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Analysis of Variance of Microspheres Blood Flow Measurements in Rabbits

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As part of a larger study on the interpretation of angiographically derived hemodynamic parameters, blood flow in several ocular tissues was measured using the radioactively labelled microspheres technique. As an unexpected secondary result, it was found that the microspheres data gave quantitative information on hyperaemic effects in the eye. This is the subject of the present paper.

The measurements were made in 13 anaesthetized pigmented rabbits. In each animal, three blood flow measurements were performed at three different ocular perfusion pressures (60–15 mmHg). The perfusion pressures of the experimental eye were varied by changing the intra-ocular pressure. The contra-lateral eye served as a control. Labelled microspheres were used as a non-recirculating blood flow indicator, enabling the estimation of regional blood flows, in this case for the iris, ciliary body, peripheral choroid and peripapillary choroid separately.

Using analysis of variance with perfusion pressure as covariate and taking into account the blood flow of the control eye, hyperaemia could be quantified in the experimental eye. Apart from a difference amongst animals, hyperaemia depended on tissue type. The amount of hyperaemia proved to be more pronounced in the anterior part of the eye, iris and ciliary body, and to decrease towards the posterior pole.

With regard to the causes of this hyperaemia one could speculate about the invasive handling (anterior eye needles) topical administration of tropicamide, in combination with the general anaesthesia.

Key words: ocular blood flow; microspheres; choroid; rabbit.

1. Introduction

In a separate paper the relation was studied between angiographically derived hemodynamic parameters and blood flow measurements using radioactively labelled microspheres (Duijm et al., 1996). An unexpected secondary finding was that the microspheres blood flow measurements gave information on ocular hyperaemia. The analysis rendering quantitative information on this hyperaemia is the subject of the present paper.

The microspheres act as a nonrecirculating blood flow indicator, and after injection in the left ventricle, they follow the blood flow and get stuck in the peripheral circulation, proportional to regional blood flow. Absolute regional blood flow can be calculated through the combination of a reference flow, from e.g. a femoral artery, and tissue radioactivity. In this study blood flow measurements were performed in rabbits under three different ocular perfusion pressure levels in the experimental eye; the other eye served as a control. Regional ocular blood flow was determined in the iris, ciliary body, peripheral choroid and peripapillary choroid separately.

The blood flow data of both experimental and control eye will be analysed using analysis of variance with the perfusion pressure as a covariate. The results, the parametrization of the differences in blood flow, were interpreted in terms of intra-individual differences in hyperaemia, as well as differences that could be attributed to tissue types.

2. Material and Methods

Material and methods were described in detail in our earlier paper (Duijm et al., 1996). This can be summarized as follows:

The experiments were performed in 13 young pigmented rabbits (Swedish Loop); pentobarbital (Mebumal, ACO, Sweden; 30 mg kg^{-1}, i.v.) was used for general anesthesia, except for two animals which received urethane. After administration of indomethacin (20 mg kg^{-1} i.v.) two anterior chamber needles (30 G) were inserted in one eye in order to raise or lower the intraocular pressure (IOP). The ocular perfusion pressure (PP) is defined as the

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TABLE I

Average baseline blood flow measurements (mean ± standard deviation), determined with microspheres, for several ocular tissues

Blood flow (mg min^{-1})	Experimental eye	Control eye
Iris	184 ± 113	126 ± 64
Ciliary body	227 ± 144	185 ± 76
Peripheral choroid	536 ± 227	495 ± 189
Peripapillary choroid	129 ± 44	120 ± 41

difference between the mean arterial pressure (MAP) and the IOP. Tropicamide (Mydriacyl 0·5%, Alcon) was topically administered in the experimental eye to induce mydriasis, since fluorescein angiograms were performed as well.

Regional blood flow was measured using radioactively labelled microspheres as described by Alm and Bill (1972). Three different labeled microspheres, diameter 15 ± 2 μm, (Co-57, Sn-113 or Ru-103, Medical Dupont, New England Nuclear) were used, enabling three blood flow measurements under three different perfusion pressure conditions in each experiment. After the experiment and killing of the animal, both eyes, experimental and control, were enucleated, and several ocular tissues were dissected: the iris, ciliary body, peripheral and peripapillary choroid (ϕ 9 mm around the optic nerve head). Blood flow in the forementioned tissues (mg min^{-1}) were calculated.

3. Results

Table I and Fig. 1 present the relation between the first of the three flow measurements in the experimental and control eye. This relation seems to be rather erratic. Figure 2 shows a strong, more or less linear dependence of flow on perfusion pressure. Since in Fig. 1 the perfusion pressure may have differed between experimental and control eye, this might have caused

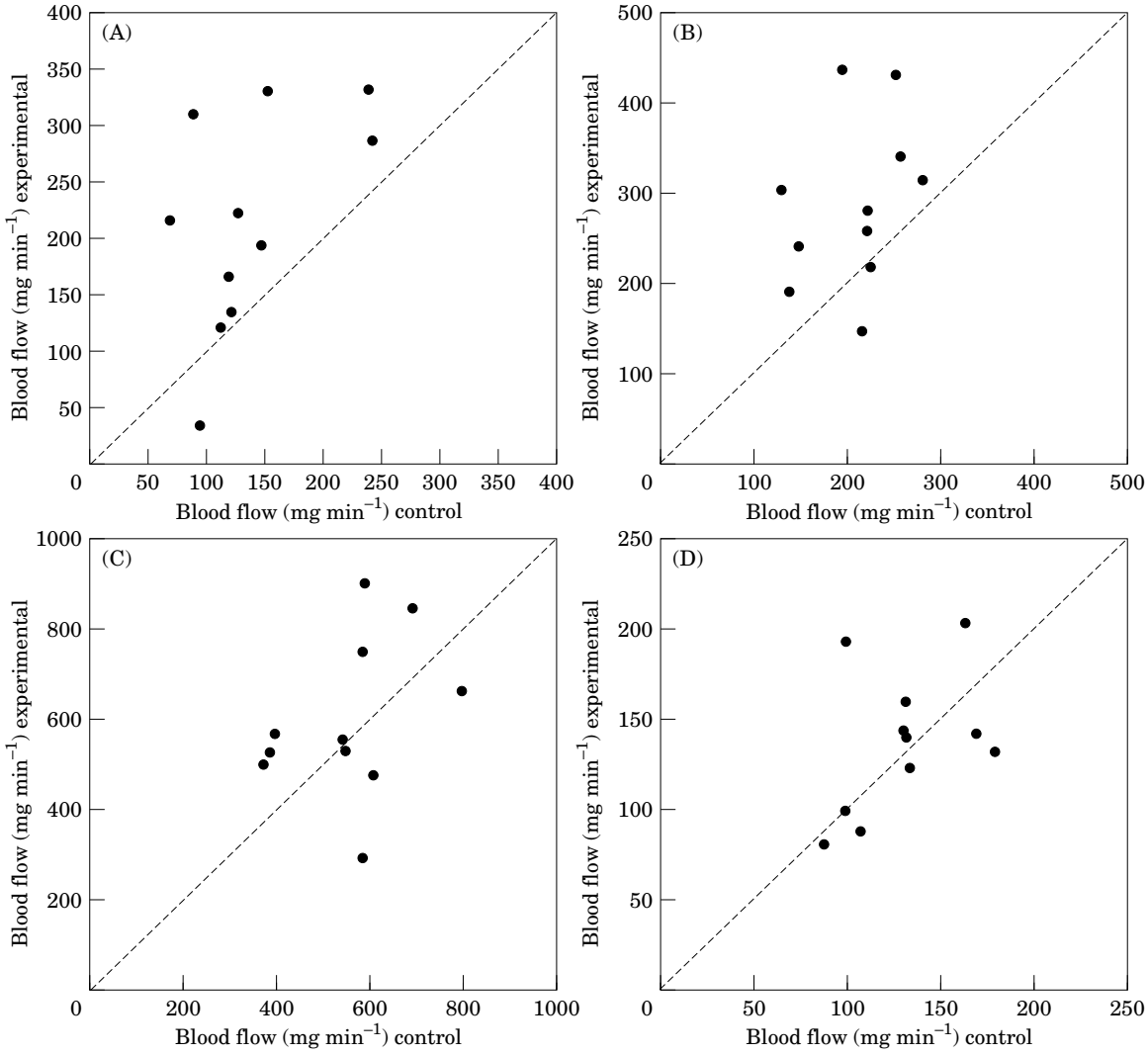

FIG. 1. Baseline blood flow values of control eye versus experimental eye for the several studied tissues (mg min^{-1}). Especially in the more anterior tissue, like iris and ciliary body the blood flow in the experimental eye seems systematically higher (A), Iris; (B), Ciliary body; (C), Peripheral choroid; (D), Peripapillary choroid.

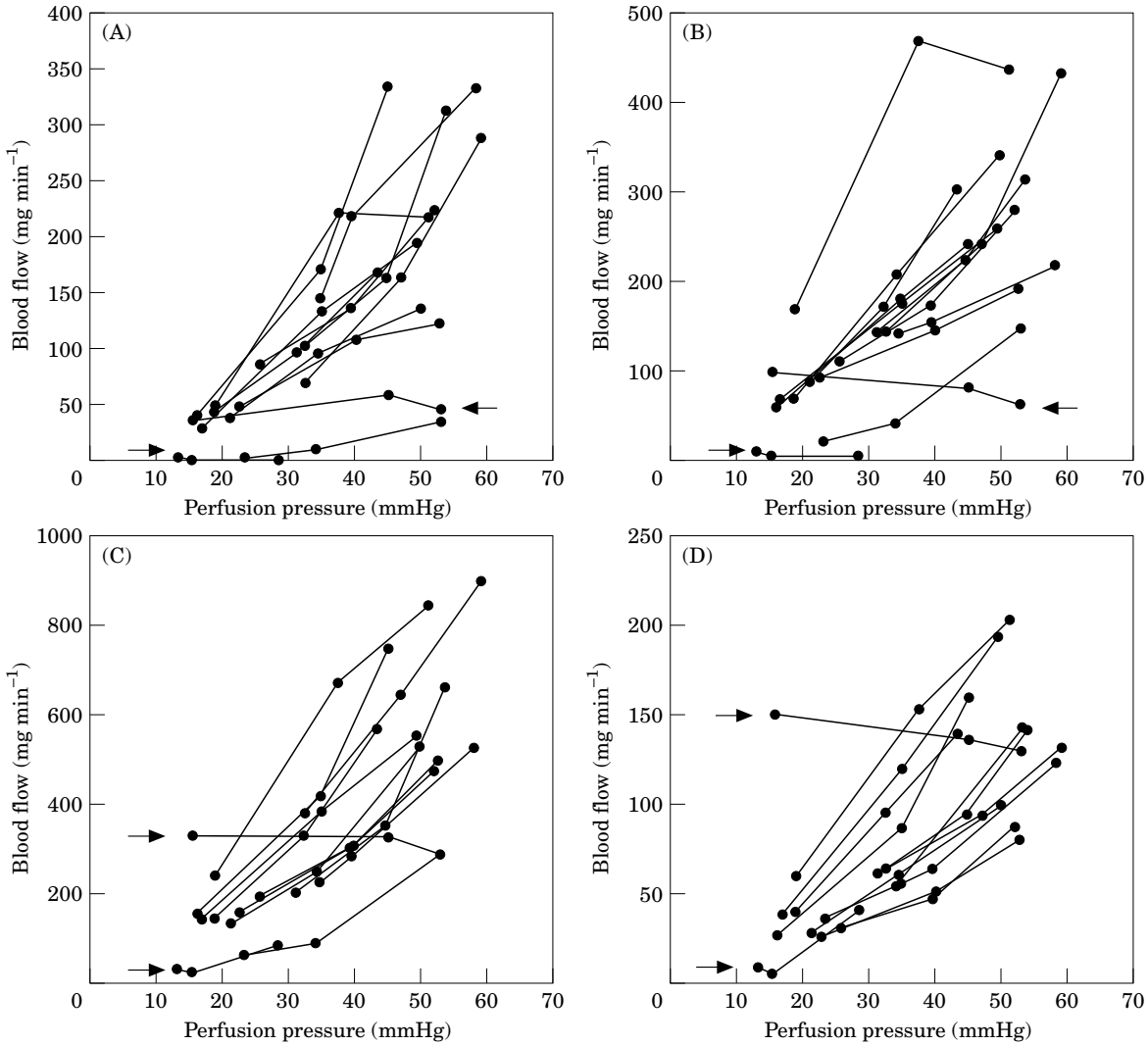

FIG. 2. Blood flow measured with microspheres of the iris, ciliary body, peripheral and peripapillary choroid (mg min^{-1}), plotted as a function of perfusion pressure (mmHg). Arrows indicating two animals not following the 'general' trend. (A), Iris; (B), Ciliary body; (C), Peripheral choroid; (D), Peripapillary choroid.

the differences in the flow measurements. To allow for pressure differences, the data were analysed using the following model. Not only the data from Fig. 1, but all data were used to fit this one-parameter model.

$$exp'_{ijk} = con_{ijk} \cdot \frac{a + b \cdot pp_{exp,ik}}{a + b \cdot pp_{con,ik}}$$

or, rewritten:

$$exp'_{ijk} = con_{ijk} \cdot \frac{\frac{a}{b} + pp_{exp,ik}}{\frac{a}{b} + pp_{con,ik}} \qquad (1)$$

where i, animal index, $i = 1, 2, 3 \ldots 11$; j, tissue index, $j = 1, 2, 3, 4$; k, measurement index, $k = 1, 2, 3$; exp'_{ijk}, predicted experimental blood flow value; con_{ijk}, measured blood flow in the control eye; pp_{ik}, perfusion pressure; a, b, parameters describing perfusion pressure term.

This model uses the 'undisturbed' blood flow values from the control eye, con_{ijk}, to predict the expected values for the experimental eye, exp'_{ijk}. The analysis works from the assumption that there is no significant difference between the left and right eye. The only differences considered are the ones in perfusion pressure, described by the term $(ab^{-1} + pp_{exp,k}) \cdot (ab^{-1} + pp_{con,k})^{-1}$. The ratio a/b describes the pressure dependence of the blood flow. This parameter was fitted by the least squares method to all 132 pairs of data (experimental and control). In Table II(a) the value of the fitted parameter a/b is given. This model analysis results in a residual error of 101·9 mg min^{-1}. Residual error is defined as

$$\text{residual error} = \sqrt{\frac{\Sigma(exp_{ijk} - exp'_{ijk})^2}{\text{degrees of freedom}}} (\text{mg min}^{-1}) \qquad (2)$$

In this case degrees of freedom (d.f.) equals 131 (132–1).

From Table I and Fig. 1 it appears that the first blood flow measurement in the control eye ($k = 1$) shows less variation than the corresponding value in the experimental eye. Moreover, on average the experi-

TABLE II

Parameter values for the models and residuals: a/b *describing influence of pressure on blood flow;* d_i *'hyperaemia' parameter, differing among the animals;* w_j *'tissue' parameter, tissue influence on hyperaemia (value ± standard error of the estimate). See the results section for further explanation*

(*a*) (*Eqn 1*)				
a/b	35			
Residual error (mg min^{-1})	101·9			
(*b*) (*Eqn 3*)				
d	−0·10	0·42	0·33	0·19
	0·13	0·68	−0·44	0·48
	−0·07	0·43	0·24	
a/b	−10·5			
Residual error (mg min^{-1})	61·9			
(*c*) (*Eqn 4*)				
	Iris	Ciliary body	Periph. ch.	Peripap. ch.
w	1·1 ± 0·002	1·7 ± 0·006	0·98 ± 0·004	0·13 ± 0·025
d	−0·07	0·43	0·33	0·16
	0·17	0·67	−0·41	0·50
	−0·03	0·35	0·25	
a/b	−10·5			
Residual error (mg min^{-1})	58·7			
(*d*) (*Eqn 5*)				
w	1·1 ± 0·002	1·7 ± 0·006	0·98 ± 0·004	0·13 ± 0·025
d	−0·10	0·40	0·26	0·07
	0·18	0·61	−0·44	0·37
	−0·08	0·29	0·13	
a/b	0·0			
Residual error (mg min^{-1})	65·5			

mental values are higher, even after correlation using Eqn 1. Particularly in the iris and the ciliary body the blood flow in the experimental eye seems systematically higher. In order to explain this we postulated that hyperaemia might exist in the experimental eye. This is not surprising considering the invasive treatment/handling of the experimental eye: the insertion of the eye needles and the application of tropicamide. To include this possible hyperaemia in the analysis, the data were further fitted using the following model:

$$exp'_{ijk} = con_{ij} \cdot (1 + d_i) \cdot \frac{\frac{a}{b} + pp_{exp,ik}}{\frac{a}{b} + pp_{con,ik}} \qquad (3)$$

where:

d_i, hyperaemia parameter.

The parameter d_i describes the hyperaemia in the experimental eye and may vary from one animal to another. It models the difference in blood flow between experimental and control eye. If d_i is zero, the blood flow in the two eyes is equal: no hyperaemia. In case of positive values for d_i the blood flow in the experimental eye is higher. In Table II(b) the fitted values of the parameters a/b and d_i ($i = 1 \ldots 11$) are given. Compared to the first model (Eqn 1), the second model provides a statistically significant improvement in the description of the data (residual error 61·9 mg min^{-1}). The relatively large change in the ratio a/b can be understood from the opposite effects in the experimental eye of the decrease in blood flow because of the decreased perfusion pressures and the increased blood flow caused by the hyperaemia.

In most animals the hyperaemia parameter, d_i, is between 1·0 and 0. This indicates that in most animals blood flow in the experimental eye is increased when compared to the control eye. In one animal the hyperaemia parameter was rather negative (−0·44). In this animal the insertion of the eye needles proved to be very difficult and eventually failed. Subsequently, the contralateral eye was taken as the experimental eye, and the experiment was completed with the 'injured eye' as a control. Hence, this finding

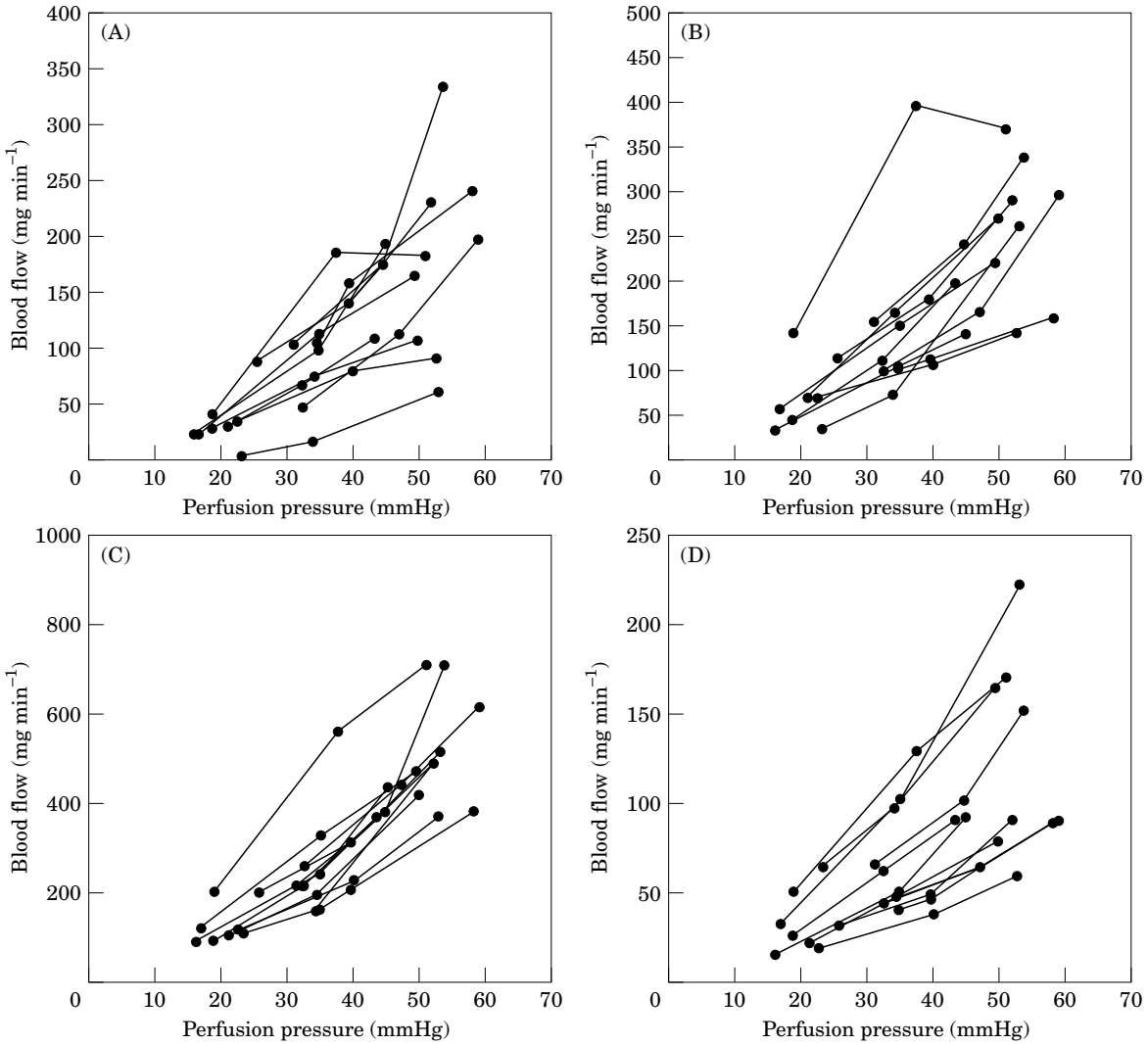

FIG. 3. For 'hyperemia' corrected blood flow for the several tissue types, measured with microspheres of the iris (mg min^{-1}), plotted as a function of perfusion pressure (mmHg). The 'raw' data were divided by $(1+d_i w_j)$, a term describing the experimental hyperaemia. (A), Iris; (B), Ciliary body; (c), Peripheral choroid; (D), Peripapillary choroid.

proved to be the exception to the rule. As other aspects of the experiment were successful, we did not exclude this animal.

The data in Table I also suggest that the 'hyperaemic effect' depends on the tissue type, e.g. iris 184 vs. 126 and peripapillary choroid 129 vs. 120 mg min^{-1} (experimental vs. control). The hyperaemia seems to be more pronounced in the anterior part of the eye and to decrease systematically towards the posterior pole. This may come as no surprise considering the site of 'injury': the anterior part of the experimental eye. To include this in the model, a parameter w_j was introduced. If this parameter is zero, the specific tissue does not show the hyperaemic effect.

$$exp'_{ijk} = con_{ij} \cdot (1 + d_i w_j) \cdot \frac{\frac{a}{b} + pp_{exp,ik}}{\frac{a}{b} + pp_{con,ik}} \qquad (4)$$

where:

w_j, tissue parameter.

Once again, statistically the introduction of this parameter significantly improved the fit, although the residual error declined only slightly to 58·7 mg sec^{-1}. From the 'regional' distribution of the tissue parameter, w_j, one can conclude that there is a decrease of hyperaemia from anterior towards posterior in the eye. Since the tissue parameter, w_j, decreases from 1·1 and 1·7 (iris and ciliary body) to 0·98 and 0·13 (peripheral and peripapillary choroid).

The ratio a/b only has a relatively small magnitude of $-10{\cdot}5$. It seems likely that the linear relation between blood flow and perfusion pressure provides only a small improvement in the description of the data compared to the assumption of a simple proportional relation between blood flow and perfusion pressure:

$$exp'_{ijk} = con_{ij} \cdot (1 + d_i w_j) \cdot \frac{pp_{exp,ik}}{pp_{con,ik}} \qquad (5)$$

Indeed, the analysis of the data using this model (Eqn 6), a model without the term a/b increases the residual error only slightly, though significantly (65·5).

In Fig. 3 the blood flow–perfusion pressure relations corrected for the hyperaemia for the several tissue types are given: the raw data divided by $(1+d_i w_j)$.

Thus, the size of the experimental bed varies because of hyperaemia (parametrized using the parameters d_i and w_j), and a correction for the experimental hyperaemia would therefore imply the division of the 'raw data' by $(1+d_i w_j)$. To correct for another source of variation, namely differences in tissue size, one could standardize by taking into account the blood flows in the control eye. Hence, the corrected blood flow, exp''_{ijk} is found by:

$$exp''_{ijk} = exp_{ijk} \frac{con_{.j.}}{con_{ijk}} \cdot \frac{1}{1+d_i w_j} \qquad (6)$$

where $con_{.j.}$ is the average of con_{ijk} over i (animal) and k (measurement index).

4. Discussion

There appears to be an evident influence of the perfusion pressure on the blood flow of the iris, ciliary body and choroid. The variation in blood flow vs. perfusion pressure (Fig. 2) is comparable with findings in literature: in cats Alm and Bill (1972) find similar flow pressure relations. In comparison with the control eye hyperaemia seems to be present in the experimental eye, and could be quantitatively estimated using a model. This hyperaemia depends on the tissue type and is more pronounced in the anterior part of the eye, especially in the iris and ciliary body. Furthermore, there exists an inter-animal difference in degree of the hyperaemia.

There are several possible causes of the experimental hyperaemia. First of all, in the experimental eye two anterior chamber needles were used. Though care was taken not to touch the iris with the needles, this may indeed have occurred. It is known that mechanical touching of the iris releases vasoactive substances, like prostaglandins. This may be in accordance with the finding that especially in the iris and ciliary body hyperaemia is strongest and, secondly, that the effect decreases towards the posterior pole. However, to prevent the release of vasodilatory prostaglandins, indomethacin was given. Indomethacin is known to block the synthesis of prostaglandins in rabbits (Neufeld, Jampol and Sears, 1972). However, not only touching the iris, but also other trauma, e.g. the needling of the eye induces a prostaglandin release, conceivably despite the pretreatment with indomethacin, and thus causing a subsequent intra-ocular pressure peak.

Another cause for the hyperaemia may be the administration of tropicamide. Tropicamide is a parasympathicolytic, cholinergic blocking substance and may induce vasodilatation in the anterior segment. However, an experimental study on whole eyes showed that application of tropicamide in the rabbit does not influence ocular blood flow measured with microspheres (Delgado, Michel and Jaanus, 1982). Also, mydriasis alone may influence the intra-ocular pressure, consequently influencing perfusion pressure and ocular blood flow.

Another cause of the increased blood flow in the experimental eye may be the combination trauma, induced by e.g. anterior chamber needles, and factors such as general anaesthesia with pentobarbital. It was found that in rabbits pentobarbital has little influence on choroidal circulation, whereas the blood flow in the anterior uvea increases (Bill and Stjernschantz, 1980). In conditions of irritation or trauma substance P and calcitonin gene-related peptide (CGRP), known vasodilators present in uveal somatosensory and autonomic nerves, may be released (Bill, 1991).

Hence, there are several potential causes for the hyperaemic effect in the experimental eye. Therefore, it is not surprising that a difference between the control and the experimental eye was found. However, in the analysis of the hyperaemia it was assumed that normally the two eyes are the same. This may seem logical, but is not necessarily true: the two eyes might differ, for example in size. Furthermore, the control intraocular pressure was not measured, and might have differed from normal as well. However, we may assume such differences to be small. Correspondingly, the analysis showed consistent behavior in all animals, and as for the exceptions explanations could be given.

It must be noticed that the study from which the data originated, was not designed to investigate hyperaemia. It was a fortunate coincidence that the data enabled us to perform this analysis. To delineate the hyperaemia and its possible causes more precisely, a study in which these factors and conditions are accurately controlled would be preferable. Furthermore, although the microspheres technique seems straightforward, it does produce its own problems and intricacies. These have been discussed elsewhere in detail (Buckberg et al., 1971; Kiel and Shepherd, 1992; Kiel, 1994; Duijm et al., 1996).

Whatever the reason for or mechanism behind the hyperaemia may be, it is reasonable to assume that the analysis, using the proposed model, properly quantifies this effect, thus enabling appropriate corrections. It may also enable investigation of the influence of blood flow modifying substances, such as medications.

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