structural moieties were necessary for activity in reducing intraocular pressure. These structural requirements included the presence of a methyl or methyl-hydroxy group at the C-9 position and the presence of intact ring structures elsewhere on the molecule. Minor configurational changes had only a small influence on activity with regard to a fall in intraocular pressure.<sup>35</sup> Both  $\Delta^9$ - and  $\Delta^8$ -THC were active in reducing intraocular pressure.<sup>35, 36</sup> Cannabidiol, cannabigerol, olivetol, and cannabichromene were all inactive and cannabinol had about half the activity of  $\Delta^9$ -THC.<sup>35, 36</sup> Some compounds ( $8\alpha$ -OH- $\Delta^9$ -THC and  $8\beta$ -OH- $\Delta^9$ -THC) were found to be active in one study<sup>35</sup> but had no activity in another study.<sup>36</sup> Oral  $\Delta^9$ -THC was also found to reduce intraocular pressure in a dose-dependent relationship from 1 to 4 hr after administration. The epoxide of THC ( $9\alpha$ ,  $10\alpha$ -EHHHC) was active orally in monkeys, whereas  $8\alpha$ ,  $9\alpha$ -EHHHC and cannabidiol were only slightly active or inactive, respectively. These authors<sup>37</sup> indicate that  $\Delta^9$ -THC is active in monkeys by the oral route, that the intraocular pressure effects appear to parallel the psychoactivity, and that most compounds related to THC that have psychoactivity show intraocular-pressure-lowering effects. Naboctate, a synthetic cannabinoid developed as an oral antiglaucoma agent, also lowered the intraocular pressure of normal rabbits when given at 20 mg/kg, as did  $\Delta^9$ -THC at the same dose.<sup>38</sup>

Several papers have been published on the effects of the topical ocular application of cannabinoids on both intraocular pressure and toxicology of the eye both in humans and experimental animals. These studies followed previous studies showing that topical  $\Delta^9$ -THC and some other cannabinoids reduced intraocular pressure, often bilaterally, following unilateral application.<sup>1, 26</sup> These initial studies were followed by an in-depth study of the toxicologic effects of two doses of topically applied  $\Delta^9$ -THC in rabbits.<sup>39</sup> A variety of tests were performed on rabbits that received either 0.1% or 1%  $\Delta^9$ -THC in 50  $\mu$ l drops of light mineral oil, or vehicle alone, six times per

day for 30 days. More statistically significant changes occurred in the 1%  $\Delta^9$ -THC-treated group than either the 0.1%  $\Delta^9$ -THC group or vehicle group. All pre- and posttreatment values for blood chemical levels, hematologic values, and urinalysis, however, fell within normally accepted limits for rabbits. The ocular changes were minor lid or conjunctival reddening that appeared to be independent of drug concentration. More pseudoeosinophils and lymphocytes were present in the treated eyes than in control eyes. The results indicate that the vehicle caused mild clinical irritation and minor pathologic responses.<sup>39</sup>

Naboctate was found to cause a fall in rabbit intraocular pressure, in both normal and water-loaded animals, after topical application at concentrations ranging from 0.2 to 0.8%.<sup>38</sup> A dose-related fall in intraocular pressure was observed with naboctate. The choice of an aqueous vehicle for naboctate revealed an intraocular-pressure-lowering capacity of this agent, whereas when used at 2% in light mineral oil the drug was ineffective. This study indicated the important role of the vehicle for the cannabinoids, as described previously.<sup>1</sup> Nabilone, another synthetic derivative, was also ineffective when applied topically to the eye in light mineral oil. Side effects with naboctate did not appear to present major problems, although there was only a small margin between those doses that reduced intraocular pressure when given orally and those doses producing side effects. Delta-8-THC has also been shown to reduce intraocular pressure in rabbits<sup>40</sup> and dogs.<sup>40, 41</sup>

Some innovative approaches to topical preparations of cannabinoids for the reduction of intraocular pressure have been reported.<sup>42</sup> Mechoulam et al. reasoned that the effects of  $\Delta^9$ -THC when applied topically were somewhat inconsistent due to the low water solubility.<sup>42</sup> They prepared a lipid-soluble compound,  $-(+)\text{-}\Delta^6\text{-THC-DMH}$ , that had a very low psychoactive effect in monkeys. Using the water-soluble maleate salt, at 0.3% in aqueous solution, in a steroid-induced glaucoma model caused a significant fall in intraocular pressure of long duration ( $> 6$  hr). It was assumed that the water-soluble ester was rapidly hydrolyzed in eye tissues and was not washed away by the tears because the lipid solubility of the metabolite caused subsequent retention in the ocular tissues. This appears to be a demonstration that the intraocular-pressure-lowering effects can be dissociated from the typical psychoactive effects of most active cannabinoids. The use of this prodrug approach, in which a relatively inactive precursor can be applied to the eye with metabolism to an active compound through enzymes located in the ocular tissues, offers a new modality that can be manipulated to provide adequate drug at the site of action.

Several studies have been reported on the ocular toxicity of either single drop or chronic application of different cannabinoids.<sup>43-45</sup> Delta-9-THC and cannabichromene were delivered unilaterally to cat eyes either as single drops or over 9 days by osmotic minipumps. When applied chronically,  $\Delta^9$ -THC caused substantial decreases in intraocular pressure, but ocular toxicity was pronounced as indicated by conjunctival chemosis, erythema, hyperemia, and corneal opacities. The latter occurred under the site of drug delivery and became apparent within 3-5 days after initiation of delivery. Cannabichromene, however, did not reduce intraocular

pressure either acutely or chronically and did not induce any ocular toxicity. Thus, with  $\Delta 9$ -THC, despite the chronic reduction of intraocular pressure by continuous, even low-level drug application over 9 days, it was accompanied by marked ocular toxicity.<sup>43</sup> This confirms the study in which drops were applied six times daily and similar toxicity was found.<sup>39</sup>

In a similar study using marijuana extract containing 21.3%  $\Delta 9$ -THC,  $\Delta 9$ -THC alone, or cannabidiol<sup>45</sup> the drugs were delivered at 100  $\mu\text{g/hr}$ , 20  $\mu\text{g/hr}$ , and 20  $\mu\text{g/hr}$ , respectively, to cat eyes using osmotic minipumps over a 9-day period. All agents caused a differential in eye pressure of 3–5 mmHg between drug-treated and control-vehicle-treated eyes. Ocular toxicity after  $\Delta 9$ -THC was, again, quite severe, with conjunctival erythema and chemosis and corneal opacification. Qualitatively similar data were obtained with marijuana extract although the effects were less marked. No ocular toxicity occurred, however, with cannabidiol. It appeared that the ocular hypotensive effect of cannabidiol was relatively dissociable from the ocular toxicity seen with both  $\Delta 9$ -THC and marijuana extract.<sup>45</sup> It may be that the other naturally occurring components of the marijuana extract modulated the  $\Delta 9$ -THC effects. Either single drops or chronic administration of cannabinol or cannabigerol was also given to cat eyes.<sup>44</sup> Cannabinol had only a modest effect on intraocular pressure acutely but both cannabinol and cannabigerol caused a significant reduction in intraocular pressure over the 9-day period. However, cannabinol, but not cannabigerol, caused conjunctival hyperemia and erythema. Combined, these studies demonstrate that certain cannabinoids can reduce intraocular pressure, at least in the cat, without causing overt ocular toxicity.

Studies on the mode of action of  $\Delta 9$ -THC have indicated a pronounced effect on outflow facility.<sup>46, 47</sup> These studies in cats indicated that neither adrenergic<sup>48</sup> nor cholinergic input to the eye was required for the mediation of the intraocular-pressure-lowering effects of  $\Delta 9$ -THC.<sup>46, 48</sup> Aqueous outflow facility, a measure of the ease of fluid exit from the eye via the normal outflow channels, was increased two- to threefold after chronic administration of  $\Delta 9$ -THC or cannabigerol, while aqueous formation rate was not significantly altered.<sup>47</sup> These data support those described earlier in the rabbit in which outflow facility was increased.<sup>1, 33</sup>

One of the difficulties in using small mammals (rabbits, cats) to examine the effects of topical cannabinoids on intraocular pressure is the determination of the dosage, given topically, in relation to body weight. Ophthalmic delivery in a 3 kg animal could provide a markedly different systemic dose level than after the application of the same size drop containing the same mass of drug to a 70 kg human.<sup>26</sup> The topical application of  $\Delta 9$ -THC at 1 or 2% concentration, in light mineral oil, to rabbit eyes resulted in maximal plasma values being reached within 1–2 hr. These levels were maintained for several hours at this plateau. Plasma concentrations of about 0.6–0.8 ng/ml and 1.4–1.6 ng/ml were found after 1 and 2%  $\Delta 9$ -THC, respectively.<sup>49</sup> These values, found between 30 min and 1 hr after topical administration, are greater than those found following the intravenous administration of 15  $\mu\text{g}$   $\Delta 9$ -THC<sup>49</sup> and are about 40 times lower than plasma levels found in volunteers after smoking a cigarette containing 10 mg  $\Delta 9$ -THC.<sup>50</sup>

To determine whether the mechanism of action of  $\Delta^9$ -THC in reducing the intraocular pressure occurred in the central nervous system, various cannabinoids were administered<sup>51</sup> into the cerebral ventricles of conscious rabbits.<sup>52</sup> The use of conscious animals negated any potential influence of anesthetics on the mediation of a subsequent response. Whereas the intravenous administration of 0.33 mg or 1 mg  $\Delta^9$ -THC reduced intraocular pressure, no effects were noted after 1 mg  $\Delta^9$ -THC was injected into the cerebral ventricles, or after perfusion of the ventricles with solutions containing up to 0.1 mg/30  $\mu$ l/min for up to 1 hr. These results indicate that the ocular hypotensive effect of  $\Delta^9$ -THC does not originate in the central nervous system.<sup>51</sup> This result may explain the temporal dissociation between the fall in intraocular pressure and the euphoric effects, since the eye effects could be initiated more quickly than the THC can enter the areas of the brain where the psychoactive effects are generated.

One study examined the effects of  $\Delta^9$ -THC on monoamine oxidase activity in mitochondrial preparations from bovine eye tissues.<sup>53</sup> Delta-9-THC in concentrations from  $10^{-8}$  to  $10^{-12}$  M stimulated enzyme activity in ciliary processes, retina, trabecular meshwork, choroid, and iris. Ciliary processes exhibited the largest increase in activity at  $1 \times 10^{-12}$  M  $\Delta^9$ -THC. While it is possible that  $\Delta^9$ -THC may affect enzyme activity by influencing membrane permeability, there may also be direct effects on the enzyme. These authors suggested that the intraocular-pressure-lowering effect of  $\Delta^9$ -THC may be associated with a stimulation of monoamine oxidase in ocular tissues that could lead to a decrease in the level of catecholamines. Certainly the studies demonstrate that an interaction exists between  $\Delta^9$ -THC and catecholamines, as has been shown in other tissues.<sup>1</sup>

Only a few studies have been reported on the effects of topical  $\Delta^9$ -THC in humans. Although one report<sup>54</sup> indicated that a small fall in intraocular pressure occurred, three further studies have shown this not to be the case.<sup>55-57</sup> With both single<sup>56</sup> and multiple drop<sup>57</sup> applications, minor conjunctival injection (hyperemia) was noted after each drop that lasted less than 60 min. Subjective responses were a sensation of minor burning and/or an increase in tear secretion. In the multiple drop study,<sup>57</sup> 5 of 28 volunteers (including 4 who used vehicle alone) dropped out of the study because of the burning sensation and lid swelling. In the 23 volunteers who completed the study no difference was found between the intraocular pressure of eyes treated with 1%  $\Delta^9$ -THC and control eyes.<sup>57</sup>

### Water-Soluble Compounds Extracted from Marijuana

#### *Pharmacology, Biochemistry, and Toxicology*

In 1981, we described the isolation from marijuana of water-soluble materials that were active in reducing rabbit intraocular pressure.<sup>58</sup> Based upon their chemical and physical characteristics, these compounds were not cannabinoids but rather pectic

polysaccharides, and therefore offered a novel approach to the use of marijuana as a source for agents active in reducing intraocular pressure. Our interests focused on the mechanism of action of these compounds that caused a marked ( $> 50\%$ ) fall in intraocular pressure seen in rabbits. Despite the use of many antagonists, including alpha- and beta-adrenergic compounds, and prostaglandin and histamine inhibitors, the fall in intraocular pressure induced by these marijuana-extracted materials was unaltered when the water-soluble materials were given intravenously to rabbits.<sup>59</sup> Furthermore, these studies showed that the ocular effects of the marijuana-derived material (MDM) were unaffected by superior cervical ganglionectomy, that MDM caused a marked reduction ( $> 75\%$ ) in aqueous humor formation rate beginning 2 hr after injection, and that MDM was ineffective in inducing an ocular response in Rhesus monkeys when the material was given intravenously.<sup>59</sup> These initial findings provided some indication of the effects of MDM both in the eye and systemically, but left many questions unanswered.

Further studies<sup>60</sup> revealed that neither adrenergic, cholinergic, aldosterone, dopaminergic, nor serotonergic antagonists affected the fall in intraocular pressure induced by MDM. In addition to the proven effect of MDM on intraocular pressure following intravenous administration of the compounds in rabbits, we also demonstrated that MDM lowered intraocular pressure after intravitreal injection in both rabbits and Rhesus monkeys. Although the magnitude of the response was the same after intravitreal as after intravenous administration of MDM (at certain dose levels, with a dose response obtained through both routes of administration), the time course was markedly different. Intravenous MDM caused an almost immediate response at high doses ( $\approx 10 \mu\text{g}$  of pure MDM/animal). After a brief increase of intraocular pressure, reaching a peak at 30 min, the pressure fell to very low values within 4 or 5 hr. Lower doses ( $< 5 \mu\text{g}$  MDM/animal) caused only a fall in intraocular pressure with the same time course as with higher doses. At all doses the intraocular pressure returned to normal by 24 hr after injection. With intravitreal MDM ( $0.0001\text{--}0.01 \mu\text{g}/\text{eye}$ ), however, the time course was extended; no change in intraocular pressure occurred for 14–18 hr before the intraocular pressure began to fall, but once it occurred it lasted for up to 4 days depending upon the dose injected.<sup>60</sup> Dexamethasone was ineffective in inhibiting the effects of either intravenous or intravitreal MDM, indicating that the ocular response was not a nonspecific response to this protein-containing compound, nor was the response likely to be mediated by prostaglandins since corticosteroids, which block the arachidonic acid cascade, did not affect the response.

Several studies were made in attempts to understand how MDM induced the ocular response, including the use of sugars in an attempt to induce antagonism of the response. This approach followed that of other studies used to identify the mechanism of action of other glycoproteins.<sup>61, 62</sup> Using this approach, mannose, glucose, and galactose, but not arabinose, when given intravenously at high concentrations (1 g/animal) resulted in a partial blockade of the MDM-induced response. While this may be a nonspecific effect, there is also the possibility that these sugars acted as

occupiers of some recognition site to which one (or more) of the moieties of MDM could attach. To determine whether the observed intraocular pressure response was specific to the marijuana-derived material, a similar plant glycoprotein, larch arabinogalactan, was tested and was without effect in the rabbit. Thus, it seems that the ocular response to MDM is specific to this type of plant extract with certain physical and chemical characteristics.<sup>60</sup>

The rate of aqueous humor formation was again shown to fall markedly, to 20% of control values, from 4 through 8 hr after intravenous MDM, while ocular blood flow measurements indicated that the iris vessels, but not those of other tissues, were dilated at both 1 and 6 hr after MDM injection.<sup>60</sup> Since these were the only times at which blood flow was determined, it was difficult to attempt to correlate this response to other ocular effects of MDM.

As indicated above in rabbits, the intravitreal response had a different time course from that occurring after intravenous injection. In addition, the effect is confined to the injected eye, indicating that the response is a local one within the eye and not due to systemic metabolism of MDM. Furthermore, whereas even high concentrations of intravenous MDM (up to 20 mg/animal) were without effect in the Rhesus monkey, intravitreal MDM (at 0.001 and 0.01  $\mu\text{g}/\text{eye}$ ) induced a dose-related fall in intraocular pressure that was of the same order of magnitude and time course as that in the rabbit. The delay in onset of the response after intravitreal injection in either the rabbit or the Rhesus monkey was probably due to the diffusion of this large molecule from the midvitreal injection site to the ciliary processes.

No effects of MDM have been noted on systemic blood pressure, body temperature, or systemic blood pH or  $\text{PCO}_2$ , despite the use of MDM concentrations that would elicit maximal intraocular pressure responses.<sup>63</sup> At very high concentrations of MDM (1 mg/rabbit) there was some systemic acidosis, a condition that is known to induce a small fall in intraocular pressure.<sup>64, 65</sup> Thus, under these conditions, there was some contribution of changes in systemic  $\text{PCO}_2$  to the fall in intraocular pressure, but this finding was only applicable to high doses and did not explain the 80% decrease in aqueous humor formation rate that occurs with far lower systemic doses of MDM.

Repeated intravenous injection of MDM caused various responses, depending upon the time of the second injection after the first injection. After a single intravenous injection the intraocular pressure returns to normal levels within 24 hr. A second systemic injection given on the day after (i.e., 24 hr) the first injection, however, resulted in almost no further change in intraocular pressure. As the time of the second injection was increased after the first injection, there was a progressively greater response to the second injection. A full response that was directly comparable to that after the initial injection occurred if 7 days elapsed between the injections.

A series of experiments were performed to examine the fate of intravenously or intravitreally injected MDM.<sup>63</sup> These experiments took two main approaches; one was *in vitro* incubation with various body fluids and the other consisted of reinjection of whole blood or plasma from animals receiving high doses of MDM into other

animals. When MDM was incubated with saline, aqueous humor, or vitreous humor *in vitro* for up to 6 hr, it always induced a fall in intraocular pressure when reinjected intravenously or intravitreally. Incubation of 24 hr, however, reduced the capacity of the MDM to induce a pressure decrease. When aqueous humor or vitreous humor was collected from animals that had been injected intravitreally with MDM 24 hr previously (and thus at a time when the intraocular pressure was low), and the aqueous and vitreous were reinjected intravitreally into fresh recipient rabbits, the intraocular pressure fell in those rabbits receiving aqueous humor but not in those receiving vitreous humor. These data indicate that MDM passes out of the eye through the aqueous humor and that it remains active during passage from the vitreous and aqueous humor to the systemic circulation. This result may also be a consequence of the small quantity of MDM needed to induce a response when injected intravitreally, indicating that sufficient MDM is present in the aqueous humor to elicit a response. That no bilateral ocular response occurred from MDM that passed through the anterior chamber and presumably into the systemic circulation is a consequence of the minor amounts being lost through this pathway relative to the intravenous doses needed to induce a response.

When MDM was incubated in whole blood or plasma for 1 hr at 37 °C, there was no response when this material was injected into recipient animals. This effect is not due to nonspecific binding to proteins since incubation with bovine serum albumin at concentrations equivalent to protein concentrations in plasma had no effect on the MDM-induced ocular effects. Incubation in whole blood or plasma appeared to lead to a breakdown or modification of MDM such that it became inactivated within 1 hr. This aspect of MDM metabolism was pursued with the reinjection of blood or plasma from one recipient of MDM that became a donor to another secondary recipient. Only when very high doses of MDM (< 2 mg/animal) were given intravenously, and only when blood was taken within 10 min of injection of the primary recipient before reinjection into the secondary recipient was there any intraocular pressure response in the secondary recipient. At longer times after the initial injection, or lower doses of MDM, there was no effect of reinjection in the second animal. These data indicated that MDM was either bound or metabolized rapidly in the blood when injected systemically.<sup>64</sup> It is of interest that given the rapid disappearance of MDM from the circulation, the eye appears to be the only organ affected by MDM.<sup>59, 60, 63</sup>

In addition to the various pharmacologic profiles of MDM effects on the eye, a variety of biochemical studies have been performed.<sup>66</sup> Both adenylate cyclase activity and sensitivity to beta-adrenergic agents were unaltered when measured 4 hr after intravenous MDM administration. Intravenous injection of large doses of MDM (200–400 µg) decreased both magnesium-dependent and anion-dependent ATPase activity, but the sodium–potassium-dependent ATPase of the iris–ciliary body was unaffected. The inhibition of the Mg<sup>+2</sup>- and HCO<sub>3</sub>-dependent ATPases was about 28%. *In vitro* treatment with MDM had no effect on any ATPase;<sup>66</sup> this result is not surprising since *in vitro* incubation also failed to induce any effect on the hydraulic

conductivity (Lp) of the isolated iris-ciliary body preparation, whereas the Lp was increased when measured 6 hr after intravenous, and 24 hr after intravitreal, MDM administration (unpublished observations). The role of the different ATPases in ciliary epithelial function is unknown at present, although there is a demonstrated link between sodium-potassium ATPases and aqueous humor inflow that argues against a direct involvement of other ATPases. The only ATPase, therefore, that appears to be involved in aqueous humor formation (sodium-potassium-dependent ATPase) was unaffected by MDM. Together with these studies, a variety of drugs were used that had been chosen to inhibit various biochemical pathways, ranging from beta-adrenergic agonism (isoproterenol), phosphodiesterase inhibition (theophylline), blockade of glycoprotein residues (mannan, sialic acid, mannose-6-phosphate), inhibition of glycoproteins (tunicamycin), and inhibition of protein synthesis (actinomycin B). None of these agents had any effect on the MDM-induced ocular response.

In attempts to discern the mode of action of MDM, light and electron microscopy was used to examine the tissue responses.<sup>67</sup> Some indications of an inflammatory reaction were obtained. Despite earlier data indicating that prostaglandins were probably not involved in mediation of the ocular response,<sup>59</sup> this additional information suggested that a closer evaluation be made of the possible role of prostaglandins. Three approaches were taken to determine the level of involvement of inflammation in the MDM-induced response: the use of anticoagulants, the use of aspirin or indomethacin, and assays of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in aqueous humor and iris-ciliary body. Neither of the anticoagulants (heparin and streptokinase) had any effect on the time course or magnitude of the ocular effects of MDM. These agents were used to inhibit the formation of thrombi in blood vessels, which had been noted in micrographs. Both aspirin and indomethacin, however, altered the time course and partially inhibited the maximal responses, of the MDM-induced fall in intraocular pressure. The initial hypertensive phase seen after intravenous MDM injection was eliminated and the intraocular pressure fall occurred earlier as well as being reduced by about 35–40%. Direct assay of prostaglandins in the aqueous humor and iris-ciliary body indicated that there was a rise in PGE<sub>2</sub> during the first 2 hr after MDM injection, but these values returned to normal for the duration of the study (up to 8 hr after intravenous MDM). After intravitreal injection of MDM, the PGE<sub>2</sub> levels remained unchanged over 24 hr despite the fall in intraocular pressure initiated at 14–18 hr. Thus, while prostaglandins appear to be involved in the hypertensive phase of intraocular pressure after intravenous administration of MDM, there was no evidence after intravitreal injection of MDM that prostaglandins were involved in the fall in intraocular pressure (no hypertensive phase was seen after intravitreal MDM<sup>60</sup>).<sup>67</sup> Whatever the role of prostaglandins in the mediation of the MDM-induced fall in intraocular pressure, it appears to be very small. It was highly likely that the only role that PGE<sub>2</sub> may play in MDM effects was in the hypertensive phase after intravenous injection of MDM. Adding to this interpretation is the fact that topically applied prostaglandins, which themselves reduce intraocular pressure when given in low doses, cause a decrease in pressure by action on outflow facility rather than on aqueous formation rate.<sup>68</sup>

MDM has been further characterized as a pectin polysaccharide<sup>69</sup> that contains mostly carbohydrate with a small amount of protein. The major sugar constituents are rhamnose, galactose, and uronic acid. The molecular weight is estimated to be about 500,000 daltons, a considerable increase over earlier estimates.<sup>1, 58</sup> The evidence indicates that this material may be a cell-wall component. Although the majority of this work has been performed with material derived from marijuana, we have recently noted that similar intraocular pressure reductions can be obtained from extracts, made in an identical manner, from other plant materials.<sup>70</sup> Little has been documented regarding any systemic effects of these compounds, but they do not appear to induce any noticeable responses other than those on intraocular pressure. Extracts from tobacco, certain flowering weeds, and some common vegetables (lettuce, spinach, and kale) all are capable of inducing large falls in intraocular pressure at low doses (between 60 and 400  $\mu\text{g}/\text{animal}$ ). The material extracted from tobacco was subjected to analysis to determine any comparisons with MDM. Tobacco extract has a higher molecular weight ( $10^6$  versus  $5 \times 10^5$  daltons), is richer in protein (25% versus 15%), and is much higher in glucosamine (50% versus 5%). Thus, the tobacco extract has a vastly different chemical composition despite being capable of inducing a large fall in intraocular pressure. At present it is not known whether the active portions of each molecule have a structural relationship, but knowledge of the similarities and differences between these chemicals would establish those inherent portions that convey the intraocular-pressure-lowering capabilities. MDM seems not to be unique because of the ubiquitous appearance of other materials from other plants derived from cell walls that reduce intraocular pressure.<sup>70</sup>

### *Morphology*

As indicated above, microscopy was used to aid in discerning the mode of action of MDM. Because rabbits were used in most of our measurements of the physiological changes induced by MDM, our original morphologic studies concerned the changes in the ciliary processes of these animals.<sup>71, 72</sup> Both intravenous and intravitreal responses were examined. Intraocular pressures were recorded at progressive periods up to the time of sacrifice.

One hour after an intravenous injection of a modest dose of MDM into rabbits, the iridial ciliary processes underwent considerable swelling (Fig. 1a, b). During the next 3–5 hr, polymorphonuclear cells collected in the capillaries of the processes, thrombi were formed, red blood cells extravasated into the stroma, and cysts formed between the pigmented and nonpigmented epithelial layers (Fig. 1c, d). The damage remained severe for several days after the initial response (Fig. 1e). As seen with electron microscopy, the early edematous changes soon involved the pigmented epithelium. By 2 hr after injection, the edematous fluid had entered the intercellular space of the epithelium, causing wide separations between the epithelial cells, and swelling of the canaliculi at the surface of the cells (Fig. 2). In some instances, the basal lamina of the epithelium had ruptured and the stroma was continuous with the

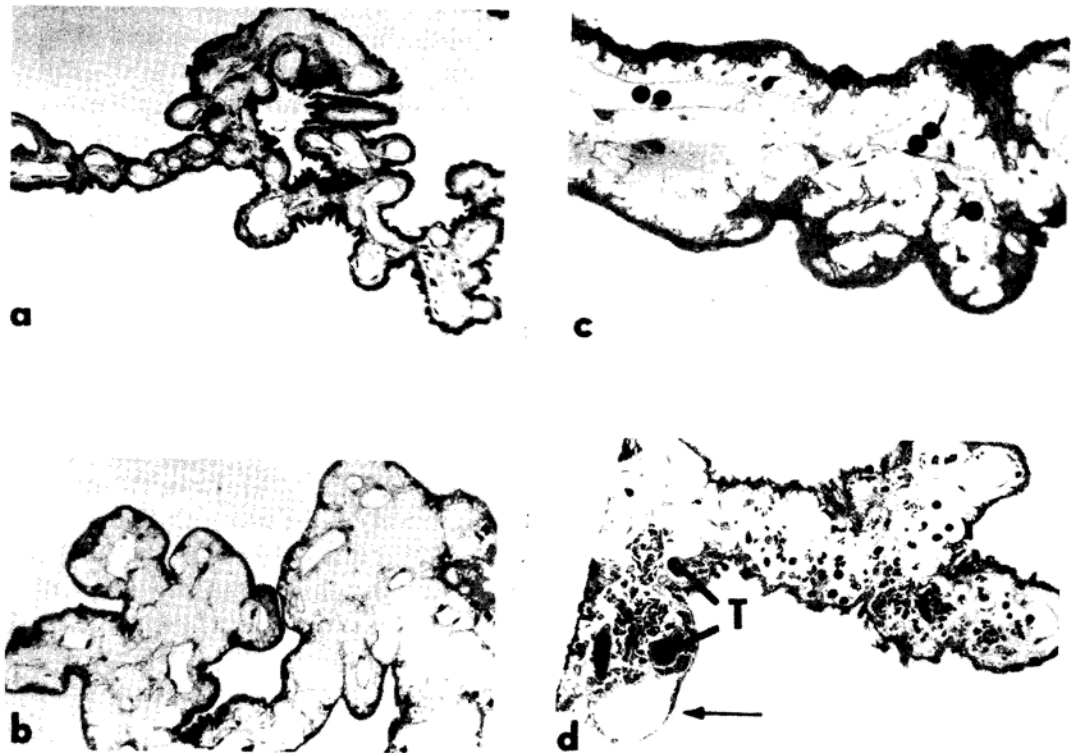


**Figure 1.** Anterior portion of rabbit ciliary processes: control and experimental. (a) A process from a control eye contains a core of highly vascular connective tissue enclosed in epithelial layers ( $\times 135$ ). (b) One hour after intravenous injection of MDM, the stroma of a process is considerably expanded with edematous fluid ( $\times 135$ ). (c) Two hours after intravenous injection of MDM, PMNs are seen adherent to capillary walls in a moderately swollen process ( $\times 370$ ). (d) Blood cells and vascular thrombi (T) in a swollen process, 5 hr after intravenous injection of MDM. The epithelial layers are separated, forming a cyst (arrow) at the periphery of the process ( $\times 185$ ).

intercellular space. Although somewhat attenuated, the nonpigmented epithelium remained intact in these tissues. Nor did the stromal capillaries show alterations at this time. Later in the injury, damaged nonpigmented cells occurred at the surface of cysts and damaged endothelial cells occurred in thrombotic capillaries.

When MDM was injected intravitreally, there was a lag of 14 hr before the onset of the response. Beginning at 14–18 hr, the processes underwent swelling in a manner similar to that seen in the intravenously injected animals. By 23 hr, thrombi were present in some of capillaries, blood cells had entered the stroma, and cysts were formed in the epithelium (Fig. 1f). In general the response to intravitreal

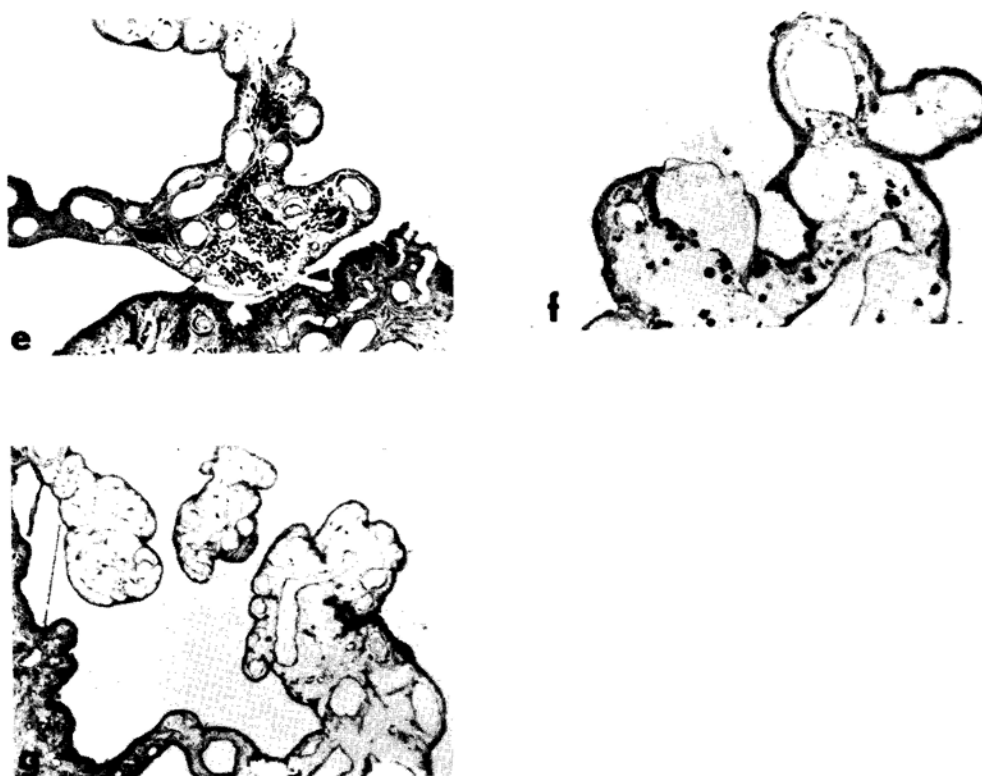


**Figure 1. (continued)** (e) Extravasated blood cells in the stroma of a process, 25 hr after intravenous injection of MDM. Note the lack of a defined epithelium in an area that may be a healing cyst (arrow-head). The process is bent over and almost touches the iris (I) ( $\times 180$ ). (f) Edema and blood cells in the stroma of a process, 23 hr after intravitreal injection of MDM. Note the ruptured cyst with material entering the posterior chamber ( $\times 165$ ). (g) A highly swollen process, 4 days after intravitreal injection of MDM ( $\times 85$ ). Reprinted by permission from ref. 71.

injections was similar to that of intravenous, however, it developed more slowly, the hematogenous phase was less pronounced, and the edematous changes lasted longer (Fig. 1g).

The occurrence of edema in the ciliary processes within 1 hr after an intravenous injection coincides with the rise in intraocular pressure that occurs in these animals. The hypertensive response is brief and may result from the sudden expansion of the edematous processes into the posterior chamber. The subsequent fall in intraocular pressure probably results from interruption of the secretory activity of the ciliary epithelium due to changed conditions in the edematous stroma and the collection of

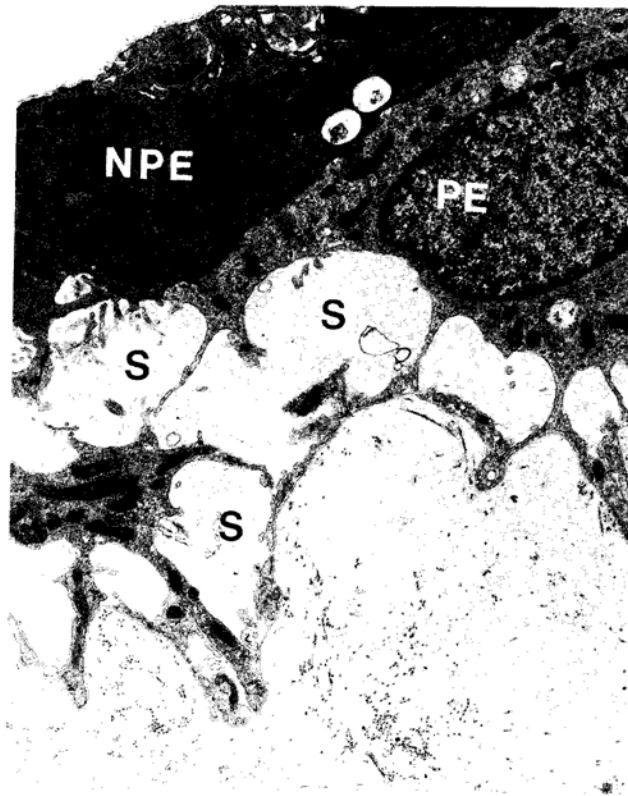


*Figure 2.* An electron micrograph of rabbit ciliary epithelium, 30 min after an intravenous injection of MDM. Edematous fluid has expanded the extracellular space (S) in the pigmented epithelial layer (PE). The nonpigmented epithelium (NPE) remains intact ( $\times 5000$ ).

fluid in the pigmented epithelial layer. The lack of a hypertensive response during tissue swelling after intravitreal injection is probably due to the slow onset of the response, allowing time for accommodation by other regulatory mechanisms in the eye.

In a second series of experiments, we examined the MDM-induced changes in monkey ciliary processes.<sup>73</sup> Of particular interest was the response to intravenous injection. Previous studies on the physiological response to intravenous injection in these animals showed no change in intraocular pressure, although intravitreal injections produced a drop in intraocular pressure similar to that in rabbits.<sup>59, 60</sup> The response to intravenous injections was studied in six squirrel monkeys. Intraocular pressures were recorded and the animals were sacrificed at 6 and 24 hr after a single injection.

Despite the lack of any significant change in intraocular pressure in these animals after intravenous injection, there were definite morphologic changes in the ciliary processes. At 6 hr, the processes were swollen to various degrees (Fig. 3a, b), but

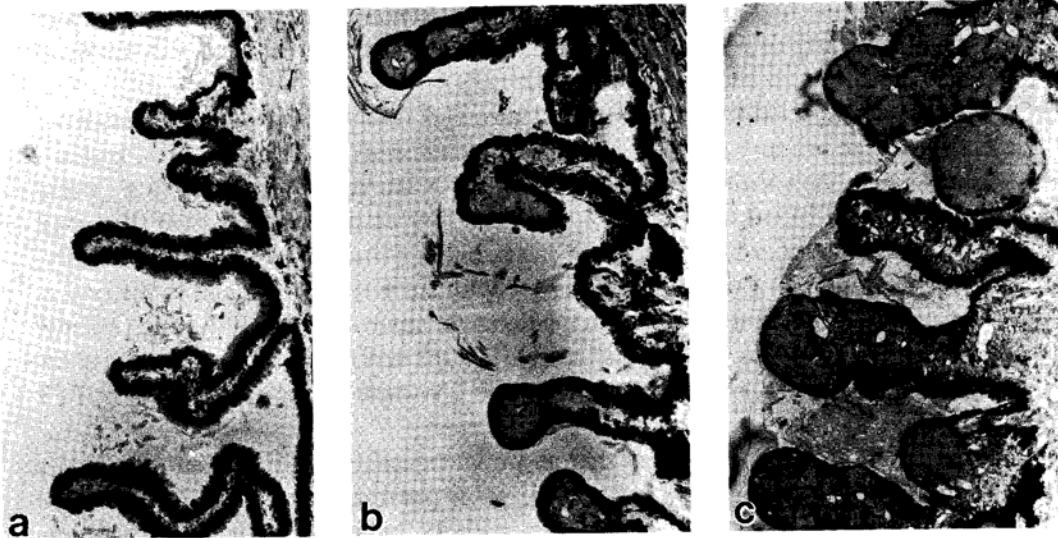


**Figure 3.** Ciliary processes in squirrel monkeys. (a) Processes in control eye ( $\times 90$ ). (b) Moderately swollen processes, 6 hr after an intravenous injection of MDM ( $\times 90$ ). (c) Severely swollen processes, 54 hr after intravitreal injection of MDM ( $\times 90$ ).

the swelling remained mild compared to that seen in rabbits. By 24 hr, the swelling was only moderately advanced over that occurring at 6 hr. The hematogenous response, so prominent in rabbits after intravenous injections, was lacking in the response of the monkeys. Examination of the swollen processes with electron microscopy showed the same edematous changes in the stroma and pigmented epithelial layer as occurred in rabbits.

Our findings concerning intravitreal injections in monkeys were based on results from three squirrel and three owl monkeys. Tissues were obtained at 24 and 54 hr after injection. All eyes studied had significantly lowered intraocular pressure. As was the case in rabbits, the major changes in monkey ciliary processes after intravitreal injection were severe swelling, cyst formation, and a moderate hematogenous response (Fig. 3c).

A third series of experiments concerned our earlier findings that the physiological response to an injection of MDM was altered if the animal had received a recent previous injection of the agent. For example, if a second intravenous injection was given to a rabbit 24 hr after the first (i.e., after the intraocular pressure had returned to normal), there was essentially no further change in intraocular pressure. Since our morphologic studies showed that damage was still present in the ciliary processes,

it was assumed that the presence of structural damage in the tissue prevented the tissue from responding to MDM. To pursue this we made repeated injections in rabbits using a variety of routes including intravenous, intravitreal, and subcutaneous and varying the time between injections.

The most unexpected finding of these experiments resulted from using subcutaneous injections as the primary injection. A single subcutaneous injection of MDM resulted in no change in intraocular pressure and only slight damage to the tissue (e.g., occasional dilation of blood vessels and a few cysts formed on the processes) (Fig. 4a). If a second injection was given 24 hr later, either intravenously or intravitreally, which when given as a primary injection would cause severe damage to the tissue, there was only a moderate increase in the damage: some slight swelling and possibly additional cysts (Fig. 4b). These data indicate that the subcutaneous injection somehow protects the tissue against the severe response known to occur after the IV or IVT injection.<sup>72</sup>

From these studies it is evident that an initial injection of MDM can influence a subsequent injection of MDM. Apparently, some pharmacologic modification of the tissue, such as receptor occupancy, occurs with the induction of only a minor morphologic response, and this modification makes the tissue refractory to further induction of MDM effects.

#### *Mode of Action of MDM*

The morphologic studies indicate that the ocular pharmacologic changes induced by MDM are correlated with a general inflammatory response in the ciliary processes. Two sites of action exist for MDM in the ciliary processes: the bilayered ciliary

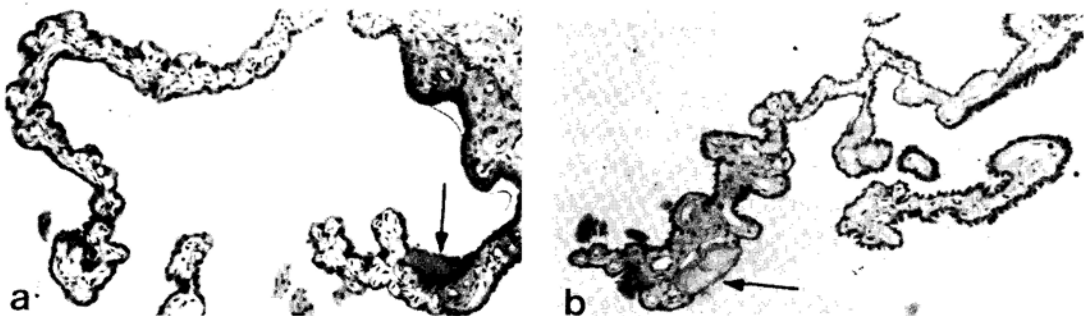


**Figure 4.** (a) Ciliary processes in a rabbit, 24 hr after a subcutaneous injection of MDM. The processes are essentially normal except for the presence of a cyst (arrow) ( $\times 60$ ). (b) Ciliary processes in a rabbit given a subcutaneous injection of MDM, 18 hr later given an intravenous injection of MDM, and sacrificed 5 hr later. The processes are slightly swollen and contain a cyst (arrow), but the damage is slight compared to that occurring after a single intravenous injection (Fig. 1d, e) ( $\times 60$ ).

epithelium or the capillary vasculature. If the reduction of aqueous humor formation, the known effect of MDM,<sup>59, 60</sup> resulted from a direct effect of the MDM on the ciliary epithelium, the drop in intraocular pressure would be correlated with the development of edema in the tissue. However, the time course of the reduction of aqueous humor inflow is such that it occurs *after* the edema occurs. The latter is noted about 30–60 min after intravenous MDM,<sup>72</sup> whereas the rapid decrease in aqueous humor inflow occurs at about 2–3 hr.<sup>60</sup> Thus, the primary effect of MDM appears to be on the vascular system, resulting in increased vascular permeability and edema. Moreover, the response seems to be specifically localized to the anterior portion of the ciliary process, an area known to be exceptionally vulnerable to insults such as toxins and immunologic disturbances. The return of the intraocular pressure to normal levels while there is still substantial injury in the tissue is difficult to explain. Perhaps the more posterior portions of the ciliary processes, which have essentially the same morphology as the anterior portions, compensate for the injured areas.

The mild morphologic response and the lack of a pharmacologic response in monkeys after an intravenous injection, while there is a severe response in the monkey after intravitreal injection and in the rabbit after both intravitreal and intravenous injections, leads to an interesting hypothesis regarding the processing of MDM in the eye. One of the main ocular differences between the two species is the permeability of the blood–aqueous barrier; primates have a much more restrictive barrier, especially to high-molecular-weight compounds such as MDM. Intravenous MDM has access to the blood vessels in the monkey and, indeed causes a slight reaction in them. Why, then, does the intravitreal route result in a much greater response? It may be that when the MDM diffuses out of the vitreous it is metabolized into smaller, more permanent, and yet still active molecules. These metabolites may then pass into the ciliary processes and react at the level of the capillaries. This would be the case in both rabbits and monkeys. Systemic MDM in the rabbit could enter the posterior chamber quite easily because of the inherent leakiness of the blood–aqueous barrier. The metabolites could then reenter the ciliary process as with the injected intravitreal material. Because of the restrictive blood–aqueous barrier, the posterior chamber in the monkey would be inaccessible to systemic MDM. The subdued morphologic response in these animals may represent the maximum effect of the nonmetabolized molecule.

The mechanism by which MDM induces the ocular response remains unknown. However, the facts that the response is localized to the ciliary processes, and that it can be antagonized by certain sugars, indicate that binding sites, probably in the capillaries, are potentially involved. The protective effect of an initial subcutaneous injection against the damage of a second intravenous or intravitreal injection may involve such binding sites. The moderate morphologic changes in the capillaries after a subcutaneous injection may represent low-level binding of MDM. If the MDM (or a metabolite of MDM) remains attached to the binding site, or if the molecular complexes are internalized by the endothelial cells, there would be fewer sites available

for reacting with the MDM of the second injection. Thus, the reaction to the second injection would be less severe. Whatever the mechanism of action of MDM, it is quite different in many aspects from either prostaglandins or cholera toxin: the latter alters adenylate cyclase and has markedly different effects on aqueous flow.<sup>74, 75</sup>

### Potential Clinical Use of Marijuana and Its Derivatives

The glaucomas are characterized by an elevated intraocular pressure, changes in visual field, a pallor of the optic disc, and cupping of the optic nerve head. Primary open angle glaucoma, which, as the name implies, occurs in an eye with an open angle between the posterior surface of the cornea and the iris, is the type of glaucoma most amenable to medical treatment. Once present, the disorder is always present and any treatment is used to arrest progression rather than cure the disturbances that have been induced. Effective treatment modalities involve the use of pharmaceutical agents or surgical procedures that prevent the progressive optic nerve damage characteristic of the disorder. While intraocular pressure reduction is one feature used to discern that a drug may be alleviating one aspect of the characteristics of glaucoma, merely reducing intraocular pressure does not necessarily prevent the progressive optic nerve damage in the glaucomatous eye, since factors other than intraocular pressure are most certainly involved.<sup>76</sup> Nevertheless, an elevated intraocular pressure appears to be a major damaging factor in chronic primary open angle glaucoma and pressure reduction would be anticipated to delay or arrest the progression of the glaucomatous visual field damage in many patients. To become a useful adjunct mode of therapy in the treatment of glaucoma, however, any drug or surgical procedure that reduces intraocular pressure must do so without causing unacceptable damage to either the eye or other organ systems while preventing optic nerve damage by reducing intraocular pressure.

Although there is considerable evidence that marijuana (and some of its derivatives when smoked, taken orally, or given intravenously) can reduce intraocular pressure, no studies indicate that either marijuana or its cannabinoid derivatives can safely lower intraocular pressure and prevent optic nerve damage in glaucoma. No studies made on glaucoma patients are of sufficient duration to allow any firm conclusion on this subject. However, the literature that does exist on marijuana use (and one must allow that the use of marijuana under most of these circumstances is not at the level sufficient to keep intraocular pressure decreased continually), indicates that many considerations of the systemic effects of marijuana must be made. Certainly the dose of marijuana or cannabinoids that will reduce intraocular pressure in short-term studies will also induce the side effects listed above that are associated with their acute use, namely euphoria, conjunctival hyperemia, systemic hypotension, and possibly dry eyes.

While no data exist concerning the long-term ocular and systemic effects of the use of marijuana by glaucoma patients, there is no reason to suspect that the chronic side effects would be different from those seen in other long-term studies of

nonglaucomatous patients. Indeed, given the estimated requirement for long-term use of marijuana to keep intraocular pressure reduced in glaucoma patients (at least 6 cigarettes per day every day of the year, for a total of nearly 2,200 "joints" per year), one would anticipate that such a continuous use of the material would lead to substantial side effects. Such considerations would bring into play several ethical questions regarding the effects of treatment for this disease: the overall health considerations for patients, the ability of patients (the vast majority of whom would be naive marijuana users) to function either at work or while driving (many studies have indicated impaired cognitive abilities<sup>17</sup>), and the risk/benefit ratio for patients.

It is important that some of the newer studies<sup>38, 41, 44, 45</sup> offer the possibility that the intraocular-pressure-reducing capability of certain cannabinoids may be dissociable from either the ocular toxicity or the euphoria-inducing effects of other cannabinoids. Consideration of such cannabinoids may lead to the development of an antiglaucoma agent that lacks many of the intolerable side effects associated with the cannabinoids in general. Certainly the use of a single, unique chemical entity is much more desirable than the use of marijuana, since this would distinguish a pharmacologically pure compound from a crude drug.<sup>14</sup> Only further studies, however, can determine whether it will be possible to obtain a complete separation of effects. The side effects that would need to be eliminated concern not only those related to any ocular toxicity but also those related to systemic responses (euphoria, etc.). Any drug would be viable only if a substantial difference existed between the low dose that would induce a useful fall in intraocular pressure (and prevent visual field loss) and that higher dose that would induce any measurable side effects. A topical form of application would also be the most desirable since this would reduce the total mass of drug needed per application, not only reducing the possibility of side effects but also providing the compound in a form less likely to be abused.

### Future Areas of Investigation

Based on the information available at present, several avenues of investigation remain viable for the pursuit of marijuana compounds that reduce intraocular pressure and have possible clinical use. In the cannabinoids, some compounds, such as cannabigerol,<sup>44</sup> reduce intraocular pressure in the cat without inducing a concomitant local toxic effect. If this is confirmed in a primate model the potential exists, because of its relatively low psychoactive properties, that this or a closely related compound could be effective as a topical agent. For other cannabinoids, the approach of a prodrug type of compound<sup>42</sup> also offers the possibility of topical use due to the conversion within the eye to a pharmacologically active compound that is effective in reducing intraocular pressure. Such an approach could significantly reduce the systemic and external ocular (irritative) side effects of cannabinoids, and could lead to use as a topical agent. Obviously, more studies are needed in a primate model to confirm this approach, particularly with extended studies in glaucoma models to

determine both long-term effects and the efficacy in preventing progression of the disorder.

With the water-soluble derivatives not only of marijuana but also of other plants,<sup>70</sup> the major difficulty still lies in their large molecular size ( $5 \times 10^5$ – $10^6$  daltons), which prevents their use as topical, or even oral, agents. In primates, the intraocular pressure is only reduced after introduction of the materials into the eye, thus considerable work is needed to examine not only the possibility of markedly reducing the molecular size without compromising efficacy but also of reducing the intraocular effects on the structural components of the eye. Certainly, based on our current hypothesis regarding the mode of action of MDM, it appears that the ocular tissues or fluids are capable of metabolizing MDM to produce a smaller yet still active compound that requires identification and characterization. If the molecular size is small enough, it could be capable of either topical or oral administration. Comparative chemical, pharmacologic, and toxicologic studies of active compounds derived from different plant sources may be of greater help in identifying the active moiety responsible for the fall in intraocular pressure. There appear to be great differences between MDM and a similar product of tobacco<sup>70</sup>: the tobacco compound is bigger ( $1 \times 10^6$  versus  $5 \times 10^5$  daltons for MDM) and contains more protein (25% versus 15%) and more glucosamine (50% versus 5%). If other compounds extracted from other plants have other differences, it may prove possible to identify the common factors that could contribute to their intraocular-pressure-reducing capacity, and develop a viable glaucoma drug. In this regard, MDM (or active derivatives) may follow the developmental pathway taken by prostaglandins. While these latter agents were originally associated only with the sequelae of severe trauma or injury to the eye, it has been appreciated more recently that low doses of prostaglandins, administered topically, can serve to reduce intraocular pressure without the induction of marked toxicologic effects.<sup>77, 78</sup> Long before such a progression can occur, however, considerable research must be undertaken with MDM and its derivatives to examine many of the basic ocular responses to these agents.

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