Relationship between the blood flow velocity in the ciliary body and the intraocular pressure of rabbit eyes

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Blood flow velocity in the ciliary body was measured with a specially designed miniature Doppler flow detector. The transducer consisted of two crystals mounted in a groove of a metal disk, 8 mm. in diameter and 3.5 mm. thick with an efficient area of 2 by 3 mm. Blood velocity in the ciliary body at various intraocular pressures was investigated in 18 anesthetized albino rabbits. The relationship between perfusion pressure (arterial pressure minus intraocular pressure) and blood velocity in the ciliary body gave a sigmoid curve. This relation re-plotted on probability paper was linear, fitting well to an integration of a binomial curve. When the Doppler shift was plotted against the intraocular pressure instead of the perfusion pressure, similar results were obtained. In order to represent the sigmoid curve, the intraocular pressure when \( \frac{df}{dp} \) was maximum, was calculated \( (P_m) \), where \( f \) is the relative Doppler shift and \( P \) is the intraocular pressure. A linear relation was found between systemic arterial blood pressure \( (P_s) \) and \( P_m \), giving a regression equation of \( P_m = 0.60P_s - 9.15 \). No reactive hyperemia was found in the ciliary body vessels of rabbits after a release of elevation of intraocular pressure.

Key words: blood flow velocity, intraocular pressure, Doppler flow detector, ciliary body, rabbit.

Measurement of blood flow in the ciliary body is of particular interest because of its physiologic significance in aqueous humor production and in the maintenance of the intraocular pressure.

Previous investigations of the blood flow were done either with thermocouples\(^1\,\,2\) or by the krypton dilution method.\(^3\) The thermocouple method required a precise control of the ambient temperature, which had great influence on the measurement. The krypton dilution method required no temperature control but it did not allow detection of a rapid change of the blood flow, since the flow was calculated from the rate of isotope dilution over a period of time. Measurement of rapid changes of blood flow without serious temperature interference may be made by the Doppler flow detector.

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Since Suzuki and Satomura\textsuperscript{4} described a Doppler flow detector for the measurement of the ocular blood flow, a few papers have been published.\textsuperscript{5,6} However, the transducers used were so large that they were placed on the upper eyelid, and detection of blood flow in a circumscribed intraocular region such as the ciliary body was by no means possible. In 1969, Schlegel and Lawrence\textsuperscript{7} used a miniature transducer for the measurement of the vortex vein flow, but it was not used for the intraocular structures. The present investigation employed a new miniature Doppler transducer designed to measure the blood flow velocity in a small intraocular region. The measurement parameters of this transducer were determined. Effects of intraocular pressure on blood flow velocity in the ciliary body are discussed in this report.

The transducer

The transducer consisted of two crystals fixed in a 3 mm. wide, 5 mm. long groove in a metal disk. The disk diameter was 8 mm. and the thickness 3.5 mm. One crystal was placed parallel to the plane of the disk; the second crystal was tilted so that its face was 135° from the first crystal (Fig. 1). The tilted crystal served as a generator of the ultrasonics, the former as a receiver. The line connecting centers of the two crystals was called the axis of the transducer. The edge of the metal disk where the first crystal was placed, was called the base edge of the transducer. The metal disk was fixed in a plexiglass ring which was shaped to conform to the scleral curvature. The transducer was operated with a transcutaneous Doppler blood velocity detector (Model 802, Parks Electronics Laboratory, Beaverton, Ore.), which consisted of the ultrasonic power oscillator (10 Mc.) and the frequency analyzer system. Doppler shift was recorded (Grass Model 7, Quincy, Mass.). The block diagram of the setup is shown in Fig. 1.

The maximum efficiency of Doppler shift is obtained when flowing fluid is placed at the intersection of the ultrasonic beam and the direction of its receiver, i.e., the focus of the transducer. The direction of the flow, whether along or at an angle to the axis of the transducer, has an effect on the result. In order to achieve a maximum measurement efficiency in animals, a model experiment was done to determine the conditions of measurement. A polyethylene tubing (PE-160, Clay Adams, N. Y.) was used as an artificial vessel in which homogenized milk flowed. The artificial vessel was placed at various distances as well as at various directions with regard to the transducer axis, and the Doppler shift was determined. As an efficient medium for the ultrasonic propagation between the trans-
In the first experiment, the artificial vessel was placed along the axis of the transducer and the distance from vessel to transducer was changed, while the flow velocity was maintained constant at 50 cm. per second (Fig. 2). A maximum Doppler shift was obtained when the vessel-transducer distance was 2 mm. A sharp drop of the Doppler shift occurred at 4 mm., and the shift was only 1/10 of the maximum at a distance of 10 mm.

In the second experiment, the artificial vessel was placed at a right angle to the axis of the transducer. The position of the vessel with regard to the base edge and also the vessel-transducer distance was changed. The Doppler shift was determined for a constant flow of 50 cm. per second (Fig. 3). When the vessel-transducer distance was 2 mm., the Doppler shift decreased as the position of the vessel receded from the base edge which was shown to be the most efficient part. The Plexiglas ring covered the margin of the transducer for 1 mm. around the circumference. Therefore, when the vessel-transducer distance was 2 mm., the most efficient part was at about 1 mm. from the base edge. When the position of the vessel was at 4 mm. from the base edge, the sensitivity was almost negligible. Therefore, the sensitive area of the transducer was 2 mm. times the width of the groove of the transducer, 3 mm.

The model experiments indicated that flow velocity in random directions could be most efficiently detected when the vessels were placed at 1 mm. from the base edge and the vessel-transducer distance was 2 mm.

In animal experiments, the measurement was through the sclera. Therefore, the effect of sclera was checked by placing a piece of the rabbit sclera between the transducer and the artificial vessel. The experiment showed that the Doppler shift decreased by about 20 per cent. This reduction however, should not give serious error in comparative changes of the blood flow, provided that the transducer position was proper and constant.

In the adult rabbit eye, the ciliary body is located about 1.5 mm. from the limbus and about 1 mm. deep from the scleral surface. Therefore, the Plexiglas ring of the transducer was so adjusted that the ciliary body could be placed at the most sensitive transducer position. The extent of the ciliary body detected was about 2 mm. wide; the flow in the ciliary body of the opposite side and the choroid could be neglected.
Fig. 3. Doppler shifts when the crystals of the transducer were applied at a right angle to the artificial vessel. The flow velocity was 50 cm. per second. The numbers in parentheses indicate the distance between the vessel and the base edge of the transducer (X).

Materials and methods

Adult albino rabbits of both sexes, weighing 2 to 3 kilograms, were anesthetized with intravenous sodium pentobarbital (30 mg. per kilogram) and an additional dose was given when necessary. In some experiments, topical anesthetic (0.5 per cent proparacaine solution) was used. Tracheotomy was done and the animal was fixed to a stand. The arterial blood pressure was measured from a femoral artery using a strain gauge pressure transducer (Statham P23Gc) and a recording system (Glass Model 7). The upper and lower lids and superior orbital margin were removed in most cases.

The anterior chamber was cannulated with two 23 gauge, 1 inch needles, each having a side opening at its midpoint with its tips plugged. The needles were immobilized with their tips penetrating into the cornea of the opposite side. Thus, injury to the iris could be avoided. One of the needles was connected to a reservoir of heparinized saline. Stepwise changes of the intraocular pressure were induced by changing the height of the reservoir. The other needle was connected to a strain gauge pressure transducer (Statham P23d) to record the intraocular pressure. The whole extraocular tubing system was filled with heparinized physiologic saline. The conjunctiva was dissected at the superior and lateral region of the limbus to expose the sclera. The Plexiglas ring of the transducer was sutured with 8-0 silk to the exposed sclera and the cornea so that the transducer was placed on the bare sclera corresponding to the ciliary body. The space between the transducer and the sclera was filled with Aquasonic 100. A few minutes were allowed to elapse in order to achieve a steady Doppler shift and intraocular pressure of 15 mm. Hg. The intraocular pressure was then increased stepwise by 10 mm. Hg and a new steady Doppler shift was reached in a few seconds and was maintained for at least 20 seconds. After the stepwise increase of the intraocular pressure to a maximum it was reduced stepwise by every 10 mm. Hg. An example of the experimental result is shown in Fig. 4.

Results

The result at a given intraocular pressure was expressed as relative Doppler
Fig. 4. Simultaneous record of Doppler shift (A), intraocular pressure (mm. Hg) (B) and arterial pressure (mm. Hg) (C).

Fig. 5. Relation between relative blood velocity and perfusion pressure. x---x increasing intraocular pressure, o---o decreasing intraocular pressure. ● result for intraocular pressure below the original level.

...shift, i.e., percentage of the initial Doppler shift obtained at intraocular pressure of 15 mm. Hg. The relative Doppler shift was plotted against the perfusion pressure (difference between the mean femoral arterial pressure and the intraocular pressure). The results for a few cases out of 18 eyes are shown in Fig. 5. The relationship between the perfusion pressure and the Doppler shift is found to have a sigmoid curve. The same results were replotted on probability paper where a linear relationship was found (Fig. 6), indicating that the perfusion pressure-Doppler shift relationship may be represented by an integration of a binominal curve. When the relative Doppler shift was plotted against the intraocular pressure...
instead of the perfusion pressure, similar results were obtained. If the relative Doppler shift is \( f \), the perfusion pressure \( P_p \), and the intraocular pressure \( P \), then the above result may be restated as that both the relationship between \( \frac{df}{dP_p} \) and \( P_p \), and between \( \frac{df}{dP} \) and \( P \) fit well to a binominal curve. The intraocular pressure, when \( \frac{df}{dP} \) is maximum, was calculated and defined as \( P_m \). The average of \( P_m \) for 18 eyes was calculated to be 48.9 ± 9.4 mm. Hg (standard deviation). The values of \( P_m \) seemed to vary depending on the systemic blood pressure and therefore they were plotted against the mean femoral arterial pressure (Fig. 7). A significant linear correlation was obtained giving a correlation coefficient of

\[
0.699 \quad (t = 3.602, \ n = 16, \ Pr \ [ |t| \geq 2.921 ] = 0.01)^* 
\]

and a regression

\[
P_m = 0.60 P_f - 9.15
\]

where \( P_f \) is the mean femoral arterial pressure.

When the intraocular pressure was reduced stepwise, the relative Doppler shift increased along the same curve as that for the increasing intraocular pressure. However, it was found that the Doppler shift of the eye, as a rule, did not return to its initial value after the experiment but was sometimes higher, sometimes lower. However, the difference usually did not exceed 5 per cent (Fig. 5).

It was studied in three rabbits whether a rapid release of the intraocular pressure caused a reactive hyperemia in the ciliary body. The intraocular pressure was raised directly from 15 mm. Hg to a certain level which was maintained for approximately 30 seconds and the pressure was then released to the original level. The changes of the Doppler shift were determined and the results are given in Fig. 8. In many cases the final Doppler shift differed slightly from the original level. However, only in a few instances did the final value exceed the original level. Therefore, rapid release of the intraocular pressure as used in this experiment did not result in a reactive hyperemia.

When the intraocular pressure was reduced below the initial level, the Doppler shift was found sometimes to increase and sometimes to decrease. Although no constant tendency was found, the difference from the original level was significant (Fig. 5).

Discussion

The Doppler flow detector used in this investigation could not distinguish the direction of flow; reversed flow was also read the same as forward. The efficiency of detecting flow velocity depends on the distance between the transducer and the vessel, and also the flow direction with

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*\( t_\alpha = \) sampling value of t-distribution.  
\( n = \) degree of freedom.  
\( Pr = \) probability.
regard to the axis of the transducer. When
the transducer is applied to the tissue hav-
ing many vessels with the flow direction
at random, one can detect the absolute
value of the average flow velocity. There-
fore, one cannot compare two different
places with different structures using the
Doppler shift. However, so far as the
Doppler shift is determined in a given
tissue, relative changes of this tissue can
be compared in terms of the absolute
average flow velocity. This flow detector
gives a measure for the flow velocity but
not the volume flow. However, if one com-
pared the result in a definite region of the
tissue where the cross-sectional area of the
vessel and transducer position remains
constant, the mean velocity changes should
be proportional to the mean volume flow.9
The change of the flow velocity of the
ciliary body induced by the intraocular
pressure can be safely compared for one
definite part and the change was expressed
as the shift relative to the original value of
the tissue.

A sigmoid curve relationship between
the intraocular pressure and the blood flow
in the eye of the rabbit similar to the
present result was reported for the ciliary
body,1-10 and for the choroid,2.11 as well
as for the vortex vein flow.7 In contrast to
the present result and above previous
demonstrations, only Friedman9 showed a
hyperbolic curve.

The difference of Friedman’s result from
others may be explained by the experiment
of Green and co-workers12 and Folkow13 on
the hind limb of the dog. They found an
equation

\[ f = c \cdot \frac{P_{m}}{P} \]

between the perfusion pressure \( P_{m} \) and
the flow \( f \), where \( c \) was a constant and
\( n \) had values between 1 and 5, generally
about 2. According to Folkow,13 the value
of \( n \) varies depending on the tone of the
blood vessels. Under good basic tone, \( n \)
was usually below 1 and the curve was
concave to the pressure axis. On the other
hand if the tone was weak, the curve was
convex to the pressure axis as \( n \) was more than 1. Therefore, the difference of the basic tone of the experimental vessels may explain the discrepancy between the sigmoid and hyperbolic curves.

When the intraocular pressure was reduced stepwise to the initial level, the final Doppler shift differed from the original value by about 5 per cent. Bill reported a similar finding on the rabbit eye when he measured blood flow of the eye using thermocouples. He could not decide if this was due to a change in the blood flow through the uvea or to slight changes in room and body temperature which could not be avoided during the experiments. Agreement between Bill's and present results indicates that his result was not due to change of the ambient temperature but of the local temperature, i.e., of the uveal blood flow.

According to Niesel, when the intraocular pressure was raised to a level less than 35 to 50 mm. Hg from the initial pressure of 15 mm. Hg and was maintained for a few minutes, a hyperemia occurred in the choroid of rabbit eyes. After a sudden release of the intraocular pressure to the initial level, he also found a reactive hyperemia. In the present experiments, a steady state of the Doppler shift was taken only for about 30 seconds at each level of the intraocular pressure. However, the Doppler shift with stepwise increase and decrease of the intraocular pressures agreed very well at a given intraocular pressure. This result suggests that a disturbing degree of reactive hyperemia did not occur during the present experiments. Also, a sudden release of the intraocular pressure to 15 mm. Hg did not cause measurable reactive hyperemia in the ciliary body region, confirming the results obtained by Bill.

At pressures below the initial pressure of 15 mm. Hg, relative Doppler shift varied more than the experimental errors; sometimes it increased, sometimes decreased. These results indicated that the vascular beds became unstable when the intraocular pressure was less than 15 mm. Hg.

The blood flow of the ciliary body should have a close relation to the rate of the aqueous humor production.\(^{16, 17}\)

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**Fig. 8.** Changes of relative blood flow velocity after sudden decrease of elevated intraocular pressure to the initial level. Each symbol and corresponding line represent experiments on one rabbit (total of three rabbits).
Bárány\textsuperscript{18} found a reduction of the aqueous humor production (F) by increasing intraocular pressure (P) and defined the pseudofacility by

\[
\frac{dF}{dP}.
\]

The pseudofacility was measured by many investigators with various techniques. Recently, Masuda\textsuperscript{14, 15} determined the change of aqueous humor production over a wide range of intraocular pressure and found that the relationship between the rate of aqueous humor production and intraocular pressure showed a sigmoid curve in living rabbit eyes as well as in monkey eyes. The relationship between pseudofacility

\[
\frac{dF}{dP}
\]

and intraocular pressure fitted to a binominal curve. The similarity of his and the present findings indicates a close relationship between blood flow in the ciliary body and aqueous humor production; the two results therefore deserve comparison. Masuda\textsuperscript{19} showed a linear correlation between the systemic blood pressure and the intraocular pressure where pseudofacility was maximum. His definition of this intraocular pressure (P\textsubscript{m}) is similar to the present definition of P\textsubscript{m}. His average of P\textsubscript{m} was found to be 27.1 mm. Hg whereas the present average was shown to be 48.9 mm. Hg. The higher P\textsubscript{m} obtained in the present experiments may be explained by the consideration that aqueous humor production depends on the more pressure-sensitive capillary blood flow than the flow in larger vessels which are included in this investigation. The relationship between systemic blood pressure and P\textsubscript{m} in both Masuda's and the present studies had very similar slopes but different intersections with the blood pressure axis. The difference of the intersection can be expected from the different average values of P\textsubscript{m}. The similarity of the relationships indicates that both the blood flow in the ciliary body and the aqueous humor production are in a similar quantitative dependence on the systemic circulation. The fitness of a binominal curve to the

\[
\frac{df}{dp}, P
\]

relationship in this study may be due to random distribution of the pressure sensitivity of the small vessels or the capillaries in the ciliary body.

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