The V/A Ratio of the Smaller Vessels of the Bulbar Conjunctiva in Diabetes Mellitus

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In patients who have had diabetes mellitus for many years, more or less pronounced vascular abnormalities in one or more organs are very common. Clinically, these abnormalities usually appear as affections of the retina, kidney, heart and limbs. There are many reasons to assume that these long-term diabetic abnormalities are manifestations of a generalized diabetic vascular disease, a diabetic angiopathy.[1-4]

Diabetic vascular disease of the retina stands apart from other vascular anomalies in diabetes because it can be directly observed and followed through its various phases by ophthalmoscopy. As early as 1890, diabetic retinopathy was a well-defined concept,[5] and it has later been analyzed in numerous studies, of which it will suffice to mention the classic works of Waite and Beetham,[6] Ballantyne[7] and Hanum.[7a]

Observations on retinopathy occupy a central position in the study of the nature of diabetic vascular disease, because this lesion is directly visible and because its ophthalmoscopic picture is characteristic. However, the special conditions which prevail in the eye due to the intra-ocular pressure make it difficult to draw conclusions about the general state of the blood vessels of the organism on the basis of observations made on the retinal vessels.

In the late 1940's, attention was directed to another vascular region, which may also be directly observed. In their studies of the so-called sludge phenomenon, Knisely et al.[8] showed that the vessels of the bulbar conjunctiva are a suitable object of in vitro studies in man. In addition, the conjunctival vessels could be considered to be more representative of the general vascular status in the organism than those of the retina.

In a series of publications, J. Ditzel and his coworkers[9-13] have reported studies of the conjunctival vessels in diabetes mellitus. The most important results of these investigations are the finding of angularity and tortuosity of the capillaries and venules, a low ratio between the diameter of the arteriole and that of the corresponding venule (a/v ratio), fusiform dilatations of the venules, and perivascular edema and "hyaline" infiltrations. However, these changes in the conjunctival vessels were observed not only in long-term diabetics but also often in patients who had suffered from diabetes for only a few years.

With one exception, the vascular abnormalities observed in the conjunctivae of diabetics by Ditzel and his associates are difficult to assess quantitatively. The exception is the a/v ratio, which may be determined with a reasonable accuracy in standardized photographs. Ditzel and St. Clair[10] developed a method for photographing the smaller vessels in the conjunctiva and described the determination of the a/v ratio in one patient. In the other publications, a/v ratios which had been estimated by direct microscopic observation are reported; these are given as 1:2, 1:3, 1:4, etc.

The following is a report of a comparative study of the ratio between the venules and the arterioles determined quantitatively in a series of patients with diabetes of shorter or longer duration and in a control nondiabetic group.

METHOD AND MATERIAL

Measurements of the diameters of the conjunctival arterioles and venules were performed by means of a measuring microscope, directly on the negatives of standardized photomicrographs of the conjunctiva taken through a microscope.

A Leitz stereobinocular microscope (Greenough type) with the tubes in the horizontal position, was used. A Contax I camera provided with a lens of the following specification: Tessar 1:2.8; F: 4.5 cm. was attached to one of the tubes of the microscope. A diaphragm 2.8 was used; exposure time 1/30 second.

Inspection of the conjunctiva and focusing on the area to be photographed were done through the other tube. The photographs were taken through a plane eyepiece 25 x and objective 4 x. The pilot light was a 12-volt incandescent electric lamp.

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The light source for the exposure was an electronic flash (Mecablitz 500) with the following specifications: light output 120 watt-seconds; color temperature 5,500° Kelvin; discharge time 1/750 second; current supply A.C., 220 volts.

The lighting setup used was a modification of the apparatus described by Bruzelius and Holm.[14] By this arrangement, in which the beam direction of the pilot light and electronic flash are identical, it is possible to avoid disturbing reflections on the photographs by reflection-free adjustment before the exposures.

During the examination, the patient was seated on a chair facing the binocular microscope. His head was immobilized by an ophthalmic head rest supporting the chin and forehead. The light source was placed to the left of the patient's head. In all cases, photographs were taken of the temporal part of the left eye as it presented itself when the patient fixed his gaze on a target. The conjunctival area to be photographed was selected by means of an ocular micrometer so that it did not include the ciliary vessels at the limbus corneae, the exposed area being at least 5 mm. from the limbus.

Eight to ten exposures were made of the conjunctiva on Adox Docupan or Agfa document films. The measurements were made directly by means of an ordinary measuring microscope with an ocular 6 x and an objective lens 3 x. The film strip was placed in a special holder which fixed the negative during measurement and allowed passage of the strip to the next area to be measured. The measuring microscope was provided with a vernier giving an accuracy of 0.01 mm.

In the individual negatives, one or more *pairs of vessels* (arteriole and accompanying venule) were selected. Five measurements were made of the arteriole and venule, and the diameters of the vessels were expressed by the average figures of these five measurements. Finally, the ratio between the diameter of the venule and that of the arteriole (v/a ratio) was calculated. The v/a ratios, stressing the importance of venular disease in diabetes mellitus, and giving always figures above 1.0, were preferred to the reciprocal a/v values used by Ditzel.

Each person was examined on one occasion only. The photographs obtained from some of the patients showed only one pair of vessels, but in most cases two or even three were to be found. Table 1 shows the results of the determinations of the v/a ratios in all the members of the two groups to be studied here. One, two or three v/a ratios are noted in columns I-III, according to the number of pairs of vessels that happened to be included on the photographs obtained.

The diameter of the arterioles varied between six and thirty-three micra and those of the venules between twelve and eighty-one micra. These absolute values were calculated by a comparison with a millimeter scale photographed in the same plane as the conjunctiva.

The patients originally consisted of eighty-eight diabetics and thirty control subjects.

In order to exclude vascular changes of old age, the series comprised only persons under fifty years of age; and in order to ensure the necessary cooperation, only persons over ten years were studied. Of the photographs taken, those from twenty patients and six controls were unsuccessful (technical faults or absence of pairs of vessels); accordingly, the final analysis comprises sixty-eight diabetics and twenty-four control subjects. No persons who had previously suffered from severe nondiabetic diseases of the eye (keratoconjunctivitis, iridocyclitis) were included in the study; at the time of the examination, none of the patients or controls showed signs of acute ocular affections.

In order to provide uniform experimental conditions at the time of the examination the following rules were observed: (1) The patient was in the examination room one half hour before the examination was performed; this room was free from draught, the temperature being 20° C. He was not allowed to smoke during this period. (2) The examination was performed at least two hours after the last meal. (3) Alcohol was not allowed during the last twenty-four hours before the examination.

In all patients and including controls, the blood-sugar level was determined by the method of Hagedorn and Norman Jensen before the examination. In the diabetics, the morning and evening urine was tested for sugar, protein and ketone bodies.

The control series consisted of randomly selected healthy persons who had not suffered from serious diseases. As a further precaution, not only blood-sugar determination, but also blood-pressure readings and ophthalmoscopy were performed in the controls. These studies showed normal findings in all.

RESULTS

The results are shown in table 1. A graph of the absolute frequencies of the various v/a ratios in the two groups studied (figure 1) gives the immediate impression that high v/a ratios are more common in diabetics than in normals. The distribution of the relative frequencies (figure 2) *rather intensifies this* impression, viz., that the distribution of the values from

TABLE 1

Age, v/a ratios and blood sugar of diabetic patients and of the persons in the control series, divided into age groups. The v/a ratios are the ratios between venular and arteriolar diameter of pairs of vessels in the conjunctiva bulbi. One, two or three pairs of vessels were available for measuring on the photographs obtained from the individual patients and have been listed in columns I-III

DIABETICS

Age 11-20 years

No.	Age	Duration (years)	Pairs of vessels I	II	III	Blood sugar mg./100 ml.
			v/a ratios			
002	13	1	2.2	4.6	1.6	165
047	13	8	2.6	1.3		72
065	15	8	2.2			104
071	15	0	1.8	1.6		108
084	15	8	3.5	2.5	2.3	92
052	16	4	2.9	2.6		435
062	16	10	1.6			246
077	16	0	1.7	1.4	1.7	348
005	17	1	1.6	3.0		171
064	17	4	3.3			280
082	17	4	3.3	2.4	1.8	212
048	19	2	1.8	4.4		274
058	20	2	1.9	2.6	1.6	
041	20	3	1.7	1.5		180

Age 21-30 years

010	21	2	3.3	2.2		467
059	21	1	1.9	2.4		129
049	22	10	1.7	2.0	2.1	36
055	22	6	1.8			157
078	23	1	1.2	2.2		328
051	24	22	2.5	1.5	1.5	110
070	24	24	2.3	2.2		202
009	25	5	1.3	1.9		122
073	26	20	2.4	1.7		278
072	27	1	1.1	1.0	4.2	216
086	30	21	1.3	1.2		200

Age 31-40 years

022	32	18	1.8	1.6		145
024	32	5	3.4	2.1	2.0	
061	32	3	2.1	3.3		140
067	32	20	1.1	1.3		67
081	32	26	1.4			262
001	35	26	3.1	1.6	2.0	299
003	35	22	2.2	1.9	2.0	184
029	35	25	3.3	1.3		258
036	35	23	1.4	1.7	1.3	50
015	36	7	1.6			110
027	36	7	2.5	1.7	1.4	149
044	36	16	2.2	1.4		101
045	36	4	2.0	1.3		231
023	37	7	1.7	1.6		264
035	37	24	2.3	1.2		164
038	37	10	3.8			236
066	37	22	1.5	1.7		279
060	39	1	1.4	1.7		142
080	39	2	2.2	2.8		167
000	40	22	1.9			173
046	40	12	2.6	4.8	2.2	74
068	40	16	2.3	1.4		222
075	40	16	2.9			124
085	40	0	1.4	1.5		165

DIABETICS

Age 41-50 years

No.	Age	Duration (years)	Pairs of vessels I	II	III	Blood sugar mg./100 ml.
			v/a ratios			
054	42	17	2.5			318
076	42	21	1.3	1.2		240
017	43	5	1.5	1.7		87
018	43	10	2.0	2.0		81
021	43	0	1.7			218
033	43	17	1.2			276
039	43	24	1.3			300
053	43	5	1.8	1.6	1.7	250
087	43	29	2.8			69
020	44	29	1.4	2.8	2.0	114
032	44	3	1.5	1.4		156
069	46	14	2.5	1.3		202
019	47	3	2.9	1.9	3.0	83
016	48	2	2.1	2.4		110
026	48	4	1.7			106
028	49	28	3.0			324
056	49	12	2.6	2.2		298
057	49	2	4.1			120
074	49	2	2.1			75

CONTROLS

Age 11-20 years

424	12	1.2	1.5		73
423	13	1.6	1.5		77
413	20	1.4	1.5		114
421	20	1.8	1.4		111
422	20	4.5	1.7		101

Age 21-30 years

412	21	1.2	1.1	2.7	104
400	22	1.9	1.8	1.4	91
404	23	1.7			79
415	23	1.3	1.2		79
416	23	2.3			112
401	25	2.1	2.3		74
405	28	1.8	2.0		116
414	29	1.6	2.1	1.4	103

Age 31-40 years

428	31	2.3			145
408	32	1.5	1.9	2.6	122
419	32	2.9	1.2	2.3	90
425	36	2.5	2.4		120
411	39	1.7	1.6		94

Age 41-50 years

403	42	2.3	1.9	1.8	97
417	42	3.3	1.0		80
429	42	1.4	1.6	2.0	135
407	44	2.1	1.4	2.2	87
410	47	2.6			82
409	48	1.4	1.4		98

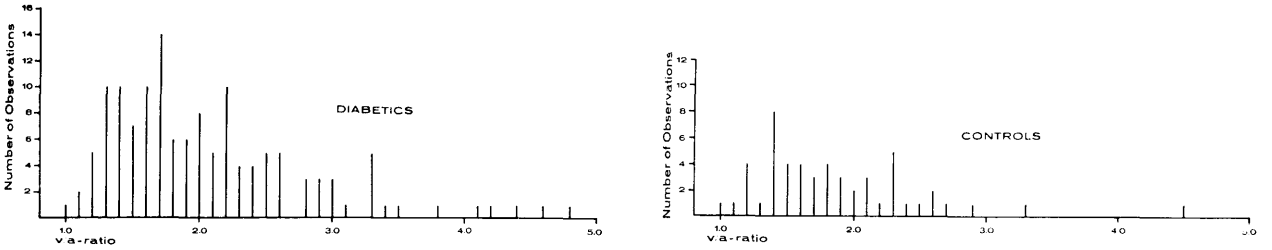

FIG. 1 (a, b). Number of observed v/a ratios in the diabetic and in the nondiabetic control group.

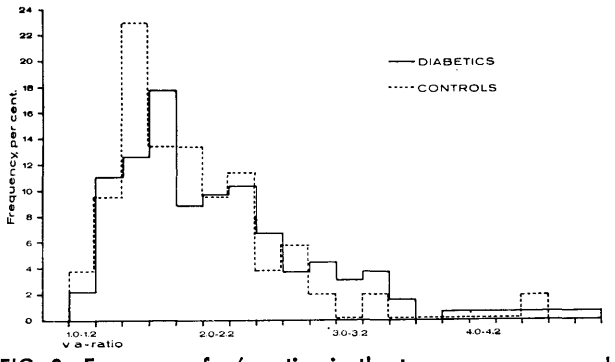

FIG. 2. *Frequency of v/a ratios in the two groups, expressed as per cent of the total number of observations in the diabetics (fully drawn line) and in the control group (stippled line). The blocks represent v/a ratio intervals of 0.2.*

the diabetics is more positively skew than that of the control group. Analysis of the v/a ratios suggests that the two groups may be characterized by a common mean, but that their variabilities are different, the diabetics being the more variable. However, before all measurements in the two groups are compared, it would seem expedient to make an attempt to eliminate the skewness of the distributions by means of a suitable transformation of the values observed. Taking the logarithms of the observations proved to make the distributions roughly symmetrical so that it may be justified to carry out a statistical analysis based on the assumption of normal or Gaussian distribution. The comparison of the means of the logarithms is as follows:

	Mean	Variance, s^2	Common standard deviation, s
Diabetics	0.2917	0.020897	0.1406
Control group	0.2520	0.016840	

Difference between means: 0.0397

Standard deviation of the difference:

$$0.1406 \sqrt{1/135 + 1/52} = 0.0229$$

$$t : 0.0397 / 0.0229 = 1.734$$

On the hypothesis that the true mean in the diabetic group must be either equal to or larger than that of the control group the difference between the means is significant (the value of t calculated lies between the 95 and the 97.5 per cent fractiles of the distribution of t or, equivalently, between the 5 and 2.5 percentage points). In the calculation of t a common variance, σ^2, has been assumed, which may be judged to be permissible, since the two s^2-values are not significantly different.

An analysis of variance was performed on the two groups in order to separate inter- and intra-individual variation, showing that whereas the interindividual variation was larger than the intra-individual variation in the diabetic group, the opposite relation characterized the control group, i.e., that different measurements in the same person of this group were more variable than the means of different persons. Although not significant this relation is so unusual that it is judged to be worth mentioning. If, instead of the above common value of s for the total distribution, a value of s calculated from the interindividual variances is used, the t value becomes lower (1.668), since s is then increased from 0.1406 to 0.1462, but still t lies between the 95 and the 97.5 per cent fractiles.

By inspection of table 1, one suspects a higher incidence of high v/a ratios in the young, but a statistical analysis of the relation between age and v/a ratio showed no significant correlation between these two characteristics, nor was any correlation between duration of diabetes and v/a ratio proved beyond doubt. At any rate nothing suggested a linear relationship between the v/a ratio and the duration of diabetes. We rather gained the impression that an initial increase was followed first by a decrease and then another increase in the v/a ratio during the first thirty years of the diabetes. This, however, cannot be taken as positively demonstrated. However, the aforementioned fact that the interindividual variation in the diabetics is larger than in normal subjects suggests that some relationship may exist between the v/a ratio and the development of the disease over the course of years. However, as indicated by the t-test above, the difference between the interindividual variances is not statistically significant in the present series.

The subdivision of our data by age and duration of

diabetes gives rather small groups. Investigations including larger number of patients are necessary to elucidate further the relation between the v/a ratio and the age and duration of diabetes.

Five of the patients had slight ketonuria at the time of examination (Nos. 010, 022, 023, 066, 077). It appears from table 1 that the v/a ratios observed in these cases were not particularly high.

DISCUSSION

The results of the present quantitative study confirm the observations reported by Ditzel et al., viz., that the v/a ratio of the conjunctival vessels is often higher in diabetics than in nondiabetics.

It is interesting that this should be demonstrable even in spite of the rather large intra-individual variation. The v/a ratio of one pair of vessels may be rather different from that of another pair of vessels in the same conjunctival bed. All the same the statistical analysis of the results obtained shows that high ratios occur more often in a group of diabetic than in a group of nondiabetic eyes.

The difference between the v/a ratio in diabetics and in nondiabetics is statistically significant, but it is, of course, not possible to diagnose or exclude diabetes mellitus by this ratio.

It should also be emphasized that the differences found in our series seem to be less pronounced than those reported by Ditzel. Ditzel and Sagild[9] found v/a ratios over 3:1 in 48 per cent of young, in 28 per cent of middle-aged and in 6 per cent of older diabetics. In control subjects ratios over 3:1 were never observed. In children, Ditzel and Duckers[11] found v/a ratios of 2:1-3:1 in 53 per cent, of 4:1 in 29 per cent, of 5:1 in 14 per cent, and ratios equal to or greater than 6:1 in 4 per cent. Only 1 per cent of the control subjects had a v/a ratio above 3:1.

Like Ditzel and his associates, we found that high v/a ratios occur both in short-term and in long-term diabetes. The question is therefore: What connection is there between the peculiarities of the conjunctival vessels in diabetics and the typical incapacitating angiopathy of long-term diabetes?

It is reasonable to assume that long-term diabetic vascular disease, characterized by the well-known histological abnormalities and clinical manifestations, represents the end result of a development which has been going on ever since the beginning of the diabetes. The relative venular dilatation observed in our patients with diabetes of a few years' duration demonstrates a vascular anomaly in this clinically latent period before the appearance of gross vascular damage.

Information about the state of the blood vessels in the first years of diabetes mellitus is still scarce. Bárány[15] studied diabetic patients without signs of vascular occlusion and often found a reduction of heat dissipation during body warming as well as of the intracutaneous Na^{24} clearance. Sigroth[16] studied skin temperature in diabetic patients during indirect heating and in post-ischemic hyperemia. Reduced vascular responses were found, and improvements of responses occurred in several cases when a better control was obtained. Butterfield[17] found that the foot blood flow, measured plethysmographically, was usually low in diabetic patients, also in patients with short duration of the disease. Finally, Steiness[18] has demonstrated that diabetic patients maintain their vibratory perception longer than normal after arrest of the blood flow to the limbs. This abnormality was observed both in patients with recent diabetes and in diabetes of long duration; it seems to be reversible in most patients. It should be mentioned also that Ditzel and Camerini-Davalos[13] have reported rapid amelioration of some of the vascular changes in the conjunctiva when a poor diabetic state was brought under control.

Further studies of the blood vessels in the early years of diabetes are needed. More information about the apparently reversible functional changes of the vascular bed in recent diabetes mellitus might help us to a better understanding of long-term diabetic vascular disease, and, eventually to appropriate measures for their prevention.

SUMMARY

The bulbar conjunctiva was photographed by a standardized experimental setup in sixty-eight diabetics and twenty-four nondiabetics. The arterioles and accompanying venules were measured on the negatives by means of a measuring microscope, following which the v/a ratio was calculated.

Statistical analysis of the numerical material obtained showed that high v/a ratios were more frequent in diabetics than in nondiabetics.

ACKNOWLEDGMENT

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SUMMARIO IN INTERLINGUA

Le Proportion Venulo-Arteriolar del Conjunctiva Bulbar in Diabete Mellite

Le conjunctiva bulbar esseva photographate per un

standardisate methodo experimental in sexanta-octo diabeticos e vinti-quatro non-diabeticos. Le arteriolas e le accompaniante venulas esseva mesurate in le photographias per medio de un microscopio mesurante, e postea le proportion venulo-arteriolar esseva calculate.

Le analyse statistic del magnitudes obtenite monstrava que alte proportiones venulo-arteriolar esseva plus frequente in diabeticos que in non-diabeticos.

ADDENDUM

Since the submission of this manuscript J. Ditzel, D. W. Beaven and A. E. Renold have published "Early Vascular Changes in Diabetes Mellitus" (*Metabolism* 9:400, 1960), containing among other things a short report of a quantitative examination of the conjunctival vessels in diabetics and nondiabetics. The difference between the distribution of v/a ratios in diabetics and in nondiabetics, calculated from their original data, is essentially similar to the one reported here (J. Ditzel, personal communication).

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The Metabolism of Adrenocortical Hormones in Pregnancy

It is widely recognized that many important alterations in metabolism occur in pregnancy which are determined by ovarian, pituitary, placental and adrenal cortical hormones. There is now unfolding a body of information indicating that the metabolism of hormones themselves often is decidedly different in the pregnant state.

Measurement of the urinary excretion of corticosteroids has usually provided important information concerning corticosteroid metabolism. An increase in the excretion of glycogenic corticoids in the latter half of pregnancy was reported by E. Venning (*Endocrinology* 39:203, 1946). Subsequent measurements of free cortisol excretion in pregnancy is consistent with this finding. A number of other workers who measured the urinary excretion of corticosteroids with chemical methods, which included both the free and the conjugated metabolic products of cortisol, found that there was no significant increase in the excretion of total corticosteroids.

When methods became available for the measurement of unconjugated 17-hydroxycorticosteroids, mainly cortisol, in the blood, a significant rise to about twice the normal level was observed during the third trimester in pregnant women. An exaggerated rise in the plasma cortisol takes place after the administration of ACTH.

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