Control of intraocular blood flow.
I. Intraocular pressure

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Measurement of intraocular blood flow has been difficult because of the size and inaccessibility of the ocular blood vessels. Recently, a method was developed using labeled microspheres for studying regional blood flow rates. Using this technique we have begun to study the various parameters which control ocular blood flow. This study confirms the use of labeled microspheres as a valid method for measuring blood flow. We have measured intraocular blood flow in the cat and have shown an inverse relationship between intraocular blood flow and intraocular pressure.

Measurement of ocular blood flow has been hampered because of the size and inaccessibility of the ocular blood vessels. Previous methods of measuring ocular blood flow have yielded a wide range of values and have not approximated normal physiologic conditions.[1-7]

Recently, a method was developed by Rudolph and Heyman[8] for the measurement of blood flow by the use of nuclide-labeled microspheres. The reliability of measuring blood flow using radioactive microspheres has been demonstrated.[9-11] Good agreement was shown for the values of cardiac output as measured by the indocyanine green dye-dilution technique, direct O_2 Fick method, and the labeled-microsphere method.[10] This technique has recently been applied to measure ocular blood flow in cat, rabbit, and monkey eyes.[12, 13] This paper will discuss the effect of intraocular pressure on ocular blood flow in cats using radioactive microspheres.

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Methods and materials

Adult cats weighing 2 to 3 kilograms and of both sexes were used in these studies. The animals were anesthetized with pentobarbital sodium, intraperitoneally, 30 mg. per kilogram. A tracheotomy was performed and the cat was maintained with a respirator. Intraocular pressure was controlled by cannulating the anterior chamber at the limbus with a 21-gauge needle connected to a saline reservoir. In each cat one eye acted as a control (sides were alternated), with its pressure maintained at 17 mm. Hg, while the pressure was altered in the opposite eye from 0 mm. Hg to five times the control pressure.

The microspheres were supplied by the Nuclear Products Division of the Minnesota Mining and Manufacturing Company, Minneapolis, Minn. Their exact composition has not yet been released, but they consist of a plastic compound containing 67 per cent carbon and 23 per cent oxygen. They are referred to as "carbonized microspheres." These carbonized microspheres had a mean diameter of 50 microns ± 5 microns, a specific gravity of 1.4, and they were labeled with strontium chloride,

Table I. Cardiac output and choroidal blood flow (ml./min.)

	Cat No. 1	*Cat No. 2*	*Cat No. 3*
Constant-injection method (Cardio-Green)	488	611	461
Sudden-injection method (microspheres)	520	555	468
Choroidal blood flow (microspheres)	2.04	2.13	1.97

Table II. Segmental ocular blood flow expressed as percentage of total ocular flow (mean value of ten cats)

Retina	2%
Choroid	65%
Ciliary body	28%
Iris	5%

Table III. Relative segmental ocular blood flow per gram of tissue per minute in control animals. These relative blood flow rates are expressed as a ratio of the blood flow to each segment compared to the blood flow of the retina

	Cat No. 1	*Cat No. 2*	*Cat No. 3*
Retina	1	1	1
Choroid	70	40	65
Ciliary body	20	14	13
Iris	6.5	4	4.5
Nictitating membrane	0.4		0.4
Kidney			18

^{85}Sr. The 50 micron microspheres were used in order to insure embolization at one pass and prevent recirculation of the microspheres. The radioactive label is incorporated in the spheres and is not merely a coating on their surface. The nuclide label is not lost from the spheres by solution in surrounding fluid.[8] The spheres, as supplied, are suspended in a solution of 20 per cent dextran with a small amount of the surfactant Tween-80, added to prevent aggregation and clumping. There are 5×10^6 spheres per 30 c.c. with a total activity of 1.0 mc.

The principle of the use of nuclide-labeled microspheres for measuring blood flow is that, if the microspheres are injected into the left heart, their fractional distribution in the peripheral vascular beds will be in proportion to the fraction of cardiac output going to each vascular bed. This is based on the assumption that the microspheres are well mixed in the left heart, are distributed in proportion to blood flow, do not escape to the venous circulation and recirculate, and that impaction in the tissue does not affect the blood-flow rate.[9]

The left hemithorax of the cat was opened and 0.5 c.c. of labeled microspheres per kilogram of body weight was injected into the left heart. Five minutes after injection the cats were killed by an intracardiac injection of pentobarbital sodium. The eyes of each cat were then enucleated. The muscle insertions and optic nerve were cut flush with the sclera and surface blood was rinsed from the globe. Using an operating microscope, each eye was then dissected into five segments: iris, ciliary body, choroid with tapetum, choroid without tapetum, and retina. The lens, cornea, and vitreous were found to be free of radioactivity and were discarded. As controls, paired kidneys, nictitating membranes, and muscles were utilized. The tissues were then air dried and weighed. Radioactivity was determined using a Packard Auto-Gamma Spectrometer. The gamma ray emission spectral curve for strontium chloride, ^{85}Sr, was determined. The optimum value was a peak at 502 KEV. A window from 450 to 550 KEV was then selected. Using this window, the background was negligible in relation to the counts of the sample.

In order to measure actual blood flow, cardiac output was measured by two methods. The first method used was a constant-injection indicator-dilution method employing indocyanine green for the dye dilution. A motor-driven syringe was used to insure constant injection. This standard method for determining blood flow is an application of the Fick method. The Fick method uses the relationship: blood flow (milliliters per minute) equals units of indicator injected per minute per concentration of indicator in samples (in units per milliliter).[14] The second method of measuring blood flow is based on our modification of the sudden-injection indicator-dilution method.[15] In this technique, a sudden injection (1 to 2 sec.) was performed into the left atrium of 1 c.c. of labeled microspheres whose total radioactivity was predetermined. Simultaneously, blood was withdrawn by means of a motor-driven syringe at a constant rate of 25 ml. per minute by means of a polyethylene catheter inserted into a femoral artery.

The sequence used in comparing these two methods was to obtain repeatable cardiac output values by means of the indocyanine green dye method and then, in the same animal, determine cardiac output by the microsphere sudden-injection method.

Results

Cardiac output was measured in three cats by sudden- and constant-injection in-

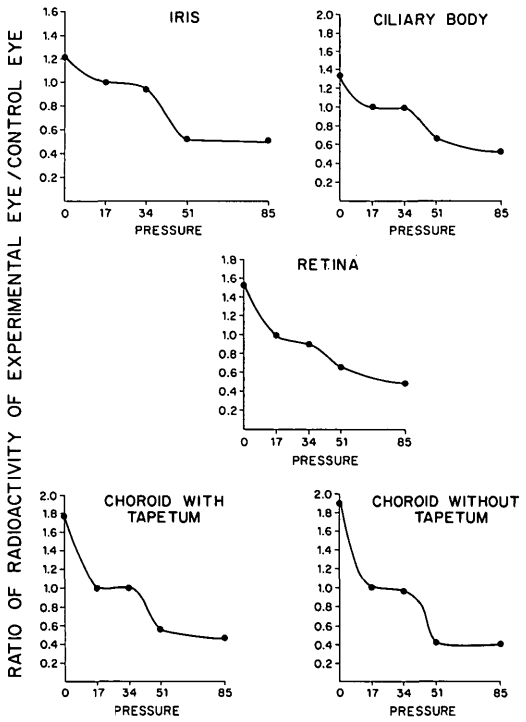

Fig. 1. Graph of the relationship between intraocular pressure and blood flow. The abscissa represents intraocular pressure measured in millimeters of Hg. The ordinate represents relative blood flow expressed as the ratio of radioactivity in the experimental tissue compared to the control tissue.

dicator-dilution methods, and it is evident that there is a close correlation between the two techniques (Table I). Using these values for cardiac output, the blood flow to the choroid was determined by measuring the percentage of the total injected labeled microspheres that were entrapped in the choroid. By this method the choroidal blood flow was approximately half of one per cent of the total cardiac output (Table I). Expressed in blood flow per gram of tissue we obtained a value of 1,300 ml. per 100 Gm. per minute for the choroid.

Relative blood flow measurements were made in ten cats. The choroidal blood flow was over three times the blood flow to the kidneys. Using the renal blood flow value (reference flow) of cats of 300 to 400 ml. per 100 Gm. per minute, obtained from the literature[16] the choroidal blood flow would be between 1,100 to 1,400 ml. per 100 Gm. per minute. The percentage of total ocular blood flow that went to subdivisions of the uvea and retina is shown in Table II. Note that 65 per cent of ocular blood flow went to the choroid, whereas the retina accounted for only 2 per cent of the total ocular blood flow.

The relative blood flow rates also demonstrate the high uveal blood flow compared to the retina. Table III represents blood flow per gram of tissue per minute expressed as a ratio of the blood flow to the retina. The choroidal blood flow is 40 to 70 times the blood flow in the retina. The remainder of the uvea also has a high blood flow in relation to the retina.

The change in blood flow produced by varying the intraocular pressure was obtained by comparing the radioactivity of each subdivision of the experimental eye to the corresponding control eye. If there was no change in blood flow, the ratio was equal to one. In three control animals, there was a variance of two to eight per cent between paired organs and vascular beds. As intra-

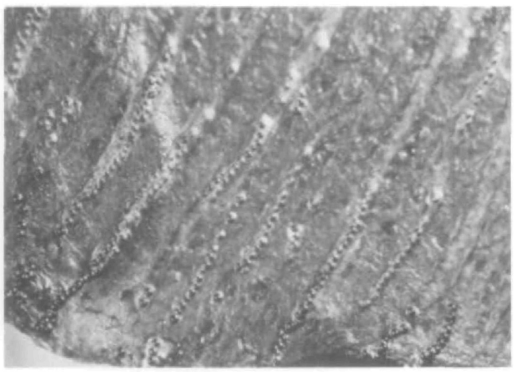

Fig. 2. A photomicrograph of the choroid, demonstrating microspheres impacted in blood vessels. (×40.)

ocular pressure was increased the blood flow to the tissues was decreased and as the pressure was decreased the blood flow increased (Fig. 1). The choroid was more responsive to pressure changes than the other tissues, especially when compared to the iris and ciliary body.

Discussion

This study confirms the value of the use of labeled microspheres in studying alterations in ocular blood flow. The principle of this method, as previously mentioned, is that, if the microspheres are injected into the left heart, their fractional distribution in the peripheral vascular beds will be in proportion to the fraction of cardiac output going to each vascular bed. This is based on the assumption that the microspheres are well mixed in the left heart, are distributed in proportion to blood flow, do not escape to the venous circulation and recirculate, and that impaction in the tissue does not affect the blood flow rate. It has been shown that spheres of 50 microns in diameter meet these criteria. If the flow to any organ (reference flow) is known, the flow to any other organ can be determined. This method has been shown to be an accurate and reliable measure of blood flow.[9] The number of microspheres that impact in the tissues is large (Fig. 2). This is important to eliminate random variation.

In this study we determined the cardiac output by a variation of the sudden-injection indicator-dilution method.[15] Each experiment can be thought of as consisting of three "systems" of blood flow. System 1 is the over-all heart-aorta-microcirculation system. System 2 is the syringe-tubing outflow, and System 3 is the uvea-retina microcirculation. The assumption used in this study is that the blood flow in all three of these "systems" is proportionately related to the number of microspheres in each system or

$$F_1/F_2/F_3 = \Sigma C_1/\Sigma C_2/\Sigma C_3$$

where F equals blood flow rate and C equals total concentration (radioactivity) of labeled microspheres in any system. These systems exist after the labeled microspheres are injected into the left heart. The labeled microspheres appear at the outflow from the system in a concentration which is a curvilinear function of time, c(t). The rate at which the microspheres leave the system at any moment, s, is the product of their concentration at that moment, c(s), and the unknown flow, F. Eventually the entire bolus, q, of injected microspheres must leave the system. If all products of c(s) and F are summed, this sum must equal the amount of injected microspheres, or,

$$q = F \int_0^\infty c(t)\ dt$$

but since the outflow rate is known (by using a calibrated motor-driven syringe) and the radioactivity of the outflow bolus can be measured, the equation can be simplified to

$$F = \frac{q}{\Sigma c} \cdot I$$

where I equals constant outflow through polyethylene sampling tube, and Σc equals total concentration (radioactivity) of labeled microspheres at sampling site. The validity of this technique was verified by previously measuring the cardiac output in each cat by the constant-injection indicator-dilution method as shown in Table I.

The observation that 65 per cent of the

total intraocular blood flow went to the choroid might be expected intuitively because of the apparent large relative volume of the choroid (Table II). These values for segmental ocular blood flow are similar to those obtained recently by Alm and Bill[13] using the same technique. More interesting, however, is the fact that per gram of tissue, the choroid has between 40 to 70 times the rate of blood flow that the retina has (Table III).

Our present study shows an inverse relationship between intraocular blood flow and intraocular pressure (Fig. 1). These results are similar to fluorescein angiography.[17] A decreased blood flow with increased intraocular pressure has been related to the pathogenesis of glaucoma,[17] but we did not perform a separate analysis of disk circulation in this study. The increase in blood flow with decreased intraocular pressure has implications for the treatment of vascular occlusive disease. It may be that lowering the intraocular pressure would be beneficial providing the increased flow is maintained.

The nonlinearity of this inverse pressure-blood flow relationship implies that there are multiple parameters to regulation of blood flow. It is evident, however, that the intraocular pressure must be controlled if valid studies are to be made of the factors that affect blood flow. We are presently using radioactive microspheres to study the contribution of the sympathetic nervous system to the vascular tonus and the homeostatic regulation of the blood flow to the eye.

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