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Measurement of Vessel Width on Fundus Photographs*

D. Bracher[1]**, M. Dozzi[2], and W. Lotmar[3]

[1] Pediatric Clinic (Director: Prof. E. Rossi)
[2] Institute of Mathematical Statistics (Director: Prof. H. Riedwyl)
[3] Eye Clinic (Director: Prof. P. Niesel) University of Berne, Switzerland

Summary. Many factors play a role in determining the width of retinal vessels, based on fundus photographs. We have tried to estimate their influence by comparing experimentally six different measuring methods, taking into account film and developer, intra- and interindividual reproducibility of results under short- and long-term conditions, and training in measuring technique. Our method of choice was tenfold projection of the negatives on a fine-grained wax layer screen and use of a very narrow marker line to be aligned with the vessel borders. Width determinations on pictures of good definition, yet low contrast, repeated by a trained observer on consecutive days, lay with a 95% probability within an interval of about 9 μm on the retina. The width so determined is, of course, relative and only a rough approximation to the absolute width, because of photographic bias, interobserver difference, and uncertainty of the refractive power of the eye. The method appears to be especially suitable for the study of intraindividual variations; however, because of photographic bias, conclusions should be drawn only when based on several pictures.

Zusammenfassung. Bei der Bestimmung von Gefäßdurchmessern in der Retina aufgrund von Fundusphotographien spielen viele Faktoren mit. Wir haben deren Einfluß durch den experimentellen Vergleich von sechs verschiedenen Meßmethoden abzuschätzen versucht, wobei Film und Entwickler, intra- und interindividuelle Reproduzierbarkeit der Ergebnisse unter Kurz- und Langzeitbedingungen, sowie Übung in Meßtechnik in Betracht gezogen wurden.

Unsere Methode der Wahl bestand in 10facher Projektion der Negative auf eine feinkörnige Wachsmattscheibe und Verwendung einer sehr feinen Strichmarke, die an die Gefäßränder angelegt wurde. Bei Bildern guter

* Supported by the Swiss National Science Foundation
** Address for offprint requests: D. Bracher, M.D., Universitäts-Kinderklinik, Inselspital, Freiburgstrasse 15, CH-3010 Bern, Switzerland

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Schärfe aber geringen Kontrasts lagen Durchmesserbestimmungen, die von einem geübten Beobachter an aufeinanderfolgenden Tagen durchgeführt wurden, mit einer Wahrscheinlichkeit von 95% innerhalb eines Intervalls von rund 9 µm auf der Retina. Der so bestimmte Durchmesser ist allerdings nur als Relativwert und erster Näherungswert des wahren Durchmessers zu betrachten, und zwar wegen möglicher photographischer Verfälschung, Meßdifferenzen zwischen verschiedenen Beobachtern und Unsicherheit bezüglich des Refraktionswertes des betreffenden Auges. Die Methode dürfte sich in erster Linie zum Studium intraindividueller Veränderungen eignen. Wegen der photographischen Einflüsse sollten aber Schlüsse nur bei Vorliegen mehrerer Bilder gezogen werden.

I. Introduction

In a recent paper we have shown that, owing to the simplification brought about by the use of a special contact lens and a hand-held camera, the fundus of newborn infants can be repeatedly photographed, even during intensive care (Bracher et al., 1975). Marked changes occurring within a few hours or days can thus be documented (Fig. 1). A quantitative assessment of such changes is of course desirable.

Several studies have been published dealing with the problem of measuring vessel width and/or lenght on fundus photographs of human adults (Kagan et al., 1967, and Hodge et al., 1969; further developed by Parr and Spears, 1974a and b, and Majewska et al., 1976).

However, in none of these investigations were all the basic properties of the system taken into account. Furthermore, we found that the proposed methods of measurement were not optimal and we could not reproduce the results reported by these authors. We therefore decided to study the problem anew. In the present paper we are concerned only with measurement of vessel width. Measurement of length variation is reported elsewhere (Lotmar et al., 1979).

II. Methods for Measuring Vessel Width

1. Basic Properties of the System

a) Fundus pictures are of limited definition. As experience shows, no further profit is obtained when the negatives are magnified more than 10- to 15-fold.

b) The objects to be measured, i.e., the vessels, are seen against a background of about the same color. Authors working with black-and-white fundus pictures have used contrasty photographic material (either orthochromatic, or panchromatic plus a green filter). It is well known, however, that material of steep gradation (high gamma), while apparently providing relatively high image definition, readily tends to falsify the position of edges. With currently available equipment it is practically impossible to standardize exposure, since reflectance of the background varies intra- and interindividually, owing to differences in the development of the pigment epithelium, blood filling of the choriocapillaris, and Hb saturation. This is especially true for fundus photography in newborns. Furthermore, optimal focusing is not always obtainable in practice, even in adults

(see e.g., Laing and Danisch, 1975). Apparent vessel width on the pictures will therefore depend on focusing, resolution of the optics (Bracher et al., 1975), exposure, and development (Fig. 1).

c) Vessels are not precision tubes but seem to vary irregularly, if only slightly, in width along their length This can easily be perceived by looking at a magnified print at an angle of about 10°. The question therefore arises as to what we understand by 'vessel width.' Note that a 'vessel' in fundus photography corresponds, strictly speaking, to the width of the erythrocyte column (Lemmingson, 1972). Irregular width fluctuations are also visible, however, on fluorescein angiograms.

d) In making measurements, short- and long-term reproducibility in one and the same observer, and differences between observers, must be taken into account.

2. Preliminary Trials

a) Densitometry

The most objective method for measuring the width of vessels on a photographic film would seem to be the use of a densitometric recorder. However, Hodge et al. (1969) have already shown that this does not work well with fundus photographs, mainly because no well-defined background base line exists. In newborns this difficulty is enhanced, since the variation in fundus reflectivity is even more pronounced than in adults. Some recordings made with a Joyce-Loebl densitometer confirmed this opinion (Fig. 2) and the method was discarded. A recent paper by Ruckdeschel and Stephan (1978) provides a glimpse of the sophistication necessary to arrive at reliable values of line width by computer evaluation of densitometer traces, and even this method works only when background density is the same on both sides of the line.

b) Split-Image Method

Huber and Handa (1967), with the aim of measuring vessel width on X-ray angiographs of the brain, applied a method derived from split-image focusing by a pair of glass wedges, as used in modern cameras. Distance between the film and the wedges is measurably varied until the left vessel border of one of the split images is aligned with the right border of the other (Fig. 3). The method is appealing because no additional marker is necessary, so image appearance is virtually unaffected.

We modified this method insofar as we used a tenfold profile projector and applied the split-image device to the projected image instead of directly to the film, and we added a vernier to the scale to read the distance of the wedges from the screen. Figure 3 shows this device[1].

Measurement of vessel width by this combination was relatively easy, rapid, and accurate on fundus pictures of high and medium contrast. We had decided, however, to use rather low-contrast film material (reasons given in section II, 1b, and II, 3), and under these conditions we found that accuracy was much impaired, especially when definition was not at its best.

c) Modified Vernier Method

Another method we tried, related to that of Huber and Handa, was as follows. The film was projected onto a transparent screen. A slit image, whose width and edge definition could measurably be varied, was projected onto the same screen in such a manner that it appeared as a prolongation of the vessel to be measured (Fig. 4), both images ending in a sharp edge at right angles to their axes and touching at these edges. The slit image thus functions as an artificial vessel that can be given the same apparent width and edge gradient (by defocusing) as the one to be measured.

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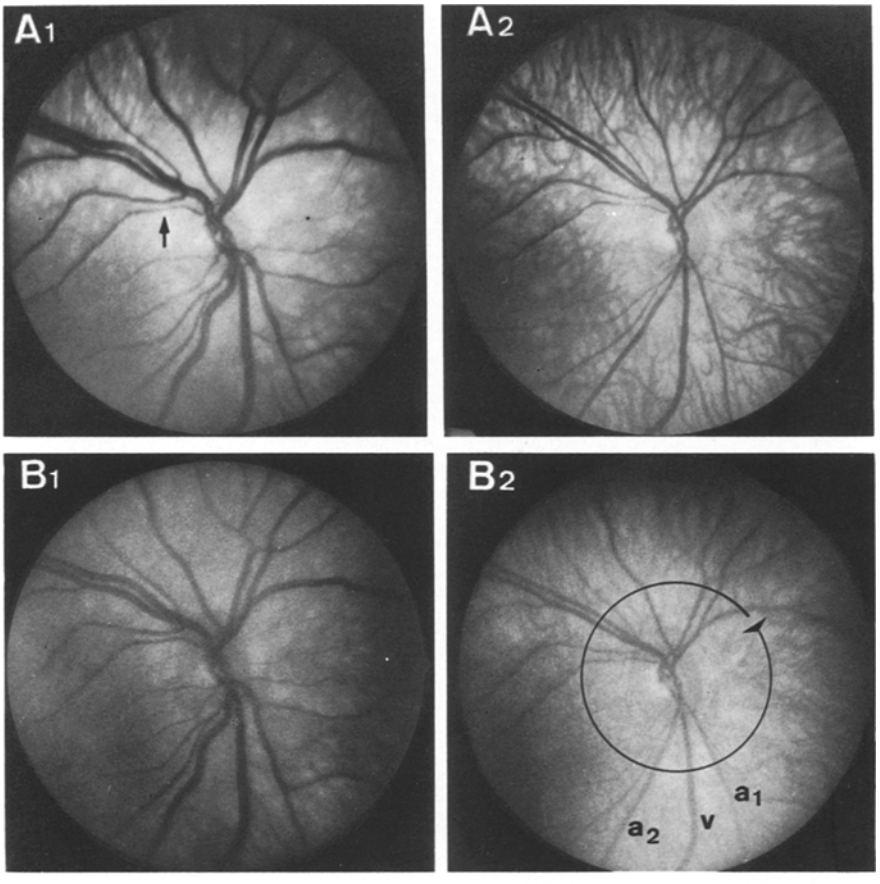
[1] We used a desk projector from Projectina Co., Heerbrugg, Switzerland. The split-image accessory was built by the same company according to our specifications



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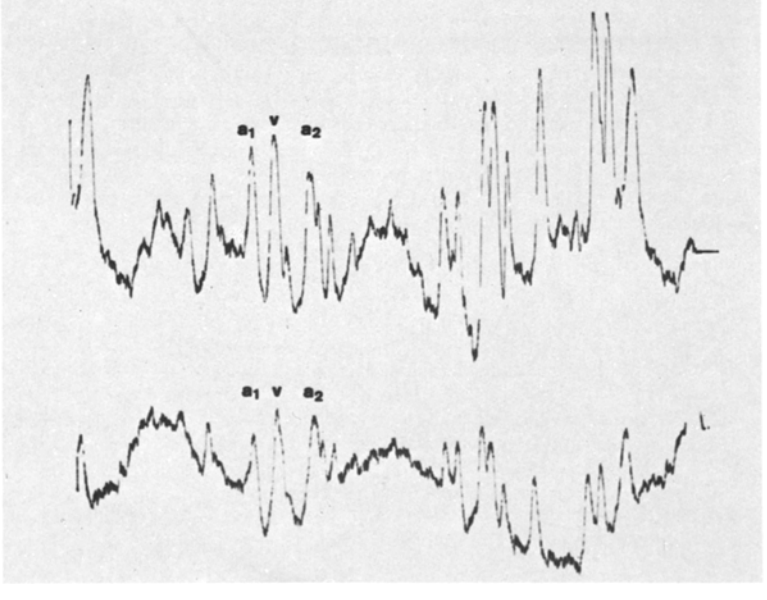

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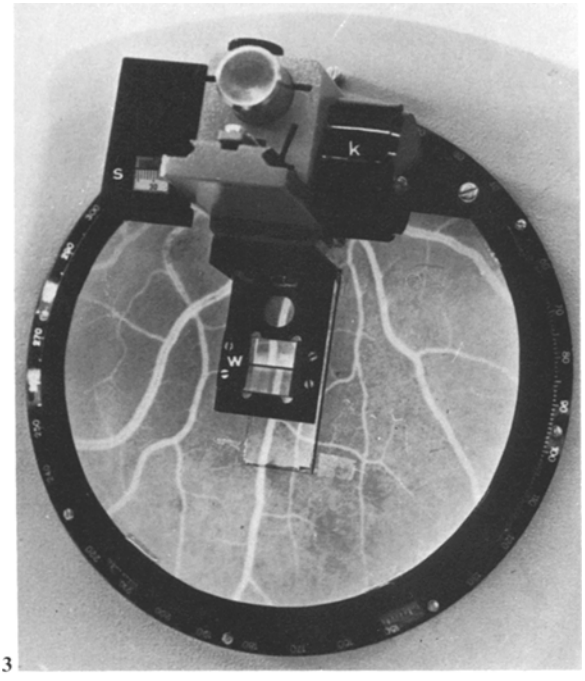

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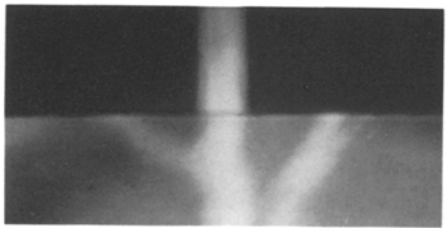

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Fig. 1 A and B. Pictures taken with a hand-held Kowa fundus camera in a postasphyctic newborn. **A** Film Agfa ortho 25 professional. **B** Ilford Pan F 135 without any filter. Both films developed in Rodinal 1:50. $\mathbf{A}_1$ $\mathbf{B}_1$ $4^1/_2$ h of life, $\mathbf{A}_2$ $\mathbf{B}_2$ 144 h of life. *Arrow* points to an artery that appears narrowed down only because high-contrast film was used.

Circle on $\mathbf{B}_2$ is the line of microdensitometric scanning of Fig. 2; *a* and *v*, artery and vein, respectively

Fig. 2A and B. Circular scanning of pictures $\mathbf{A}_1$ and $\mathbf{B}_1$ of Fig. 1 with a Joyce-Loebl densitometer. Slit width was 8.3 μm, slit length 10 μm. *a* and *v*, arteries and veins, respectively. Irregularity of base line and differences in height ratios, even of closely neighboring vessels, are evident

Fig. 3. Accessory to tenfold desk projector for measuring vessel width on projected picture by split-image technique. Lateral displacement by wedges is proportional to the distance from the screen. *w* wedges, *k* height control knob, *s* scale with vernier to read distance

Fig. 4. View of screen in modified vernier method (see text).
Lower half: projected fundus picture. *Upper half:* slit image of variable width and edge definition aligned with vessel

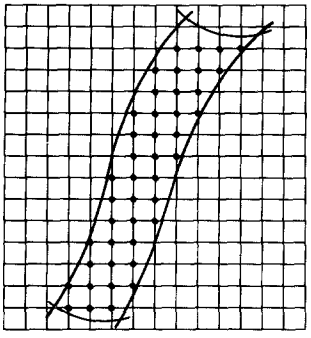


Fig. 5. Principle of morphometric measurement. Glass plate with square grid is superimposed on a magnified print. Intersection points lying on the vessel are counted

This device may therefore be described as a kind of modified vernier method. With fundus pictures of high contrast it gave quite promising results. However, when the background of the fundus pictures was not nearly black but differed only slightly from the bright vessels, as in our case, it became apparent that the black surroundings of the slit image interfered with precise measurement. It would have been necessary to lighten this part of the field in a controllable manner, which is possible, but not easy. To have achieved convenience of measurement would have required the construction of a rather sophisticated, and correspondingly expensive, apparatus, not generally available. So, to our regret, we decided to abandon this method.

d) Measuring Microscope

Hodge et al. (1969) found that the best of the four methods they compared was to use a microscope with a screw micrometer eyepiece whose marker was a rectangular cross bisected by the vessel border, but measurement with a caliper on a projected image was only slightly less accurate. The latter method was, however, much less fatiguing. Our own trials supported this view.

e) Morphometry

Another method we tried was morphometry, especially prompted by the width fluctuations of the vessels as mentioned previously (II, 1c). A morphometric method covering a certain lenght of a vessel could be expected, in principle, to yield a more representative value of width than a measurement on only one or a few sites. We used a glass plate with a photomechanically generated square grid of 0.9×0.9 mm mesh and 10 µm line width, which was superimposed on a tenfold-magnified print (Fig. 5), but were unable to standardize this method. The decision to count an intersection point of the grid was, of course, easy when it lay well within the vessel, but difficult when it lay near the border. The ratio of inner to border points is higher for large vessels; accordingly, reproducibility was better in large vessels. Also, it was often difficult to find a section of sufficient lenght suitable for measurement (no crossing with other vessels, no variation in definition). Moreover, the measured value has to be corrected for tortuosity. Since reproducibility was rather low we abandoned this method.

3. Method Adopted

Finally we decided to use a desk profile projector at tenfold magnification, as Parr and Spears have done, but to pay special attention to the problems of granularity of the screen and the form of the marker to be used. In this we profited from experience in engineering metrology. For a screen we used a thin wax layer (0.2 mm) between two microscope slides (Lotmar, 1954). This device is characterized by a much finer grain than that of a ground-glass screen or a translucent

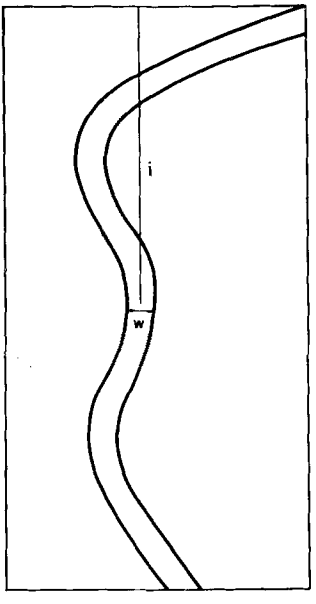


Fig. 6. Principle of measurement of method adopted. Film is moved so that first the right border and then the left border of the vessel comes to lay at the tip of the index *i* (on Figure in medium position). Width *w* is the difference between the two positions as read on the micrometer screw of the profile projector

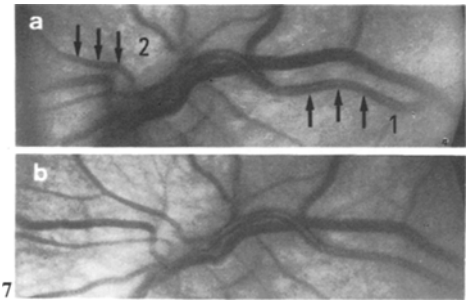
paper, and at the same time by a distribution of the transmitted light over a relatively large angle.

As regards the *measuring mark,* our experience was that a density gradient, especially one of low contrast and moderate definition, like those at the vessel borders in our fundus pictures, is best located by the tip of a black line whose width is not far from the limit of resolution. The underlying principle is again to interfere as little as possible with the appearance of the object to be measured. The next best device then is probably cross hairs at 45° to the vessel axis. We tried both markers, engraving the corresponding pattern on one of the inner glass surfaces of the wax screen. This was done with a diamond point (glass cutter's tool). The resulting furrow was blackened by graphite and had a width of about 90 µm, corresponding to a visual angle of 1.2″ of arc at a distance of 250 mm. The problem of measuring marks has been discussed, for example, by Lehmann (1960). The wax screen was placed on the clear glass plate of the profile projector and surrounded by translucent paper (see Lotmar et al., 1979). The film covered by a block of Transpex lay on the stage, which could be rotated and displaced in two directions by micrometer screws. In preliminary trials, reproducibility was the same for the line marker and for cross hairs at 45°. However, we found the line marker more convenient and applied it throughout the measurements described below.

Film. After what has been said previously (II, 1 b), we decided to use low-contrast material, in an attempt to minimize artifacts. For this we selected Ilford Pan F film, used without any filter, since it seemed to meet best the two desirable requirements, namely, a wide range of linearity and relatively high resolution at low contrast (70 lines/mm, Bracher et al., 1975).

Pictures of newborns under intensive care (Figs. 7 and 9) were taken with a contact lens and the Kowa RC-2 fundus camera by one of the authors, and developed in Rodinal 1:50. The pictures of five healthy adults (Fig. 11) were taken with a Zeiss fundus camera by the photographer of the University Eye Clinic on two films, one developed in Rodinal 1:50, the other in Microphen.

Measuring Procedure. All measurements were made in the same darkened room after an adaptation of about 3 min. Luminance of the retinal background on the screen varied between 7.5 and 72.5 cd/m^2. Arteries were about 1.5 × and veins about twice as bright as the retinal backgrounds. For



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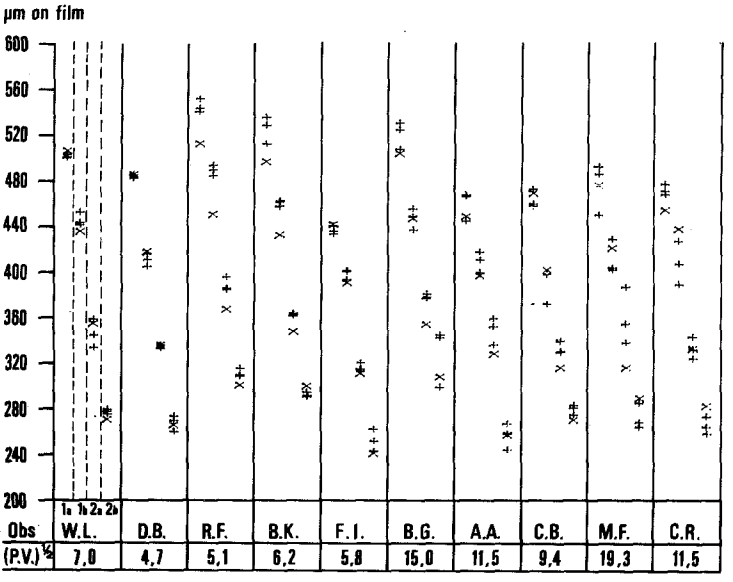

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Fig. 7 a and b. Cuttings of pictures taken with a Kowa camera in a postasphyctic newborn, **a** at 4 $^1/_2$ and **b** at 48 h of life. *Arrows* point to sites of measurement

Fig. 8. Width determination in arteries of Fig. 7 repeated three times on the same day or within a few days (+), and a fourth time after about a month (×) by ten different observers. *1 a, 1 b, 2 a, 2 b* arteries of Fig. 7, *Obs*: observers (for degree of training see text). $(P.V.)^{1/2}$= square root of pooled variance for the + measurement (in µm on the film negative)

every vessel, three sites of good definition were chosen on a sixfold-magnified print and marked with arrows. These sites were then identified by inspection on the projected film picture. Measurement itself is exemplified by Fig. 6. The vessel width at each site was measured four times and the arithmetic mean taken. Throughout this paper, a *determination of a vessel width* signifies this mean of 12 (3 × 4) measurements.

Reproducibility. First, widths of a larger and a smaller artery of the same eye photographed at two different times (Fig. 7) were determined by ten observers three times within a week, and a fourth time about 1 month later (Fig. 8). Observers 1–3 were trained, observers 4–6 were technicians of the hematological laboratory (not trained, but used to work with optical equipment), and observers 7–10 were neither trained nor used to work with optical equipment.

Second, widths of ten arteries, both of newborns (Fig. 9) and adults (Fig. 11), were determined by the same observer in four and three sequences, respectively (Figs. 10 and 12). Within one sequence, determinations were repeated four times on consecutive days. The sequences were separated by 1–2 months.

Statistics. Whenever homogeneity of variance allowed, it analysis of variance methods were applied. Otherwise, nonparametric methods were chosen (Kruskal-Wallis test). Homogeneity of variance itself was tested by using Bartlett and Cochran tests or by using the F test.

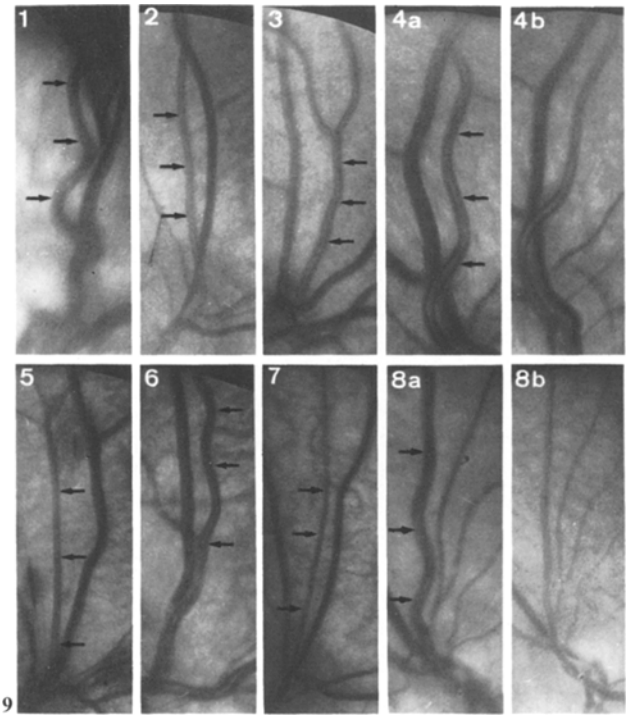
III. Results

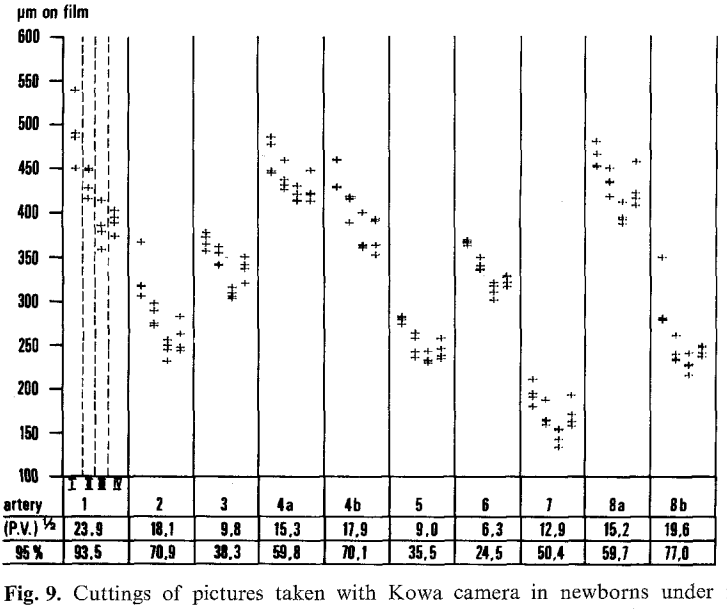
For all arteries shown in Figure 7, the measured widths (Fig. 8) differ significantly between observers ($0.002 < P < 0.004$). Between observer 3 and observer 5, there are differences of up to 100 μm for the same artery on the film negative, corresponding to about 30 μm on the retina. Interobserver agreement is better for differences and ratios (Table 1). For the same artery measured at different times (1a/1b, 2a/2b), no significant differences between observers are found. For differences and ratios between different arteries, there are significant differences between observers, but at higher P values than for measured widths. Pooled variances vary between observers and are clearly related to training (Fig. 8). When observer D.B. of Fig. 8 repeated measurements on ten arteries shown in Fig. 9, a systematic and significant shift ($P < 0.01$) was demonstrated in sequences separated by intervals of 1–2 months (Fig. 10): determinations on all ten arteries resulted in sequence I > sequence II > sequence III < sequence IV. A similar systematic shift between sequences is also demonstrated in Fig. 12 for the determinations of arteries of human adults shown in Figure 11 (sequence I > sequence II < sequence III); it is significant for both developers ($P < 0.001$). The interval comprising width determinations made by the same observer on consecutive days with a 95% probability varies considerably among pictures of newborns (Fig. 11), from 24.5 μm on the film negative, corresponding to 7.7 μm on the retina (see Lotmar, 1976) for artery 6, up to 93.5 μm on film and 29.2 μm on the retina for artery 1. The 95% interval for the pictures of adults (Fig. 13) may be given for all pictures simultaneously by 23.1 μm 9.3 μm when developed with Microphen and 32.1 μm [12 (0.9) μm] when developed with Rodinal. Determinations of width on the Rodinal-developed pictures resulted in significantly smaller values for artery 1 ($P < 0.001$), while those for artery 4 were significantly larger ($P = 0.003$) than on Microphen-developed pictu-

Table 1

Difference	P value	Difference	P value	Ratio	P value	Ratio	P value
1a–1b	0.23 ns	1a–2a	0.009	1a/1b	0.22 ns	1a/2a	0.03
2a–2b	0.12 ns	1b–2b	0.007	2a/2b	0.09 ns	1b/2b	0.04
1a–2b	0.01	1b–2a	0.014	1a/2b	0.07 ns	1b/2a	0.03

P values for differences and ratios between arteries of Fig. 8, as determined by ten observers (Fig. 9)

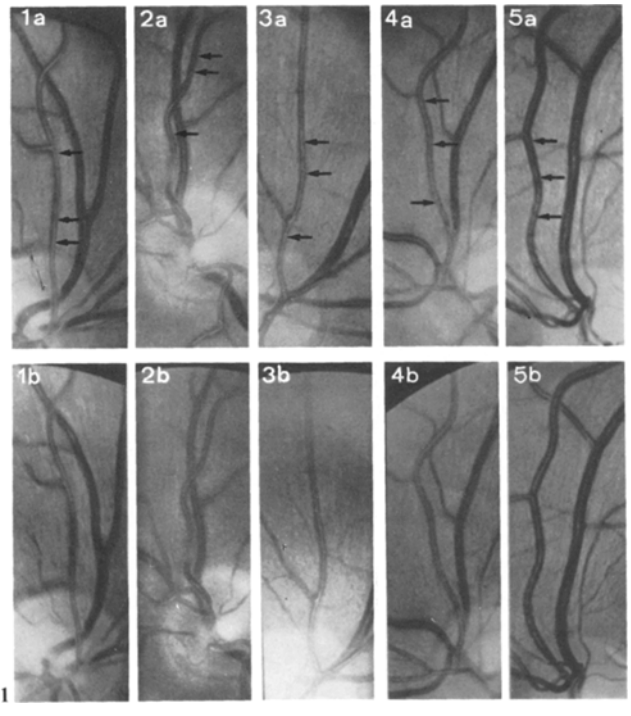




artery	1	2	3	4a	4b	5	6	7	8a	8b
(P.V.) ½	23,9	18,1	9,8	15,3	17,9	9,0	6,3	12,9	15,2	19,6
95 %	93,5	70,9	38,3	59,8	70,1	35,5	24,5	50,4	59,7	77,0

Fig. 9. Cuttings of pictures taken with Kowa camera in newborns under intensive care. *4 and 8* eye. At different times eyes photographed at different times a and b

Fig. 10. Width determination in arteries of Fig. 9, repeatedçby observer D.B. of Fig. 8 in four sequences I–IV. Sequences are 1–2 months apart; four determinations (+) on consecutive days within a sequence. $(P.V.)^{1/2}$ = square root of pooled variance, 95% = lenght of 95% interval for individual sequences, both in µm on the film negative, corresponding to 3.2 × the values on the retina (Lotmar, 1976)



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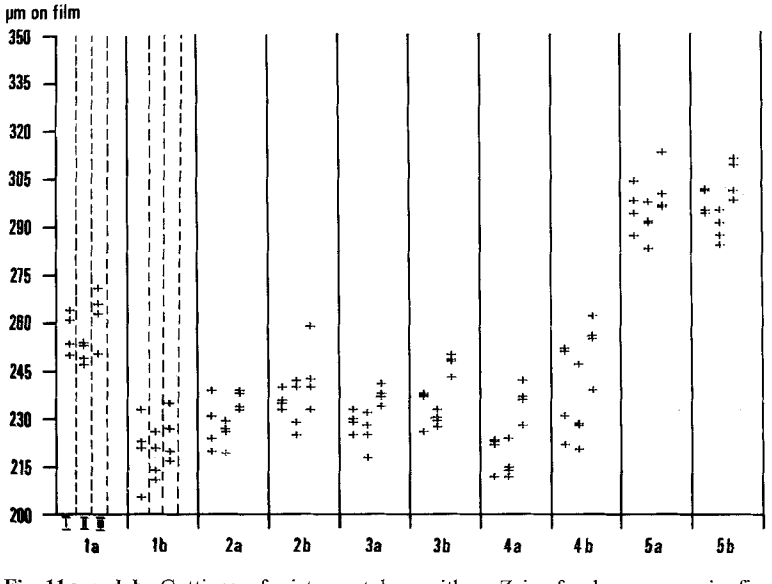

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Fig. 11 a and b. Cuttings of pictures taken with a Zeiss fundus camera in five healthy adults. *1–5* Ilford Pan F 135 without any filter. **a** developed in Microphen, **b** in Rodinal

Fig. 12. Width determination in arteries of Fig. 11, repeated by observer D.B. of Fig. 8 in three sequences I–III

res. Arteries 2, 3 and 5 did not vary significantly. Pooled variance for Microphen-developed pictures is significantly smaller than for Rodinal-developed pictures ($P=0.02$)[2].

IV. Discussion

We made arbitrary decisions on several points:

Film, Filter, and Developer. As stated previously (see section II, 1, and Figs. 1 and 2), reflectance of the fundus varies intra- and interindividually and tends to bias vessel appearance because of under- or overexposure. On the other hand, photographic material of low contrast reduces the reproducibility of measurements, as shown by the significantly greater pooled variance for the Rodinal-developed pictures. Since it is neither possible to measure real width nor easy to build a realistic model eye, we do not see how to investigate the problem of choosing optimal material. All we can say from our investigation is that there is no systematic shift between Rodinal- and Microphen-developed pictures, but we do not know whether the significant differences between arteries 1a–1b and 4a–4b (Fig. 12) are real or artifacts generated by the photographic process.

Variation of *definition* not only influences reproducibility but is also likely to shift the localization of vessel borders outward or inward, as the case may be. Hence, when diameters of arteries of different definition are compared, the results may be biased. Trials to find an objective criterion of definition through densitometry or the modified vernier method previously described had to be abandoned. It has to be judged by inspection whether picture definition is satisfactory, and it is desirable that authors keep their films available (see relation between definition of arteries of Fig. 9 and pooled variance in Fig. 10).

As sites of measurement, we select points which are well defined in relation to other structures of the fundus. We accept a lack of precision when we transfer these sites, as marked on the print, to the picture on the screen. On the evidence of Figure 1, we feel that it would be self-deceptive to use sophisticated methods to reidentify a given site on a vessel after it has undergone a pathophysiological change. We found no significant differences in reproducibility for more peripheral compared with peripapillary sites; this agrees with the theoretical prediction of the limited relevance of astigmatism (Bracher et al., 1975).

How Best to Express Width. Because of the width fluctuations of the vessels, we feel that it is not correct from the physiological aspect to speak of vessel width when it has been measured at one site only. In preliminary trials we found that reproducibility was improved when measurements at three sites were evaluated as one batch, compared with measurements at one site only. We investigated whether reproducibility could be further improved by taking the

[2] A detailed statistical evaluation is available on request from the second author's address

Table 2

	Present paper		Hodge et al.			
	Microphen	Rodinal	*P*	*L*	*C*	*D*
(Pooled variance)$^{1/2}$	2.361	3.279	2.700	2.781	2.269	2.748
Length of 95% interval	9.254	12.854	10.584	10.902	8.894	10.772

Square roots of pooled variance (μm on retina) and length of 95% interval (μm on retina) for data of Fig. 12, compared with data based on these reported by Hodge et al. (1969). *P*=dial caliper on profile projector, *L*=microscope eyepiece with a line, *C*=microscope eyepiece with a cross, *D*=densitometric measurement

mean from 5 or 7 sites, or by measuring 6 or 12 times at a site instead of 4 times, but found no improvement.

Comparison with other methods is only conclusive when the same observer tries both methods on the same pictures. There is no significant difference between the pooled variances of our measurements on Microphen-developed films and the results shown by Hodge et al. (1969) (Table 2). However, Hodge et al. used highly selected material (6 pictures of which 4 were fluorescein injection and 2 taken *with* a green filter), and it can be assumed that our method would give better results on such pictures. In the most recent paper by Majewska et al. (1976) it is unfortunately not stated if and how they overcame the problems mentioned in section II, 2a, of the present paper, with the digital image transformer they used.

V. Conclusions

In measurement of vessel width on fundus photographs by the method described, differences of up to 9 μm on the retina can be caused by measurement errors, even in pictures of good definition; bias by the photographic process entails further uncertainty. Consistent results can be expected for intraindividual changes

– either documented in collectives, e.g., adults suffering from hypertension before and after treatment,

– or in individual cases, when the change in width is documented by several pictures both before and after a pathophysiological event.

All pictures should be made with the same filter-film-developer combination (which should guarantee a sufficient range of linearity), and all comparative measurements should be made by the same observer on the same day, or corrected by frequent reference to a standard picture.

In interindividual comparison, differences in branching of vessels must be taken into account; a corresponding method has been proposed by Parr and Spears (1974a, b).

Absolute figures on width of the central retinal vessels are a rough approximation because of possible bias by the photographic process, uncertainty of

the refractive power of the eye (unless bulbus length is measured), and inter- and intraobserver differences in evaluation.

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