evidence that indomethacin did not interfere with preformed PG synthesis. The specificity of action that indomethacin exhibited is further supported by the lack of activity of dexamethasone which is yet known as a very potent anti-inflammatory agent. PG release is part of the inflammatory process and dexamethasone as well as other steroids can act by different mechanisms.

This limited study on ocular disposition of indomethacin appears to justify the use of the topical route of administration as compared with the oral route. A better biological response was obtained topically with 40 times less drug than after oral dosing; moreover, blood levels were very low after instillation, and thus decreased the risk of systemic side effects. Orally, indomethacin elicited an inhibitory response with a small amount recovered from the aqueous humor, but certainly enough drug was present at the site of action of PG synthetase.

AA, although a powerful irritant, did not affect the penetration of indomethacin, not only through anterior segments of the eye after ocular instillation, but also and particularly through the ciliary processes after its oral administration, which is an additional evidence of the protection of the blood aqueous barrier by indomethacin.

To conclude, this testing procedure is very useful as a model for routine in vivo investigations on ocular PG synthetase.

We are in debt to Prof. M. Sears for his continuous interest in our experimental work. We thank Mrs. D. Alix, Mrs. M. Chatrousse, and Mrs. L. Coulbault for their skilful technical assistance.

From the MSD-Chibret Research Institute, 200, Bd. E. Clémentel, 63018 Clermont Ferrand Cédex, France. Submitted for publication April 17, 1975.

Key words: arachidonic acid, prostaglandin E2, rabbit, intraocular pressure, indomethacin, dexamethasone, topical administration, ocular penetration.

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The circadian rhythm of the intraocular pressure in the New Zealand White rabbit. RAANANAH SWIRSKY KATZ, PAUL HENKIND, AND ELLIOT D. WEITZMAN.

Most physiologic functions in man as well as in animals have been shown to have a circadian (approximately 24 hour) rhythm. It has been found in both the normal and glaucomatous human eye that the intraocular pressure, which is one of these circadian functions, varies considerably over a 24-hour period. Because of the extensive use of the rabbit in glaucoma research, a detailed study of the intraocular pressure over a 24-hour period was undertaken. Using hourly applanation tonometry for 25 consecutive measurements in ten New Zealand White rabbits (20 eyes), a circadian rhythm of the rabbit intraocular pressure was found. The pressures were lowest at night and rose to their highest values during the day. In addition, the data suggest that the maximum intraocular pressure may be biphasic with maximum values occurring between 0900 and 1100 and 1600 and 1900. Therefore, studies involving the New Zealand White rabbit must take into consideration these 24-hour pressure changes when intraocular pressure measurements are made.

Biologic periodicity is a feature of almost all animal and plant life. Most of these rhythms are described as circadian, i.e., they approximate a 24-hour period. The intraocular pressure (IOP) is one of the many bodily functions in man which follows a 24-hour variation. This idea, originally proposed by Sider-Huguenin in 1898 was further investigated by Maslenikow in 1904 in both glaucomatous and normal human eyes. In general, it was found that there was a tendency...
In 24-hour IOP variation toward an increase in the IOP in the morning with a sharp decline in the afternoon. Köllner in 1916 was the first to describe these daily fluctuations of IOP in the glaucoma patient as a "diurnal rhythm."

In 1958, Ericson, studying the IOP diurnal variation of the normal human eye, also concluded that the highest IOP's occurred between 8 A.M. and 12 noon and decreased to their lowest values at midnight. More recently, Henkind, Leitman, and Weitzman utilizing the technique of hourly or more frequent IOP measurements during a 24-hour period, have described in detail the general pattern of this diurnal variation. They found that the lowest IOP, in both normal and glaucomatous human eyes, occurred at about 3 A.M. and then rose to a peak at approximately 11 A.M. The peak values reach a plateau which persists until approximately 5 P.M. after which the IOP slowly decreases to a 3 A.M. low. In addition, short-term fluctuations of IOP were noted in both normal and glaucomatous eyes, ranging from 3 to 9 mm Hg within hourly measurements. Further studies have correlated the 24-hour IOP with the circadian rhythm of cortisol demonstrating a phase shift of several hours, peak IOP lagging behind peak cortisol secretion.

The rabbit is used extensively in eye research, particularly in the study of glaucoma. It is therefore of considerable interest to determine whether the rabbit eye also demonstrates the phenomenon of 24-hour IOP variation and, if so, to describe these variations in greater detail. For this reason,
we have studied the diurnal variation of the IOP in the New Zealand White (NZW) rabbit (albino eye).

**Materials and methods.** Ten New Zealand White male rabbits weighing from 2.3 to 4.5 kilograms were obtained from Camm Research, Wayne, N. J. All of the rabbits were healthy and their eyes were clinically normal. All animals were housed in the animal care room for a minimum of seven to ten days prior to the experiment. Both artificial (fluorescent) light as well as natural illumination was the source of light for the light:dark (L:D) schedule. The lights were turned on every morning at 0700 and off at 1630. Sunset during the time when these experiments were done was 1730. Therefore, the L:D schedule for these rabbits was 0700 to 1730 light: 1730 to 0700 dark.

The animals were removed from the animal care unit for the 25 hours of experimentation and placed in a specially equipped examination room. Each rabbit was caged separately. Room temperature ranged between 25° C. and 27° C. Food and water were provided ad libitum. The L:D schedule for the 24-hour period of the experiment was 0730 to 2330 light: 2330 to 0730 dark.

The IOP was measured each hour in both eyes for 25 consecutive measurement hours with a Mackay Marg applanation tonometer No. 12. One observer (R S K) performed all of the measurements. The rabbit was removed from the cage for each hourly measurement. One drop of Proparacaine 0.5 per cent diluted 1:5 with saline was then instilled into each eye immediately prior to each hourly set of applanations. During a measurement the rabbit sat unrestrained on a table. Great care was taken not to touch the animal in the area of the orbit during the pressure determination. In one half of the animals the right eye was applanated first. In all cases at least ten separate measurements were made for each eye. Only tracings which were considered "legible" by the standards of the Mackay Marg "Tonometer Manual" were used. The average of five to ten such acceptable tracings was used in compiling the data. In five instances (2 per cent of the total recordings) it was necessary to discard the hour's reading for a particular rabbit. Measurement of both eyes took approximately three to four minutes. The rabbits were returned to their cages after each measurement. The tracings were not read immediately, but rather were collected and then read at random to insure that no bias was present in regard to the preceding and succeeding measurements.

The pupillary diameters were observed and any major change of size was noted during the procedure.

Although the rabbit is a nocturnal animal in his natural environment, the domesticated rabbit used in experimentation had adjusted very well to the laboratory schedule of activity. In general, while in their cages, the rabbits were noted to be in a sleeping posture during most of the night (0100 to 0700) and were only transiently
Fig. 3. Mean per cent deviation from the mean of five New Zealand White rabbits (10 eyes) studied over the same 24-hour period.

aroused when each hourly measurement was made. During the daytime the rabbits were found to be awake.

Results. A significant 24-hour variation of the intraocular pressure was found in 18 of the 20 eyes studied. The well known congruence of IOP change from one eye to the other was clearly demonstrated in all of the rabbits. On the average, the IOP was within 1 mm. Hg for both eyes. Because this is within the range of experimental error it was considered valid to combine the data from the two eyes.

The 24-hour curve of the rabbit IOP is not a smooth function varying daily. Major pressure changes were found to occur over short time periods and in almost all cases were the same for both eyes (Fig. 1, A and B). The greatest hourly increase in IOP was 7.5 mm. Hg (from 8.5 to 16 mm. Hg) and occurred between 0700 and 0800 (rabbit No. 2068). The greatest hourly decrease in IOP was 4.5 mm. Hg (16 to 11.5 mm. Hg) and this occurred during two time intervals in a single rabbit (No. 2061) between 0500 and 0600 and again between 0800 and 0900.

The curve of the mean values of the IOP for the 20 NZW eyes (ten rabbits) demonstrated that the time of the minimum IOP was between 1900 and 2100 hours (Fig. 2). It should be emphasized that the individual curves showed a more pronounced diurnal variation (Fig. 1, A and B).

Analysis of the time of peak values and of second highest values of IOP demonstrated a clustering during the 24-hour period around two time intervals. These were from 0800 to 1100 and 1600 to 1900. Furthermore, if the maximum value occurred during one of these two intervals, then the second highest value for IOP occurred during the second of the two intervals.

It was noted on several occasions that the IOP would rapidly decrease when pupillary constriction occurred. One rabbit showed a decrease in IOP from 16 to 12 mm. Hg. This decrease occurred within 60 seconds as the pupil constricted.

The data are expressed in a graph of "mean per cent deviation of five NZW rabbits (10 eyes) measured in the same 24-hour period" vs. "time" (Fig. 3). This demonstrates that the IOP deviated above the mean between 0800 and 1100 and 1700 and 1800. The IOP deviated below the mean between 1900 and 2100. This supports the conclusions drawn from the curve pattern of absolute 24-hour IOP measurements.

Finally, to test the reproducibility of the observed diurnal curve, we repeated the experiment over a second 24-hour period separated by three weeks on two of the rabbits (No. 2068 and No.
Fig. 4. Graph of results of both the original and repeat studies done on rabbit No. 2060 (NZW). Circles represent the original 24-hour IOP curve done on Feb. 11, 1975, for the right eye only (OD). Triangles represent the data obtained from a repeat study done on March 6, 1975. Similar results were obtained for the left eye.

Discussion. We have found a circadian rhythm of IOP in the New Zealand White rabbit similar to that found in man. In this study there appears to be a curve with peak pressures occurring between 0800 and 1100 and 1600 and 1900. The minimum IOP is reached during the evenings between 1900 and 2100.

Eleftheriou7 has evaluated the circadian rhythm of blood and brain biogenic amines in the NZW rabbit. In his rabbits, maintained on an 0600 to 1900 light:1900 to 0600 dark schedule, he noted that the plasma corticosteroids, norepinephrine, and glucose all reach their peak with the onset of darkness, i.e., 1900. Weitzman and co-workers8 have described the circadian rhythm of the plasma cortisol production in man and have related it to several bodily functions, the IOP being one of them. They show that there is a correlation of peak plasma corticosteroid production and the intracocular pressure. The rabbit might serve as a model for the study of the relationships between the circadian rhythm of corticosteroid and norepinephrine secretion and IOP.

Since eyes with higher IOP's appear to have larger diurnal pressure variations, the study of the circadian rhythm of IOP in the congenitally glaucomatous rabbit may provide a natural amplification of the normal diurnal pressure variation. This is particularly relevant to glaucoma in the human eye for which the circadian rhythm of IOP has been worked out in great detail.

The rabbit is used extensively in the study of glaucoma both as a model of the normal eye and as a subject for steroid-induced glaucoma. It is also used in evaluating the pharmacologic treatment of glaucoma as well as the success of various surgical techniques. Due consideration of the normal diurnal variations of the IOP in the rabbit should now be given in interpreting the results of studies in which the rabbit is used.

From the Departments of Ophthalmology and Neurology, Albert Einstein College of Medicine, and Montefiore Hospital Medical Center, Bronx, N. Y. Assisted by National Institutes of Health Grant No. EY 01224, and an unrestricted grant for publication April 28, 1975. Reprint requests: Dr. Henkind.

Key words: intraocular pressure (IOP), circadian rhythm, glaucoma, rabbit intraocular pressure, tonometry.

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Note on the distribution of Iodine-123-labeled indocyanine green in the eye. XVIII.* A. ANSARI, R. M. LAMBRECHT, S. PACKER, H. L. ATKINS, C. S. REDVANLY, and A. P. WOLF.

Indocyanine green was excitation labeled with radioactive iodine-123 and the distribution of the labeled dye in the eye structures of animals was determined.

Indocyanine green (ICG), a water-soluble tri-carbocyanine dye, has been considered for in vivo studies of the ocular dynamics of choroidal flow patterns, retinal circulation times, and retinal blood flow.1-6 We have recently developed a method to label ICG with carrier-free iodine-123,7 and have evaluated 123I-ICG for dynamic studies of the hepatobiliary system. Tissue distributions with time and loading dose studies in mice, rabbits, and dogs have indicated that excitation labeled 123I-ICG behaves like unlabeled ICG, and that the liver clearance curves may be useful for determining the functional status of the hepatobiliary system.8

The purpose of this study was to determine what affinity 123I-ICG has for choroidal structures and other ocular components at various times after intravenous administration of 123I-ICG. By virtue of the radioactive label of 123I it is possible to follow the distribution of 123I-ICG at trace levels which cannot be determined by conventional spectrometric methods. In particular, we wished to establish whether ICG had a significant affinity for pigmented tissue, and to determine whether ICG labeled with 123I (Tv = 13.3 hours) would be of use in localizing ocular melanoma.

Methods. The ICG was labeled with 123I by the reactive 123I atoms and ions formed by the 123Xe (β, EC) 123I nuclear transformation, i.e., excitation labeling.9 The 123Xe was produced by the 125Te (α, 3n) 123Xe or the 127I (p, 5n) 123Xe nuclear reactions. Briefly described,6 the method consisted of removing the adulterant bulk stable sodium iodide from U.S.P. ICG, (supplied by Hyson, Wescott, and Dunning, Inc., by passage of an alcohol solution of the dye through a column of AG-11A ion retardation resin, 50 to 100 mesh, (Bio-Rad Laboratories). The repurified dye (0.5 to 2.0 mg.) was dried and then exposed to 123Xe (Tv = 2.1 hours) for 2 to 6 hours to excitation label9 the ICG. Subsequently, the organically bound 123I was removed from the labeled 123I-ICG by the same chromatographic method described. The specific activity of the 123I-ICG > 1 mCi per milligram. The 123I-ICG was dissolved in the U.S.P. sterile aqueous solvent (HW & D) prior to intravenous injection. There are at least two radiiodinated products in the 123I-ICG.8

For the experiments the loading dose (i.e., the number of milligrams of ICG per kilogram of body weight) was < 0.1 mg. per kilogram. Loading dose effects with 123I-ICG are not significant at this low level.9 The animals were killed at time intervals after intravenous (dogs, rabbits) or intraperitoneal (hamsters) administration of the 123I-ICG. Subsequently, the tissues were dissected out, weighed, and assayed for radioactivity.

Results and discussion. Table I summarized the distribution of the 123I-ICG in the various ocular tissues of three dogs killed at 1 minute, 1 hour, and 20 hours. The bulk of the 123I-ICG remaining in the eye of the dog is in the vitreous. The per cent of the injected dose in the total fluid is 0.029 per cent at 1 hour and 0.0158 per cent (i.e., only 50 per cent less) at 20 hours. The choroid accumulated the 123I-labeled compound to about 0.002 per cent of the injected dose and the percentage increased at 20 hours. The fraction of the 123I associated with the cornea, iris, lens, retina, and sclera were less than 0.0001 per cent at 1 and 60 minutes, but also increased slightly at the later time.

The low affinity of the ocular structures for 123I-ICG, and its presence principally in the vitreous, suggests that the rationale for using ICG (not radioactive) for studies of ocular hemodynamics is valid. Ocular infrared absorption curves and the ocular clearance kinetics of ICG are obtained during the first 4 to 60 seconds after intravenous administration in humans. The total concentration of 123I-ICG in the dog eye at one