Image splitting—a technique for measuring retinal vascular reactivity

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SUMMARY The observation of changes of pial vessel calibre has withstood the test of time in assessing cerebrovascular reactivity. A recent refinement has been the adoption of the image splitting television technique of Baez, which allows accurate and rapid measurements of vessel calibre to be made in situ. This method has been successfully applied to the eye, where the retinal vessels are readily visible through the pupil. Results are presented of preliminary experiments in normal persons, in whom an induced increase in intraocular pressure was associated with retinal vasodilatation.

Direct observations of pial arterial calibre have been used to measure cerebrovascular reactivity for many years. More recent studies have used photographic methods,¹ which have also been widely used in studies of retinal vascular responses,² but a recent refinement in pial vessel studies has been the adoption of the image splitting technique of Baez.³ This allows accurate and rapid measurement of arterial calibre to be made in situ.

This report describes the application of this technique to the eye, where it provides, as in the brain, a rapid and accurate method of measuring changes in arterial calibre.

Materials and methods

The system consists of a standard Zeiss fundus camera through which the fundus is visualised by means of a low-light-level television camera (Ikegami). This camera contains a silicon intensifier target (SIT) tube. The image of the retinal arterioles is passed from the fundus camera through an image splitting eyepiece (Vickers), and the split image received by the television camera is displayed on a monitor. The shearing screw of the eyepiece which controls the degree of image splitting is connected to a 10-turn potentiometer and in turn to a digital readout of resistance. In this way frequent measurements of shear (which is directly proportional to vessel calibre) were obtained in arbitrary units. The fundus is illuminated with the standard observation light of the fundus camera on position 2.

Pupils were dilated with 2 drops of tropicamide 1% instilled 20 and 10 minutes before measurement.

The accuracy of the method (intraobserver error) was first assessed by making 50 measurements of shear on a first-order arteriole near the optic disc in a human volunteer. One observer determined the end point by observing the point between overlap and separation of the optically split image on the television monitor. A second observer recorded each measurement from the digital meter. Inter-observer error was assessed by 3 trained observers making 10 measurements of a single retinal arteriole in a second volunteer.

A further 7 volunteers were used in 11 experiments in which 5 measurements were made at resting intraocular pressure followed by another 5 measurements after increasing the intraocular pressure to 30–50 mmHg. Initial attempts to increase and maintain the intraocular pressures with a suction cup were not reliable. With this method the rise in pressure was unstable while the suction resulted in distortion of the globe, which degraded the quality of the image. The method finally employed used a Mackay-Marg tonometer mounted on a jig which could be adjusted by a micrometer screw to compress the temporal sclera of the eye and so increase the intraocular pressure.⁴ The tonometer was connected to an analogue meter from which continuous readings of increase in intraocular pressure were monitored, and the intraocular pressure was kept at a sustained level. Arterioles were selected at random, but all were near the disc and either first- or second-order retinal arterioles.

Results

INTRAOBSERVER ERROR

The results of 50 measurements made on a second-order arteriole by one observer gave a mean of 76

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units (SD 2.67), with 95% confidence limits of ± 0.77 ($t=2.014$, 45 degrees of freedom). Five random measurements of the same arteriole gave a mean of 76.7 units (SD 2.64), and 95% confidence limits of ± 3.276 ($t=2.776$, 4 degrees of freedom). Usually this number of measurements could be obtained in 10–15 seconds.

INTEROBSERVER ERROR

Table 1 shows the means and standard deviations obtained by 3 observers measuring the diameter of the same retinal arteriole 10 times. One-way analysis

Table 1 *Image splitting: means and standard deviations of 10 separate readings of arteriole diameter by each of 3 observers*

Observer	Mean (units) (n=10)	SD*
1	62.2	3.35
2	65.4	2.41
3	63.9	3.84

*SD = standard deviation.

of variance confirmed that there was no significant difference among the observed means (62.2, SD 3.35; 65.4, SD 2.41; 63.9, SD 3.84) and variances.

CHANGES WITH INCREASED INTRAOCULAR PRESSURE

When intraocular pressure was elevated to a level of 30–45 mmHg and maintained at that level for 2 minutes, variable vasodilatation was noted. When vasodilatation was measured against perfusion pressure (taken as brachial artery mean blood pressure minus intraocular pressure) an autoregulatory response was apparent in 7 out of the 11 subjects. (Levels of significance $<0.001\%$ to $<1\%$; Student's t test). In one subject (2) vasodilatation occurred in the face of an increase in perfusion pressure.

Discussion

It has been found possible to adapt the method of image splitting to the measurement of changes in retinal arteriolar diameter in man. With the standard observation light of the Zeiss fundus camera it is

Table 2 *Changes in arteriolar diameter in response to the alterations of retinal perfusion pressure induced by raising intraocular pressure. (a) Baseline measurements, (b) after raising intraocular pressure for each subject*

Subject	Intraocular pressure (mmHg)	Mean arterial blood pressure (mmHg) (arbitrary units)	Perfusion pressure (mmHg)	Mean arteriolar diameter (arbitrary units)	Change in perfusion pressure (mmHg)	Change in arteriolar diameter	% Level of significance of change in arteriolar diameter (t test)
1 (a)	14	108	94	56.5	-10	+ 8.15	NS*
(b)	24	108	84	64.65			
2 (a)	18	78	60	84.2	+ 15	+ 12.20	$<1\%$
(b)	28	103	75	96.4			
3 (a)	16	105	89	91.3	-31	+ 10.30	$<0.1\%$
(b)	50	108	58	101.6			
4 (a)	18	117	99	78.3	-37	+ 8.40	$<1.0\%$
(b)	56	117	62	86.7			
5 (a)	12	103	91	74.2	-12	-3.0	NS
(b)	28	107	80	71.2			
6 (a)	20	108	88	98.5	-11	+ 0.50	NS
(b)	30	107	77	99.0			
7 (a)	18	102	84	92.2	-32	+ 19.50	$<0.01\%$
(b)	50	102	52	111.7			
8 (a)	16	116	100	53.6	-2	+ 14.2	$<1.0\%$
(b)	30	128	98	67.8			
9 (a)	16	103	87	89.9	-12	+ 22.8	$<0.1\%$
(b)	30	105	75	111.7			
10 (a)	12	97	85	79.8	-33	+ 21.8	$<0.001\%$
(b)	40	92	52	121.6			
11 (a)	14	95	81	97.3	-26	+ 16.10	$<0.01\%$
(b)	38	93	55	113.4			

*NS = not significant.

not possible to view the fundus with a standard television camera. A low-light-level camera incorporating an SIT tube must be used to obtain a good quality image. Although photographic techniques for measuring retinal vessel diameter, originally used by Hickam and Frayser and more recently by Tsacopoulos and David⁵ provide accurate measurements, rapid consecutive measurements cannot be made in view of recirculating fluorescein. With the image splitter readings of arterial diameter may be obtained within 20 seconds. This is a considerable advantage for measurement of calibre changes, which may occur quite rapidly. The original work on the image splitting technique showed that its accuracy was an order of magnitude greater than the resolving power of the optical system used to obtain the image.⁶ This is an additional advantage in subjects who may have opacities of the media. The experiments in which retinal vascular reactivity to changing perfusion pressure was measured in humans represent a small series which confirms previous work using a photographic method.⁷

The ideal system for the study of retinal vascular physiology would be a noninvasive technique which would allow measurements of retinal blood flow to be accurately and rapidly determined. Current methods for measuring blood flow are invasive and complicated, and although they have yielded important information in animal experiments none has yet evolved as an acceptable clinical tool. If flow measurements are regarded as first-order information, then studies on retinal vessel calibre must be

regarded as second-order information. If one supposes that changes in retinal vessel calibre may be used as an index of retinal perfusion, albeit less accurate than direct measurements of flow,⁸ then this technique provides a noninvasive, rapid, and accurate method of obtaining information on the reactivity of the human retinal vascular bed.

The authors acknowledge the support of the National Health and Medical Research Council of Australia, the Lions Save-Sight Foundation (Western Australia) Inc., and the Australian Foundation for the Prevention of Blindness (Western Australian Division) Inc.

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