Experimental chlorpromazine cataracts

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Chronic ingestion of large doses of chlorpromazine (CPZ) by human beings has been associated with the production of corneal and lens opacities, and less frequently with chorioretinal pigmentation, and a peculiar purplish coloration of exposed skin and conjunctiva. When guinea pigs were fed large doses of CPZ and exposed to ultraviolet light, lens opacities were consistently produced that resemble those in human patients on chronic high doses of CPZ. They developed in albino and pigmented animals. A progressive increasing deposition of CPZ with increased treatment was demonstrated in the lens and other tissues of treated guinea pigs, and occurred in albino and pigmented animals. The respiratory metabolism of lens epithelium of CPZ-treated animals was reduced by a statistically significant amount in this study, when compared to control values. Sodium succinate was shown to stimulate respiration in CPZ-treated, but not control, lens epithelium (both exposed to glucose substrate). It appears that CPZ alters respiratory mechanisms by effecting a metabolic block at some site preceding succinate. On the basis of this study, the CPZ cataract has no apparent relation to melanin. It may represent foci of denatured protein resulting from the interaction of light with the drug, a photosensitizing agent, and lens protein, or possibly deposits of drug within the lens.

Key words: Drug-induced cataract, chlorpromazine, ultraviolet light, lens epithelium, lens fibers, lens capsule, respiration, succinates, sodium, melanin.
light—a Sylvania F 15 T 8, 15 watt, fluorescent, ultraviolet lamp with a peak emission frequency of 3,650 Å, and essentially no radiation below 3,200 Å, placed 25 cm. above the back of each animal. Each animal received 30 mg. of CPZ by mouth each day. Control guinea pigs received the same diet and same ultraviolet light exposure, but did not receive CPZ.

One group of 30 animals received CPZ daily and ultraviolet light exposure continuously for a period of 12 months. Nineteen of these animals survived and were examined with a Haag-Streit slit lamp, at magnifications of 10× and 16×, one and three months after cessation of therapy. In an effort to interpret objectively the slit lamp findings, the result of each examination was recorded as a drawing of any opacity seen before the treatment status of the animal was known to the examiner. The animals were then painlessly put to death; the eyes were removed, and the corneas and lenses isolated. Other tissues were taken from each animal and frozen for later chemical analysis.

In a second group, 24 guinea pigs were treated similarly for four months. Slit lamp examinations were performed prior to treatment, and at subsequent two-week intervals. At the end of the treatment period the animals were killed and specimens of several tissues were taken from each animal and frozen for later chemical analysis.

**Chemical studies.** The tissues taken from the treated and control animals were examined for material behaving as CPZ by ultraviolet absorption. The tissues were blotted dry with filter paper, then weighed and homogenized in four volumes of water. The homogenates were treated with trichloroacetic acid, followed by 10 per cent sodium hydroxide, and the tissue homogenates were extracted with n-heptane and isooamyl alcohol, according to the method of Wechsler and Forrest. The extracts were then treated with hydrogen peroxide to convert CPZ and any of its metabolites to the sulfoxide. The quantity of CPZ sulfoxide present was calculated from the absorption at 340 ms with the use of a Zeiss PMQII or a Beckman DU spectrophotometer.

Other in vitro studies were performed to determine the affinity of melanin and tissue homogenates for CPZ. The rate and extent of diffusion of melanin and CPZ through a dialysis membrane was determined. Melanin was either purchased from a commercial source (Pierce Chemical Co., P.O. Box 117, Rockford, Ill. 61105), or prepared according to the method of Schnitli. In this method of melanin synthesis, histamine dihydrochloride is exposed to a stream of air in the presence of ferric chloride and ascorbic acid. Dialysis bags were prepared from cellophane tubing ¼ inch in diameter. Each tube was filled with 1 ml. of an aqueous suspension of CPZ (1 mg. per milliliter) and 1 ml. of water, or 1 ml. of a suspension of melanin (1 mg. per milliliter), and dialyzed for periods of time up to 135 minutes against 200 ml. of water at 25° C. The amount of CPZ remaining in each bag after dialysis was then determined. In this manner the rate of passage of CPZ through the membrane was determined alone and in the presence of melanin. Dialysis was also performed with equal concentrations of CPZ on both sides of the dialysis membrane. After dialysis the total CPZ content on both sides of the membrane was determined. By means of these procedures the rate of dialysis of CPZ was measured alone or in the presence of tissue homogenates, such as lens or uveal tissue of pigmented or albino guinea pigs. The rate of dialysis of CPZ was also measured with the use of solutions of bovine serum albumin of the same protein content as the tissue homogenates. Rates of dialysis were determined as a function of pH, temperature, and concentration of sodium chloride and urea. Treated guinea pig eyes were fixed and stained with the Fontana stain, and compared with control eyes. CPZ solution was permitted to dry on a glass slide and was stained by the Fontana technique following exposure to ultraviolet light and after no exposure.

**Metabolic studies.** The rate of oxygen consumption of lens epithelium and capsule and of anterior lens fibers were measured according to the Cartesian diver technique of Lindstrom-Lang. Our modification of this procedure has been described. For the measurement of respiratory rates, red and black guinea pigs that had been treated with CPZ and exposed to ultraviolet light for 12 months and also untreated controls were killed by injection of 50 mg. of sodium pentobarbital into the heart. The eyes were enucleated immediately and the lens exposed by removing the cornea from the eye. The anterior lens capsule and lens epithelium were removed as one specimen from the central portion of the lens, corresponding to the area of lens opacities seen with the slit lamp. Subepithelial lens tissue was also taken from the anterior cortical areas near the visual axis.

The lens tissue was placed in 1 to 2 ml of Ringer’s phosphate buffer containing 200 mg. per cent of glucose inside a Cartesian diver of approximately 25 ml total volume. A layer of KOH absorbed the CO₂ produced by the tissue. Measurements were made at 37.00 ± 0.005° C. Changes in pressure within the diver were measured to an accuracy of 1 mm. of Brodie’s solution (1 atmosphere = 10,000 mm. of Brodie’s solution). In some of the experiments a baseline rate of oxygen consumption was recorded, following which a 0.5 ml side drop of 0.2M sodium succinate was added to the lens tissue layer, in an effort to exaggerate any difference between the
respiration of the control and treated animal lens tissue. At the conclusion of the measurements of oxygen consumption, the lens sample was removed from the diver, washed with distilled water to remove any salt, dried in an oven at 105° C. to constant weight, and weighed on a microbalance sensitive to 0.05 μg. Oxygen consumption was calculated as:

\[ Q_{o_2} = \frac{\text{mL O}_2 \text{ consumed}}{\text{mg. anhydrous tissue/hr.}} \]

**Results**

**Animal studies.** Of the original group of 36 guinea pigs, 19 survived 12 months of CPZ ingestion and ultraviolet light exposure. All of the animals had macroscopic corneal abrasions which morphologically appeared to be the result of trauma (two or more animals were housed in each cage), but could possibly be related to ultraviolet exposure. Consequently, slit lamp evaluation for possible corneal opacities was difficult. However, no definite granular opacities were recognized in the deep stroma; a faint diffuse stromal haze was noted in the corneas of a few animals housed individually. Definite cortical and subcapsular lens opacities could be seen with the slit lamp in all animals, male and female, red, black, and albino. The most frequent lesion was a stellate veil-like opacity formed by very fine powdery white dots in the anterior cortex near the visual axis. A similar lesion was present much less frequently in the posterior lens cortex. These opacities were morphologically similar, but more prominent than the opacities in animals treated for only four months. The density of the opacities was not marked, and they could not be seen without magnification. They might be overlooked with a rapid scan with the slit lamp; however, when the faint delicate pattern was described, every observer skilled in using a slit lamp readily identified the lesions. Similar cortical lens opacities were absent in control animals. Nuclear lens opacities were present in both control and treated guinea pigs and could not be distinguished.

Twelve of the original group of 24 guinea pigs survived four months of treatment. These animals also had macroscopic corneal abrasions. No definite granular corneal opacity was seen when each animal was examined with a slit lamp. Various small opacities (punctate, geographic, dense and veil-like) were observed to appear in the nuclear region of both control and treated animal lens. It was not possible to identify any unique characterizing feature of the nuclear opacities in the CPZ-treated animals. A characteristic faint opacity was seen to develop in the anterior lens cortex of the treated animals. The most common lesion was a stellate opacity near the visual axis composed of hundreds of separate miniscule white dots in the anterior lens cortex near the visual axis, arranged in veil-like patterns (Fig. 1). The earliest lesions were detected in half of the female animals six weeks after institution of the treatment. After the same time interval, no definite lens opacity was seen in treated males. Lens opacities were consistently identified earlier in treated females. Following nine weeks of treatment, typical anterior cortical lens opacities were identified in more than half of the males. After 12 to 16 weeks, anterior cortical lens opacities were present in more than 90 per cent of the treated females and in approximately 70 per cent of the treated males.

![Fig. 1. Diagrammatic representation of the opacities that developed in lenses of guinea pigs receiving 30 mg. of CPZ per day for 3 to 12 months.](http://iovs.arvojournals.org/pdfaccessashx?url=data/journals/iovs/933003/ on 04/15/2017)
Table I. Chlorpromazine content of guinea pig tissues (µg of CPZ per gram of wet tissue)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Duration of treatment (30 mg./day/animal)*</th>
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<tr>
<td></td>
<td>3 mo.</td>
</tr>
<tr>
<td>Cornea</td>
<td>17</td>
</tr>
<tr>
<td>Lens</td>
<td>6</td>
</tr>
<tr>
<td>Retina</td>
<td>10-67</td>
</tr>
<tr>
<td>Uvea</td>
<td>01-59</td>
</tr>
<tr>
<td>Liver</td>
<td>7</td>
</tr>
<tr>
<td>Kidney</td>
<td>4</td>
</tr>
<tr>
<td>Heart</td>
<td>0*-10</td>
</tr>
<tr>
<td>Lung</td>
<td>0* - 13</td>
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*A range of values is reported for assays from guinea pigs of different pigmentation. Values represent duplicate determinations on tissue pooled from 2 to 5 animals of a single color.

No detectable CPZ was found in albino tissue.

Similar cortical lens opacities were not seen in control animals. In two control animals, a small (1 to 2 mm.) aggregate of fine dustlike opacities developed at the interface between the anterior cortex and nucleus of the lens, and was misinterpreted as early CPZ opacities before the treatment status of the animal was known. These opacities were seen only at the interface, and did not extend into the cortex and subcapsular region as did the opacities in all treated animals.

Chemical studies. After three months of treatment, the amount of material acting as CPZ by ultraviolet absorption in the cornea and lens was very low, and at the limit of detection by this technique (Table I). After four months of treatment (Fig. 2), the amount of CPZ in ocular tissues had increased to 5 to 40 µg of CPZ per gram of wet cornea and lens, and this increased to 60 to 83 µg of CPZ per gram of wet tissue after 12 months of treatment. It should be emphasized that CPZ was present in the lens of treated albino guinea pigs.

While 120 and 170 µg of CPZ per gram of wet tissue were determined in the uveal tissue of treated black and red animals, respectively, after four months of treatment, no detectable CPZ was found in the uvea of the albino animals treated for the same time. The heart, lung, liver, and lens of treated albino animals, however, had significant deposits of CPZ.

No detectable CPZ was present in the control animals.

The rate of dialysis of CPZ from a cellophane bag was reduced in the presence of both synthetic and natural melanin (Fig. 3). If the rate of dialysis of CPZ from a mixture of melanin and CPZ gives a measure of the affinity of these two substances, it is unaffected by concentrations of sodium chloride or urea up to 3M, or by changes in pH between 3 and 10. The rate of dialysis of CPZ from a cellophane bag was also reduced when homogenates of lens or uveal tract from treated black guinea pigs were added; however, this did not occur when homogenates of uvea or lens from treated albino animals were used (Table II).

Staining of the guinea pig eyes by the Fontana technique revealed irregular intense purple to black coloration of the cornea and lens of treated and control animals that could not be differentiated. When the Fontana stain was applied to CPZ on a slide, a similar intense purple to black coloration was produced with and without prior ultraviolet light exposure. This staining technique is clearly not specific for identification of melanin in tissues.

Metabolic studies. The rate of oxygen consumption of lens epithelium and capsule was reduced in guinea pigs treated...
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![Graph]

Fig. 2. Average chlorpromazine content of guinea pig tissue after treatment for 4 months.

![Graph]

Fig. 3. Dialysis rate of chlorpromazine in the presence of synthetic melanin (see text). A similar curve was obtained with natural melanin.
Table III. Rate of oxygen consumption of guinea pig lens tissue

<table>
<thead>
<tr>
<th></th>
<th>Control ($Q_o$)</th>
<th>Chlorpromazine treated ($Q_o$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lens epithelium + capsule</td>
<td>1.28 (17) t</td>
<td>0.63 (19) t</td>
</tr>
<tr>
<td>Lens epithelium + capsule + 0.1M succinate</td>
<td>1.03 (11)</td>
<td>0.83 (11)</td>
</tr>
<tr>
<td>Anterior cortical lens fibers</td>
<td>0.34 (5)</td>
<td>0.26 (3)</td>
</tr>
<tr>
<td>Anterior cortical lens fibers + 0.1M succinate</td>
<td>0.48 (5)</td>
<td>0.34 (3)</td>
</tr>
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"at" of O$_2$ consumed per milligram of dry tissue per hour. t Number of experimental determinations.

with CPZ and ultraviolet light for one year, when compared to untreated controls (Table III). The average $Q_o$ was 1.28 $\mu$l of O$_2$ per milligram of tissue per hour for the control guinea pigs, and 0.63 for the treated animals. The difference is statistically significant ($p < 0.01$).

The oxygen consumption of lens epithelium and capsule from CPZ-treated guinea pigs was measured before ($Q_o = 0.26$) and after ($Q_o = 0.34$) adding 0.2M succinate to the tissue medium. The difference is significant ($p < 0.05$).

The difference between $Q_o$ of lens epithelium of control guinea pigs before and after adding 0.2M succinate was not statistically significant.

The difference between $Q_o$ of anterior cortical fibers before and after adding 0.2M succinate was not statistically significant.

The difference between $Q_o$ of anterior cortical fibers from control and treated guinea pigs was not statistically significant.

In additional experiments CPZ was added after a baseline $Q_o$ for lens epithelium had been obtained. An immediate reduction of respiration rate was demonstrated for concentrations of CPZ greater than $10^{-6}$ to $10^{-8}$M.

### Discussion

Conjunctival and skin pigmentation and corneal and lens opacities in human subjects who were receiving chronic CPZ therapy were described initially by Greiner and Berry in 1964. Many patients with similar eye and skin findings have been reported subsequently by other authors. Corneal and lens opacities have occurred in 27 to 76 per cent in different studies, whereas conjunctival and skin pigmentation has been observed less frequently ( < 1 per cent).

In human patients receiving chronic CPZ therapy, the exposed skin may acquire a metallic purple to gray coloration. Brown conjunctival coloration occurs within the palpebral fissure only in those patients with skin coloration. Ptosis and miosis occur commonly. Deficient tearing has been demonstrated by the Schirmer test. Corneal involvement has consisted of punctate peratitis, or Hudson-Stahli lines (considered to be significant because they occurred in young adults), or slight cloudiness of the epithelium and Bowman layers in the interpalpebral area, or granular opacities in the posterior portion of the stroma, near Descemet's layer, within the interpalpebral area.

Gonioscopy yields normal results. The pupils dilate poorly, but light and near response are normal. Vision may be normal or decreased, while color vision, ERG, and intraocular pressure are generally normal in these chronically treated patients. A few patients who are receiving chronic CPZ therapy have developed a fine granular pigmentation of the retina, some patients with retinopathy have developed attenuation of retinal vessels, or constricted visual fields, or possibly optic atrophy. One patient with retinopathy had normal dark adaptation and normal EOG.

In this study, when CPZ was fed to guinea pigs, lens opacities were consistently produced that resemble those in treated human patients. Corneal, conjunctival, and chorioretinal changes were not apparent in this study.
While the number of experimental animals in this study is small, there appeared to be an increase in the death rate among the animals receiving CPZ. CPZ may, in some unknown way, predispose some animals to a premature death. This impression is not inconsistent with observations in human subjects receiving CPZ, when sudden, unexplained deaths have occurred in apparently healthy young adults.²³, ²⁴

Some clinical studies have shown that more than half of the human patients receiving a 1,000 Gm. total dose of CPZ will develop lens opacities that can be seen with the slit lamp.²⁵ If it is assumed that an average patient weighs 70 kilograms, lens opacities appear in man when a total dose of approximately 14 Gm. of CPZ per kilogram has been ingested. More than half of the guinea pigs treated with 30 mg. of CPZ daily for four months have developed lens opacities in this study—or when approximately 7 Gm. of CPZ per kilogram has been ingested. While such calculations are only approximate, they suggest that lens opacities occur in this experimental animal at total dosages comparable to those used in human patients, when body weight is taken into account.

Lens opacities demonstrated in guinea pigs treated with CPZ are morphologically similar in size, shape, location, and fine detail, as seen by slit lamp examination, to those that occur in man. In view of this striking similarity, the opacities may have a common origin. Since CPZ cataracts developed regularly in albino animals, the cataracts cannot represent an accumulation of melanin pigment.

One possible explanation for these opacities would be that a compound, perhaps CPZ or a metabolite, is actively transported or diffuses into the anterior lens subcapsular area, accumulates, and causes the opacities seen with the slit lamp.

A second explanation is also tenable. It has been well documented that protein exposed to ultraviolet light in the presence of a photosensitizing agent will be denatured, followed by flocculation of the denatured protein.²⁵ CPZ is a known photosensitizer and has been demonstrated in this report to accumulate in the lens of treated guinea pigs. Thus the lens opacities seen in CPZ-treated guinea pigs exposed to ultraviolet light may represent foci of flocculated denatured protein. This interpretation is supported by the location of lens opacities within the pupillary area, and by the observations of DeLong.²⁶ He has been able to reproduce our findings of cortical and subcapsular lens opacities in all guinea pigs treated with CPZ and exposed to ultraviolet light. However, he also treated a group of guinea pigs with CPZ but did not expose them to ultraviolet light. The characteristic CPZ opacities were infrequent in this group of animals. This suggests that both CPZ and irradiation are important for the development of the experimental CPZ cataract. If a similar mechanism operates in man, a variation in the quantity of irradiation may play a role in determining the variable incidence in numerous clinical reports.

No attempt was made to determine the amount of ultraviolet light incident upon and absorbed by the lens in these experiments. Kinsey²⁷ has shown that 84 per cent of incident 3,600 A light reaches the anterior lens of albino rabbits, and 4.2 per cent is transmitted to the anterior vitreous, so a significant amount of 3,600 A light probably is incident upon the guinea pig lens in these experiments.

CPZ is metabolized in man to form several compounds.²⁸, ²⁹ The main pathways are sulfoxidation of the phenothiazine nucleus, or hydroxylation at the 7 or 3 position, followed by conjugation with glucuronic or sulfuric acid. CPZ, CPZ sulfoxide, and 7-hydroxychlorpromazine glucuronide have been identified in body tissues and the urine of human subjects receiving chronic CPZ therapy. In this study, absorption at 340 mμ of lens extract treated to form the sulfoxide was used as the criterion to identify CPZ. This is not a unique identification, and these or other known metabolites of CPZ may be included in this determination. No attempt to identify such metabolites was made. No material
acting as CPZ was detected in any control animal.

Histologic examination of guinea pig eyes with Fontana’s silver stain demonstrated fine granular material in the lens cortex and nucleus in both treated and control animals. While the Fontana stain has generally been interpreted as a stain for melanin in CPZ studies, it has not been demonstrated to be specific for melanin, and, in fact, will stain CPZ granules on a slide.

The distribution in tissues of material behaving as CPZ falls into two categories. The first is typified by pigmented structures, such as the uveal tissue, where there is a correlation between melanin content and retention of CPZ by tissues. Such an association has been suggested by others, and in the present study was shown both in vivo and in vitro. Relatively large quantities of a CPZ-like material were present in the uveal tract of red and black animals, whereas none was found in the uveal tract of albinos. The uveal tract in pigmented species contains large numbers of melanin-producing cells. There are none in albinos. It is possible that, in the uveal tract of pigmented animals, CPZ and melanin form charge transfer complexes where CPZ is the electron donor and melanin is the electron acceptor.

Tissues in a second category, of which kidney, liver, and possibly lens serve as examples, probably accumulate CPZ by diffusion or transport. The melanin content of these tissues is practically nonexistent, hence melanin-CPZ complexes cannot account for the large quantities of CPZ-like material detected in them. CPZ accumulation in lens in vivo probably arises from CPZ that enters via the aqueous circulation. It is particularly interesting that homogenates prepared from lens of albino animals showed no ability to retain CPZ even though the drug deposition occurs in vivo. It is not clear why homogenates of lens from black guinea pigs retarded the dialysis of CPZ, for melanin apparently is not present in this tissue. It should also be noted that there was a continuous accumulation of CPZ within the lens with longer periods of treatment.

The respiratory metabolism of lens epithelium in CPZ-treated animals was reduced by a statistically significant amount in this study. As CPZ or a structurally similar metabolite has been demonstrated in these lenses, and is absent in control lenses, it would appear that CPZ or a metabolite exerts a direct toxic effect on the respiratory metabolism of lens epithelium. As sodium succinate will stimulate respiration in CPZ-treated, but not control, lens epithelium (both exposed to glucose substrate), it would seem that CPZ effects a metabolic block at some site preceding succinate.

It has been proposed that the primary and perhaps only action of CPZ is to alter the permeability of membranes throughout the body. Michon and Lambert have demonstrated an increased permeability of the lens to rubidium in the rabbit. Thus altered lens permeability may be the mechanism which facilitates the entry of CPZ or a metabolite into the lens. In addition, however, CPZ exerts a direct metabolic action on energy-producing mechanisms and probably acts as a photosensitizing agent in the lens.

It should be noted specifically that the chronic CPZ cataracts demonstrated in this report have no apparent relationship to the acute CPZ cataracts obtained following intraperitoneal injection of CPZ in rats or to the “dehydration cataracts” which develop when animal eyelids are kept open.

REFERENCES


