STUDIES ON THE EYE

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VOLUME II

SECTION II

AUTONOMIC NERVE FUNCTION

2.1 THE EFFECTS OF SYSTEMIC AUTONOMIC NERVE STIMULATION ON THE EYE

2.11 THE EFFECTS OF SYSTEMIC AUTONOMIC NERVE STIMULATION ON INTRAOCULAR PRESSURE

PAPER 23

TITLE: INTRAOCULAR PRESSURE RESPONSES TO SYSTEMIC AUTONOMIC STIMULATION.

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RUNNING TITLE:

Autonomic intraocular pressure responses.

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SUMMARY

The intraocular pressure (IOP) responses to two standard tests of autonomic nerve function were studied in 15 healthy subjects, mean age 33 years (range: 20-64). Both tests are based on cardiovascular reflexes: 1) The rise in diastolic blood pressure provoked by sustained isometric muscle contraction, a response mediated by the sympathetic nervous system, 2) The heart-rate responses to the Valsalva manoeuvre, a response mediated by the parasympathetic nervous system. During sustained isometric exercise, the mean IOP decreased significantly from baseline values of 12.60 mmHg (SEM 0.55) to 11.26 mmHg (SEM 0.32, $p<0.05$) while mean diastolic blood pressure increased by 20.80 mmHg (range: +12 to +53 mmHg). However there was no direct correlation between them ($r= -0.05$). Post exercise the mean IOP remained significantly lower than baseline values for 5 minutes ($p<0.01$). During the Valsalva manoeuvre, there was a significant increase in IOP of 7.20 mmHg from a baseline of 12.80 mmHg (SEM 0.45) to 20.00 mmHg (SEM 0.78, $p<0.001$); during the recovery period, mean IOP was significantly lower than baseline values at 12.13 mmHg (SEM 0.39, $p<0.05$). The mean Valsalva ratio was normal at 1.68 (range: 1.2 - 2.6). The possible mechanisms involved in these responses are discussed.

Key words:

Intraocular pressure, isometric exercise, Valsalva manoeuvre, Autonomic nerve function tests.

INTRODUCTION:

The role of the autonomic nervous system in the regulation of intraocular pressure is well recognised (1-6). Current medications prescribed in the treatment of glaucoma - guttae adrenaline, guanethedine, timolol and pilocarpine - act primarily via autonomic effects in the anterior segment of the eye. More recently, raised intraocular pressure has been associated with autonomic dysfunction (7). A number of simple well established tests of autonomic nerve function based on cardiovascular reflexes allow accurate assessment of the autonomic nervous system (8-13). The aim of the present study was to assess the intraocular pressure responses in normal subjects to systemic autonomic stimulation.

The integrity of the sympathetic nervous system may be assessed by diastolic blood pressure responses to sustained isometric muscle contraction. The forearm muscles are utilised for this purpose and the experimental model is sustained handgrip (12). The cardiovascular response to the sustained handgrip is reflex in nature (14) and is thought to be initiated by stimuli from the exercising muscle (15). The rise in blood pressure is mediated partly by a heart-rate dependant increase in cardiac output (16) and

partly by peripheral vasoconstriction mediated through alpha-adrenergic receptors of the peripheral autonomic nervous system (17). A rise of <10 mmHg diastolic blood pressure is an abnormal response, >16 mmHg normal and borderline if <16 mmHg and >10 mmHg (13).

The Valsalva manoeuvre stimulates a generalised autonomic response, involving both parasympathetic and sympathetic nervous systems, producing specific, well-defined effects on heart-rate and blood pressure. Parasympathetic nerve function may be assessed by heart-rate responses to the Valsalva manoeuvre (13). There are four phases in the haemodynamic responses to the Valsalva manoeuvre. Phase 1 is the onset of expiratory straining when the heart rate slows; it speeds up in response to the decreased venous return and falling blood pressure (Phase 2). Acute release of intrathoracic pressure (Phase 3) causes an increasing tachycardia due to a further drop in cardiac output secondary to increased pulmonary venous capacitance. Phase 4 (period of recovery) follows with a reflex bradycardia as blood pressure increases secondary to increasing cardiac output into a peripheral vasoconstricted vasculature. The change in heart rate during the Valsalva was used as an index of response and expressed as the Valsalva ratio. This is the ratio of the longest R-R interval after the Valsalva to the shortest R-R interval during the Valsalva. A ratio of <1.10 is defined as abnormal; >1.20 as normal and borderline if between 1.20 and 1.10 (13).

PATIENTS AND METHODS:

15 healthy untrained volunteers were studied following informed consent. The mean age of the group was 33 years (range: 20-64 years) and 5 were male. The subject sat at the slit-lamp throughout the study.

1. Diastolic blood pressure response to sustained isometric muscle contraction.

Each subject performed 3 sustained isometric muscle contractions using the dominant arm to establish their maximum voluntary contraction (MVC). Each maximum grip was sustained for less than 2 seconds. The handgrip dynamometer was devised by one of us (D.W.H) and built in the department. An inbuilt electronic audible alarm representing 33% MVC was used for test contractions to alert the subject to weakening grip. 3 baseline intraocular pressures (IOP) were recorded from one eye (whichever was preferred by the subject) using slit-lamp applanation tonometry after topical corneal anaesthesia with Benoxinate (0.4%) and sodium fluorescein (0.25%). 3 baseline blood pressures were recorded from the non-exercising arm using an automatic sphygmomanometer (COPAL-model UA-231).For the study 33% MVC was held for 2-2.5 minutes, during which time 3 measurements of IOP and blood pressure were made at defined intervals as

shown in Figure 1. On recovery 5 measurements of IOP and blood pressure were recorded at 1 minute intervals.

2.Valsalva manoeuvre.

After a rest of 10-15 minutes, the subject was instructed to perform the Valsalva manoeuvre by blowing into a tube connected to a pressure transducer and to maintain an expiratory airway pressure of 35-40 mmHg for 15 seconds. The heart rate was monitored throughout this study by a continuously recording electrocardiograph. All subjects were in sinus rhythm prior to commencing the Valsalva manoeuvre. 3 baseline IOP were recorded in the same eye and 3 more during the Valsalva manoeuvre as described in Figure 2. On recovery 5 measurements of IOP were made at 1 minute intervals.

STATISTICAL ANALYSIS:

1.Diastolic blood pressure response to sustained isometric muscle contraction:

Blood pressure and intraocular pressure at each specified period were recorded and the means and standard error of the means (SEM) calculated. Differences between peak change and mean baseline values and between mean recovery values and mean baseline values were then analysed for the group using

Wilcoxon paired signed rank test; statistical significance was defined as $p<0.05$.

2.Valsalva manoeuvre:

The means and the SEM of IOP and individual Valsalva ratios were calculated. Differences between peak change and mean baseline values and between mean recovery values and mean baseline values were analysed using the Wilcoxon paired signed rank test; statistical significance was defined as $p<0.05$.

RESULTS

1.Diastolic blood pressure response to sustained isometric muscle contraction:

All subjects maintained the handgrip at 33% MVC for 2 to 2.5 minutes. The mean rise in diastolic blood pressure during this static exercise was 20.80 mmHg (range: +12 to +53 mmHg), indicating a normal response (Figure 3). Table 1 gives the individual changes in blood pressure and IOP in the 15 subjects. IOP (mean ± SEM) decreased significantly from a baseline of 12.60 ± 0.55 mmHg to 11.26 ± 0.32 mmHg before handgrip was released ($p<0.05$). There was no direct correlation between the rise in diastolic blood pressure and the decrease in IOP ($r= -0.05$, $p>0.05$). Although diastolic blood pressure returned to near baseline levels within the first minute of recovery, IOP remained significantly lower

than baseline values for at least 5 minutes ($p<0.01$),(Figure 3).

2. Valsalva manoeuvre:

There was a significant increase in mean IOP during the Valsalva manoeuvre of 7.20 mmHg (56%) from a baseline of 12.80 ± 0.45 mmHg to 20.00 ± 0.78 mmHg at 15 seconds ($p<0.001$) (Figure 4). IOP remained lower than baseline values during the recovery period: mean recovery IOP was 12.13 ± 0.39 mmHg ($p<0.05$). The mean Valsalva ratio was normal at 1.68 ± 0.11 in all subjects. Table 2 gives the individual changes in IOP during this test with the individual Valsalva ratios.

DISCUSSION

Diastolic blood pressure response to sustained isometric muscle contraction:

These results show that during generalised sympathetic nerve stimulation, effected by sustained isometric muscle contraction, there is a significant fall in IOP which remains below baseline levels for at least 5 minutes post exercise. No direct correlation however was found between the fall in IOP and the rise in diastolic blood pressure.

A complex study by Marcus et al (18) on the effects of sustained handgrip on IOP reported a trend similar to that in our study. They studied 6-7 subjects performing 50%, 20% maximal and 20% submaximal MVC. Close inspection of the data shown in their Figures 1-3, demonstrate that there was a small but definite rise in IOP soon after commencing the handgrip contraction, although this point was not discussed in their text. This initial rise although not statistically significant, was also seen in our study (Figure 3) and may reflect the sudden increase in blood pressure at the beginning of the handgrip contraction. We have shown a post exercise decrease in IOP similar to that demonstrated by Marcus et al and other authors (18-21). The exact mechanism for this is unclear; increase in serum osmolarity, a decrease in pH and an increase in serum lactate have all been put forward as possible explanations. Stewart et al (20) have shown that no significant alterations in the facility of aqueous outflow or in episcleral venous pressure occurs following exercise, thereby presumably excluding these factors as a possible influence. Marcus et al demonstrated that the post exercise hypotension 1) could be delayed by the application of a sphygmomanometer cuff on the exercising arm raised above systolic pressure (18) and 2) was associated with increased blood lactate, decreased pH and increased serum osmolality (21). Both these observations indicate that some metabolic factor may be responsible for the post exercise ocular hypotension.

Unlike our experience Marcus failed to demonstrate any significant change in IOP during the exercise period. This may have been due to the differences in timing of IOP measurements in their study as compared to ours.

The graphic illustrations of the IOP changes in the study by Marcus et al suggest that there was a direct correlation between the intensity of the handgrip contraction and the degree of post exercise ocular hypotension though with similar blood pressure responses. This would again support a local factor as a possible cause of the post exercise ocular hypotension. Moreover the absence of a direct association between blood pressure changes and post exercise decrease in IOP could be interpreted as contributory evidence to support this metabolite theory.

Another possibility to be considered is the release of sympathomimetic amines during handgrip contraction. In our study, mean diastolic blood pressure increased immediately on starting the 33% MVC from a baseline of 78 mmHg to 99 mmHg at 2-2.5 minutes. Astrand et al (22) showed that heart rate and blood pressure increases were higher after static exercise than dynamic exercise. This cardiovascular response is largely attributable to an increase in sympathetic adrenergic vasomotor tone, resulting in constriction of resistance vessels (15,22). Thus even moderately strong isometric muscle contractions have been found to result in a

marked increase in arterial blood pressure (16). Hence one may postulate that because of the increased sympathetic tone, plasma adrenaline levels will be elevated and this may be effective in lowering IOP during handgrip. It may also explain the post exercise ocular hypotension observed both in our study and other studies, in view of the time required for the breakdown and re-uptake of circulating adrenaline. Exercise is recognised to increase plasma adrenaline levels significantly (23).

Valsalva manoeuvre

The Valsalva manoeuvre is accompanied by a variety of complex haemodynamic events and involves both sympathetic and parasympathetic autonomic responses. The most likely mechanism for the significant rise in IOP during this manoeuvre is secondary to the effect of increased intrathoracic pressure on the great veins of the head and neck. Raised venous pressure is transmitted to the ocular veins and indeed a relationship exists between venous pressure and intrathoracic pressure: IOP = 6.51 + 0.82 venous pressure (24). This effect would probably override the effect of the falling blood pressure which occurs simultaneously.

By contrast to our results in which all subjects showed a rise in IOP, Rosen et al (25) observed 2 principal patterns of ocular pressure changes during during the Valsalva manoeuvre which they described as 1) the positive pattern, where the primary change was an increase in IOP and 2) the negative pattern where the IOP fell during the Valsalva. It is however possible for a subject to artificially maintain an expiratory airway pressure of 40 mmHg by inadvertently occluding the mouthpiece or tube with the tongue, but this would clearly not represent an intrathoracic pressure of 40 mmHg and by definition would not be a Valsalva manoeuvre. In this situation the IOP would fall after a brief initial rise - simulating the negative pattern described above. Monitoring the heart rate changes during the Valsalva manoeuvre would be an objective indicator of the subject's performance, but was unfortunately omitted in the study by Rosen et al (25).

The low IOP observed after the Valsalva manoeuvre, which was maintained for at least 5 minutes, is difficult to explain. Episcleral venous pressure is significantly raised during the Valsalva manoeuvre, thereby decreasing the facility of outflow and precipitating elevation of IOP. Upon termination of the manoeuvre, acute decrease in intrathoracic pressure will inevitably be accompanied by decreased episcleral venous pressure, increased outflow of aqueous and therefore lowering of IOP. If the changes in IOP were determined

exclusively by the systemic blood pressure, rapid homeostasis of IOP would be anticipated. In fact, stabilisation of IOP required a period in excess of 5 minutes, suggesting an additional contributory factor - perhaps humoral or neurogenic - in the observed sequence of events. During phase 2 of the Valsalva manoeuvre where intrathoracic pressure is raised, blood pressure falls secondary to a decreased venous return; immediately on cessation of the Valsalva manoeuvre (phase 3), there is a further drop in blood pressure with increasing tachycardia due to the increased pulmonary capacitance. The falling blood pressure in phases 2 and 3 stimulates baroreceptors in the carotid sinus and aortic arch, and afferent impulses pass via the glossopharyngeal and vagus nerves to the medulla. Subsequent activation of the efferent sympathetic nerves causes peripheral vasoconstriction and may be associated with increased circulating adrenaline levels, with potentially significant effects in the post-Valsalva ocular hypotension.

The present study has demonstrated a significant association between stimulation of systemic autonomic cardiovascular reflexes and IOP, although the exact role of the autonomic nervous system in these acute IOP changes is unclear in view of the complex haemodynamic changes that occur; some possible mechanisms have been proposed. A greater understanding may be obtained by studying the IOP responses

to autonomic stimulation in patients with systemic autonomic dysfunction. This work is in progress.

ACKNOWLEDGEMENTS

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Table 1

Individual changes in diastolic blood pressure and intraocular pressure in response to sustained handgrip (HG).

Subject number	Age	Baseline diast.*BP	BP rise during HG	Baseline IOP*	IOP change during HG	Recovery IOP*
1	61	69	+21	13	-1	9
2	54	99	+49	13	-1	11
3	20	68	+22	13	-2	10
4	21	77	+15	13	-1	12
5	20	68	+22	8	+2	8
6	21	76	+16	12	-2	10
7	21	81	+14	16	-4	12
8	21	76	+16	13	-3	11
9	21	72	+23	13	-3	10
10	31	89	+11	8	+2	8
11	23	75	+20	13	-1	12
12	31	66	+20	14	-2	11
13	22	76	+25	12	-2	11
14	64	84	+21	15	-1	12
15	64	91	+17	13	-1	11

* Mean values, see text for details.

Table 2

Individual changes in intraocular pressure during the Valsalva manoeuvre.

Subject number	Age	Valsalva ratio	Baseline IOP*	IOP rise during Valsalva	Recovery IOP*
1	61	1.2	13	+4	11
2	54	1.3	14	+12	14
3	20	1.8	13	+7	12
4	21	1.5	13	+6	13
5	20	1.8	9	+11	10
6	21	2.0	12	+4	11
7	21	2.0	14	+8	13
8	21	1.6	14	+9	12
9	21	1.6	13	+7	11
10	31	2.0	10	+14	10
11	23	2.6	13	+2	13
12	31	1.3	13	+5	11
13	22	1.2	11	+7	12
14	64	1.2	16	+6	15
15	64	2.2	14	+6	14

* mean values, see text for details.

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LEGENDS:

FIGURE 1.

Protocol for the handgrip study.

FIGURE 2.

Protocol for the Valsalva study.

FIGURE 3.

Changes in intraocular pressure (IOP) and diastolic blood pressure (BP), during the handgrip (HG) study.

FIGURE 4.

Changes in intraocular pressure (IOP) and heart rate during the Valsalva study.

Figure 1

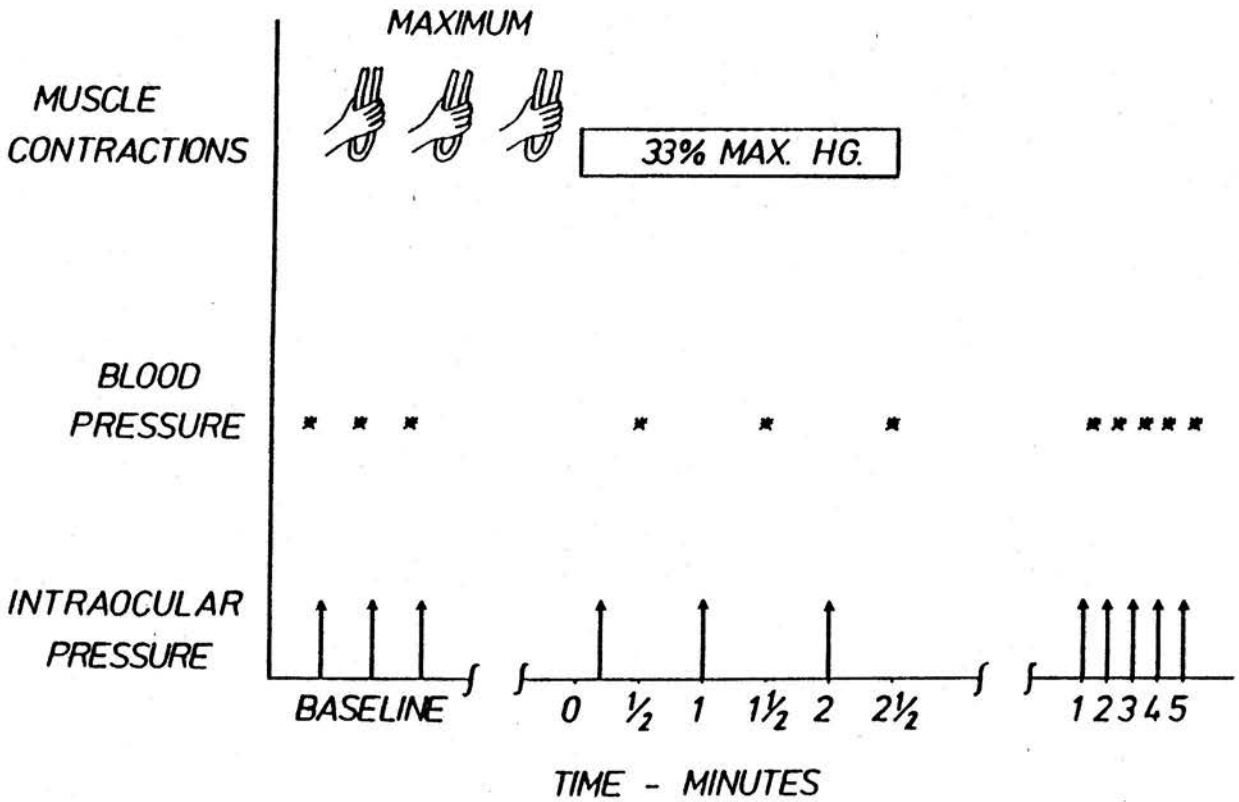

MAXIMUM
MUSCLE CONTRACTIONS
33% MAX. HG.
BLOOD PRESSURE
INTRAOCULAR PRESSURE
BASELINE
0
½
1
1½
2
2½
1 2 3 4 5
TIME - MINUTES

Figure 2

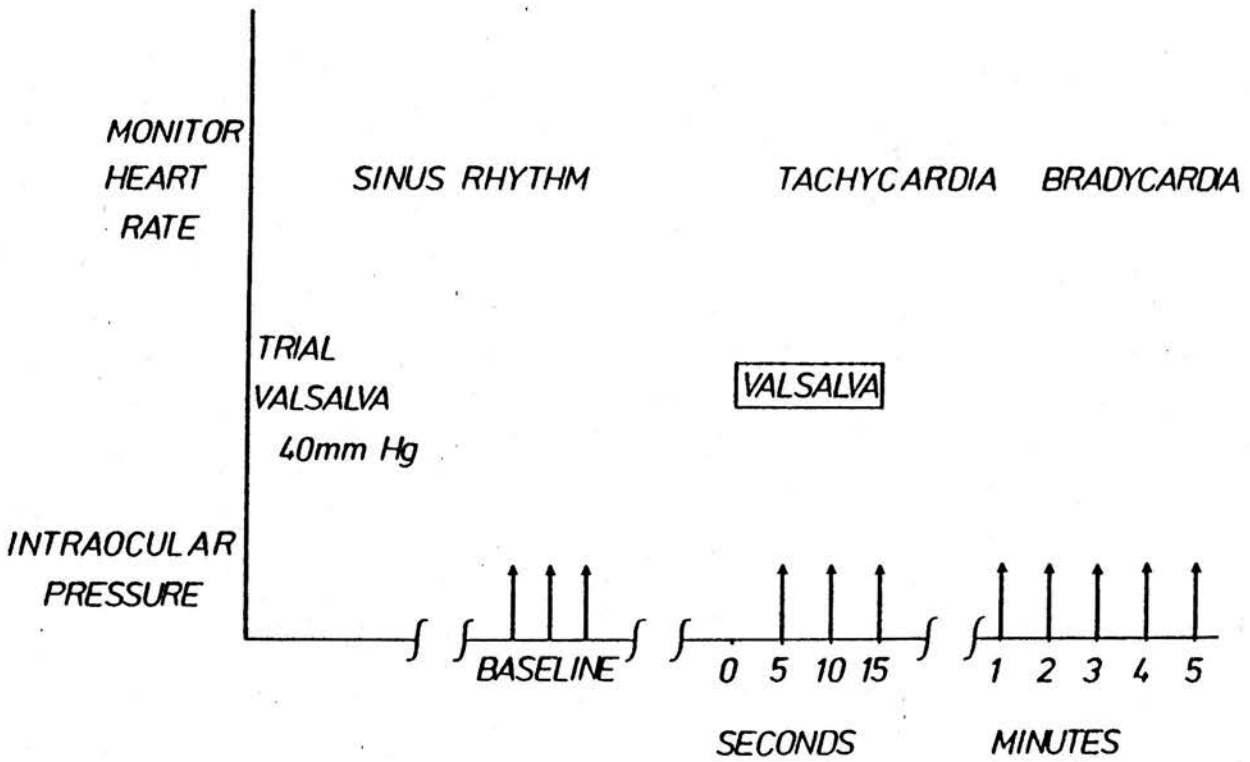

MONITOR
HEART
RATE
SINUS RHYTHM
TACHYCARDIA
BRADYCARDIA
TRIAL
VALSALVA
40mm Hg
VALSALVA
INTRAOCULAR
PRESSURE
BASELINE
0 5 10 15
1 2 3 4 5
SECONDS
MINUTES

Figure 3

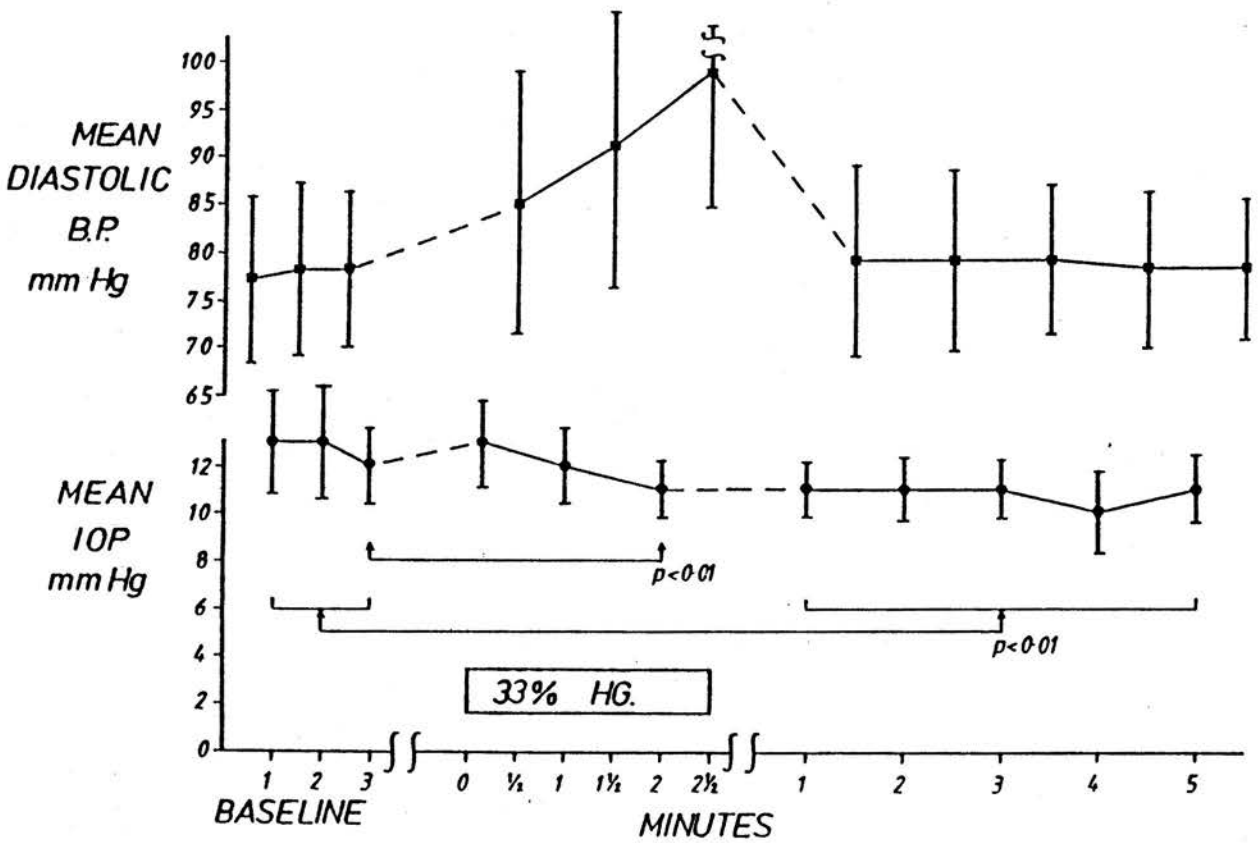
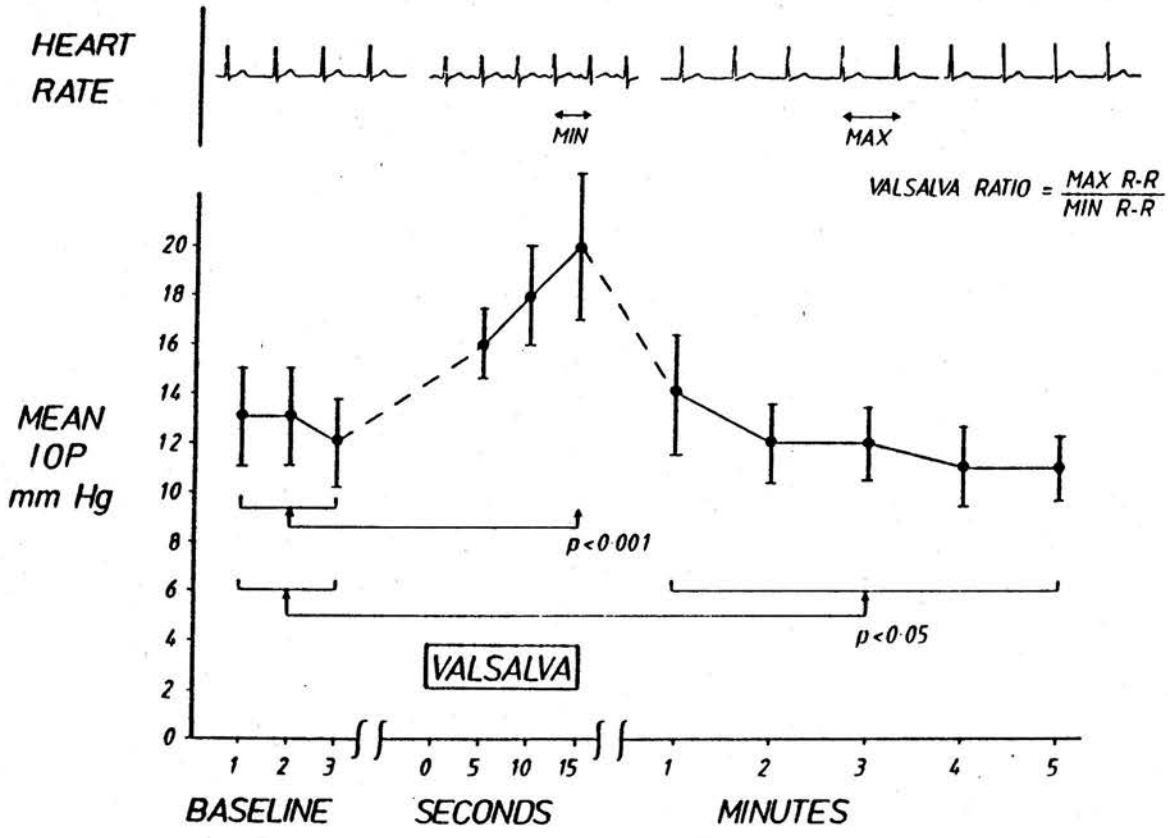

MEAN
DIASTOLIC
B.P.
mm Hg
100
95
90
85
80
75
70
65
MEAN
IOP
mm Hg
12
10
8
6
4
2
0
p<0·01
p<0·01
33% HG.
1 2 3
BASELINE
0 ½ 1 1½ 2 2½ 1 2 3 4 5
MINUTES

Figure 4


HEART RATE
MIN
MAX
VALSALVA RATIO = MAX R-R / MIN R-R
MEAN IOP mm Hg
20
18
16
14
12
10
8
6
4
2
0
p<0·001
p<0·05
VALSALVA
1 2 3
0 5 10 15
1 2 3 4 5
BASELINE
SECONDS
MINUTES

PAPER 24

TITLE:

INTRAOCULAR PRESSURE RESPONSES TO SYSTEMIC AUTONOMIC STIMULATION IN DIABETES MELLITUS

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SUMMARY:

The acute changes in intraocular pressure during sustained handgrip contraction (2.5 minutes duration) and the Valsalva manoeuvre (15 seconds duration), both standard tests of autonomic nerve function were studied in 14 diabetic patients and 14 similar aged control subjects.

During sustained handgrip contraction, diastolic blood pressure increased by 16.35 ± 1.87mmHg in the diabetic patients and 21.36 ± 2.66mmHg for the control group. Mean intraocular pressure decreased by 0.71 ± 0.43mmHg in the diabetics ($p<0.05$) and 0.64 ± 0.27mmHg ($p<0.01$) in the control group.

There was no correlation between the blood pressure and the intraocular pressure responses in either group.

On release of handgrip contraction, mean recovery intraocular pressure over 5 minutes was significantly lower than mean baseline values for the two groups: control, baseline 14.78 ± 0.49 to 14.14 ± 0.67 ($p<0.001$), and diabetic, 14.57 ± 0.65 to 13.86 ± 0.72 ($p<0.001$).

During the Valsalva manoeuvre, there was a significant rise in intraocular pressure in the control (+7.85 ± 0.75 mmHg, $p<0.001$) and the diabetic group (+7.93 ± 1.18 mmHg, $p<0.001$). 5 minutes after release of intrathoracic pressure, mean recovery intraocular pressure remained significantly below baseline values for the two groups. The Valsalva ratios were in the normal range for the control group (1.21 to 2.2) while 2 diabetics had abnormal ratios.

Key words: intraocular pressure, sustained isometric exercise, Valsalva manoeuvre, diabetes mellitus.

Intraocular pressure responses to systemic autonomic stimulation in diabetes mellitus.

Introduction:

An association between diabetes mellitus and glaucoma (both chronic open angle glaucoma and ocular hypertension), has been recognised for many years, although the exact nature of the relationship has not been fully defined. Increased prevalence of diabetes has been demonstrated in open angle glaucoma (1,2) ocular hypertension (3,4) and closed angle glaucoma (4).

Diabetes is the commonest cause of autonomic neuropathy in the United Kingdom, and it has been suggested that anterior segment autonomic dysfunction may be a significant predisposing factor in the pathogenesis of raised intraocular pressure.

The acute intraocular pressure responses to systemic autonomic stimulation have been established in normal subjects (awaiting publication), using two standardized tests of autonomic nerve function:

1) sustained handgrip as a test of sympathetic nerve function (6)

The cardiovascular responses to the sustained handgrip are reflex in nature (6) and are thought to be initiated by

stimuli from the exercising muscle (7).The rise in blood pressure is mediated partly by a heart-rate dependent increase in cardiac output (8) and partly by peripheral vasoconstriction mediated through alpha adrenergic receptors of the peripheral autonomic nervous system (9). A rise of $\leqslant$10 mmHg diastolic blood pressure is an abnormal response, $\geqslant$16 mmHg normal and borderline if between 10 - 16 mmHg (10).

2) Valsalva manoeuvre as a test of parasympathetic nerve function (11)

The cardiovascular responses during the Valsalva manoeuvre are well established. There are four phases to the haemodynamic responses to the Valsalva manoeuvre. Phase 1 is the onset of expiratory straining when the heart rate slows; it speeds up in response to the decreased venous return and falling blood pressure (Phase 2). Acute release of intrathoracic pressure (Phase 3) causes an increasing tachycardia due to a further drop in cardiac output secondary to increased pulmonary venous capacitance. Phase 4 (recovery period) follows with a reflex bradycardia as blood pressure increases secondary to increasing cardiac output into a peripheral vasoconstricted vasculature. The change in heart rate during the Valsalva was used as an index of response and expressed as the Valsalva ratio. This is the ratio of the longest R-R interval after the Valsalva to the shortest R-R interval during the Valsalva. A ratio of $\leqslant$1.20 is defined as abnormal and normal if $\geqslant$ 1.21 (10).

The aim of the present study was to examine the effect of diabetes mellitus on aqueous dynamics by measuring the intraocular pressure responses to equivalent systemic autonomic stimuli in a comparable group of patients with diabetes .

Patients and methods:

After informed consent had been obtained, intraocular pressure responses to systemic autonomic stimulation were assessed in 14 patients with diabetes mellitus: 8 Insulin dependent (IDDM), mean age ± standard error of the mean (SEM): 50 ± 3.22 years) and 6 Non insulin dependent diabetics (NIDDM), (59 ± 1.93 years). Mean duration of diabetes was 12.43 ± 2.43 years (range:1-29 years): IDDM (15.1 ± 3.22 years), NIDDM (8.83 ± 3.44 years).
These were compared with 14 similar aged control subjects (61.35 ± 2.67 years: range:43 to 78 years). The male:female ratio was 11:3 in both groups. No subject included in the study was taking any medication known to interfere with autonomic nerve function. Autonomic status was determined by a series of autonomic function tests based upon cardiovascular reflexes (Table 1).

1.Intraocular pressure responses to sympathetic stimulation.

Each subject performed three maximum handgrip isometric muscle contractions,sustained for less than 2 seconds each,

using the dominant arm on a strain-gauge handgrip dynamometer, to establish their mean maximum voluntary contraction (MVC). An adjustable electronic audible alarm set at 33% MVC was used during the experiment to alert the subject to weakening grip. Three baseline intraocular pressures (IOP) were recorded from one eye (whichever was preferred by the subject) using slit-lamp applanation tonometry. Three baseline blood pressures (BP) were recorded from the non-exercising arm using an automatic sphygmomanometer (COPAL UA-231) with an inflatable cuff. For the study, 33% MVC was held for 2.5 minutes during which time 3 measurements of IOP were taken at 1, 2 and 2.5 minutes and 3 BP measurements at .5, 1.5 and just before release of handgrip at 2.5 minutes. During recovery five measurements of IOP and BP were recorded at one minute intervals.

2. Intraocular pressure responses to parasympathetic stimulation.

After an interval of 10-15 minutes, the subject was instructed to perform the Valsalva manoeuvre and maintain an expiratory airway pressure of 35-40 mmHg for 15 seconds, with continuous electrocardiograph recording throughout the study. Three baseline IOP were recorded in the same eye and three more during the Valsalva manoeuvre at 5, 10 and 15

seconds. During recovery five measurements of IOP were made at one minute intervals.

All subjects in the control and diabetic groups were in sinus rhythm and maintained an expiratory airway pressure of 35 - 40 mmHg for 15 seconds, verified on chart recording.

Statistical analysis:

Means and standard error of the means (SEM) for BP and IOP were calculated for the two groups. Differences in mean BP and IOP responses within each group and between the two groups, during the handgrip and Valsalva studies were analysed using the Student t test.

Results:

1. Autonomic status:

The results of autonomic nerve function tests in the diabetics are shown in Table 2. Only 2 diabetics had definite evidence of autonomic neuropathy, with early autonomic dysfunction in 4 other diabetics.

a). Sustained isometric muscle contraction.

The rise in diastolic blood pressure (BP)(mean ± SEM) during sustained handgrip contraction, for the control group was 21.36 ± 2.66mmHg; range: 12-49 mmHg (Figure 1). The blood pressure response was borderline in 2 subjects and normal in all remaining subjects.

The mean rise in diastolic blood pressure in the diabetic group was 16.35 ± 1.87 mmHg; range:7 to 28mmHg) (Figure 2). The blood pressure responses were abnormal in 4 subjects and borderline in 4 subjects. The individual results of both groups are shown in Tables 3 and 4.

There was no significant difference in the mean rise in diastolic blood pressure during handgrip contraction between the two groups ($p>0.05$).

Post handgrip contraction, mean recovery diastolic BP over 5 minutes for each group (controls, 88.98 ± 0.96mmHg); diabetics, 81.60 ± 0.25mmHg) were not significantly different from the corresponding mean baseline values: controls, 90.78 ± 2.44mmHg; diabetics, 85 ± 2.57 ($p>0.05$).

During the 2.5 minute sustained handgrip contraction, mean IOP decreased by 0.64 ± 0.27mmHg from a baseline of 14.78 ± 0.49mmHg to 14.14 ± 0.67mmHg ($p<0.05$) in the control group, and by 0.71 ± 0.43mmHg from 14.57 ± 0.65mmHg to 13.86 ± 0.72mmHg ($p>0.05$) in the diabetics.

There was no correlation between the rise in diastolic blood pressure and the decrease in IOP during handgrip contraction in either group: control subjects, $r= -0.52$ ($p>0.05$) and diabetics, $r= -0.33$ ($p>0.05$).

On release of handgrip contraction, mean recovery IOP over 5 minutes (control, 11.71 ± 0.29mmHg; diabetics, 12.21 ± 0.86mmHg remained significantly lower than the corresponding

baseline values (control, 14.78 ± 0.49mmHg; diabetics, 14.57 ± 0.65mmHg for both groups ($p<0.001$).
There was a small difference of 0.71 mmHg in the post-exercise ocular hypotension between the two groups ($p>0.05$):(control, -3.07 mmHg, diabetics, -2.36 mmHg).

b) Valsalva Manoeuvre

During the Valsalva manoeuvre there was a significant rise in mean IOP of 7.85 ± 0.75mmHg from a mean baseline IOP of 14.36 ± 0.33mmHg to a peak IOP at 15 sec Valsalva of 22.21 ± 0.73mmHg ($p<0.001$) for the control group; and a similar rise of 7.93 ± 1.18mmHg from a mean baseline IOP of 14.35 ± 0.58mmHg to a peak value of 22.28 ± 1.06mmHg ($p<0.001$) observed in the diabetic group (Figures 3 and 4). There was no significant difference in the rise in IOP during the Valsalva manoeuvre between the two groups ($p>0.05$).

Following a 5 minute recovery interval, mean IOP for the control group (13.51 ± 0.75mmHg) and diabetic group (13.34 ± 0.39) were significantly lower than the corresponding respective mean baseline values: control, 14.36 ± 0.33mmHg and diabetics: 14.35 ± 0.58 ($p<0.01$). There was no significant difference in the post Valsalva ocular hypotension between the two groups ($p>0.05$). Individual results of both groups are shown in Tables 5 and 6.
All subjects in the control group had a normal Valsalva ratio, with a mean ratio of 1.51 ± 0.09, range: 1.2 to 2.2.

In the diabetic group, the Valsalva ratio was abnormal in 2 subjects (Tables 2 and 6), with a group mean Valsalva ratio of 1.41 ± 0.06, range: 1.0 to 1.7.

Discussion:

Sustained isometric muscle contraction in normal subjects causes a decrease in intraocular pressure which remains significantly lower than baseline values for at least 5 minutes post exercise (awaiting publication). This effect was also demonstrated in diabetic patients. Similarly there was no correlation, in either diabetic patients or normal subjects, between the decrease in intraocular pressure and the rise in diastolic blood pressure. The difference of 0.71 mmHg in the post-handgrip ocular hypotension between the control group and the diabetic patients was not significant at the 5% level.

The mean Valsalva ratio was similar in the two groups but 2 diabetics had an abnormal Valsalva ratio. In our previous study (awaiting publication) we found a significant rise in IOP during the Valsalva manoeuvre which then remained below baseline levels for at least 5 minutes after the recovery. This was also seen in the control and diabetic groups in this study; and there was no significant difference in the mean rise in IOP between the two groups ($p>0.05$). This is not particularly surprising as the rise in IOP during the Valsalva manoeuvre is almost certainly due to the mechanical

effect of the raised intrathoracic pressure on the great veins of the neck, transmitted to the ocular venous pressure and hence IOP (13). The diabetics had lower ratios than the control group, yet there was no significant difference in mean decrease in IOP post Valsalva.

It has been postulated that activation of the sympathetic nervous system during the handgrip contraction and phase 3 of the Valsalva manoeuvre may increase circulating adrenaline (12) which could theoretically contribute to the post-handgrip and post-Valsalva ocular hypotension, present for at least 5 minutes after termination of the manoeuvre. The demonstration of definite autonomic dysfunction in only two diabetic patients precludes any assessment of the effects of autonomic neuropathy on the present results. Interestingly, a study by Hilsted et al (14) showed that diabetics with autonomic dysfunction required greater exercise stress in order to reach a given plasma catecholamine level than their control group.

The present study has demonstrated similar intraocular pressure responses to systemic autonomic stimulation - by sustained isometric exercise and Valsalva manoeuvre - in normal subjects and diabetic patients with intact autonomic nerve function, suggesting that diabetes per se does not have a significant effect on the responses of aqueous dynamics to systemic autonomic stimulation. The effect of

autonomic neuropathy on intraocular pressure responses in these patients remains to be evaluated.

Acknowledgements:

We thank the patients of Guy's Hospital for their cheerful cooperation in this study and Dr. D.J.Ewing, University of Edinburgh for advice relating to autonomic nerve function assessment. The project was aupported by a grant from the Royal National Institute for the Blind. L.P.Lanigan holds a Smith & Nephew Fellowship in Ophthalmology.

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Table 1

Normal, Borderline and abnormal values for cardiovascular autonomic nerve function tests (D.J.Ewing et al, 1986).

	Normal	Borderline	Abnormal
Immediate heart-rate response to standing (30:15 ratio)	≥1.04	1.01-1.03	<1.00
Blood pressure response to standing (fall in systolic BP)	≤10 mmHg	11-29 mmHg	>30 mmHg
Blood pressure response to sustained handgrip	≥16 mmHg	11-15 mmHg	≤10 mmHg
Heart rate response to Valsalva manoeuvre (Valsalva ratio)	>1.20		≤1.20

Table 2

Diabetic autonomic nerve function tests

Subj. No.	Age	Sex	Type of DM	Duration of DM (yrs)	DiastBP rise during HG	Valsalva ratio	BPchange L/S	Heart-rate (30:15)	Autonomic status
1	56	M	IDDM	1	+15[a]	1.7	+25	0.83[c]	E
2	56	M	IDDM	18	+14[a]	1.4	-24[a]	1.28	N[b]
3	56	F	MODM	6	+7[c]	1.1[c]	+29	0.85[c]	D
4	60	M	IDDM	9	+26	1.6	-20[a]	1.00	E
5	34	M	IDDM	13	+26	1.5	+2	1.16	N
6	60	M	MODM	2	+19	1.6	-10	1.18	N
7	55	F	IDDM	17	+13[a]	1.5	-4	1.23	N
8	53	F	MODM	11	+9[c]	1.6	-20[a]	1.08	N[b]
9	42	M	IDDM	25	+10[c]	1.2[c]	0	1.00[c]	D
10	54	M	IDDM	29	+10[c]	1.6	-40[c]	1.03	N[b]
11	43	M	IDDM	9	+28	1.3	-5	1.19	N
12	58	M	MODM	4	+17	1.5	+8	1.19	N
13	60	M	MODM	5	+23	1.0[c]	-30[c]	1.06	E
14	67	M	MODM	25	+12[a]	1.2[c]	0	1.00[c]	E

DM diabetes mellitus
IDDM insulin dependent diabetes
NIDDM maturity onset diabetes
HG handrip
BP blood pressure
L/S lying to standing

E early autonomic dysfunction
N normal
[b] query normal
D definite autonomic dysfunction
[c] abnormal
[a] borderline

Table 3.

Responses during Handgrip study - Control Group

Subject Number	Age	Baseline diastolic BP*	BPrise during HG	Baseline IOP*	IOPchange during HG	Recovery IOP*
1	56	90	+14	14	0	11
2	78	84	+17	16	+1	13
3	68	101	+17	16	0	13
4	69	90	+19	18	0	14
5	43	81	+17	14	0	11
6	44	90	+12	14	-1	11
7	60	93	+38	15	-3	10
8	73	97	+17	18	0	16
9	58	101	+20	12	-2	10
10	61	69	+21	13	-1	9
11	54	99	+49	13	-1	11
12	64	91	+17	13	-1	11
13	64	84	+21	15	-1	12
14	67	101	+20	16	0	12

* mean values
BP blood pressure
IOP intraocular pressure

Table 4

Responses during the Handgrip study - Diabetic Group.

Subject Number	Age	Baseline diastolic BP[a]	BPrise during HG	Baseline IOP[a]	IOPchange during HG[a]	Recovery IOP[a]	Autonomic status
1	56	88	+15	18	-2	18	E
2	56	81	+14	12	-2	10	N[b]
3	56	99	+7	16	-1	14	D
4	60	86	+26	14	0	11	E
5	34	84	+26	12	-2	10	N
6	60	76	+19	18	-2	15	N
7	55	104	+13	18	0	18	N
8	53	90	+9	12	+3	9	N[b]
9	42	88	+10	12	-2	9	D
10	54	79	+10	15	+2	12	N[b]
11	43	92	+28	14	-2	9	N
12	58	73	+17	12	0	10	N
13	60	69	+23	17	-1	15	E
14	67	81	+12	14	-1	11	E

[a] mean values
BP blood pressure
IOP intraocular pressure
HG handgrip
D Definite autonomic dysfunction
[b] query normal
E early autonomic dysfunction

Table 5

Responses during Valsalva study - Control Group

Subject Number	Age	Valsalva ratio	Baseline IOP*	IOP rise during Valsalva	Recovery IOP*
1	56	1.3	14	+8	12
2	78	1.3	15	+7	14
3	68	1.6	14	+6	13
4	69	1.4	16	+8	15
5	43	1.3	13	+7	13
6	44	1.3	13	+9	13
7	60	1.6	14	+12	13
8	73	1.3	16	+4	16
9	58	1.3	13	+13	13
10	61	2.2	13	+14	11
11	54	1.3	14	+12	14
12	64	2.2	14	+6	14
13	64	1.21	16	+6	15
14	67	1.8	16	+8	13

* mean values
IOP intraocular pressure

Table 6

Responses during the Valsalva study - Diabetic Group

Subject Number	Age	Valsalva Ratio	Baseline IOP[a]	IOPrise during	Recovery IOP[a]	Autonomic status
1	56	1.7	16	+9	16	E
2	56	1.4	12	+7	11	N[b]
3	56	1.1	15	+6	14	D
4	60	1.6	14	+8	12	E
5	34	1.5	12	+18	12	N
6	60	1.6	18	+6	16	N
7	55	1.5	17	+9	18	N
8	53	1.6	13	+5	10	N[b]
9	42	1.2	12	+16	11	D
10	54	1.6	14	+10	14	N[b]
11	43	1.3	14	+4	12	N
12	58	1.5	12	+5	10	N
13	60	1.0	18	+2	17	E
14	67	1.2	14	+6	13	E

[a] mean values
BP blood pressure
IOP intraocular pressure
E early autonomic dysfunction
N normal
[b] query normal
D definite autonomic dysfunction

LEGENDS FOR FIGURES:

FIGURE 1: Control Group: BP and IOP responses (mean ± SEM) during sustained handgrip.

FIGURE 2: Diabetic Group: BP and IOP responses (mean ± SEM) during sustained handgrip.

FIGURE 3: Control Group: IOP (mean ± SEM) responses during Valsalva

FIGURE 4: Diabetic Group: IOP (mean ± SEM) responses during Valsalva.

Figure 1

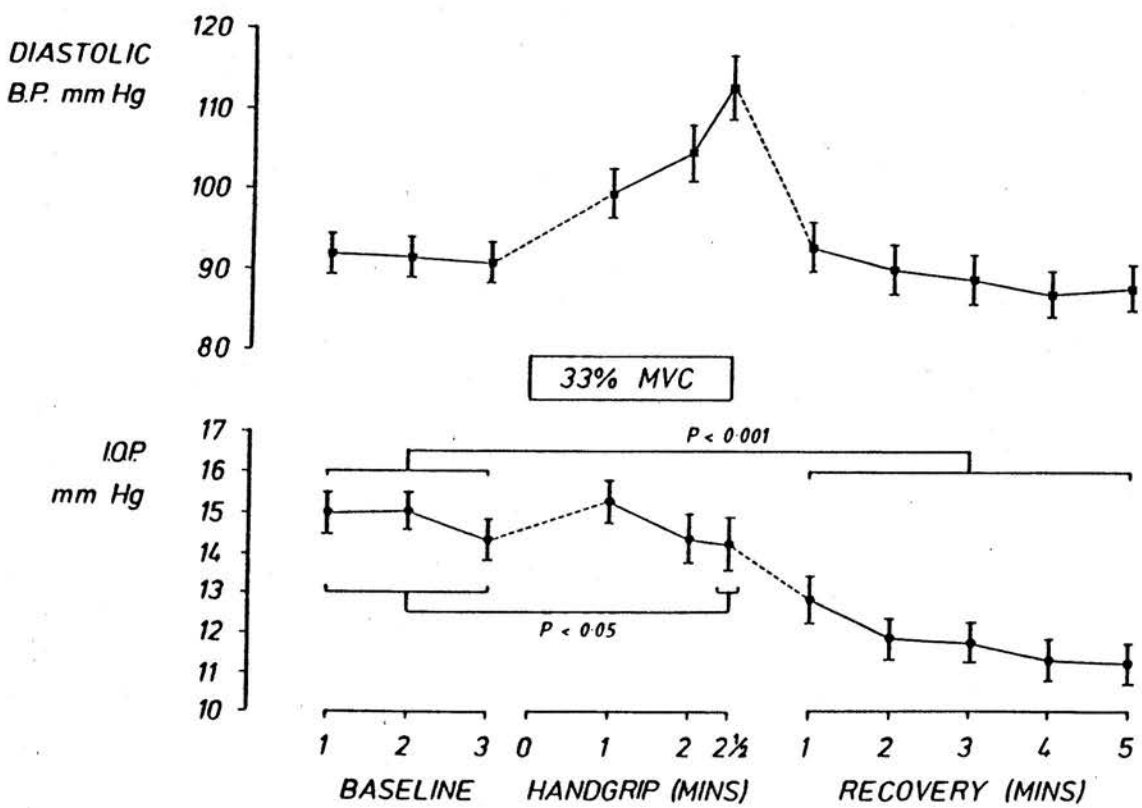

DIASTOLIC
B.P. mm Hg
120
110
100
90
80
33% MVC
I.O.P.
mm Hg
17
16
15
14
13
12
11
10
P < 0·001
P < 0·05
1 2 3 0 1 2 2½ 1 2 3 4 5
BASELINE
HANDGRIP (MINS)
RECOVERY (MINS)

Figure 2

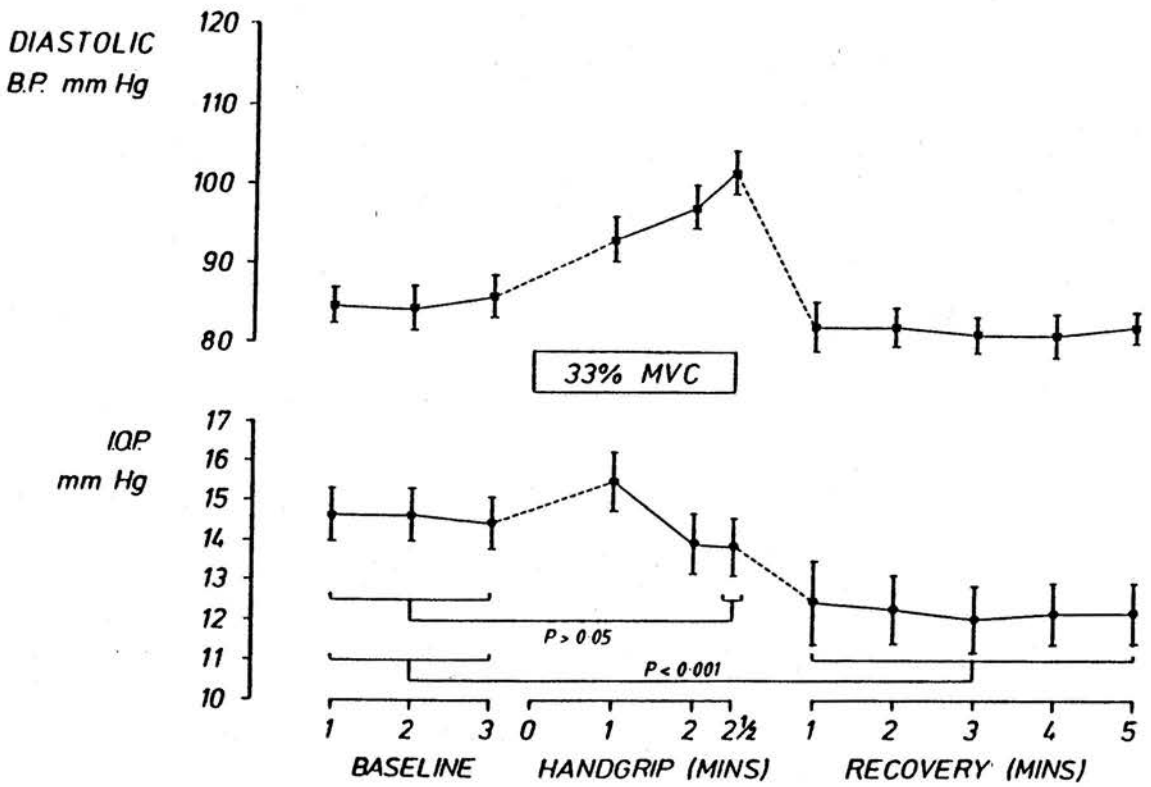

DIASTOLIC
B.P. mm Hg
120
110
100
90
80
33% MVC
I.O.P.
mm Hg
17
16
15
14
13
12
11
10
P > 0·05
P < 0·001
1 2 3 0 1 2 2½ 1 2 3 4 5
BASELINE
HANDGRIP (MINS)
RECOVERY (MINS)

Figure 3

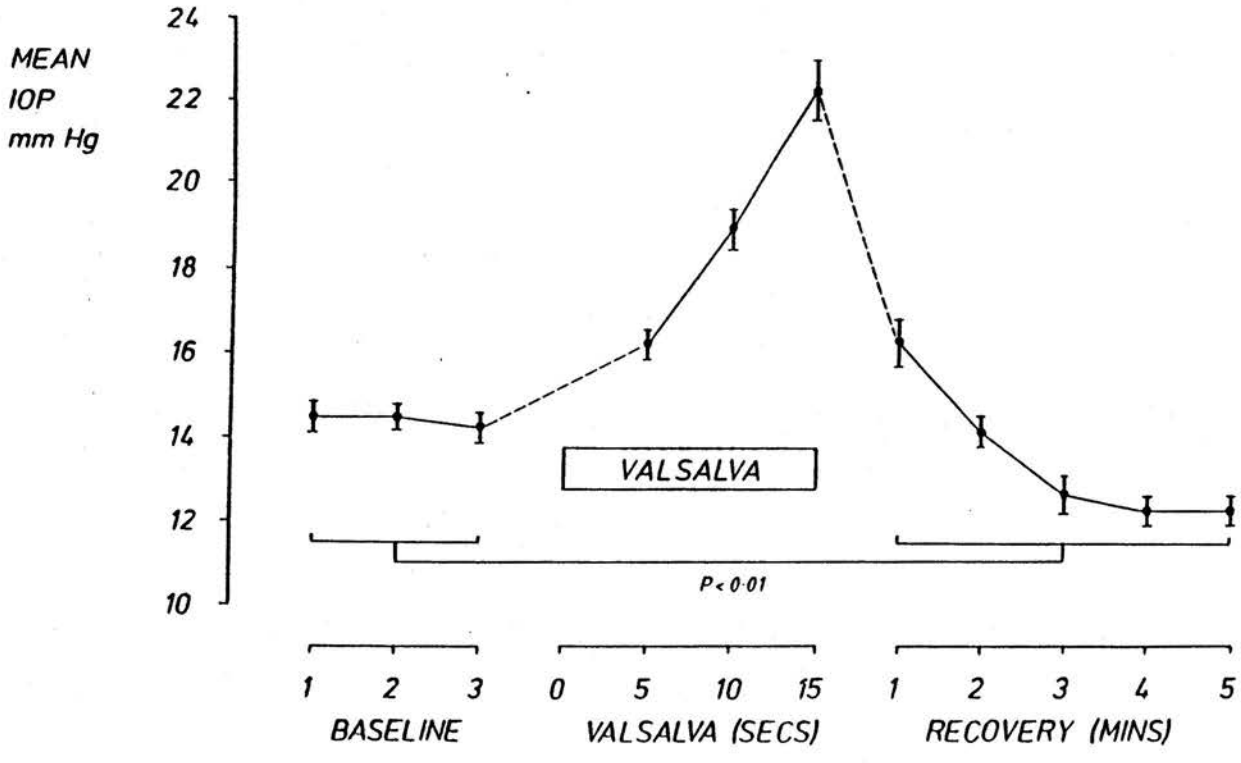
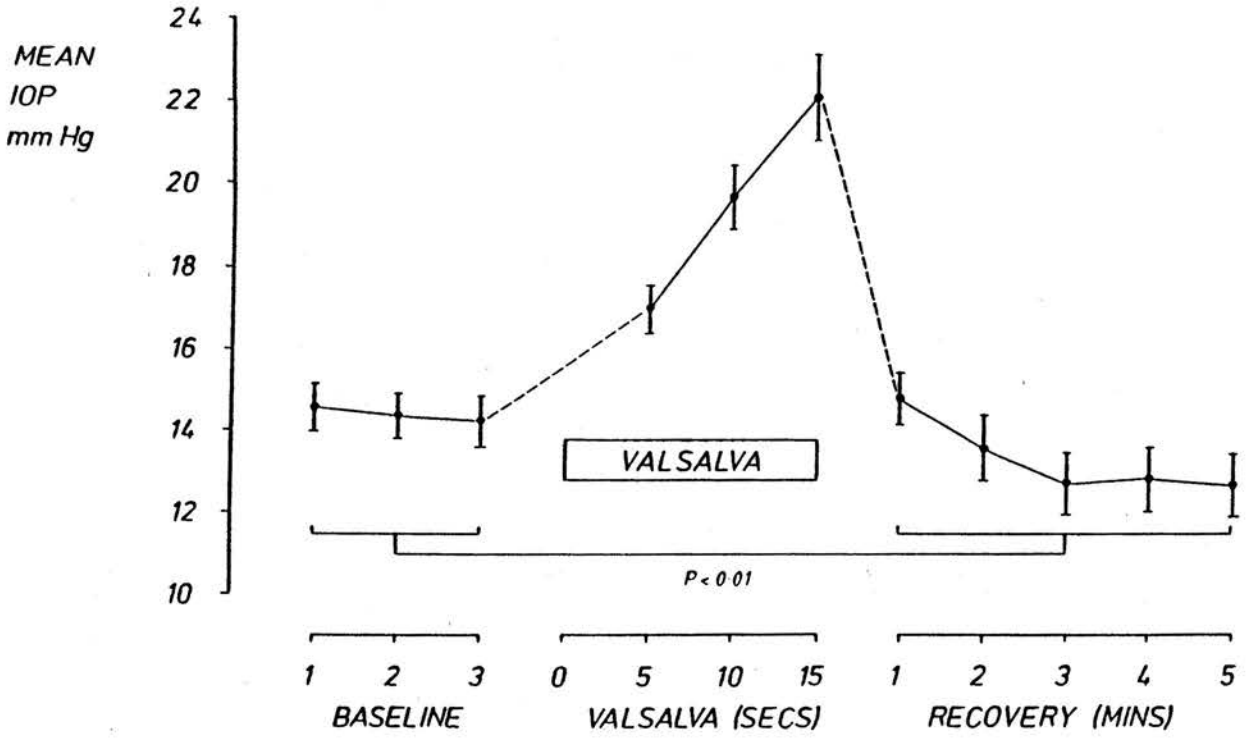

MEAN
IOP
mm Hg
24
22
20
18
16
14
12
10
VALSALVA
P<0·01
1
2
3
0
5
10
15
1
2
3
4
5
BASELINE
VALSALVA (SECS)
RECOVERY (MINS)

Figure 4


MEAN
IOP
mm Hg
24
22
20
18
16
14
12
10
VALSALVA
P < 0·01
1
2
3
0
5
10
15
1
2
3
4
5
BASELINE
VALSALVA (SECS)
RECOVERY (MINS)

2.12 THE EFFECTS OF SYSTEMIC AUTONOMIC NERVE STIMULATION ON THE RETINAL CIRCULATION

PAPER 25

Eye (1988) 2, 412–417

Retinal Circulation Responses to Systemic Autonomic Nerve Stimulation.

LUMINA P. LANIGAN, CHARLES V. CLARK, DAVID W. HILL

London

Summary

The retinal vessel calibre responses to controlled stimulation of the autonomic nervous system were studied in 10 healthy subjects, using sustained isometric muscle contraction as stimulus. Each subject was studied twice using different mydriatic agents, (1) g.tropicamide 1% a parasympatholytic agent and (2) g. phenylephrine 10% a sympathetic agonist. In the tropicamide study, there was a mean arteriolar constriction of 8.1% (SEM 1.67, $p<0.001$) and venule constriction of 3.7% (SEM 0.85, $p<0.001$) with a mean rise in diastolic blood pressure of 27.4 mmHg (SEM 2.95, range: 13-45 mmHg). When g.phenylephrine 10% was used, there was a mean arteriolar constriction of 8.6% (SEM 1.68, $p<0.001$) and venule constriction of 4.8% (SEM 1.22, $p<0.001$) with a mean rise in diastolic blood pressure of 29.2 mmHg (SEM 2.56, range: 17-44 mmHg). There was no significant difference in retinal vessel calibre in the recovery phase compared to baseline phase ($p>0.05$) or between the two mydriatic agents on vessel responses ($p>0.05$). There was no correlation between the rise in diastolic blood pressure and the degree of retinal vessel constriction, during handgrip contraction in either study. This study has demonstrated a significant association between retinal vessel calibre and systemic autonomic nerve stimulation. The possible mechanisms for the retinal vessel constriction observed in this study are discussed.

The retinal circulation is autoregulated[1] and therefore has the capability to maintain constant blood flow despite changes in perfusion pressure whether induced by changes in intraocular pressure or arterial blood pressure.[2-4] The mechanisms which control this autoregulation are poorly understood.[5]

The aim of the present study was to assess the responses of the retinal circulation to controlled stimulation of the autonomic nervous system in normal subjects. A number of well established tests based on cardiovascular reflexes allow accurate assessment of autonomic nerve function.[6-10] Sustained handgrip provides an isometric muscle contraction which provokes a sympathetic response with reflex rise in diastolic blood pressure.[9,11] This pressor response is presumed to be initiated by stimuli from the exercising muscle.[12] The rise in diastolic blood pressure is partly due to peripheral vasoconstriction secondary to activation of the alpha adrenergic receptors of the peripheral autonomic nervous system[13] and partly to a heart-rate dependent increase in cardiac output.[14] A diastolic blood pressure rise of ≤ 10 mmHg in response to sustained handgrip is abnormal, a rise of ≥ 16 mmHg is

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normal and the response is borderline if between 10 and 16 mmHg.[15]

Patients and Methods

After informed consent had been obtained, 10 healthy subjects were included in the study: their mean age was 30 years (range 23-35 years) and 6 were male. Each subject was studied seated at a fundus camera on two occasions: one pupil was dilated (whichever was preferred by the subject) with g.tropicamide 1% (a parasympatholytic agent) for the first study and g. phenylephrine 10% (a sympathetic agonist) for the second. Retinal vascular responses to sympathetic nerve stimulation were measured separately using pupillary dilating agents with different autonomic effects, to determine the effect of topical autonomic agonists on retinal vessel responses. All photographs were taken by the same observer using a wide angle Canon fundus camera (CF 60-S) which was triggered during the diastolic phase of the pulse cycle, using a finger-tip pulse detector coupled to an adjustable time delay. Five photographs were taken in quick succession at approximately one second intervals at each phase.

Baseline photographs were taken followed by 3 baseline measurements of blood pressure recorded from the non-exercising arm (Fig. 1) using an automatic sphygmomanometer (COPAL-UA 231). The subject was then instructed to perform 3 maximum handgrip contractions, using the dominant arm, to establish the maximum voluntary contraction (MVC). The duration of each maximal contraction lasted less than 2 seconds. A laboratory built strain guage handgrip dynamometer with continuous chart recording was used; it had an adjustable audible alarm set at 33% of each subject's MVC. The subject was then instructed to perform a sustained handgrip at 33% MVC for 2.5 minutes. During this exercise period 3 phases of fundus photographs were taken at 1, 2 and 2.5 minutes; three blood pressure measurements were recorded at 0.5, 1.5 and 2.5 minutes (Fig. 1). On recovery one phase of fundus photographs and a final blood pressure reading were taken at five minutes after release of sustained handgrip contraction. All photographs were recorded on Ilford FP4 film with a red free filter and developed in PQ Universal (1 +19) for 12 minutes at 20°C with sensitometric control.[16]

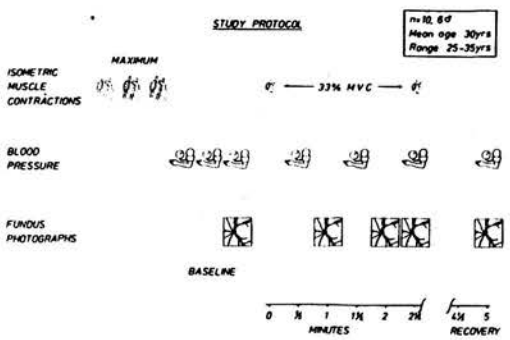


Fig. 1. *Protocol of handgrip study.*

Analysis:

The films were analysed using the Quantimet 800 Image Analyser (Cambridge Instruments). This technique has been developed in the Department of Ophthalmology, Royal College of Surgeons of England.[16] Seven–eighteen suitable vessel sites were measured in each subject comprising similar numbers of arterioles and venules nominally within the calibre range: 60 – 170μm (calculated on the basis of the Gullstrand Schematic Eye). A typical fundus photograph illustrating sites of measurement in one subject is shown in Figure 2. All calibre measurements of these vessel sites were stored on floppy disc for later retrieval and statistical analysis which included calculation of means and standard error of the means (SEM) and one way

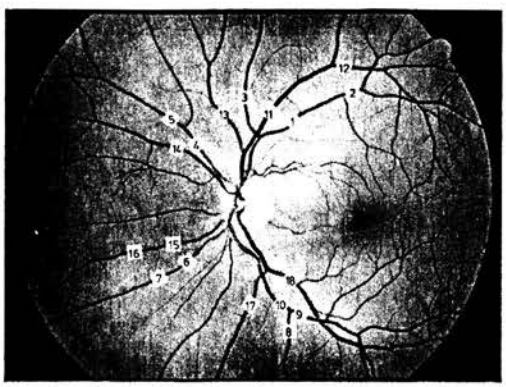


Fig. 2. *Typical fundus photograph with measurement sites.*

analysis of variance (A.N.O.V) within replications and between the phases for each site. Percentage change in mean retinal vessel calibre between phase 1 (pre-test) and phase 4 (following 2.5 minutes of sustained handgrip) for each subject was calculated; significance was assessed by the student t for paired data,2 tailed test.

Results

The individual results of variation in retinal vessel calibre during sustained isometric muscle contraction are shown in Tables I and II. Group mean values are as follows:

(i) Pupil dilation with g.tropicamide 1%

In response to sustained handgrip, arteriolar calibre (mean ± SEM) decreased by 8.1% ±1.67 ($p<0.001$) and venule calibre decreased by 3.7% ±0.85 ($p< 0.01$); diastolic blood pressure increased by 27.4 ±2.95 mmHg (range: 13 – 45 mmHg).

(ii) Pupil dilation with g.phenylephrine 10%

In response to sustained handgrip, arteriolar calibre decreased by 8.6% ± 1.68 ($p<0.001$) and venule calibre decreased by 4.5% ± 1.84 ($p<0.05$); diastolic blood pressure increased by 29.2 ± 2.56 mmHg (range: 17-44 mmHg).

There were no significant differences in retinal vessel calibre responses to sustained handgrip or diastolic blood pressure between the dilating agents ($p>0.05$). However inadequate mydriasis often resulted when g.phenylephrine 10% was used in subjects with dark irides. There were no significant

Table I *Variation in retinal vessel calibre during sustained isometric muscle contraction in 10 normal subjects, following pupil dilation with g.tropicamide 1% (Mean* +SEM).

Subject Number	*Variation in arterial calibre %*	*sig.*	*Variation in venule calibre %*	*sig*	*Rise in dias BP %, mmHg*
1	−8.10 ± 1.63	p<0.001	−0.50 ± 2.39	ns	28 23
2	−4.38 ± 2.85	ns	−7.14 ± 3.50	p<0.05	28 21
3	−14.42 ± 2.66	p<0.01	−1.50 ± 2.76	ns	17 13
4	−9.10 ± 4.45	p<0.01	−7.75 ± 1.74	p<0.01	40 27
5	−11.70 ± 1.04	p<0.001	−4.13 ± 1.02	p<0.01	36 27
6	−13.44 ± 2.38	p<0.001	−4.22 ± 1.40	p<0.05	42 33
7	−6.09 ± 1.35	p<0.05	0 ± 2.45	ns	35 23
8	−5.44 ± 0.87	p<0.001	−2.00 ± 1.13	ns	64 45
9	+3.25 ± 5.28	ns	−3.75 ± 5.71	ns	46 39
10	− 11.75 ± 3.04	p<0.01	−5.77 ± 2.24	p<0.05	32 23

Table II *Variation in retinal vessel calibre during sustained isometric muscle contraction in 10 normal subjects, following pupil dilation with g.phenylephrine 10%. (Mean +SEM)*

Subject Number	*Variation in arterial calibre %*	*sig.*	*Variation in Venule calibre %*	*sig.*	*Rise in Dias BP %,mmHg*
1	−5.60 ± 2.83	p<0.05	−0.28 ± 1.72	ns	31 26
2	−6.25 ± 4.85	ns	−6.14 ± 2.48	p<0.05	35 26
3	−14.11 ± 8.06	ns	−17.00 ± 9.65	ns	51 37
4	−3.10 ± 3.26	ns	−5.98 ± 1.48	p<0.01	43 30
5	−15.25 ± 1.33	p<0.001	−5.44 ± 3.00	ns	62 44
6	−14.00 ± 4.54	ns	−1.33 ± 2.51	ns	34 24
7	−7.63 ± 1.89	p<0.01	+0.85 ± 1.74	ns	25 17
8	+0.75 ± 4.28	ns	+4.00 ± 0.97	p<0.01	41 32
9	−7.71 ± 2.19	p<0.05	−7.40 ± 4.66	ns	44 35
10	−12.77 ± 2.33	p<0.001	−6.57 ± 2.64	p<0.05	26 21

changes in either retinal vessel calibre or blood pressure in the recovery phase compared to the control phase ($p>0.05$). The reduction in venule calibre during these studies was less than the arterioles but nevertheless statistically significant. There was no correlation between the rise in diastolic blood pressure and the degree of retinal vessel constriction in either the tropicamide study ($r=+0.51$, $p>0.10$) or the phenylephrine study ($r=-0.187$, $p>0.50$).

Individual vessel calibre changes at 18 sites (Fig. 2), in a typical subject are shown in Table III. This demonstrates the variability of the individual vessel responses at different sites during sustained isometric muscle contraction. In this subject there was an overall mean arteriolar constriction of 11.7% (SEM 1.04, $p<0.001$) and venule constriction of 4.1% (SEM 1.02, $p<0.01$) with a mean rise in diastolic blood pressure of 27 mmHg.

In the tropicamide 1% study a total number of 90 arteriolar sites were measured of which 58 showed significant change ($p<0.05$, one way A.N.O.V) and 81 venule sites (46 significant, $p<0.05$). In the phenylephrine 10% study a total of 81 arteriolar sites (22 significant, $p<0.05$) and 69 venule sites (19 significant $p<0.05$) were measured.

Figure 3 shows the percent change in mean vessel calibre and diastolic blood pressure for all subjects through phases 2-5 (sustained handgrip contraction), compared to the mean baseline values in phase 1.

Table III *Individual retinal vessel calibre changes during sustained isometric muscle contraction in a single subject.*

Subject No. 5 Age 29 G. Tropicamide 1%
Rise in diastolic BP − +27mmHg or +36%

		Percent change in vessel calibre (phase 4 compared with phase 1)
Arterial sites:		
	No 1	− 12
	2 *	− 13
	3 *	− 11
	4	− 16
	5	− 6
	6 *	− 14
	7	− 6
	8 *	− 14
	9 *	− 12
	10 *	− 13
Venule sites:		
	No 11	− 5
	12	− 5
	13	− 2
	14	− 5
	15	+ 1
	16	− 9
	17 *	− 5
	18	− 3

* significant sites A.N.O.V. ($p<0.05$)

Discussion

The present study has shown consistent responses of the retinal vasculature to controlled sympathetic nerve stimulation, resulting from a well established test, isometric muscle contraction produced by sustained handgrip. These responses are consistent

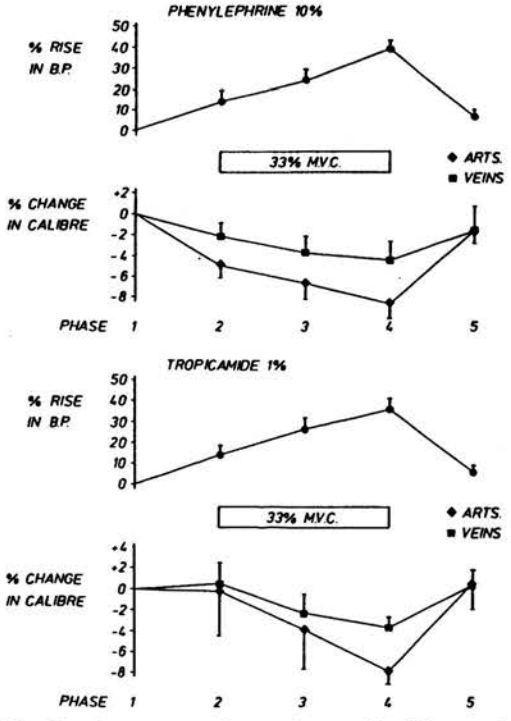


Fig. 3. *Percentage change in vessel calibre and diastolic blood pressure (mean ± SEM) in phases 2–5, compared with phase 1.*

with autoregulation, in the face of the rising diastolic blood pressure which accompanies sustained handgrip (Fig. 3), since reduction in retinal arteriolar calibre implies an increase in peripheral resistance, which theoretically varies inversely as the fourth power of the vessel radius, according to Poiseuille's Law. The relationship between brachial blood pressure and ophthalmodynamometric retinal artery pressure in population studies is well known, and the consistent relationship when blood pressure was raised by isometric exercise was demonstrated by Robinson et al.[2]

There is extensive evidence for autoregulation in the retinal circulation[3,4,17,18] including responses to acute rises in blood pressure produced by systemic infusions of angiotensin and noradrenaline;[19,20] though the latter experiments have shown varying results. Retinal vessel constriction in the present study occurred within 30 seconds of commencing the sympathetic stimulation and coincided with the rise in diastolic blood pressure. With sustained stimulation during the period of handgrip both retinal vessel response and diastolic blood pressure elevation increased (Fig. 3); this would suggest a temporal relationship between autonomic nerve stimulation and retinal vessel response. Vascular smooth muscle tone may be influenced in three ways: neurogenic, myogenic or humoral. As yet there is no evidence of autonomic innervation of the retinal vessels, forward of the lamina cribrosa.[21–23] The retinal arterioles near the optic disc have 5 to 7 layers of smooth muscle.[22] Russell[18] reported that a 16% increase in retinal arteriolar calibre occurred, immediately following reduction of retinal perfusion pressure, produced by compression of the globe with a spring dynamometer: similar results were obtained both in patients with Horner's Syndrome and normal subjects. He argued that these observations pointed to the improbability of involvement of the sympathetic nervous system; and suggested a myogenic response due to altered transmural pressure (Bayliss effect, [24]). There is however some uncertainty about the status of these patients since the degree and level of denervation was not defined.

In the sympathectomised eyes of cats Kaskel et al.[25] have shown that acute elevation of the blood pressure, provoked by ligation of the aorta, caused overperfusion of the retina and breakdown of the blood retinal barrier: Bill et al.[26] confirmed this and demonstrated that sympathetic stimulation under conditions of acute arterial hypertension prevented retinal overperfusion.

There are therefore, indications that regulation of the retinal blood flow in response to altered perfusion pressure, may be brought about by both myogenic and neurogenic means. In the present study, though the diastolic blood pressure rise and retinal artiolar constriction were contemporaneous (Fig. 3), there was no correlation between the magnitude of the blood pressure rise and the extent of the arteriolar constriction, suggesting that parallel but independent responses were occurring. Had the mechanism of constriction been solely myogenic, then a degree of correlation between blood pressure and retinal vessel response might be expected.

This study has demonstrated a significant association between retinal vessel calibre and systemic autonomic nervous stimulation. The exact role of the autonomic nervous system remains to be defined, however the present results suggest that autonomic effects are a significant integral feature in the autoregulation of retinal blood flow.

Grateful thanks are due to Mr. A. Crabtree, for photographic assistance and colleagues at Moorfields Eye Hospital who participated in the study. L. P. Lanigan holds a Smith & Nephew Fellowship in Ophthalmology. The project was supported by a grant from the Royal National Institute for the Blind. We thank Dr D. J. Ewing, University of Edinburgh, for advice relating to autonomic nerve function assessment.

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PAPER 26

TITLE: Reproducibility of retinal vessel responses to systemic autonomic nerve stimulation.

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RUNNING TITLE:

Autonomic retinal vascular responses.

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Summary

The reproducibility of retinal vessel responses to systemic autonomic nerve stimulation was studied in a group of 9 normal subjects (mean age 23.7 ± 1.61 years, range: 20 to 32 years; 8 were male). Each subject was studied twice under similar experimental conditions. Retinal vessel calibre changes during 33% maximum handgrip contraction were measured using the Quantimet Image Analyser; vessel calibre measurements were taken from identical retinal vessel sites in each subject during both tests.

There was no significant difference in both the group mean arteriolar responses between test 1 and test 2 (−6.4 ± 1.84%; −5.5 ± 1.00%) and the group mean venule responses (−4.6 ± 0.71%; −3.5 ± 0.78%); $p>0.10$. Although there was variation in mean retinal vessel responses for the 9 subjects between test 1 and test 2, there was no significant difference between the tests in 8 of the 9 subjects. The mean rise in diastolic blood pressure provoked by the sustained handgrip contraction for the group was 22.11 ± 1.54 mmHg (range: 16 to 30 mmHg) for test 1 and 22.44 ± 1.26 mmHg (range: 15 to 27 mmHg) for test 2; there was no significant difference between these two responses.

Mean retinal vessel responses to sustained handgrip contraction are therefore reproducible and may be utilised effectively in either comparisons of different groups of subjects, or single individuals on different occasions.

Key words:

Autonomic nerve function tests, Systemic autonomic nerve stimulation, retinal autoregulation, retinal vessel calibres.

Reproducibility of retinal vessel responses to systemic autonomic nerve stimulation

Introduction

Maintenance of constant blood flow within the microcirculation by autoregulation is a well-recognised feature (Johnson, 1960) and has been demonstrated in the retinal circulation (Riva, Sinclair and Grunwald, 1981), however, the mechanisms by which this homeostatic autoregulation are controlled have not been defined (Ernest, 1980).

Myogenic, humoral and neurogenic effects have been proposed - both individually and synergistically - as primary determinants of retinal blood flow. Recently a significant association between retinal vessel calibre and systemic autonomic nervous stimulation has been demonstrated in normal subjects (Lanigan, Clark and Hill, 1988). In response to controlled stimulation of the sympathetic nervous system - by sustained isometric contraction of the forearm muscles (Ewing, Irving, Kerr, Wildsmith and Clarke, 1974) - vasoconstrictive responses of the retinal vasculature have been observed, with mean arteriolar constriction of 8.1% and a mean venule constriction of 3.7% in a group of 10 young healthy subjects. The results suggested that autonomic effects were a significant integral feature in the autoregulation of retinal blood flow.

The aim of this study was to assess the reproducibility of retinal vessel responses to systemic autonomic nerve stimulation by sustained handgrip, to establish the degree of intra- and inter-individual variation.

Patient and methods.

Reproducibility of retinal vessel responses to systemic sympathetic nerve stimulation was assessed in 9 healthy volunteers from the hospital medical staff (mean age 23.7 ± 1.61, range 20 to 32 years). The male : female ratio was 8:1. Informed consent was obtained from all subjects prior to participating in the study.

Each subject was studied seated at the Canon fundus camera (CF 60-S) with one pupil dilated with g.tropicamide 1%. All photographs were taken by the same observer using the Canon fundus camera which was triggered during the diastolic phase of the pulse cycle, using a finger-tip pulse detector coupled to an adjustable time delay. Five photographs were taken in quick succession at approximately one second intervals at each phase of the investigation. Baseline photographs were taken followed by 3 baseline measurements of blood pressure recorded from the non-exercising arm using an automatic sphygmanometer (COPAL-UA 231). The subject was then instructed to perform 3 maximum handgrip contractions using the dominant arm, to establish the maximum voluntary contraction (MVC). The duration of each maximal contraction lasted less than 2 seconds. A laboratory built strain gauge handgrip dynamometer with continuous chart recording was used; it had an adjustable audible alarm set at 33% of each subjects MVC. The subject was then instructed to perform a sustained handgrip at 33% MVC for 2.5 minutes. During this exercise period 3 phases of fundus photographs were taken at 1, 2 and 2.5 minutes; three blood pressure measurements were recorded at 0.5, 1.5 and 2.5 minutes. On recovery one phase of fundus photographs and a final

blood pressure reading were taken at five minutes after release of sustained handgrip contraction.

Each subject was studied twice on the same day; each session was separated by at least half an hour. Care was taken to ensure that conditions for both tests were similar for all subjects:

1. the same fundus was studied on each occasion; the pupil (whichever was preferred by the subject) was dilated with g. tropicamide 1%.
2. the same arm (the dominant arm) was used to maintain the handgrip contraction in each test.
3. the same level of 33% maximum handgrip contraction was used for each test; the first reading was used as the standard for the two tests.
4. fundus photographs were taken by the same observer for each individual and individual pairs of films were processed together.
5. the same retinal vessel sites (range 9 to 15 sites) were measured during both tests for each subject. All vessel calibre measurements were made on a double-masked basis by a single observer.

All photographs were recorded on Ilford FP4 film with a red free filter and developed in Universal PQ (1+19) for 12 minutes at 20 C with sensitometric control.

The films were analysed using the Quantimet 800 Image analyser (Cambridge Instruments). 9 to 15 suitable vessel sites were measured in each subject comprising similar numbers of arterioles and venules. A typical fundus photograph illustrating sites of measurement in a subject is shown in Figure 3. All calibre measurements of these vessel sites were stored on floppy disc for later retrieval and statistical analysis.

Percentage calibre change for each vessel site, for each subject in each test was calculated from the control phase (pre-test) to phase 4 (peak handgrip). The mean percentage calibre change for arterioles and venules for each subject individually was then calculated (Tables 1 and 2); the one sample t-test was used to assess significance of the differences in individual mean vessel responses between the control phase and phase 4. The repeatability of the test was assessed by:

1. comparing the mean vessel responses in test 1 with test 2 for each individual using the 2-tailed student t-test
2. comparing the group mean vessel responses in test 1 and test 2 for all 9 subjects, using the 2-tailed student t-test.
3. assessing the degree of correlation between the retinal vessel responses and blood pressure responses in test 1 and test 2.

Statistical significance was taken as $p<0.05$ for all analyses.

Results.

1. Arteriolar responses:

The group mean arteriolar responses (mean ± SEM) for test 1 and 2 were −6.14 ± 1.84% and −5.5 ± 1.00% respectively; there was no significant difference between these group mean responses ($0.5>p>0.2$). While the group mean difference between the two tests was only −0.92 ± 0.81%, the individual mean arteriolar responses for test 2 varied from +3.8% to −6.8% compared to test 1. However there was no statistically significant difference in individual mean arteriolar response between the tests in 8 of the 9 subjects. The individual mean arteriolar responses for the two tests are shown in Table 1. There was a significant correlation between the individual mean arteriolar responses for the two tests: $r= +0.833$; $p<0.01$; Figure 1.

2. Venule responses:

The group mean venule responses (mean ± SEM) for test 1 and test 2 were −4.6 ± 0.71% and −3.5 ± 0.78% respectively; there was no significant difference between these group mean venule responses ($p>0.10$). The group mean differences between the two tests was −1.2 ± 0.92%. Individual mean venule responses between the two tests varied from +6.6% to −6.6%, but the difference was not statistically significant in 7 out of the 9 subjects. The individual mean venule responses for the two tests are shown in Table 2. There was no correlation between the individual venule responses for test 1 and test 2: $r = -0.36$; $p>0.10$; Figure 2.

3. Individual variation:

Differing degrees of retinal vessel responses were seen at the same sites during the two tests in each individual. Table 3 demonstrates this variation in subject no. 1 who had calibre changes measured in 8 arteriolar and 7 venule sites (Figure 3). The mean rise in diastolic blood pressure differed minimally during the two tests: +18 mmHg in Test 1 and +21 mmHg in Test 2.

4. Blood pressure responses:

All 9 subjects in the study had a normal pressor response to sustained handgrip in both tests: Tables 1 and 2. The mean diastolic blood pressure rise for the group during test 1 was 22.11 ± 1.54mmHg (range: 16 - 30 mmHg), and 22.44 ± 1.26 mmHg (range: 15 - 27 mmHg) for test 2. There was no significant difference between the pressor responses in both tests, $p>0.05$.

There was no correlation between the retinal vessel responses and diastolic blood pressure elevation for either test 1 (arterioles, $r = +0.14$, $p>0.50$; venules, $r = +0.01$, $p>0.50$), or test 2 (arterioles, $r = -0.24$, $p>0.50$; venules, $r = -0.36$, $p>0.10$).

Discussion.

Assessment of the reproducibility of retinal vessel responses to systemic autonomic stimulation by sustained handgrip is inevitably limited by independent variables which cannot be standardised: initial retinal vessel calibres and diastolic blood pressure responses to the controlled stimulus. Elevation of diastolic blood pressure during the two tests of retinal vessel response to sustained handgrip contraction was not identical in each individual varying by −7 mmHg to +6 mmHg. Although isometric muscle contraction was maintained at a controlled level of 33% MVC, the resultant stimulus due to blood pressure elevation differed slightly between the two tests at individual subject level. Nevertheless there was no significant difference in the group means either of retinal vessel calibre change or of diastolic blood pressure rise for the two tests as shown by the paired t-test. Examination of the results shows a contemporaneous association between the differences in diastolic blood pressure and retinal vascular constriction in the two tests, however this was not statistically significant ($r = +0.55$, $p>0.05$). Deductions regarding reproducibility of retinal vessel responses to a standardised sympathetic stimulus are therefore restricted by the inability to maintain a reproducible level of blood pressure elevation. It is interesting that mean retinal arteriolar and venule constrictive responses during test 2 were decreased by an approximately equivalent degree (0.92% and 1.2% for arterioles and venules respectively). Although this difference was not statistically significant, impairment of the vasoconstrictive response in the second

test suggests the development of fatigue in the retinal vascular reflex response to this stimulus.

The variability of retinal vessel responses at different vessel sites within the individual (Table 3) is not particularly surprising as each vessel site has individual characteristics: initial vessel calibre, amount of smooth muscle (Hogan and Feeney, 1963) and degree of autonomic innervation (Furukawa, Nomura and Kurumoto, 1984; Forster, Ferrari-Dileo and Anderson, 1987; Ruskell, 1972). The range of normal responses to sustained handgrip contraction is likely to preclude defining a single observation of a single vessel site in one individual as abnormal, however there were no significant differences between the mean arteriolar and venule responses for test 1 and test 2 in 8 of the 9 subjects. Furthermore group mean vessel responses between test 1 and 2 were not significantly different, suggesting that repeated estimates of mean retinal vessel responses are reproducible, both within a single individual and in a group of subjects.

The results of this study imply that mean retinal vessel vasoconstrictive responses to controlled systemic sympathetic nerve stimulation (by sustained handgrip contraction) demonstrate a statistically acceptable level of reproducibility and that this test may therefore be effectively utilised in comparisons either of different groups of subjects, or of single individuals at different times.

Acknowledgements.

We thank colleagues at Moorfields Eye Hospital who participated in this study. The project was supported by a grant from the Royal National Institute for the Blind. L.P.Lanigan held a Smith & Nephew Fellowship in Ophthalmology.

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Table 1.

Individual mean percentage arteriolar calibre change and diastolic BP rise during sustained handgrip contraction for Test 1 and 2.

Subj.No.	% arteriolar calibre change				Diast BP rise
	Test 1	(sig)	Test 2	(sig)	mmHg
1	-3.8%	$p<0.05$	-6.9%	$p<0.01$	+21
2	-3.6%	$p<0.05$	-3.1%	$p<0.02$	+15
3	-6.8%	$p<0.01$	-3.8%	ns	+24
4	-4.0%	ns	-3.4%	$p<0.01$	+20
5	-2.5%	ns	-6.3%	$p<0.05$	+26
6	-1.5%	ns	-1.9%	ns	+27
7	-18.0%	$p<0.05$	-12.0%	ns	+24
8	-5.0%	$p<0.05$	-4.9%	ns	+25
9	-11.6%	$p<0.05$	-7.0%	$p<0.01$	+25

Diast BP : diastolic blood pressure

sig : significance

ns : not significant (one sample t-test).

Table 2.

Individual mean percentage venule calibre change and diastolic BP rise during sustained handgrip contraction for Test 1 and 2.

Subj.No.	% venule calibre change				Diast BP rise
	Test 1	(sig)	Test 2	(sig)	mmHg
1	-4.3%	$p<0.02$	-3.3%	ns	+18
2	-5.6%	$p=0.05$	+0.3%	ns	+16
3	-4.0%	ns	-4.8%	ns	+30
4	-8.6%	$p<0.001$	-3.9%	$p<0.01$	+25
5	-1.0%	ns	-7.6%	$p<0.01$	+19
6	-2.6%	ns	-1.1%	ns	+27
7	-5.8%	ns	-2.4%	ns	+20
8	-4.6%	ns	-5.4%	ns	+24
9	-5.3%	ns	-3.0%	ns	+20

Diast BP : daistolic blood pressure

sig : significance

ns : not significant (one sample t-test).

Table 3

Retinal vessel responses at each site for subject no.1 during test 1 and test 2 (15 sites measured).

	TEST 1	TEST 2	mean diast BP rise
Arteriole Site No.	-4%	-2%	Test 1: +18 mmHg
	-1%	+1%	Test 2: +21 mmHg
	-1%	-4%	
	-9%	-3%	
	-15%	+2%	
	-6%	-7%	
	-5%	-10%	
	-14%	-7%	
mean	-3.8%	-6.9%	
Venule Site No.	-6%	-8%	
	-10%	-9%	
	0%	-6%	
	+1%	0	
	-3%	-3%	
	-2%	-2%	
	-3%	-2%	
mean	-4.3%	-3.3%	

LEGENDS:

Figure 1: Correlation between individual mean retinal arteriolar responses in Test 1 and Test 2.

Figure 2: Correlation between individual mean retinal venule responses in Test 1 and Test 2.

Figure 3: Fundus photograph of a subject (No 1) showing 8 arteriolar and 7 venule sites selected for calibre measurement.

Figure 1

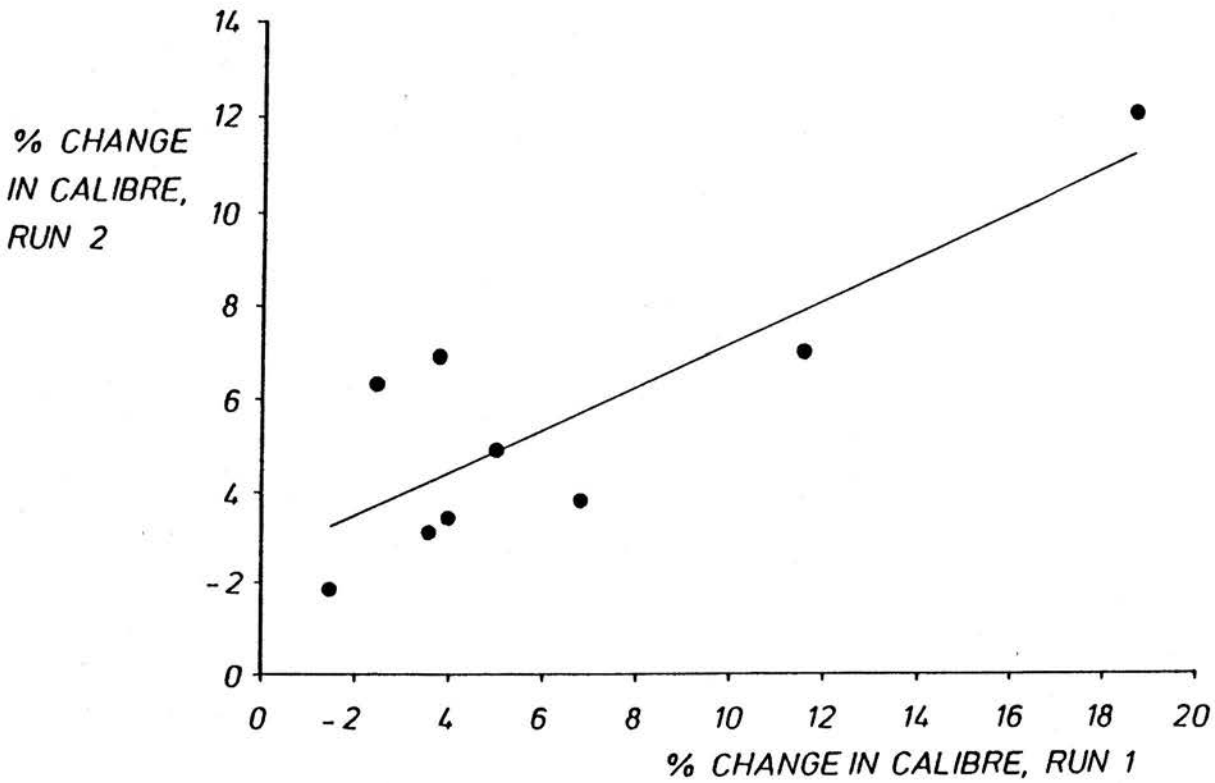

% CHANGE IN CALIBRE, RUN 2
14
12
10
8
6
4
-2
0
0
-2
4
6
8
10
12
14
16
18
20
% CHANGE IN CALIBRE, RUN 1

Figure 2

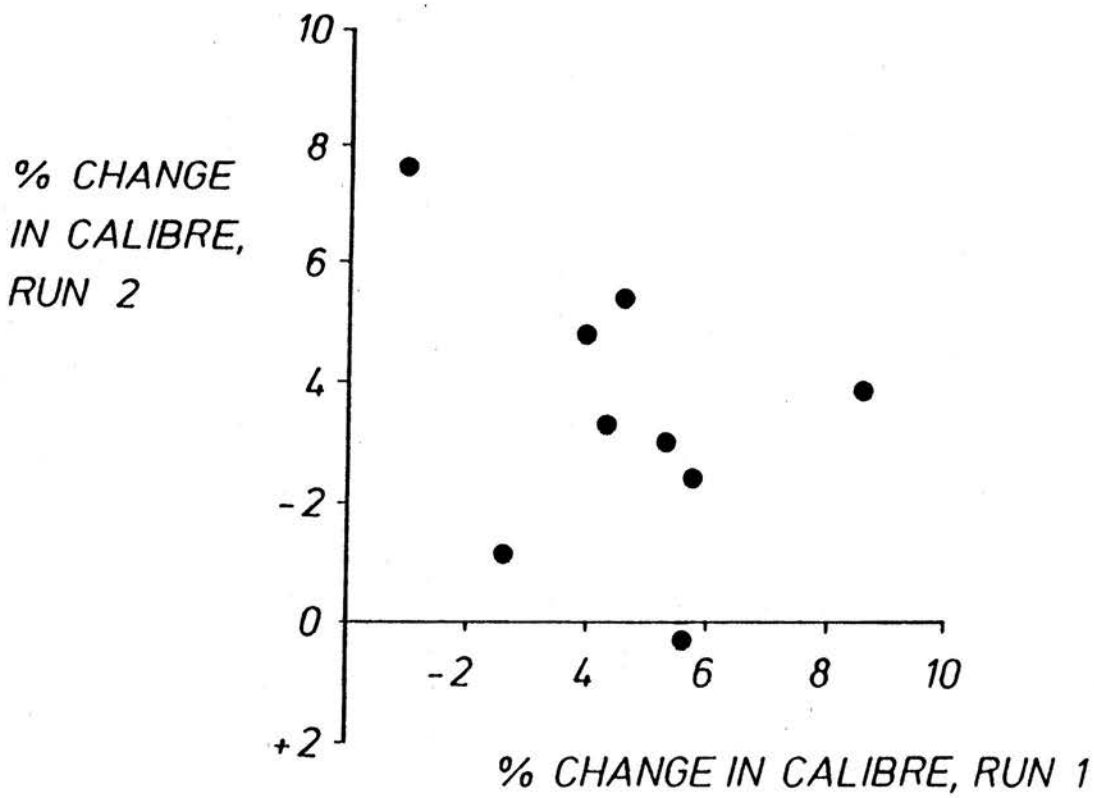

% CHANGE IN CALIBRE, RUN 2
10
8
6
4
-2
0
+2
-2
4
6
8
10
% CHANGE IN CALIBRE, RUN 1



Figure 3

PAPER 27

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Responses of the Retinal Circulation to Systemic Autonomic Stimulation in Diabetes Mellitus

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Summary

The retinal vessel calibre responses to systemic sympathetic stimulation, were studied in 22 randomly selected diabetic patients (mean age ± SEM: 54.7 ± 2.59 years, range 25-73; 13 IDDM, 9 NIDDM; 4 females), using sustained isometric muscle contraction as the stimulus. At a different session the integrity of the autonomic nerve function in these diabetic patients was assessed using 3 standard tests of autonomic nerve function, based on cardiovascular reflexes. Diabetic patients with an intact autonomic nervous system: Group 1, (n=11, mean age: 54.9 ± 4.55 years, 7 IDDM 4 NIDDM) showed a mean arteriolar constriction of 9.2% (SEM 2.89, $p<0.01$) and a mean venule constriction of 5.1% (SEM 1.73, $p<0.02$), for a mean rise in diastolic blood pressure of 23.7 mmHg (SEM 2.19 range: 13-33). There were no significant mean retinal vessel responses however, in diabetics with autonomic dysfunction (Group 2): mean arteriolar constriction of 1.2% (SEM 1.38 $p>0.05$) and venule constriction of 2.1% (SEM 1.38, $p>0.05$); for a mean rise in diastolic blood pressure of 19.8 mmHg (SEM 4.49, range: 2-50). There was no correlation between the rise in diastolic blood pressure and the retinal arteriolar constriction in the 2 groups (Group 1: $r=0.45$, $p>0.1$ and Group 2: $r=0.56$, $p>0.05$). Duration, type and control of diabetes were not significantly different between the 2 groups. The severity of retinopathy was slightly worse in Group 2 compared to Group 1. These results point to an association between autonomic neuropathy and failure of regulation of retinal blood flow.

Autoregulation of the microcirculation is a well recognised phenomenon[1] and has been demonstrated in the retinal circulation.[2] Neurogenic, myogenic and humoral factors may be involved in the homeostatic maintenance of the retinal blood flow during changes in perfusion pressure, however, the definitive mechanism has not been established. Recently, a significant association has been demonstrated between retinal vessel calibre and systemic autonomic nerve stimulation, with consistent responses of the retinal vasculature to generalised sympathetic nerve stimulation.[3] These results are particularly relevant to patients with diabetes, as diabetes mellitus is the commonest cause of autonomic neuropathy in the United Kingdom;[4] 20-40% of diabetics have evidence of autonomic nerve dysfunction on initial presentation.[5,6] Several studies have shown a significant association between autonomic nerve dysfunction and retinopathy in patients with diabetes mellitus.[7–9] Cardiovascular autonomic neuropathy has been demonstrated in 75% of diabetic patients with proliferative retinopathy[7] and ocular autonomic neuropathy (involving the anterior segment of the eye) in 57% of this

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group.[8] The aim of this study was to assess the responses of the retinal circulation on systemic autonomic stimulation in patients with diabetes mellitus, using sustained handgrip as a test of sympathetic nerve function.[10] The cardiovascular responses to sustained handgrip are reflex in nature[11] and are thought to be initiated by stimuli from the exercising muscle.[13] There is a rise in blood pressure, mediated partly by a heart rate dependent increase in cardiac output[14] and partly by peripheral vasoconstriction mediated via alpha adrenergic receptors of the peripheral autonomic nervous system.[12]

Patients and Methods

Twenty-two diabetic patients, (mean ± standard error of the mean (SEM) age: 54.7 ± 2.59 years; range: 25-73 years; 4 females) were included in the study following informed consent: 13 Insulin dependent diabetics (IDDM) (mean age 51.7 ± 3.97 years) and 9 non insulin dependent diabetics (NIDDM) (mean age 59.1 ± 2.23 years). These patients were subdivided into two equal groups based on the results of autonomic function assessment as defined by Ewing *et al.*, 1986 (Table I).[15] Group 1 consisted of 11 patients with no evidence of autonomic nerve dysfunction (mean age 54.9 ± 4.55 years): 7 IDDM (mean age 51.0 ± 6.66 years) and 4 NIDDM (mean age 61.8 ± 3.12 years); Group 2 comprised 11 patients with autonomic dysfunction (mean age 54.5 ± 2.77 years), 6 IDDM (mean age 52.5 ± 4.48 years) and 5 NIDDM (mean age 57.0 ± 3.08 years). The mean duration of diabetes in this series was 12.2 ± 1.83 years; mean duration of diabetes in patients with autonomic dysfunction (Group 2: 13.2 ± 2.77 years) was not significantly different from diabetics with no evidence of autonomic dysfunction (Group 1: 11.3 ± 2.50 years, $0.1>p>0.05$). Long term control of diabetes was assessed by glycosylated haemoglobin estimation in all subjects.

Autonomic nerve function and retinal vasculature responses to autonomic stimulation were assessed by the following methods:

Autonomic Nerve Function:

(i) Immediate heart rate response to standing.[15] The heart rate was determined from a continuously recorded electrocardiograph, and the ratio of the longest R-R interval around the 30th beat after standing to the shortest R-R interval around the 15th beat calculated (30:15 ratio).

(ii) Systolic blood pressure response to standing.[15] This test is performed by measuring the blood pressure in the supine position and after 2 minutes standing. The difference in systolic blood pressure is taken as the measure of the postural blood pressure change.

(iii) Diastolic blood pressure response to sustained handgrip.[10]
3 baseline blood pressures were recorded from the non-exercising arm. Each patient then performed a sustained handgrip using the dominant arm, at 33% maximum voluntary contraction for 2.5 minutes. A blood pressure read-

Table I *Normal, borderline and abnormal values for cardiovascular autonomic function tests (DJ Ewing et al, 1986).*

	NORMAL	BORDERLINE	ABNORMAL
Immediate heart rate response to standing (30:15 ratio).	≥ 1.04	1.01 – 1.03	≤ 1.00
Blood pressure response to standing (fall in systolic BP)	≤ 10 mmHg	11 – 29 mmHg	≥ 30 mmHg
Blood pressure response to sustained handgrip (rise in Diastolic BP)	≥ 16 mmHg	11 – 15 mmHg	≤ 10 mmHg

ing was taken just before release of the handgrip. The rise in diastolic blood pressure to sustained handgrip was taken as the difference between the diastolic blood pressure after 2.5 minutes of sustained contraction and the mean baseline diastolic blood pressure.

Retinal Circulation Responses to Systemic Autonomic Stimulation

Each patient was studied seated at the fundus camera: one pupil was dilated (whichever was preferred by the subject) with g. tropicamide 1% and g. phenylephrine 10%. All photographs were taken by the same observer using a wide angle Canon camera (CF 60-S), which was triggered during the diastolic phase of the pulse cycle. Five photographs were taken in quick succession, at approximately one second intervals at each phase. Baseline photographs were taken, followed by three baseline blood pressure measurements recorded from the non-exercising arm using an automatic sphygmomanometer (COPAL - UA 231). The subject was then instructed to perform three maximum handgrip contractions using the dominant arm to establish their maximum voluntary contraction (MVC). A laboratory-built strain gauge dynamometer with continuous chart recording was used with an adjustable audible alarm, set to alarm below 33% of each patient's MVC. The patient was then instructed to perform a sustained handgrip at 33% MVC for 2-2.5 minutes; three blood pressure measurements were recorded at 0.5, 1.5 and 2.5 minutes and three phases of fundus photographs were taken at 1, 2 and 2.5 minutes. On recovery one final phase of fundus photographs and one blood pressure reading were taken at five minutes after release of handgrip contraction. All photographs were recorded on Ilford FP4 film, with a red free filter and developed in PQ Universal (1+19) for 12 minutes at 20°C with sensitometric control.[16]

All films were analysed using the Quantimet 800 Image analyser (Cambridge Instruments). This technique has been established in the department (R.C.S).[16] Ten to eighteen suitable vessel sites were selected in each subject comprising similar numbers of arterioles and venules nominally within the calibre range: 64-174 μm, calculated on the basis of the Gullstrand Schematic Eye. A typical fundus photograph illustrating sites of measurement is shown in Figure 1.

Vessel calibres at these sites were measured and the means, standard error of the means (SEM) and one way analysis of variance (ANOV) for each site, within replications and between the phases, were calculated. Percentage change in mean retinal vessel calibre between phase 1 (pre-test) and phase 4 (following 2.5 minutes of handgrip) for each subject was calculated; significance was assessed by the student t test for paired data (2 tailed).

Least significant differences between phase means, at those sites where a significant ANOV was demonstrated, were calculated by applying the 't' distribution to the within phase replication variance.[16]

Results:

The results of autonomic function assessment and retinal vessel responses to systemic autonomic stimulation for each individual patient in Groups 1 and 2 are shown in Tables II and III respectively.

Group 1: Diabetic patients with normal autonomic nerve function

In response to sustained handgrip, arteriolar calibre (mean ± SEM) decreased by 9.2 ± 2.89%, $p<0.01$ and venule calibre decreased by 5.1 ± 1.73%, $p<0.02$; diastolic blood pressure increased by 23.7 ± 2.19 mmHg (range: 13-33 mmHg). Mean variations in retinal vessel calibre and diastolic blood pressure for each subject in Group 1 are shown in Figure 2.

No significant correlation was present between the elevation of diastolic blood pressure and retinal vessel constriction (Table III, $r=0.45$; $p>0.1$.)

ii. *Group 2: Diabetic patients with autonomic dysfunction.*

In response to sustained handgrip, arteriolar calibre decreased by 1.2 ± 1.38% and venule calibre decreased by 2.1 ± 1.38%, neither change being significant at the 5% level. Diastolic blood pressure increased by 19.8 ±

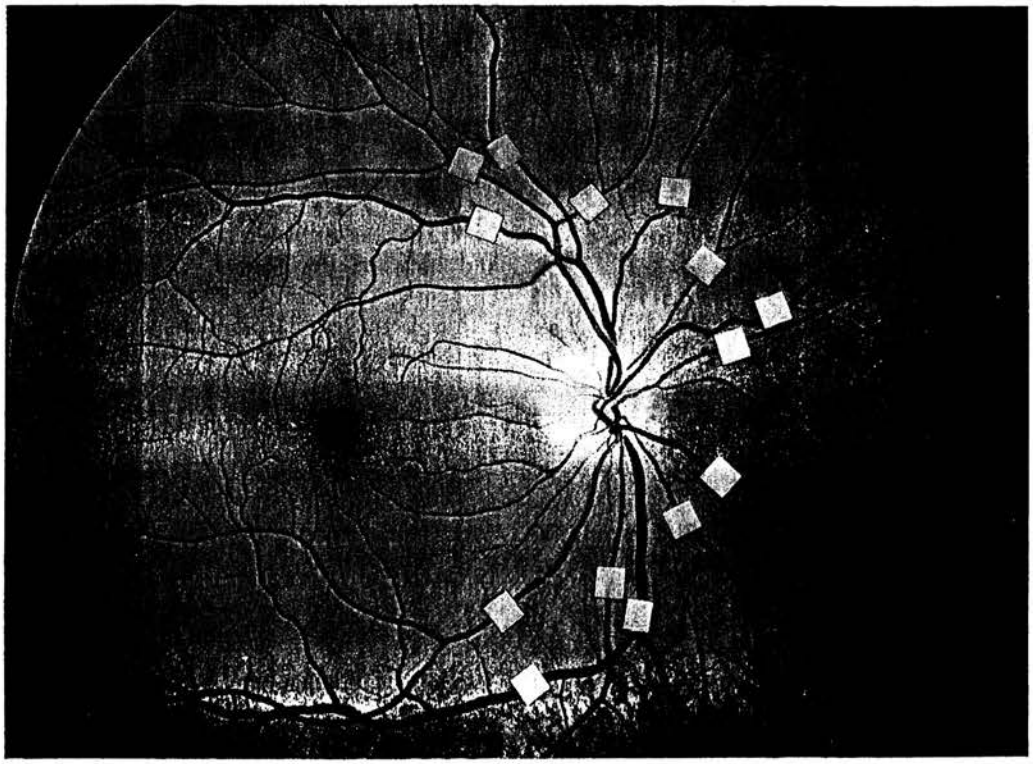


Fig. 1. *Typical fundus photograph with selected sites of measurement.*

Table II *Group 2: Diabetics with autonomic dysfunction*

Subj. no.	*sex*	*age*	*diab type*	*duration of diab.*	*Retinop. grade*	*autonomic nerve function tests*			*no. of abn/ borderline tests*	*mean retinal vessel change %*	
						30:15	*SBP L/S*	*DBP HG*		*Art*	*Ven*
1	M	54	IDDM	29yrs	1	1.03	−40	+7	2/*1	−1	−8
2	M	67	NIDDM	25yrs	2	1.00	0	+12	1/*1	−3	−8
3	M	60	NIDDM	5yrs	2	1.06	−30	+18	1	+2	+0.3
4	F	56	NIDDM	6yrs	2	0.85	+29	+12	1/*1	−4	−4
5	F	53	NIDDM	11yrs	3	1.08	−20	+2	1/*1	−11	−8
6	M	49	NIDDM	9yrs	0	1.06	−35	+37	1	+2	+2
7	M	71	IDDM	12yrs	2	1.00	−42	+23	2	+7	0
8	M	56	IDDM	1yr	0	0.83	+25	+17	1	−3	−0.2
9	M	42	IDDM	25yrs	3	1.00	0	+34	1	−2	−4
10	M	41	IDDM	9yrs	1	1.00	−20	+50	1/*1	+2	+2
11	M	51	IDDM	13yrs	2	0.95	−30	+6	3	−2	+2

*: borderline results for autonomic nerve function tests.
SBP L/S: postural change in systolic BP from lying to standing
DBP HG : rise in diastolic blood pressure to sustained handgrip.

Table III *Group 1: Diabetics with intact autonomic nervous system*

Subj. no.	*sex*	*age*	*diab type*	*duration of diab.*	*Retinop. grade*	*autonomic nerve function tests*			*no. of abn/ borderline tests*	*mean% retinal vessel change*	
						30:15	*SBP L/S*	*DBP HG*		*Art*	*Ven*
1	M	71	NIDDM	7yrs	0	1.18	+20	+15	*1	−19	−6
2	M	58	NIDDM	4yrs	0	1.19	+8	+28	0	−4	−3
3	M	60	NIDDM	2yrs	0	1.18	−10	+20	0	−9	−3
4	M	58	NIDDM	2yrs	0	1.17	−15	+30	*1	−4	−2
5	M	67	IDDM	25yrs	2	1.18	−4	+33	0	−1	−5
6	M	60	IDDM	14yrs	0	1.23	−5	+29	0	−8	−4
7	F	55	IDDM	17yrs	1	1.23	−4	+29	0	−6	−3
8	M	25	IDDM	25yrs	0	1.20	−6	+17	0	−33	−22
9	M	73	IDDM	6yrs	1	1.15	+2	+17	0	+2	−3
10	M	43	IDDM	9yrs	1	1.19	−5	+30	0	−10	−3
11	M	34	IDDM	13yrs	3	1.16	+2	+13	*1	−9	−2

*: borderline results for autonomic nerve function tests
SBP L/S: postural change in systolic blood pressure from lying to standing
DBP HG : rise in diastolic blood pressure to sustain handgrip

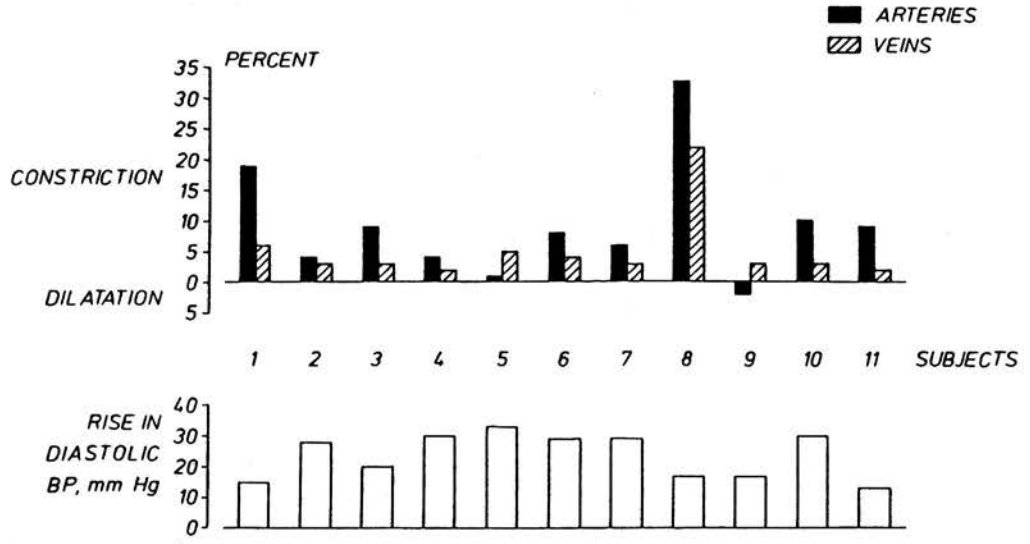


Fig. 2 *Change in mean retinal vessel calibre and the rise in diastolic blood pressure for each diabetic in Group 1, from control to 2.5 minutes handgrip contraction.*

4.49 mmHg (range: 2-50 mmHg). Mean variations in retinal vessel calibre and diastolic blood pressure for each subject in Group 2 are shown in Figure 3.

No significant correlation was present between the elevation in diastolic blood pressure and retinal vessel constriction (Table II, $r=0.56$; $0.1>p>0.05$).

Mean arteriolar calibre changes in response to sustained handgrip were significantly lower in diabetics with autonomic dysfunction than in those with intact autonomic nerve function (t test difference of means, $0.05>p>0.02$). There was no significant difference in venule calibre changes between the 2 Groups ($p>0.1$).

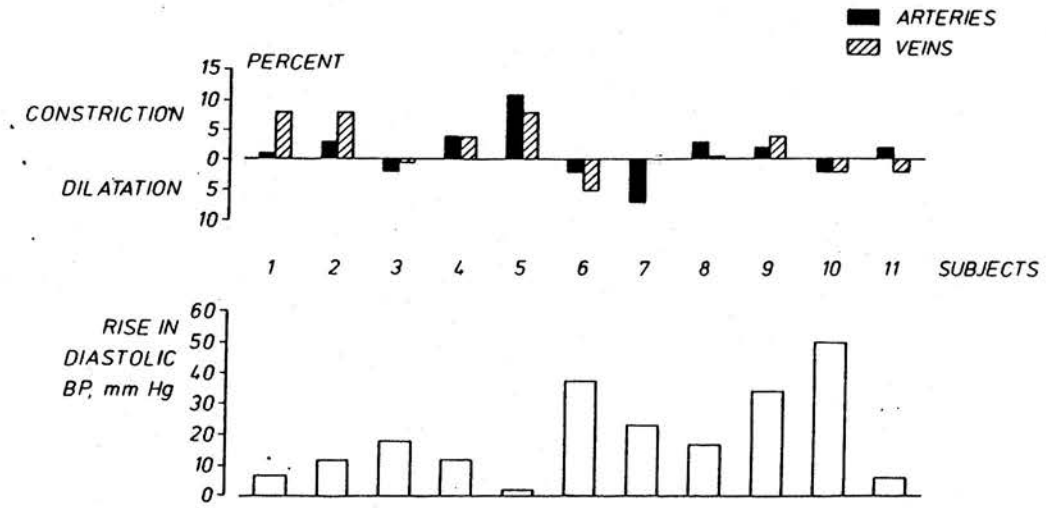


Fig. 3 *Change in mean retinal vessel calibre and the rise in diastolic blood pressure for each diabetic in Group 2, from control to 2.5 minutes handgrip contraction.*

iii. *Individual results:*
Background variance is an inevitable feature of a multistage measurement process as used in this study to assess retinal vessel calibre changes: this varies from site to site depending largely upon the clarity of the image; the use of replication photographs permits an evaluation of the variance. In all, 90 arterial sites in Group 1 and 88 in Group 2 were measured and the spread of the within replication variances taken from the individual site analyses of variances examined. One subject No.4, Group 2 (mean calibre change: arterial −4%, venous −4%) had markedly less consistent measurements over all 10 sites, due to photographic difficulties and was excluded from this calculation. Of the remaining 21 subjects the individual site within replication variances, transposed in terms of percent calibre change, gave a cumulative frequency distribution of the least significant differences (LSD) between the phase means, at the 95% level, shown in Table IV. The cumulative frequency distributions for the two groups of diabetic subjects (with exclusion of subject 4, Group 2) were very similar.

These results indicated the need in this study for relatively large calibre changes in order to attain significant individual differences, however significant calibre constrictions occurred between phase 1 (control) and phase 4 (last photographs during handgrip) in 25 of the 90 sites in Group 1, but only 8 of the 88 sites in Group 2, thus confirming the impression of reduced arteriolar response in Group 2 which is conveyed by the subject mean arteriolar calibre changes (Tables II, III).

iv. *Handgrip performance.*
The subject maximum handgrip tests showed a similar strength of grip in both groups (Group 1, mean 45.2, standard deviation (SD) ±12.8; Group 2, mean 57.0, SD±15.1, arbitrary units, strain gauge recording). The time weighted mean grip force during the test was well maintained in both groups, being +11.5%, SD±7.2 and +10.7%, SD±12.6 in groups 1 and 2 respectively, above the target level (33% MVC). In no instance did the time weighted mean fall significantly below the target level, the lowest level being −1%.

Table IV *Least Significant Differences at 95% Probability Level*

Individual arteriolar sites	
Difference interval %	*cumulative frequency %*
1 – 10	46
11 – 20	77
> 20	100

v. *Diabetic retinopathy.*
The incidence of diabetic retinopathy was determined by ophthalmoscopy at the time of pupil dilatation for calibre studies, assisted by inspection of the calibre measurement photographs; results were expressed in 4 categories:

0 ... no retinopathy
1 ... mild background retinopathy (<10 haemorrages)
2 ... severe background retinopathy (>10 haemorrages and/or exudates
3 ... Proliferative retinopathy.

Findings for the two groups are shown in Table V. The numbers are too small for statistical testing but the trend suggests a greater severity in Group 2.

vi. *Glycosylated haemoglobin*
There were no significant differences in glycosylated haemoglobin (mean ± SEM) between patients in Group 1 (8.0 ± 0.76%) and Group 2 (9.3 ± 0.55%).

Discussion

The retinal vessels have an intrinsic ability to dilate or constrict in order to maintain constant blood flow to the retina. Sustained handgrip causes a significant elevation in diastolic blood pressure (a response mediated by the sympathetic nervous system), implying a concomitant increase in retinal perfusion. Retinal vessel constriction occurs in normal subjects in response to the systemic sympathetic stimulation precipitated by this stimulus.[3] Retinal vascular responses to autonomic stimulation in diabetics with intact autonomic function (within the limits of sensitivity of these tests) were similar to those of a group of 11 normal subjects of similar age (unpublished observations; mean age 53.9 ± 2.55 years; range: 42-65), showing an arteriolar constriction of 7.4% (Group 1: 9.2%); and venous constriction of 3.8% (Group 2: 5.1%). By contrast, in Group 2 diabetic patients with autonomic dysfunction, the mean arteriolar constriction (1.2%) was significantly lower than in Group 1 ('t' test, difference of means, 2.49, $0.05>p>0.02$), though the performance in the handgrip test was similar in the two groups. The venous response was also reduced to a constriction of 2.4%. Neither of these group 2 mean changes represents a significant departure from the baseline values; though on ANOV occasional arteriolar sites showed significant constriction (8 out of 88 sites as compared to Group 1, 25 out of 90). These results indicate a very considerable loss of retinal vascular response in Group 2, the recorded changes being due largely to random error, except perhaps in subject No. 5.

Table V *Prevalence of Diabetic Retinopathy*

Category	*Group 1*	*Group 2*
0	6	2
1	3	2
2	1	5
3	1	2

There was no correlation between elevation of diastolic blood pressure and the degree of retinal vessel constriction in either group or in our unpublished study of normal subjects of similar age range (quoted above), who sustained a mean diastolic blood pressure rise of 21.3 ± 1.70 mmHg, not significantly different from groups 1 and 2. In Group 2 diabetics with autonomic dysfunction, there is a dissociation of effects with virtual loss of the retinal vessel response, but a similar mean diastolic blood pressure rise to that in Group 1; though the variance of Group 2 is significantly greater (F ratio 4.16, $0.025>p>0.01$) indicating a greater spread of blood pressure responses. These two factors, lack of correlation between blood pressure and vessel constriction and dissociation of effects in autonomic neuropathy are evidence for independent responses of blood pressure and retinal vessels to a generalised sympathetic stimulus. The relationship of diastolic blood pressure and retinal arteriolar calibre response is seen graphically in Figure 4.

An association has already been established between proliferative retinopathy and autonomic nerve function in diabetes.[7-9] The present study includes only 3 patients with proliferative retinopathy, too small a group for valid comment upon this association; although it is interesting to note (Table V) that of 22 randomly selected diabetic

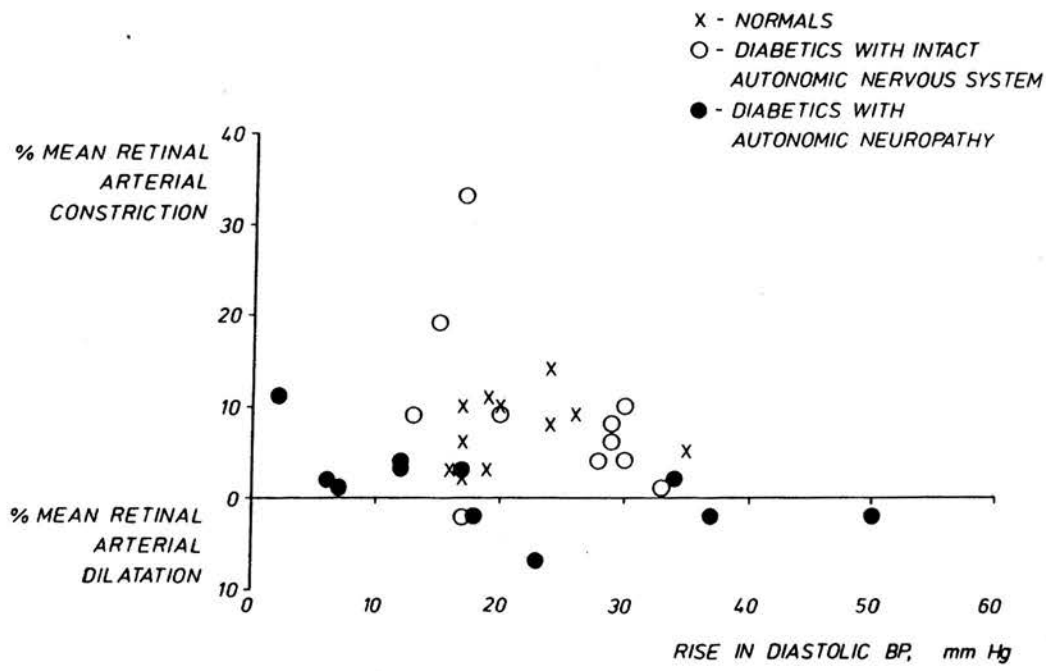


Fig. 4. *This scattergram shows the relationship between diastolic blood pressure rise and mean retinal arteriolar constriction during sustained handgrip contraction in 3 groups of subjects: diabetics without demonstrable autonomic neuropathy (Group 1), diabetics with autonomic neuropathy (Group 2) and normal subjects of similar age range. Note the dissociation between the two responses, the greater variability of arteriolar responses in Group 1 as compared to the normal and the virtually absent vessel calibre response in Group 2.*

patients, 6 of the 11 subjects with intact autonomic function (Group 1) had no evidence of retinopathy, compared to only 2 of the 11 subjects with autonomic dysfunction (Group 2); furthermore severe background retinopathy was present in only 2 subjects of the first group as compared to seven of the second, which supports the general association between autonomic neuropathy and diabetic retinopathy.

Autonomic neuropathy may interfere with autoregulatory mechanisms.[18,19] Several studies have demonstrated abnormalities in the retinal circulation in diabetic patients.[20–25] Rhie *et al*[21]. showed that retinal vascular reactivity to norepinephrine and angiotensin II in diabetics with retinopathy was abnormal compared to control subjects and diabetics without retinopathy, and Sinclair *et al.*[26] concluded that retinal vascular autoregulation decreases as diabetic retinopathy increases, with complete loss of autoregulation in patients with proliferative retinopathy.

Duration, type and control of diabetes were not significantly different between the two groups in the present study and therefore the results cannot be explained as a direct manifestation of diabetic severity. The only significant difference between these otherwise equivalent groups of diabetic patients was the integrity of the autonomic nerve function.

This study establishes, that in response to sustained isometric muscle contraction, there is a dissociated loss of retinal vascular reactivity, which occurs in association with clinical evidence of autonomic neuropathy. The retinal vascular response is not linked to the rise in diastolic blood pressure which occurs during the isometric muscle contraction and its mechanism requires further investigation.

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PAPER 28

IMPAIRED AUTOREGULATION OF THE RETINAL VASCULATURE AND MICROALBUMINURIA IN DIABETES MELLITUS

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SUMMARY.

The relationship between microalbuminuria and retinal vessel responses to sustained handgrip contraction was studied in a group of 20 diabetic patients. The diabetics were divided into two groups based on their albumin excretion rates (AER): Group 1 (AER ≤10mcg/min) consisted of 10 diabetic patients, mean age 55.8 ± 3.9 years (mean ± SEM); 5 IDDM and 5 NIDDM. Group 2 (AER >10mcg/min) comprised 10 diabetic patients: mean age 56.8 ± 3.04 years; 6 IDDM and 4 NIDDM. Both groups were similar in that there were no significant differences between mean age, type of diabetes, mean duration of diabetes, glycaemic control or mean resting blood pressures. Group 2 diabetics had a higher incidence of autonomic dysfunction than Group 1, based on the results of 4 standard tests of autonomic nerve function. There were significantly decreased retinal vessel responses to sustained handgrip contraction in Group 2 diabetics (mean arteriolar constriction 0.1 ± 0.32%, and mean venule constriction 1.0% ± 0.99%) compared with Group 1 diabetics (mean arteriolar constriction 6.9 ± 1.69%, and mean venule constriction 4.2 ± 0.05%). Retinopathy was slightly worse in Group 2.

The implications of the association of microalbuminuria (AER >10mcg/min) and loss of retinal vessel reactivity to sustained handgrip contraction are discussed.

KEY WORDS:

Sustained isometric muscle contraction, retinal autoregulation, diabetes mellitus, microalbuminuria, autonomic nerve function, retinal vessel calibres.

Impaired autoregulation of the retinal vasculature and microalbuminuria in Diabetes Mellitus.

Introduction:

Retinal vessel responses to sustained isometric muscle contraction have recently been quantified (1). The changes in vessel calibre probably reflect an autoregulatory response to the simultaneous rise in systemic blood pressure. By contrast, the diabetic retinal circulation shows impaired autoregulation with complete loss occurring in proliferative retinopathy (2).

An association between proliferative retinopathy and proteinuria is recognised in diabetic patients (3-6). Functional haemodynamic changes have been proposed as aetiological factors in the development of these complications (7,8). As a direct corollary, one may postulate a relationship between proteinuria and impaired retinal autoregulation in patients with diabetes mellitus.

We therefore studied the relationship between microalbuminuria and retinal vessel responses to sustained isometric muscle contraction in a group of diabetic patients.

Patients and methods

20 diabetic patients (mean ± standard error of the mean (SEM)), (18 male; mean age; 56.3 ± 2.4 years; range: 34 to 73 years) were included in the study following informed consent and Ethical Committee approval. The 11 Insulin dependent diabetics and 9 non-insulin dependent diabetics were subdivided into 2 groups: based on the results of albumin excretion rates (AER). The AER were determined using a modified immunoassay technique (with single antibody assay and polyethylene glycoprecipitation) to measure albumin concentration (9). Excretion rates were calculated from from one or more timed overnight samples of urine collection. The upper limit of normal for AER from overnight samples was set at 10 mcg/min (10) and patients with such values were included in Group 1. Diabetics with AER greater than 10 mcgm/min were defined as Group 2.

Group 1 (AER $\leq$10 mcgm/min) consisted of 10 diabetic patients: mean age: 55.8 ± 3.9 years, range: 34-71 years: 5 IDDM and 5 NIDDM. Group 2 comprised 10 diabetic patients: mean age 56.8 ± 2.04 years, range: 41 to 73 years; 6 IDDM and 4 NIDDM.

The mean duration of diabetes mellitus was 13 ± 2.86 years (range: 2 to 25 years) and 10.3 ± 2.61 years (range: 1 to 29 years) for Groups 1 and 2 respectively.

Long-term control of diabetes was assessed by glycosylated haemoglobin estimation in all subjects. Diabetics with autonomic nerve dysfunction were identified by the following methods: (a) diastolic blood pressure rise of less than or

equal to 10 mmHg in response to 33% maximum sustained handgrip contraction (b) immediate heart-rate response to standing: 30:15 ratio less than or equal to 1.00 (c) a decrease in systolic blood pressure in response to standing from the supine position of greater than or equal to 30 mmHg (d) Heart-rate variation inresponse to a Valsalva ratio of less than 1.21. The degree of autonomic dysfunction was classified as early, definite and severe, according to standard criteria (11).

Retinal vessel responses to systemic autonomic stimulation.

The protocol was similar to that described previously (1). Each patient was studied seated at the fundus camera with one pupil dilated (whichever was preferred by the patient) with g.tropicamide 1% and g.pheneylephrine 10%.

3 baseline blood pressures were taken from the non-exercising arm using a sphygmomanometer with an automatic inflatable cuff (COPAL UA 231). The subject was instructed to perform 3 maximum handgrip contractions with the dominant arm to establish their maximum voluntary contraction (MVC). The subject then performed a sustained handgrip contraction at 33% MVC for 2-2.5 minutes; 3 BP measurements were taken at 0.5, 1.5, and 2.5 minutes during handgrip contraction and 3 phases of fundus photographs

(each consisting of 5 replications) were taken at 1, 2 and 2.5 minutes. On release of the handgrip a final BP reading and a single phase of fundus photographs were taken at 5 minutes. All diabetic patients maintained a 33% maximum voluntary contraction for 2.5 minutes.

Analysis:

All films were analysed using the Quantimet 800 Image analyser (Cambridge Instruments) a technique established in this department (12). 10 to 18 suitable vessel sites were selected in each patient comprising approximately equal numbers of arterioles and venules nominally within the calibre range (64 - 174um) calculated on the basis of the Gullstrand Schematic Eye. Vessel calibres at these sites were measured and the means, standard error of the means (SEM) and one way analysis of variance (ANOV) for each site within replications and between the phases were calculated. Percentage change in mean retinal vessel calibre between phase 1 and phase 4 (at 2.5 min handgrip) were calculated for each subject; significance was assessed by the student t test for paired data (2 tailed).

Linear regression analysis was applied to assess any correlation between microalbuminuria and mean retinal arteriolar responses

Results

The two diabetic groups were very similar in all respects except retinopathy and autonomic status; Table 1.

1. Retinal vessel responses:

Mean retinal vessel responses to sustained handgrip were significantly greater in Group 1 than Group 2; mean arteriolar constriction ($p<0.02$) and mean venule constriction ($p<0.02$). In Group 1 (AER $\leq$10 mcgm/min), there was a mean arteriolar constriction of 6.9 ± 1.69% ($p<0.01$) and mean venule constriction of 4.2 ± 0.05% ($p<0.001$). Group 2 (AER > 10 mcgm/min) showed a mean arteriolar constriction of 0.1 ± 0.32% and mean venule constriction of 1.0 ± 0.99%. The individual mean retinal vessel responses are shown in Figures 1 and 2.

2.Blood pressure responses:

There were no significant differences in the mean rise in diastolic blood pressure during sustained handgrip contraction between the two groups: Group 1 +22.6 ± 2.0 mmHg, Range 12-34; Group 2 +23.4 ± 4.29 mmHg, Range 6-50 mmHg.

There was no correlation between the blood pressure responses and the retinal vessel responses in either group.

3. Autonomic status (Table 2 and 3).

3 diabetics in Group 1 had definite autonomic dysfunction based on autonomic nerve function tests, while in Group 2 (AER >10 mcg/min) 3 had definite autonomic dysfunction and 4 early dysfunction.

4. Glycaemic control

There was no difference in the degree of glycaemic control between the two groups as assessed by HbA1 values: Group 1 9.09 ± 0.83%, and Group 2 8.38 ± 0.56%.

5. Retinopathy.

Retinopathy was assessed by ophthalmoscopy and inspection of the fundus photographs. The degree of retinopathy was slightly worse in group 2 compared to Group 1 (Table 4). Retinopathy was graded as follows:

0.....no retinopathy.

1.....mild background (<10 haemorrhages)

2.....severe background (>10 haemorrhages ± exudates).

3.....proliferative retinopathy.

6. Relationship between microalbuminuria and retinal vessel responses.

Subject No. 7 in Group 2 had an AER of 69.7 mcg/min and was a complete outlier compared to the rest of the AER values in this group. Excluding this subject from the linear regression analysis, there was a significant correlation between loss of retinal artery responses and microalbuminuria ($r = 0.579$; $p<0.001$), (Figure 3).

Discussion.

The present study demonstrates significantly decreased retinal vessel responses to systemic autonomic nerve stimulation in diabetic patients with microalbuminuria. The groups studied were reasonably equivalent, with no

significant differences in mean age, type of diabetes, mean duration of diabetes, glycaemic control or mean resting blood pressures.

Significant retinal vessel constriction occurs in normal subjects during systemic autonomic stimulation (1). The vessel constriction probably represents an autoregulatory response to the elevation in systemic blood pressure during sustained handgrip contraction. The present study has shown a significant loss of retinal vessel reactivity to sustained handgrip contraction in diabetics with microalbuminuria (AER >10 mcg/min). Excluding subject No.7 in Group 2, there is a significant correlation between retinal vessel responses and microalbuminuria, confirming an association between these diabetic complications.

There was a higher incidence of autonomic dysfunction in Group 2 (7 diabetics, of which 3 had definite autonomic neuropathy and 4 mild autonomic dysfunction) compared with Group 1 (3 diabetics had definite autonomic dysfunction). Autonomic neuropathy has previously been shown to affect renal function by reducing splanchnic vascular resistance with consequent increase in renal blood flow (13) and urinary sodium excretion (14). The higher incidence of autonomic dysfunction in Group 2, may be an important factor related to the increased AER in this group, although the association of autonomic dysfunction and microalbuminuria

may equally merely reflect an advanced stage in the diabetic microvascular disease, as patients with diabetic autonomic neuropathy are also at a higher risk of developing abnormal renal function.

The degree of retinopathy, however, was actually slightly worse in Group 1 compared to Group 2 (Table 4), although the difference between the groups was not significant. This may reflect the small size of the group studied and the patchy nature of diabetic vascular disease. Sinclair et al (2) have demonstrated an inverse relationship between retinal vessel autoregulation and diabetic retinopathy, with complete loss of autoregulation occurring in proliferative retinopathy. However in the present study, the group with maximum loss of retinal vessel response (ie impaired autoregulation) (AER >10mcg/min; Group 2) showed less severe retinopathy. This apparent paradox was also present in the study by Osei et al (15), reporting abnormal retinal artery responses to cold pressor testing in patients with no clinical retinopathy. Furthermore 50% of the diabetics with microalbuminuria studied by Barnett et al (4), did not have any retinopathy.

It has been proposed that diabetics with higher glomerular filtration rates early in their disease course are more likely to develop nephropathy suggesting an association between early haemodynamic changes and late glomerulopathy (16). Haemodynamic changes have also been implicated in the

development and/or progression of diabetic retinopathy (17-19). Ipsilateral carotid artery stenosis and renal artery stenosis afford protection from retinopathy and diabetic glomerulosclerosis respectively (20,21). It is noteworthy that nephropathy has been reported in at least 47% of diabetics with advanced proliferative retinopathy (5) and, conversely, Deckert et al (22) have shown that at some stage during the development of nephropathy, retinopathy is an almost inevitable concomitant feature.

Microalbuminuria is both an early predictor of diabetic nephropathy (16,23) and has been proposed as the most effective non-ocular indicator of visual loss in diabetes (5). The observed association between retinal vessel reactivity and microalbuminuria may be a manifestation of coincidental natural history, however it may equally support the hypothesis of a causal relationship between haemodynamic changes and diabetic nephropathy. Impaired autoregulation of the retinal vasculature may represent an early prognostic indicator of the alteration in haemodynamics proposed as a significant factor in the pathogenesis of nephropathy and retinopathy.

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Table 1.

Clinical features of Groups 1 and 2 diabetics.

	Group 1. AER $\leq$10mcg/min n=10	Group 2. AER >10mcg/min n=10.
Sex M:F	9:1	9:1
Age (years)	55.8 ± 3.87	56.8 ± 3.1
Duration of DM (years)	13 ± 1.27	10.3 ± 2.6
Systolic BP	145 ± 5.8	151 ± 3.7
Diastolic BP	85 ± 3.3	85 ± 3.0
Retinopathy (No)	7	7
HbA1%	9.1 ± 0.8	8.4 ± 0.6
Aut. Dysfunction (No)	3	7
Type of DM	5 IDDM	6 NIDDM
Ret. artery change %	-6.9 ± 1.69	-0.1 ± 0.32
Ret. venule change %	-4.2 ± 0.55	-0.99 ± 0.99
Diast. BP rise to HG	+23 ± 2.9	+23 ± 4.3

mean ± SEM.

Table 2.

Blood pressure and mean retinal vessel responses and autonomic status for Group 1 diabetics : AER $\lesssim$10mcg/min.

Subj. No.	Age	Sex	Type DM	Dur. DM	resting BP	Auton. status	Retino. grade	AER mcg/min	Rise in DBP	Retinal vess calibre cha	
										Art.%	Ven.%
1.	67	M	NIDDM	25	130/76	D	2	1.6	+12	-3	-8
2.	56	F	NIDDM	6	182/93	D	2	8.5	+12	-4	-4
3.	42	M	IDDM	25	142/80	D	3	7.5	+34	-2	-4
4.	58	M	NIDDM	4	153/82	N	0	6.3	+28	-4	-3
5.	60	M	NIDDM	2	126/63	N	0	9.5	+20	-9	-3
6.	67	M	IDDM	25	142/95	N	2	9.5	+33	-1	-5
7.	60	M	IDDM	14	161/99	N	0	0.3	+29	-8	-4
8.	71	M	NIDDM	7	147/84	N	1	4.2	+15	-19	-6
9.	43	M	IDDM	9	145/90	N	1	2.6	+30	-10	-3
10.	34	M	IDDM	13	118/88	N	3	6.4	+13	-9	-2

Table 3.

Blood pressure and mean retinal vessel responses and autonomic status for Group 2 diabetics : AER >10mcg/min.

Subj. No.	Age	Sex	Type DM.	Dur. DM.	resting BP.	Auton. status	Retino. Grade	AER mcg/min	Rise in DBP	Retinal vess calibre cha Art. %	Ven. %
1.	54	M	IDDM	29	151/86	E	1	22.8	+7	-1	-8
2.	60	M	NIDDM	5	150/95	D	2	11.7	+18	+2	+0.3
3.	49	M	NIDDM	9	145/95	E	0	22.3	+37	+2	+2
4.	71	M	NIDDM	12	169/80	D	2	19.5	+23	+7	0
5.	56	M	IDDM	1	166/96	E	0	13.9	+17	-3	-0.2
6.	41	M	IDDM	9	138/78	E	1	14.4	+50	+2	+2
7.	51	M	IDDM	13	138/76	D	2	69.7	+6	-2	+2
8.	73	M	IDDM	6	164/67	N	1	13.3	+17	+2	-3
9.	55	F	IDDM	17	148/88	N	1	10.8	+29	-6	-3
10.	58	M	NIDDM	2	139/87	N	0	25.0	+30	-4	-2

Table 4.

Severity of retinopathy in the two diabetic groups.

Retinopathy Grade	Group 1 (AER ≤10mcg/min)	Group 2. (AER >10mcg/min).
0...no retinopathy	3	3
1...mild background	2	4
3...severe background	3	3
4...proliferative	2	0

LEGENDS:

Figure 1: Individual mean retinal vessel and blood pressure responses during handgrip contraction for Group 1 (AER$\leq$10mcg/min).

Figure 2: Individual mean retinal vessel and blood pressure responses during handgrip contraction for Group 2 (AER>10mcg/min).

Figure 3: Correlation between mean retinal artery responses during handgrip contraction and AER.

Figure 1

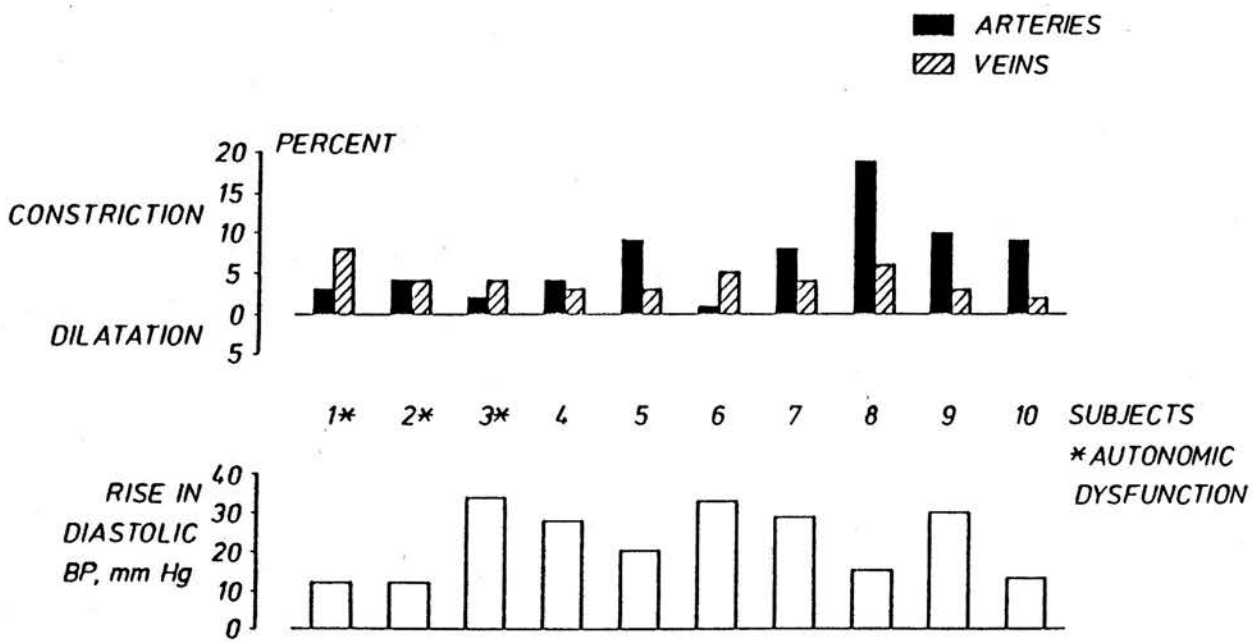

ARTERIES
VEINS
PERCENT
CONSTRICTION
DILATATION
20
15
10
5
0
5
1*
2*
3*
4
5
6
7
8
9
10
SUBJECTS
*AUTONOMIC DYSFUNCTION
RISE IN DIASTOLIC BP, mm Hg
40
30
20
10
0

Figure 2

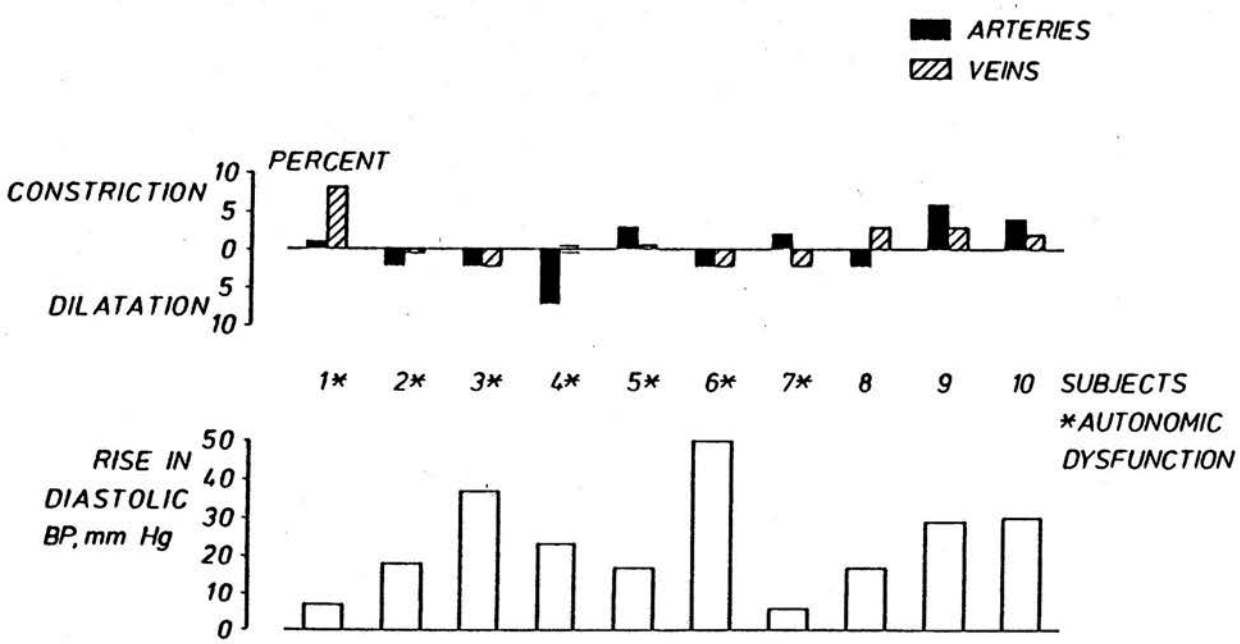
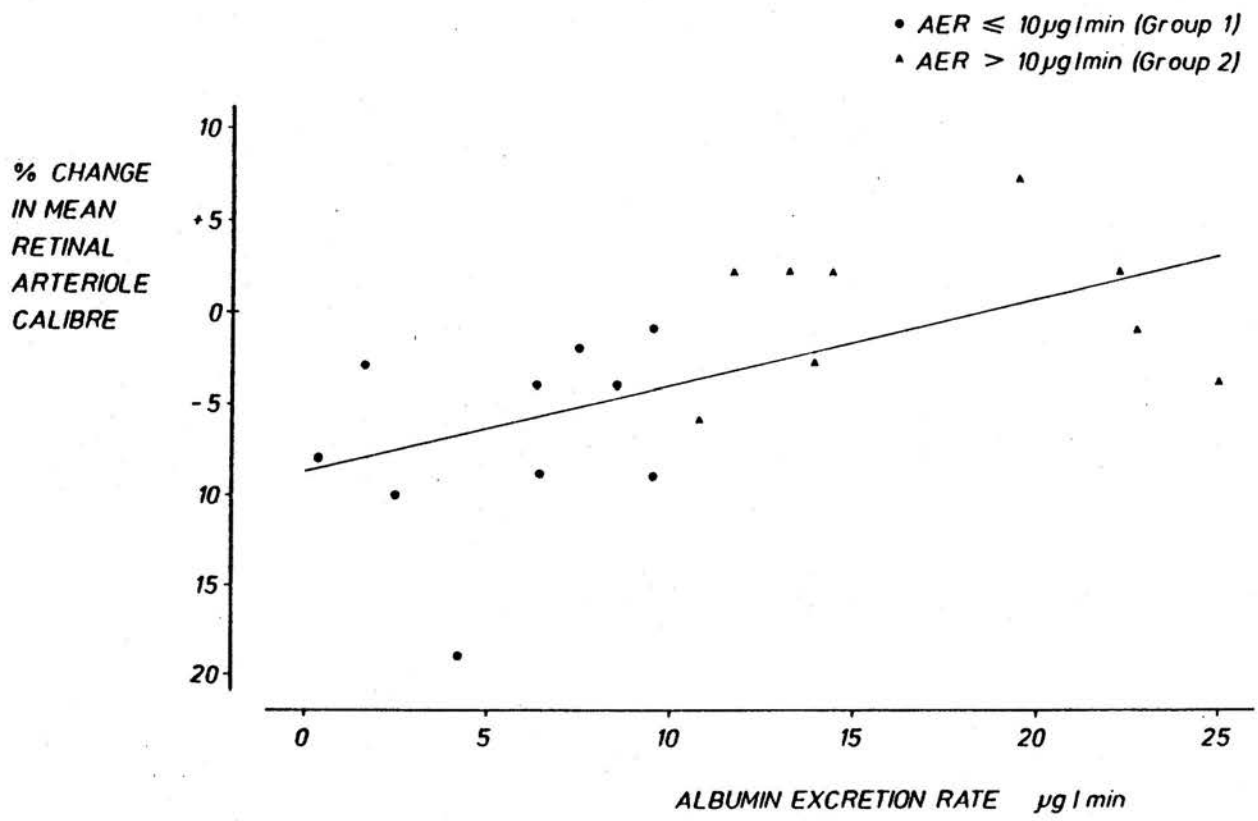

ARTERIES
VEINS
PERCENT
CONSTRICTION
DILATATION
10
5
0
5
10
1*
2*
3*
4*
5*
6*
7*
8
9
10
SUBJECTS
*AUTONOMIC DYSFUNCTION
RISE IN DIASTOLIC BP, mm Hg
50
40
30
20
10
0

Figure 3


• AER ≤ 10µg/min (Group 1)
▲ AER > 10µg/min (Group 2)
% CHANGE IN MEAN RETINAL ARTERIOLE CALIBRE
10
+5
0
-5
10
15
20
0
5
10
15
20
25
ALBUMIN EXCRETION RATE µg/min

PAPER 29

TITLE:

The effect of cervical sympathectomy on retinal vessel responses to systemic autonomic stimulation.

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SUMMARY:

The retinal vessel calibre responses to systemic sympathetic stimulation, were studied in 9 patients (8 male; mean age: 31.7 years; range: 19-58 years) with unilateral disruption of their cervical sympathetic tract. All patients had ipsilateral decreased / absent facial sweating and a Horners syndrome, evidence of unilateral sympathetic denervation. Both eyes of each patient were studied and the results were analysed in two groups: the group of 9 sympathectomised eyes and the control group of unaffected fellow eyes. During handgrip contraction there was a significant difference in the mean retinal arteriolar constriction (mean ± SEM) between the group of sympathectomised eyes (4.6 ± 0.89%) and control eyes (7.1 ± 1.13%), $p<0.01$. Similarly, there was a significant difference in mean venule constriction during sustained handgrip contraction between the group of sympathectomised eyes (1.5 ± 0.67%) and control eyes (4.9 ± 0.98%), $p<0.05$. There was no significant difference in the mean rise in diastolic blood pressure between the two groups: control eyes +27.9 ± 2.38 mmHg and sympathectomised eyes +27.8 ± 2.25 mmHg. There was no correlation between the blood pressure and retinal vessel responses in either group.

These results suggest that the sympathetic nervous system plays an integral role in retinal blood flow regulation.

KEY WORDS:
autonomic nerve function tests, cervical sympathectomy, retinal vessel calibre, sustained isometric exercise.

The effect of cervical sympathectomy on retinal vessel responses to systemic autonomic stimulation.

Introduction.

Until comparatively recently, there have been conflicting reports both supporting and denying the existence of autonomic innervation to the retinal vasculature. In 1963, Laties and co workers (1), using a fluorescent histochemical method, reported that the nerve supply to the central retinal artery in the rabbit did not extend beyond the lamina cribrosa on entering the globe. Similar results were obtained from studies involving the retinal arterioles of both New and Old World monkeys and cats (2). By contrast, Seitz had previously demonstrated vasodilatation of the rabbit pre-retinal vessels after bilateral superior cervical ganglionectomy in 1953, suggesting that sympathetic nerves were present in the pre-retinal vessels of the rabbit eye (3). In a study of the neuropathy of the retina and the optic nerve in diseased human eyes, Matsuyama (1961) demonstrated nerve fibres running parallel with retinal vessels using light microscopy (4); some of the nerve fibres followed the same direction as the retinal blood vessels prompting the hypothesis of centrifugal nerve fibres in the retina, but the origin of these nerve fibres was not discussed. Unmyelinated nerve fibres with vesicles have subsequently been demonstrated in the retinal vessel adventitia (5,6). Adrenergic nerve fibres have also been observed in the wall of human retinal vessels on the optic disc beyond the lamina cribrosa and for several millimetres proximally on the retinal surface surrounding the optic disc using transmission electron microscopic (7) and fluorescent examination (8). Furukawa et al (9), using scanning electron microscopy and transmission electron microscopy, have detected unmyelinated nerve fibres with nerve endings filled with vesicles in retinal arterioles in man, rabbit and rat, supporting the concept of autonomic innervation to the retinal vasculature.

Recent reports have shown a consistent response of the retinal vessels to systemic autonomic nerve stimulation in normal subjects, with a mean constriction of retinal arterioles of 8.1% and retinal venules of 3.7%,

in a control group (10). By contrast, retinal vasoconstriction in response to sustained isometric muscle contraction was significantly impaired in patients with diabetes mellitus (11), the commonest cause of autonomic neuropathy in the United Kingdom (12). The aim of the present study was to assess the retinal vessel responses to systemic sympathetic nerve stimulation in patients with unilateral disruption of cervical sympathetic nerve innervation.

Patients and methods.

9 patients with unilateral disruption of the cervical sympathetic tract were included in the study: 8 were male, mean age 31.7 years, range 19 - 58 years. 7 were victims of motorcycle accidents and had complete unilateral brachial plexus injuries (avulsion of cervical nerve roots, confirmed on surgical exploration several weeks after the accident). The remaining 2 patients had a surgical sympathectomy performed for relief of intractable pain (cervical neuritis and malignant schwannoma of the median nerve). Unilateral decreased or absent facial sweating on the affected side and unilateral Horner's syndrome (indicating sympathetic denervation) were present in all patients. The mean time interval from the date of their accidents to the date of this study was 2.2 years; with a range of 1 to 4 years.

All patients were studied following informed consent. The protocol (10) was approved by the hospital ethical committee. Each patient was studied seated at the fundus camera with both pupils dilated with guttae tropicamide 1%. All photographs were taken by the same observer using a wide angle Canon camera (CF 60-S) which was triggered during the diastolic phase of the pulse cycle. Each phase of photographs consisted of five photographs were taken in quick succession, at approximately one second intervals. A baseline phase of photographs was taken, followed by three baseline blood pressure measurements recorded from the non-exercising arm using an automatic sphygmomanometer (COPAL - UA 231). The patient was then instructed to perform three maximum handgrip contractions to establish his maximum voluntary contraction (MVC). A laboratory-built strain-gauge dynamometer with continuous chart recording was used with an adjustable audible alarm, set to alarm below

33% of each patient's MVC. The patient was then instructed to perform a sustained handgrip at 33% MVC for 2.5 minutes; three blood pressure measurements were recorded at 0.5, 1.5 and 2.5 minutes and three phases of fundus photographs were taken at 1, 2 and 2.5 minutes during the handgrip contraction. On recovery one final phase of fundus photographs and one blood pressure reading were taken five minutes following release of handgrip contraction. All photographs were recorded on Ilford FP4 film with a red free filter and developed in PQ Universal (1+19) for 12 minutes at 20 C with sensitometric control (13).

As two patients had an above elbow amputation and another had the affected arm in a plaster cast (nerve transfer procedure done 4 weeks previously), the blood pressure readings were taken from the exercising arm, which was not always the dominant arm for obvious reasons; this convention was followed for all patients in order to standardise the procedure.

Both eyes of each patient were studied with a 15 minute interval between studies. The photographer randomly selected the order of the eyes to be studied for each patient. This permitted the sympathectomised eye to be compared with the control eye within each patient.

Analysis:

All films were analysed using the Quantimet 800 Image analyser (Cambridge Instruments) (13). 16 to 18 suitable vessel sites were selected from each fundus photograph for each eye, comprising approximately equal numbers of arterioles and venules. A sample fundus photograph illustrating typical sites of measurement is shown in Figure 1. The following parameters were calculated for all calibre measurements: mean, standard error of mean (SEM) and one way analysis of variance within replications and between the phases for each site. Percentage change in mean retinal vessel calibre between phase 1 (control) and phase 4 (peak handgrip contraction) was calculated. Differences between individual mean vessel responses (unaffected and sympathectomised eyes) and between the two groups (control and sympathectomised groups) were assessed by the Wilcoxon signed rank test, with the level of statistical significance being taken at $p<0.05$ for both arterioles and venules.

Results:

The results of this study were analysed in two groups: the group of 9 sympathectomised eyes and the control group of 9 unaffected fellow eyes. A total of 166 retinal vessel sites were measured in both the control group (78 arteriolar and 78 venule sites) and in the sympathectomised group (82 arteriolar and 74 venular sites).

During the exercise period, there was a significant difference in the mean arteriolar retinal vessel constriction (mean ± SEM) between the group of sympathectomised eyes (4.6 ± 0.89%) and control eyes (7.1 ± 1.13%), $p<0.01$. Similarly there was a significant difference in mean venule constriction during sustained handgrip between the group of sympathectomised eyes (1.5 ± 0.67%) and control eyes (4.9 ± 0.98%), $p<0.05$, Figure 2. The individual mean retinal vessel responses to sustained handgrip contraction for the sympathectomised and control fellow eyes, with the corresponding rise in diastolic blood pressure, are shown in Figures 3 and 4; the range of retinal vessel responses to sustained handgrip contraction within the individual precluded statistically significant differences (between control and sympathectomised eyes), except for subject no. 4 (Figure 4).

Figure 5 demonstrates the sequential change in mean retinal vessel calibre through phases 2 to 5 (handgrip contraction and recovery) compared to phase 1 (baseline), for sympathectomised and control eyes. While there was a gradually increasing degree of retinal vessel constriction with continued stimulation for both arteries and venules in both groups, there is nevertheless a definite reduction in the magnitude of the responses for the sympathectomised eyes.

There was no significant difference in the mean rise in diastolic blood pressure ($p>0.05$) between the two groups: control eyes, +27.9 ± 2.38 mmHg and sympathectomised eyes, +27.8 ± 2.25 mmHg. There was no correlation between the blood pressure rise and the retinal arteriolar responses (controls, $r= +0.30$: sympathectomised eyes, $r= -0.22$ or the venule responses (control eyes, $r=0.0003$: sympathectomised eyes, $r= -0.33$) in either group.

Discussion:

Histological studies have confirmed the existence of autonomic receptors on retinal blood vessels, although the effects of these receptors are not known. Nerve endings on pre-retinal arterioles (fewer on venules), distributed from the optic disc to the periphery and containing axonal varicosities (diameter 0.5 - 1.5 u) with vesicles, were demonstrated by Furukawa in 1987 in the rabbit eye (14). Using scanning electron microscopy, transmission electron microscopy and fluorescence histochemical examination, he showed an inverse relationship between both the number of nerve endings on arterioles and the diameter of the arteriole with distance from the optic nerve. The nerve endings were noted to disappear following superior cervical ganglionectomy, suggesting that sympathetic nerves originating in the superior cervical ganglion innervated pre-retinal vessels in the rabbit eye.
Further evidence for autonomic innervation of the retinal vessels comes from a study by Forster, Ferrari-Dileo and Anderson (15), demonstrating the presence of autonomic binding sites in mammalian retinal vessels. The exact location of these sites on the vessels were not defined, however, as retinal vessel homogenates were used. High affinity alpha-1 and alpha-2 adrenergic binding sites were demonstrated both in the vessel homogenates and in the retinal tissue. This is not surprising as the retina is known to contain noradrenaline and is involved in its synthesis (16). In addition alpha adrenergic receptors can be stimulated by dopamine, which is present in high concentrations in the retina. Furthermore catecholaminergic amacrine cells are also present in the retina (1).

The demonstration of alpha-1 and alpha-2 high-affinity binding sites - presumably receptors - is also not surprising, as post-synaptic alpha receptors are present in peripheral and central aortic arteries as a mixed population of alpha-1 and alpha-2 receptors. Both subserve contractile responses, but the mechanism of each receptor is different. The alpha-2 receptor is negatively coupled to adenylate cyclase and is critically dependent on extracellular calcium ions to elicit its vasoconstrictor response (17,18), whereas the alpha-1 type appears to be

dependent on intracellular calcium ions and phosphatidylinositol turnover (19,20).
The presence of adrenergic receptors in the retinal vessels may permit a contractile response to low concentrations of adrenergic agonists. As workers have recently suggested the presence of adrenergic innervation of the retinal vessels, there is thererfore an inevitable potential for stimulation from the autonomic nervous system. Indeed the normal concentration of circulating catecholamines is in the nanomolar range, which is sufficient to stimulate alpha-1 and alpha-2 receptors and thus maintain smooth muscle tone efficiently.

The mechanism by which intraluminal catecholamines affect receptors on smooth muscle receptors is not known as retinal arterioles lack fenestrations and possess tight junctions in the endothelium. This suggests that receptor stimulation could only occur if there were a breach in the inner blood retinal barrier. An alternative explanation would be that the alpha receptors were located in the endothelium, rather than the muscle layer. Indeed evidence for alpha-2 receptors in the luminal layers of peripheral (mesenteric and renal) and central (aortic) arteries exists (21, 22); these alpha-2 receptors promote a relaxing response or at least attenuate a pre-existing contractile one. A third explanation might be the presence of receptors in all layers of the retinal vessels, with differing effects depending upon the integrity of the pre-existent vascular anatomy.

The results of the present study demonstrate that cervical sympathectomised eyes show a significant decrease in group mean retinal vessel responses to systemic autonomic stimulation compared with fellow eyes with an intact sympathetic innervation. While these observations would tend to support recently reported work which has demonstrated adrenergic binding sites in mammalian pre-retinal vessels (15) and autonomic nerve endings in retinal vessels (14), interpretation of the results requires caution. Although mean retinal vessel responses to autonomic stimulation were significantly reduced in sympathectomised eyes, the arterial response was still present to a significant degree (a constriction of 4.6%). This suggests that these retinal vessels may have

an incomplete cervical sympathetic denervation - as indeed sympathetic nerves have a great propensity for regeneration - or that the sympathetic nerves are not the only factor responsible for autoregulatory responses. As retinal vessel responses to sustained handgrip may be an autoregulatory response to the rise in systemic blood pressure, both factors are important considerations.

Comparisons of retinal vessel responses in each sympathectomised eye with the corresponding fellow control eye for each individual patient demonstrated no significant differences in all but one patient. This may be due to the heterogeneous nature of the variables involved. For example although many sites were measured in each fundus, arteriolar and venule sites were selected randomly and cannot be assumed to be similar or identical to retinal sites in fellow eyes. Therefore although 7 out of 9 sympathectomised eyes showed a decrease in mean retinal arteriolar response to systemic autonomic stimulation (Figure 3) when compared to their fellow unaffected eyes, the difference was not statistically significant, probably for the aforementioned reasons.

The results imply that the sympathetic nervous system exerts a significant effect - either directly or indirectly - on the smooth muscle tone of the retinal vasculature. Denervation may cause loss of tone and altered haemodynamics, and may therefore represent an important aetiological factor in the pathogenesis of diabetic retinopathy. Abnormal haemodynamics are well-recognised in the diabetic circulation (23-25); the effects of denervation in the retinal vasculature probably occurs as an integral feature of a generalised autonomic dysfunction. Indeed, Neubauer et al, showed considerable reduction in cardiovascular tissue concentrations of catecholamines in diabetic subjects, reflecting the extent of sympathetic denervation in these patients (26). Furthermore, a loss of significant retinal vessel responses to autonomic stimulation has recently been documented in diabetics with autonomic neuropathy, whilst retinal vessel responses to equivalent stimuli were normal in diabetic subjects with intact autonomic nerve function (11).

The present study demonstrates a significant reduction in group mean retinal vessel responses to systemic autonomic nerve stimulation in sympathectomised eyes compared to control eyes, with the inevitable implication that impairment of vascular reflexes is related to disruption of cervical sympathetic innervation to these vessels. The results support the hypothesis that autonomic reflexes of the retinal vasculature may be a significant contributing factor in the effective autoregulation of retinal blood flow.

Acknowledgements.
We are grateful to the patients for their cheerful participation in the study and to the Royal National Institute for the blind for supporting the study. L.P.Lanigan held a Smith & Nephew Fellowship in Ophthalmology.

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LEGENDS.

FIGURE 1: Fundus photograph showing measurement sites.

FIGURE 2: Individual mean vessel responses during handgrip contraction for control and sympathectomised eyes.

FIGURE 3: Mean vessel responses during handgrip contraction in sympathectomised eyes compared with fellow control eyes for each subject.

FIGURE 4: Individual retinal vessel responses with corresponding blood pressure responses during handgrip contraction.

FIGURE 5: Sequential change in retinal vessel constriction in sympathectomised and control eyes, during handgrip contraction.

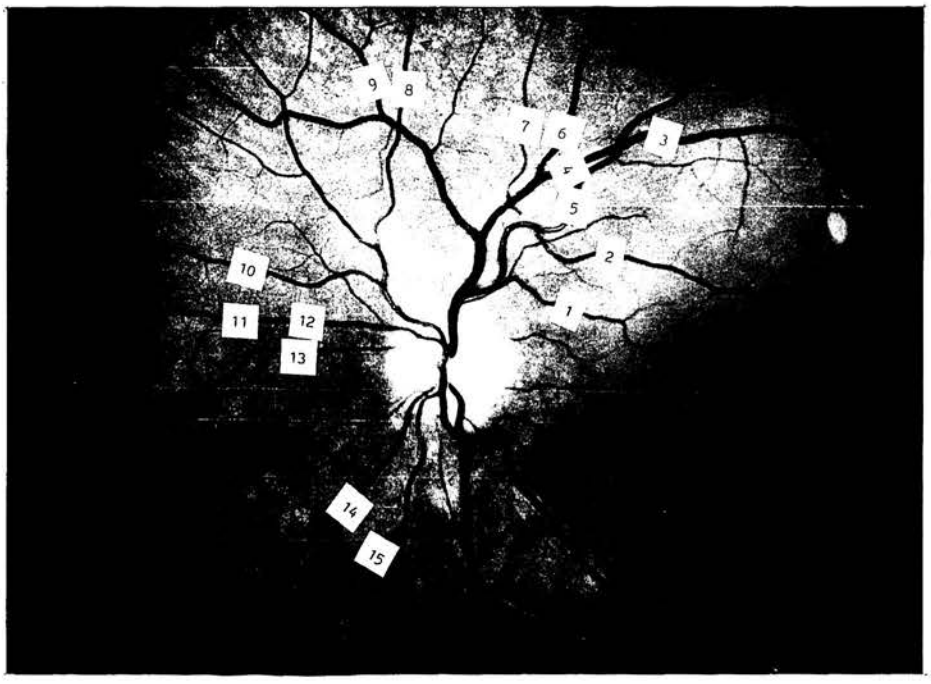


Figure 1

Figure 2

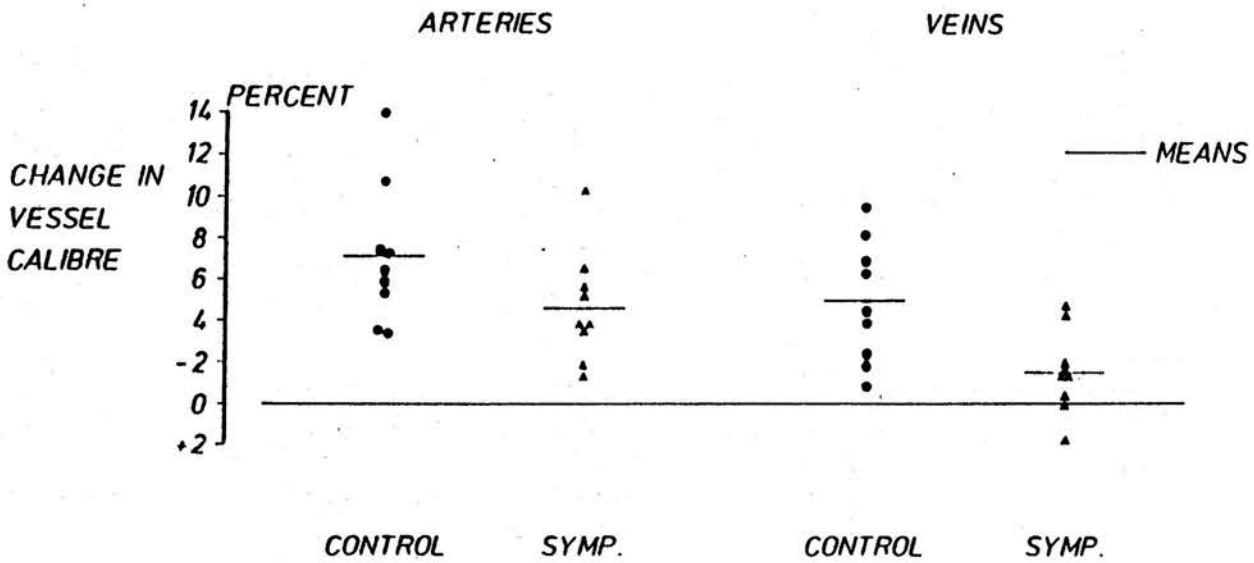

ARTERIES
VEINS
PERCENT
CHANGE IN VESSEL CALIBRE
14
12
10
8
6
4
-2
0
+2
MEANS
CONTROL
SYMP.
CONTROL
SYMP.

Figure 3

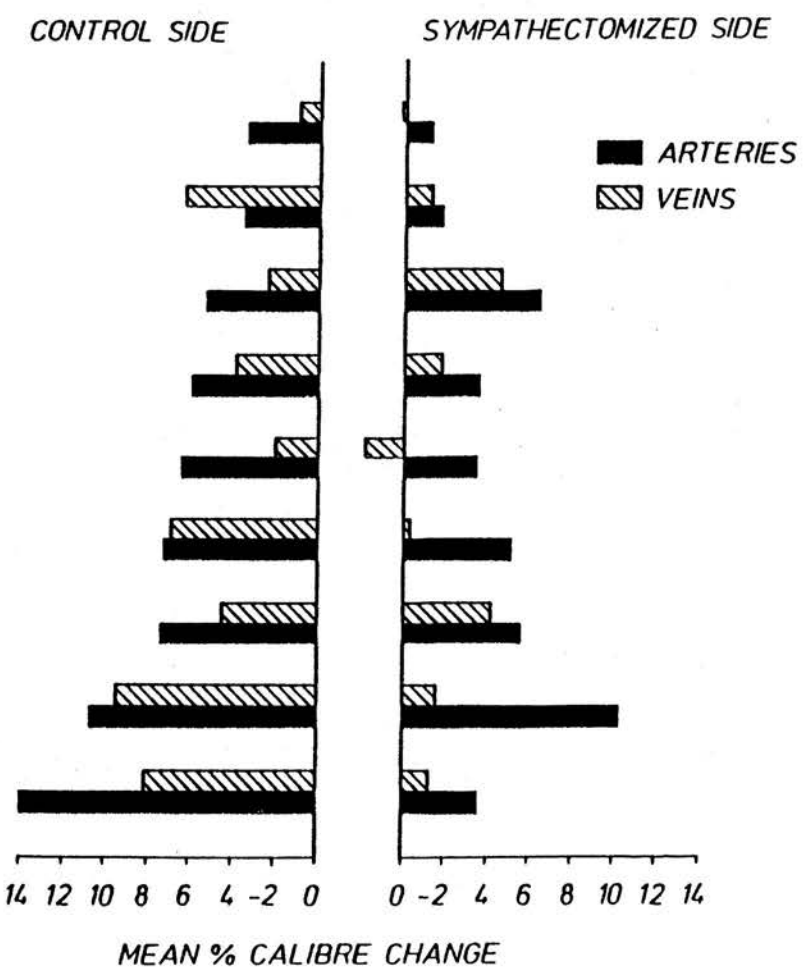

CONTROL SIDE
SYMPATHECTOMIZED SIDE
ARTERIES
VEINS
14 12 10 8 6 4 -2 0
0 -2 4 6 8 10 12 14
MEAN % CALIBRE CHANGE

Figure 4

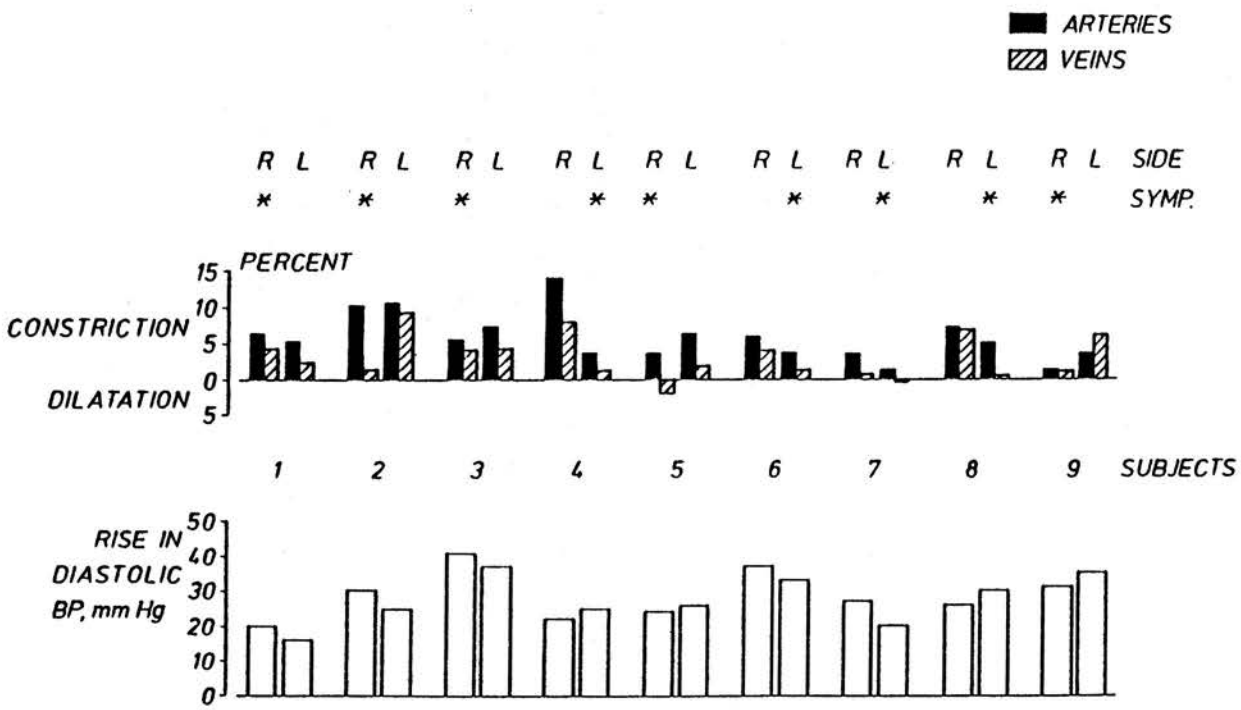
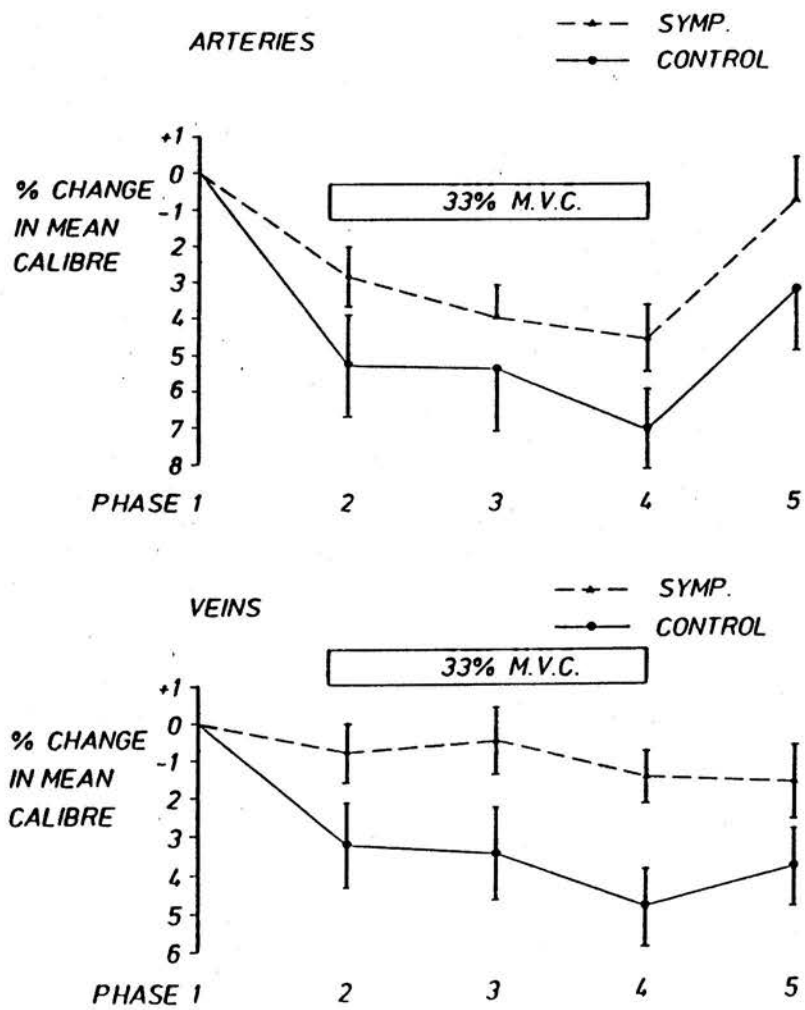

ARTERIES
VEINS
R L R L R L R L R L R L R L R L R L SIDE
* * * * * * SYMP.
PERCENT
CONSTRICTION
DILATATION
15
10
5
0
5
1 2 3 4 5 6 7 8 9 SUBJECTS
RISE IN DIASTOLIC BP, mm Hg
50
40
30
20
10
0

Figure 5


ARTERIES
SYMP.
CONTROL
% CHANGE IN MEAN CALIBRE
+1
0
-1
2
3
4
5
6
7
8
33% M.V.C.
PHASE 1
2
3
4
5
VEINS
SYMP.
CONTROL
33% M.V.C.
% CHANGE IN MEAN CALIBRE
+1
0
-1
2
3
4
5
6
PHASE 1
2
3
4
5

2.2 THE EFFICACY OF AUTONOMIC NERVE FUNCTION

PAPER 30

AUTONOMIC NEUROPATHY IN PROLIFERATIVE DIABETIC RETINOPATHY

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SUMMARY

Autonomic nerve function was assessed in 28 diabetic patients with proliferative retinopathy and 61 age- and sex-matched control subjects, using a series of tests based upon cardiovascular reflex responses to standardised stimuli. Autonomic neuropathy was present in 75% of diabetics with proliferative retinopathy, compared with 5.2% of the control group. The implications of a significant association between cardiovascular autonomic neuropathy and proliferative diabetic retinopathy are discussed.

INTRODUCTION

Autonomic neuropathy has an insidious onset which precludes accurate identification due to the compensatory mechanisms inherent within the autonomic nervous system. Until comparatively recently, tests of autonomic function have been extremely difficult to interpret and almost impossible to quantify. A series of simple, non-invasive tests based upon cardiovascular reflexes have been developed during the past 15 years, permitting accurate, objective and reproducible measurement of autonomic nerve function (1,2,3,4,5,6). As in all reflex assessments, the subject's output reaction is measured following application of a standardised input stimulus. Results are based upon blood pressure and heart-rate responses to a variety of stimuli, the relative importance of each component of the reflex having been determined by selective pharmacological autonomic blockade. Evidence to date confirms that these tests reflect damage elsewhere in the autonomic nervous system, and represent generalised autonomic nerve function (7,8,9).

The aim of the present study was to assess systemic autonomic control of cardiovascular function in diabetic patients with abnormal retinal microvascular proliferation - proliferative retinopathy.

PATIENTS AND METHODS

After informed consent had been obtained, cardiovascular autonomic nerve function was assessed in 28 diabetic patients with proliferative retinopathy (mean age 50.8 ± 14.3 years) and 61 age- and sex-matched control subjects (mean age 53.1 ± 10.1 years). The diabetic group comprised 21 subjects with IDDM (mean age 46.0 ± 12.9 years) and 7 subjects with NIDDM (mean age 65.8 ± 6.5 years). The mean duration of diabetes was 20.9 ± 8.8 years: IDDM (23.6 ± 7.3 years); NIDDM (12.6 ± 7.9 years). The control group consisted of hospital staff and patients attending an ophthalmic casualty department who were subsequently determined to have no detectable abnormality. Subjects with medical disorders predisposing to autonomic nerve dysfunction, or taking medication with effects on the autonomic nervous system, were excluded from the study. A standard series of cardiovascular autonomic function tests are shown in table 1. These are simple, non-invasive tests, without known side-effects, however there are potential complications in this particular group of patients which required modification of the standard methods. Valsalva manoeuvre and raising blood pressure - as occurs in the diastolic blood pressure response to sustained isometric muscle contraction - involve a rare, potential risk of vitreous haemorrhage in subjects with retinal neovascularisation, and therefore these tests were omitted. The test regimen employed in proliferative retinopathy was therefore:

Parasympathetic nerve function

i. Heart-rate variation during deep breathing

There are many different techniques of assessing respiratory sinus

arrhythmia (10,11,12,13); one of the standard methods is to request the subject to breathe at a rate of 6 deep breaths/minute, whilst recording a continuous electrocardiogram. In normal subjects, a relative tachycardia occurs during inspiration, with a relative bradycardia during expiration. Maximum and minimum R-R intervals are measured and expressed as beats/minute, and the final result is the mean of the differences between maximum and minimum heart-rates.

ii. Immediate heart-rate response to standing

During alteration in posture from lying to standing a characteristic cardiovascular reflex response occurs under vagal control, consisting of an initial tachycardia, maximal at about the 15th beat after standing, followed by a relative bradycardia around the 30th beat. R-R intervals are measured to determine the shortest around the 15th beat and longest at about beat 30, and the result expressed as the 30:15 ratio.

iii. Immediate heart-rate response to lying

A similar, though distinctly separate reflex occurs during alteration in posture from standing to lying. A tachycardia occurs during the 3rd-4th beat after commencing the manoeuvre; the reflex is represented by the ratio of the longest R-R interval during the 5 beats before lying to the shortest during the 10 beats after lying down.

Sympathetic nerve function

Systolic blood pressure response to standing

A fall in blood pressure is precipitated by gravitational pooling

of blood in the lower extremities. Significant fluctuations in blood pressure during alteration in posture is prevented by an intact baroreflex arc, effecting peripheral and splanchnic vasoconstriction via sympathetic efferent nerves. The immediate difference between systolic blood pressure lying and standing is the postural change in blood pressure.

All subjects were in normal sinus rhythm, an essential prerequisite to cardiovascular autonomic function assessment.

Long-term glycaemic control was assessed by glycosylated haemoglobin, using a micro-column technique (14), and an estimation of renal function was obtained by measurement of plasma creatinine (15).

Statistical analysis

Comparisons were made between the results of the control group and diabetic patients with proliferative retinopathy; significance was assessed by Student's unpaired t test. Results are expressed as mean ± SEM.

RESULTS

The results of cardiovascular autonomic function assessment in 28 diabetic patients with proliferative retinopathy and 61 age- and sex-matched control subjects are shown in table 2. Results of all parasympathetic nerve function tests were significantly lower in diabetics with proliferative retinopathy than the control group, with significance at the 0.1% level. Sympathetic nerve function was not significantly different. Age-adjusted normal tolerance intervals in the control group were calculated for each individual test by linear regression analysis; the data were fitted according to various mathematical models using the computer programme SPSSx on an IBM 4341 computer to obtain a normal distribution on age, thus permitting determination of tolerance intervals (16). Values below the 2.5th percentile were arbitrarily designated abnormal. In every test of autonomic nerve function, abnormal values were significantly elevated in diabetics with proliferative retinopathy (table 3).

Autonomic dysfunction is defined as an abnormal result of at least one autonomic function test. By this criterion, the prevalence of parasympathetic neuropathy in proliferative diabetic retinopathy was 67.9%, with sympathetic neuropathy in 28.6% of patients, compared with values in the control group of 2.6% respectively. Neuropathy involving both parasympathetic and sympathetic nerves was present in 21.5% of diabetics with proliferative retinopathy; no member of the control group had evidence of both parasympathetic and sympathetic neuropathy.

Glycosylated haemoglobin was moderately elevated in diabetics with proliferative retinopathy (10.3 ± 0.42%; normal range 6.0-8.5%). Serum creatinine levels were not excessively abnormal in the diabetic group (104.5 ± 8.31 μmol/l; normal range 55-110 μmol/l); abnormal results were present in only 5 diabetic patients.

DISCUSSION

Clinical assessment of autonomic nerve function based upon cardiovascular reflex tests provides an accurate and quantifiable measurement of a nervous system previously virtually impossible to isolate. Heart-rate is determined by the autonomic nervous system, and represents a balance between sympathetic and parasympathetic effects (17,18). Although each test may be used individually, the application of a series of tests provides a detailed assessment of systemic autonomic nerve function.

Diabetes mellitus is the commonest cause of autonomic neuropathy in this country; approximately 17-30% of randomly selected diabetics have abnormal autonomic function, a prevalence confirmed by several large studies (5,19,20,21). By definition, this population will include patients with proliferative retinopathy, and therefore the prevalence in diabetics without retinopathy is proportionately less. Cardiovascular autonomic neuropathy is present in 75% of diabetic subjects with proliferative retinopathy, which is the highest prevalence of secondary autonomic neuropathy recorded to date. Glycaemic control and renal function were not excessively abnormal in these patients, and therefore this could not be considered merely a manifestation of severity of diabetes. Similarly, duration of diabetes was relatively unremarkable.

In conclusion, this study has demonstrated a significant association between cardiovascular autonomic neuropathy and proliferative diabetic retinopathy. The exact nature of this relationship

remains to be defined, however severe impairment of autonomic control of the cardiovascular system may be a contributing factor to the development of retinal neovascularisation in diabetes mellitus.

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Table 1

CARDIOVASCULAR AUTONOMIC FUNCTION TESTS

Parasympathetic nerve function

i. Heart-rate variation during deep breathing

ii. Immediate heart-rate response to standing

iii. Immediate heart-rate response to lying

iv. Valsalva manoeuvre

Sympathetic nerve function

i. Systolic blood pressure response to standing

ii. Diastolic blood pressure response to sustained isometric muscular contraction

Table 2

RESULTS OF CARDIOVASCULAR AUTONOMIC FUNCTION ASSESSMENT IN 28 DIABETIC PATIENTS WITH PROLIFERATIVE DIABETIC RETINOPATHY AND 61 AGE- AND SEX-MATCHED CONTROL SUBJECTS

(Group mean values ± SEM)

	Control group	PDR	Significance
Parasympathetic nerve function			
i. Heart-rate variation (beats/min)	18.24 ± 0.93	10.08 ± 1.42	$p < 0.001$
ii. 30:15 ratio	1.17 ± 0.01	1.07 ± 0.01	$p < 0.001$
iii. S:L ratio	1.21 ± 0.01	1.12 ± 0.01	$p < 0.001$
Sympathetic nerve function			
Postural hypotension (mmHg)	8.36 ± 1.42	11.59 ± 2.99	$p > 0.05$

Table 3

THE PROPORTION OF DIABETICS WITH PROLIFERATIVE RETINOPATHY AND CONTROL SUBJECTS WITH ABNORMAL RESULTS OF CARDIOVASCULAR AUTONOMIC NERVE FUNCTION, DEFINED BY THE AGE-ADJUSTED NORMAL TOLERANCE LIMIT AT THE LOWER 2.5th PERCENTILE

	Control group	PDR
Parasympathetic nerve function		
i. Heart-rate variation	1.3%	64.3%
ii. 30:15 ratio	2.6%	11.1%
iii. S:L ratio	1.3%	25.0%
Sympathetic nerve function		
Postural hypotension	2.6%	28.6%

PAPER 31

Eye (1988) 2, 96–101

Ocular Autonomic Nerve Function in Proliferative Diabetic Retinopathy

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Summary

Ocular autonomic nerve function was assessed in 28 diabetic patients with proliferative retinopathy (PDR) and 61 age- and sex-matched control subjects, by measurement of the pupil cycle time and determination of autonomic denervation hypersensitivity of the iris. Sustained pupil cycle time was absent in 88.5 per cent of PDR compared with 9.8 per cent of control subjects ($p<0.001$). Pupil constriction in response to 2.5 per cent methacholine—indicative of parasympathetic denervation hypersensitivity—was significantly increased in PDR ($p<0.001$), whilst pupil dilation in response to 0.5 per cent phenylephrine—indicative of sympathetic denervation hypersensitivity—was also significantly higher in PDR ($p<0.001$). Pupil reflexes were abnormal in 88.5 per cent of diabetics with proliferative retinopathy, with established autonomic denervation hypersensitivity in 57 per cent of patients.

The principle of denervation hypersensitivity—defined as an increased response of a tissue to a chemical neurotransmitter or agonist following deprivation of its nerve supply[1]—may be applied in the assessment of ocular autonomic nerve function. Pupil diameter represents the relative balance of autonomic activity in the anterior segment of the eye; sympathetic nerves effect pupillary dilation via the dilator pupillae and parasympathetic nerves effect pupillary constriction via the sphincter pupillae. The assessment of parasympathetic denervation hypersensitivity is facilitated by measurement of the pupil diameter after topical application of 2.5 per cent methacholine, a parasympathetic agonist, which constricts Adie's tonic pupils whilst normal pupils may remain unaffected by concentrations of 15 per cent.[2,3] Concentrations of sympathetic agonists diagnostic of sympathetic denervation hypersensitivity have not been definitively established; 1 to 2 per cent phenylephrine[4,5] and 0.1 per cent adrenaline[6] have been proposed for this purpose, although the latter drug has given conflicting results.[4] Assessment of ocular autonomic function is augmented by measurement of pupil cycle time, which enables the integrity of the pupillary reflex arc to be quantified accurately and objectively by a simple, non-invasive technique.[7] In subjects with normal iris musculature, pupil cycle time is directly dependent upon nerve conduction velocity, synaptic delay, and the number of nerve impulses transmitted.

The aim of this study was to assess and quantify ocular autonomic nerve function in diabetic patients with proliferative retino-

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pathy by a series of tests based upon pupil responses to light, and to autonomic mediators recognised to be effective in the determination of ocular autonomic denervation hypersensitivity.

Patients and Methods

Ocular autonomic nerve function was assessed in 28 diabetic patients with proliferative retinopathy (mean aged 50.8 ± 14.3 years): 21 IDDM (mean age 46.0 ± 12.9 years) and 7 NIDDM (mean age 65.8 ± 6.5 years). Mean duration of diabetes was 20.9 ± 8.8 years: IDDM (23.6 ± 7.3 years); NIDDM (12.6 ± 7.9 years). They were compared with a control group of 61 age- and sex-matched subjects (mean age 53.1 ± 10.1 years) who were drawn from fit hospital staff and patients attending an ophthalmic casualty department subsequently determined to have no detectable abnormality. No subject had any medical disorder or were taking any medication known to interfere with autonomic function. Any individual eye was excluded if there was a history of trauma, disease, or operation on the eye, or current treatment for any eye disorder. The study was approved by the local Hospital Advisory Ethical Committee, and each patient was informed of the details of the tests.

(i) *Pupil cycle time*

Edge-light pupil cycle time was measured according to the following method. The patient was seated at a slit-lamp (Zeiss 30SL, Carl Zeiss, Welwyn Garden City, England) in a sealed room with low, constant background illumination (20 lux), and requested to fixate straight ahead on a distant object at 6 metres. A 0.5 × 6 mm slit-beam was positioned horizontally and elevated until it just overlapped the inferior margin of the pupil, then focused on the iris-lens border. The pupil constricted, then reflexly dilated in response to removal of afferent stimulation by iris blocking the light. Thirty consecutive pupil cycles were timed by stop-watch; the result divided by 30 is the duration of a single cycle of the pupillary reflex arc. The pupil cycle time was measured 4 times for each eye separately (i.e. 4 × 30-cycles) and the result averaged.

(ii) *Autonomic denervation hypersensitivity*

(a) Parasympathetic nerves

Pupil diameters were recorded photographically under standardised lighting conditions (luminance 20 apostilbs) using an ophthalmic head-rest and a scale positioned against the lower eyelid in the perpendicular plane of the iris. One drop of 2.5 per cent methacholine chloride solution was placed in the conjunctival sac of one eye, and one drop of sterile normal saline solution concurrently placed in the conjunctival sac of the other eye. The choice of the eye to be tested was selected randomly. Forty-five minutes later, a second photograph was taken.

(b) Sympathetic nerves

One month later, the assessment of sympathetic denervation hypersensitivity was performed using the same technique. A pupil photograph was taken, as described. One drop of 0.5 per cent phenylephrine hydrochloride was placed in the conjunctival sac of the previously untested eye and one drop of sterile normal saline solution placed in the conjunctival sac of the other eye. A second pupil photograph was taken forty-five minutes later.

The photographic slides were projected on to a white screen at five metres, producing a final magnification of ×17. Horizontal pupil diameters were measured to an accuracy of ±0.5 mm; these were corrected to actual values by comparison with relative magnification of the scale. Measurements of actual pupil diameters were therefore accurate to ±0.03 mm.

Parasympathetic denervation hypersensitivity was expressed as the 2.5 per cent methacholine ratio:

$$\frac{\text{horizontal pupil diameter 45 minutes post-test}}{\text{horizontal pupil diameter pre-test}}$$

Sympathetic denervation hypersensitivity was measured in a similar manner, and expressed as the 0.5 per cent phenylephrine ratio:

$$\frac{\text{horizontal pupil diameter 45 minutes post-test}}{\text{horizontal pupil diameter pre-test}}$$

Assessments were performed on a double-

masked basis, with neither the examiner nor the subject being aware which was the tested eye.

Renal function was assessed by plasma creatinine,[8] and long-term glycaemic control was determined by measurement of glycosylated haemoglobin, using a micro-column method.[9]

Statistical analysis

Comparisons were made between the results of the control group and diabetic patients with proliferative retinopathy; significance was assessed by Student's unpaired t test, and χ^2 test with Yates' correction. Results are expressed as mean ± SEM.

Results

(i) *Pupil cycle time*

Sustained pupillary oscillation for 120 consecutive cycles in response to edge-light stimulation was absent in a significant percentage of eyes examined; pupil cycle time was absent in 88.5 per cent of diabetics with proliferative retinopathy compared with 9.8 per cent of control subjects ($p<0.001$). Consequently, measurement of pupil cycle time was possible in a proportionately decreased number of eyes: PDR 11.5 per cent; control group 90.2 per cent. There were no significant differences in pupil cycle time between those diabetics in whom pupil cycle time was present (1,063 ± 49 ms) and the control group (971 ± 14 ms), however this represents only 11.5 per cent of the diabetic patients.

(ii) *Parasympathetic denervation hypersensitivity*

2.5 per cent methacholine ratios were significantly lower in diabetic patients with proliferative retinopathy (0.84 ± 0.03) than the control group (0.95 ± 0.01) ($p<0.001$). Pupillary constriction resulting from methacholine stimulation of the sphincter pupillae in a diabetic patient with proliferative retinopathy is shown in Figure 1.

Age-adjusted normal tolerance intervals have been established for 2.5 per cent methacholine ratio;[10] Log_{10} (2.5 per cent methacholine ratio) depends significantly on age ($r = -0.40$; $p<0.001$). The age-adjusted normal tolerance limit, arbitrarily set at the lower 5th percentile of the log normal distribution after regression on age, was applied to the results of diabetics with proliferative retinopathy, to determine the proportion of subjects outwith the normal limit. By this criterion, parasympathetic neuropathy was present in 36 per cent of diabetics with proliferative retinopathy, compared with 6.4 per cent of the control group.

(iii) *Sympathetic denervation hypersensitivity*

0.5 per cent phenylephrine ratios were significantly higher in diabetic patients with proliferative retinopathy (1.35 ± 0.05) than the control group (1.17 ± 0.03) ($p<0.001$). Pupil dilation following phenylephrine stimulation of the dilator pupillae in a diabetic with proliferative retinopathy is shown in Figure 2.

Age-adjusted normal tolerance intervals for pupil response to 0.5 per cent phenylephrine have not been previously defined; 0.5 per cent phenylephrine ratios in the control group were therefore assessed for dependence on age by regression analysis. The data were fitted according to various mathematical models using the computer programme SPSSx to obtain a normal distribution on age, thereby permitting determination of tolerance intervals.[11] Linear regression analysis was calculated from:

$$RLP_{0.5} = (\log_{10}P_{0.5} - (-0.05084 + 0.00213 \times \text{age}))/0.05214$$

$RLP_{0.5}$ = standardised residual log (0.5 per cent phenylephrine ratio)

Log_{10} (0.5 per cent phenylephrine ratio) was shown to depend significantly on age ($r = 0.64$; $p<0.001$) (Fig. 3). The age-adjusted normal tolerance limit, arbitrarily set at the upper 95th percentile of the log normal distribution after regression on age, was then applied to the results obtained from diabetics with proliferative retinopathy, to determine the proportion of subjects outwith the normal limit. By this criterion, sympathetic neuropathy was present in 38.4 per cent of diabetics with proliferative retinopathy compared with 5.0 per cent of the control group.

Serum creatinine levels were not excessively abnormal in the diabetic group (104.5 ±



Fig. 1. *Pupil constriction following instillation of 2.5 per cent methacholine chloride solution into the left eye of a diabetic patient with proliferative retinopathy, demonstrating parasympathetic denervation hypersensitivity.*



Fig. 2. *Pupil dilation following instillation of 0.5 per cent phenylephrine hydrochloride solution into the right eye of a diabetic patient with proliferative retinopathy, demonstrating sympathetic denervation hypersensitivity.*

8.31 μmol/l; normal range 55–110 μmol/l), with abnormal results in only 5 diabetic patients. Glycosylated haemoglobin was moderately elevated in diabetics with proliferative retinopathy (10.3 ± 0.42 per cent; normal range 6.0–8.5 per cent).

Discussion

Providing its structural integrity is intact, the iris facilitates direct measurement of autonomic efficacy in the anterior segment of the eye. Pupil cycle time permits rapid and accurate assessment of the pupillary reflex arc; the procedure is without side-effects and involves no expense, and is therefore directly applicable to the clinical situation. Recent studies have established that pupil cycle time is a particularly sensitive measure of dysfunction in the parasympathetic efferent limb of the pupillary reflex arc,[12] however definitive anatomical localisation of defects requires more specific tests; this is provided by isolation of the primary determinants of pupil diameter—the sympathetic and parasympathetic nervous systems—by using minute concentrations of chemical agonists which only effect changes in pupil diameter in the presence of increased receptor density at the postsynaptic receptor site[13,14]—a characteristic feature of autonomic denervation hypersensitivity. Impaired parasympathetic innervation of the anterior segment is demonstrated by 2.5 per cent methacholine[2,3]; as responses to methacholine are significantly age-related, comparison with an age-adjusted normal tolerance interval is essential. The type and optimal concentration of agonist to be utilised in the assessment of sympathetic denervation hypersensitivity has not been established, however phenylephrine, a direct-acting sympathomimetic amine, which effects mydriasis by alpha-adrenoceptor stimulation of the sympathetically-innervated dilator pupillae,[15] is ideally suited for this specific purpose. The exact concentration of phenylephrine to be used in the evaluation of sympathetic denervation hypersensitivity is not as important as comparison with an age-adjusted normal tolerance interval; this has been established for 0.5 per cent phenylephrine, which may therefore be applied effectively in this context.

The results of the present study have shown significant ocular autonomic dysfunction in diabetic patients with proliferative retinopathy, thereby providing further evidence of an association between autonomic neuropathy and proliferative diabetic retinopathy. Parasympathetic neuropathy was present in 36 per cent of diabetics, with sympathetic neuropathy in 38.4 per cent of subjects, compared with a prevalence in the control group of 6.4 per cent and 5 per cent respectively. Ocular autonomic neuropathy involving either parasympathetic or sympathetic nerves was present in 57 per cent of diabetics compared with only 6.4 per cent of control subjects. Abnormalities in the pupillary reflex arc, with impairment or absence of sustained pupil cycling in response to light, was present in 88.5 per cent of diabetics with proliferative retinopathy; this may represent the earliest manifestation of ocular autonomic nerve dysfunction. One may reasonably postulate

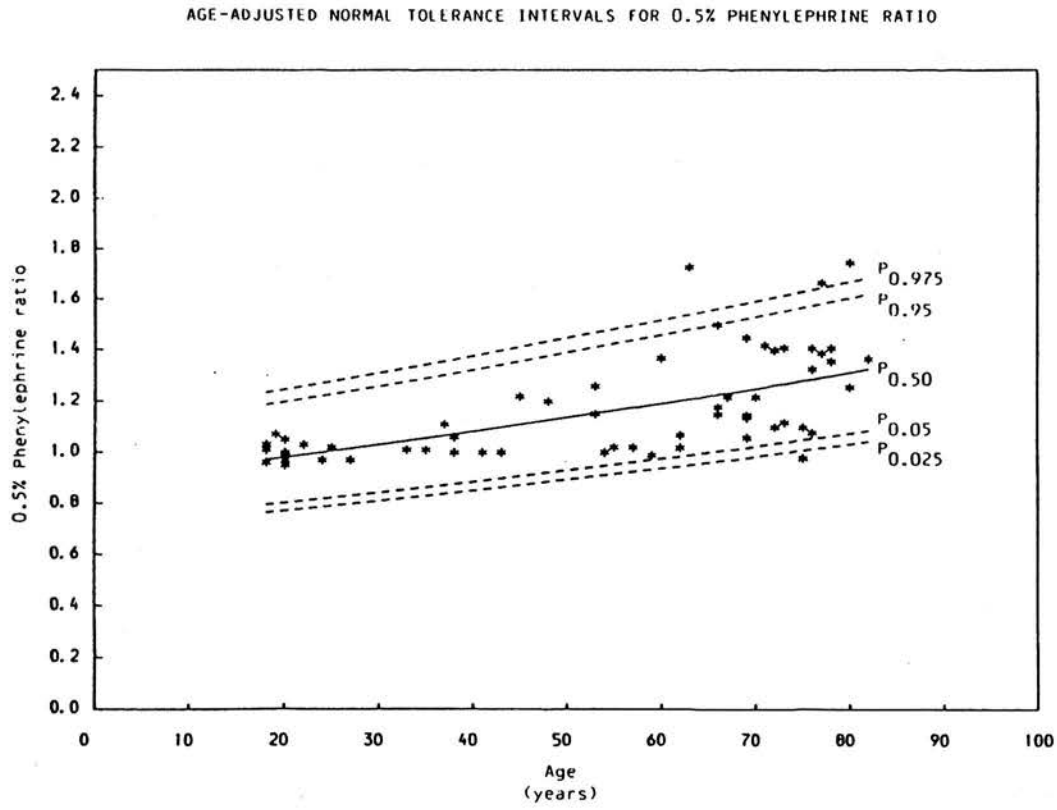


Fig. 3. *Age-adjusted normal tolerance intervals for 0.5 per cent phenylephrine ratio, with 2.5th, 5th, 95th, and 97.5th percentiles.*

that assessment of iris dynamic function over prolonged periods of stimulation is more likely to reveal the prodromal signs of impaired efficacy prior to the development of specific changes in iris structure—for example, increased density of postsynaptic receptors, which is primarily determined by tests of denervation hypersensitivity.

Autonomic neuropathy is not characteristically a localised disorder. In diabetes, tests of ocular autonomic function correlate closely with autonomic function tests based upon cardiovascular reflexes.[12] As systemic cardiovascular autonomic neuropathy has been demonstrated in 75 per cent of diabetics with proliferative retinopathy,[16] the presence of ocular autonomic neuropathy in 57 per cent of these patients is not surprising. Glycaemic control and renal function were not excessively abnormal in this group, duration of diabetes was not unduly prolonged, and therefore this is unlikely to represent a direct manifestation of severity of diabetes.

Previous studies have established a significant association between cardiovascular autonomic neuropathy and proliferative diabetic retinopathy,[16,17] with direct correlation between the degree of cardiovascular autonomic neuropathy and the severity of retinopathy.[17] Evidence of severe impairment of ocular autonomic nerve function in diabetics with proliferative retinopathy raises the inevitable question of whether this association, which cannot be explained exclusively in terms of diabetic severity, represents merely a coincidental natural history,[17] or perhaps a more significant link between these diabetic complications.

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PAPER 32

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Ocular autonomic function in progressive autonomic failure

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Key words: autonomic neuropathy, denervation hypersensitivity, pupil cycle time, progressive autonomic failure

Abstract. Ocular autonomic function was assessed in 4 patients with progressive autonomic failure (PAF) and age-matched control subjects, by measurement of the pupil cycle time, and determination of autonomic denervation hypersensitivity of the iris. Pupil cycle time was abnormal in all patients with PAF; sustained pupil cycling was absent in 5 of the 8 eyes tested of the PAF patients, compared with only 16 eyes from 70 control subjects. Pupil constriction in response to 2.5% methacholine, indicative of parasympathetic denervation hypersensitivity, was significantly increased in patients with PAF ($p < 0.001$), whilst pupil dilation in response to 0.5% phenylephrine, indicative of sympathetic denervation hypersensitivity, was also significantly higher in the PAF patients ($p < 0.001$). The results suggest that ocular autonomic function may provide a sensitive early indicator of generalised autonomic dysfunction.

Introduction

Denervation hypersensitivity is defined as an increased response of a tissue (the effector) to a chemical neurotransmitter or agonist after the tissue has been deprived of its nerve supply [1]. This principle may be applied in the assessment of ocular autonomic integrity. Parasympathetic nerves effect pupillary constriction via the sphincter pupillae, and sympathetic nerves effect pupillary dilation via the dilator pupillae. Pupil diameter is easily and accurately measured, and reflects the relative balance of autonomic activity in the anterior segment of the eye. Measurement of pupil diameter after topical application of methacholine, a parasympathetic agonist, and phenylephrine, a sympathetic agonist, can be used to assess denervation hypersensitivity in the eye [2, 3, 4, 5]. These techniques of ocular assessment may be augmented by measurement of edge-light pupil cycle time, a simple and quantifiable way to assess the efferent parasympathetic limb of the pupillary light reflex arc [6].

The aim of this study was to assess and quantify ocular autonomic

function in patients with progressive autonomic failure (PAF) by a series of tests based upon pupillary responses to light, and to autonomic mediators recognised to be effective in the determination of ocular autonomic denervation hypersensitivity.

Methods

Ocular autonomic function was assessed in 4 patients with PAF (aged 62, 66, 66, 75 years). They were compared with a control group of 70 subjects (mean age 66.1 ± 8.2 years: range 50–89 years) who were drawn from fit hospital staff and patients attending an ophthalmic casualty department. Pupil cycle time was measured in all 70 control subjects, and autonomic denervation hypersensitivity was assessed in 22 control subjects (mean age 67.9 ± 6.0 years: range 56–76 years). None of the control subjects had any known medical disorder or were taking any medication that might interfere with autonomic function. Any individual eye was excluded if there was a history of disease, trauma, or operations on that eye, or current treatment for any eye disorder. The diagnosis of PAF was made from the clinical history, and results from a standard series of five cardiovascular reflex tests. The tests used were the heart rate responses to the Valsalva manoeuvre, deep breathing and standing up, and the blood pressure responses to standing up and sustained handgrip. We have described the techniques in detail elsewhere, together with normal ranges [7]. The results were grossly abnormal in all four subjects (Table 1). Subjects with known secondary causes of autonomic dysfunction (e.g. diabetes) were excluded. Three of the four subjects presented with postural hypotension, and the fourth subject had symptoms of multiple system atrophy, with postural hypotension, nystagmus, intention tremor, dysarthria, ataxia, bladder problems and impotence. The study was approved by the Local Hospital Advisory Ethical Committee, and each patient agreed to participate after being informed of the details of the tests.

Pupil cycle time

This was determined as described in detail previously [6]. The technique involves shining a horizontal slit-beam of light on the inferior margin of the pupil, thereby inducing a cycle of constriction and dilation which can be simply and accurately timed with a stop-watch. The duration of a single cycle of the pupillary reflex arc is represented by the average of 100 cycles and is expressed in milliseconds.

Table 1. Cardiovascular autonomic function in 4 patients with progressive autonomic failure.

Patient No.	Sex	Age	Cardiovascular autonomic function tests				
			Valsalva ratio	Heart rate variation during deep breathing (max-min heart rate) (beats/min)	30:15 ratio	Systolic BP response to standing (mmHg)	Diastolic BP response to sustained handgrip (mmHg)
1	M	66	1.00	6	0.97	−26	5
2	M	66	1.13	3	1.08	− 2	8
3	F	62	1.06	4	1.05	−28	2
4	F	75	1.04	1	1.04	−56	2
Normal values			⩾1.21	⩾15	⩾1.04	⩽−10	⩾16

Autonomic denervation hypersensitivity

Parasympathetic involvement. One drop of 2.5% methacholine chloride solution was placed in the conjunctival sac of one eye and one drop of sterile normal saline solution concurrently placed in the conjunctival sac of the contralateral eye. The choice of the eye to be tested was selected randomly. Pupil diameters of both eyes were recorded photographically under standardised lighting conditions (luminance 20 apostilbs) using an ophthalmic headrest and a scale positioned against the lower eyelid in the perpendicular plane of the iris before, and 45 minutes after the drops were put in the eyes.

Sympathetic involvement. Two hours later, the assessment of sympathetic denervation hypersensitivity was performed using the same technique. One drop of 0.5% phenylephrine hydrochloride was placed in the conjunctival sac of the previously untested eye and one drop of normal saline placed in the conjunctival sac of the contralateral eye. Pupil photographs were taken before, and 45 minutes after putting the drops in the eyes.

The photographic slides were projected on to a white screen at five metres, producing a final magnification of $\times 17$. Horizontal pupil diameters were measured to an accuracy of ± 0.5 mm, and corrected to actual values by comparison with relative magnification of the scale. Effectively, measurements of actual pupil diameters were accurate to ± 0.03 mm.

Parasympathetic denervation hypersensitivity was expressed as the 2.5% methacholine ratio:

$$\frac{\text{horizontal pupil diameter 45 minutes post-test}}{\text{horizontal pupil diameter pre-test}}$$

Sympathetic denervation hypersensitivity was measured in a similar manner, and expressed as the 0.5% phenylephrine ratio:

$$\frac{\text{horizontal pupil diameter 45 minutes post-test}}{\text{horizontal pupil diameter pre-test}}$$

All assessments were performed on a double-blind basis, with neither the subject nor the examiner being aware which was the tested eye.

Anterior chamber dimensions

The volume of the anterior chamber was measured in all eyes of patients with PAF by a photogrammetric technique (8). Standard age-adjusted

normal reference values were used for comparison. Angle-assessment was made by the van Herick method [9], according to the Shaffer classification [10]. Intraocular pressure was measured in all subjects by Goldmann applanation tonometry.

Statistical analysis

Comparisons were made between the results of the control group and patients with PAF. Significance was assessed by Student's unpaired t test. Results are expressed as mean ± SD.

Results

Pupil cycle time

Pupil cycle times in the 4 patients with PAF are shown in Table 2. Sustained pupillary oscillation for 100 cycles in response to light was absent in 5 of the 8 of eyes examined, compared with only 16 eyes of 70 control subjects. Age-adjusted normal tolerance intervals have been established for pupil cycle time [11]; the normal tolerance limit was arbitrarily set at the upper 95th percentile, and applied to the PAF results. By this criterion, pupil cycle time was abnormal in all 8 eyes of the PAF patients. In eyes where it was measurable, pupil cycle time was significantly higher in the PAF patients (1279 ± 144 ms) than the control group (971 ± 136 ms).

Pupil diameter

There were no significant differences between pre-methacholine test pupil diameter in the control group (4.42 ± 0.94 mm) and the PAF patients (4.05 ± 1.74 mm). Similarly, there were no significant differences between pre-phenylephrine test pupil diameter in the control group (4.63 ± 1.04 mm) and the PAF patients (3.81 ± 1.42 mm). Following application of 2.5% methacholine, pupil diameter was significantly lower in the PAF patients (2.83 ± 1.42 mm) than the control group (4.07 ± 1.03 mm)($p < 0.05$). In response to 0.5% phenylephrine, pupil diameter was significantly higher in the PAF patients (6.37 ± 1.39 mm) compared with the control subjects (5.02 ± 1.12 mm)($p < 0.05$)(Table 2).

Parasympathetic denervation hypersensitivity

Figure 1 shows the individual pupil responses to 2.5% methacholine

1234.

Table 2. Results of ocular autonomic function assessment in 4 patients with progressive autonomic failure.

Patient No	Pupil cycle time (ms)		Pupil diameter (pre 2.5% M) (mm)	Pupil diameter (2.5 M) (mm)	2.5% M ratio	Pupil diameter (pre 0.5% P) (mm)	Pupil diameter (0.5 P) (mm)	0.5% P ratio	Anterior chamber volume (μl)		Angle measurement		Intraocular pressure (mmHg)	
	Right eye	Left eye							Right eye	Left eye	Right eye	Left eye	Right eye	Left eye
1	1445	No cycling	2.45	2.22	0.91	2.52	4.37	1.73	136.8	121.4	Wide	Wide	14	16
2	No cycling	No cycling	6.37	4.96	0.78	5.70	7.48	1.31	200.1	172.1	Wide	Wide	14	15
3	No cycling	No cycling	4.35	2.15	0.49	4.07	7.11	1.75	136.8	124.1	Wide	Wide	14	10
4	1191	1201	3.04	2.00	0.66	2.96	6.53	2.20	152.0	152.2	Wide	Wide	15	17

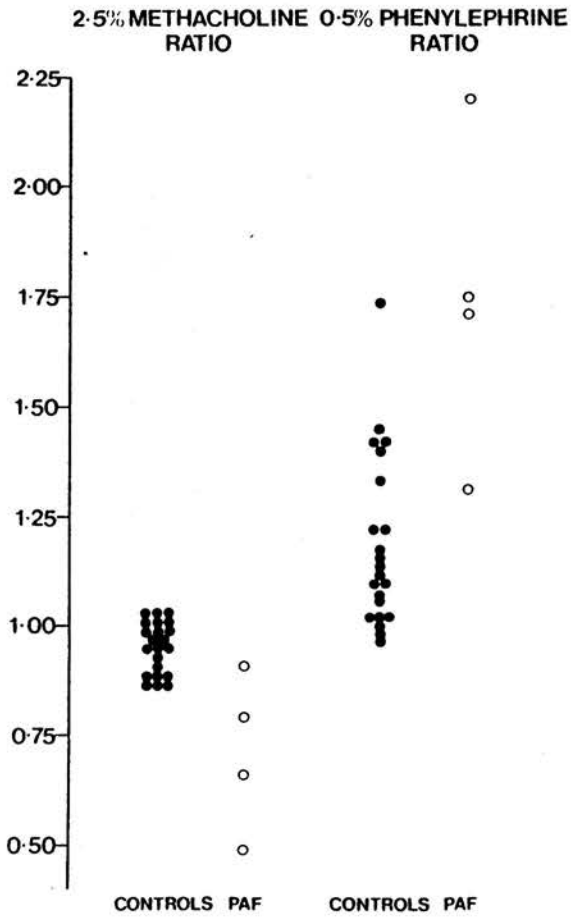


Fig. 1. Pupil responses to 2.5% methacholine and 0.5% phenylephrine in 4 patients with PAF and 22 age-matched control subjects.

chloride in the PAF patients and age-matched control subjects. 2.5% methacholine ('2.5% M') ratios were significantly lower in patients with PAF (0.71 $\pm$ 0.18) than the control group (0.95 $\pm$ 0.05)($p < 0.001$)(Table 2). This is demonstrated in Fig. 2, with significant pupillary constriction in response to methacholine in the right eye of a patient with PAF. Age-adjusted normal tolerance intervals have been established for the 2.5% methacholine ratio [12]; the normal tolerance limit was arbitrarily set at the lower 5th percentile, and applied to the PAF results. By this criterion, ocular parasympathetic neuropathy was present in 3 of the 4 PAF patients (subjects 2, 3 and 4).



Fig. 2. Pupil constriction in the right eye of a patient with PAF following application of 2.5% methacholine, demonstrating parasympathetic denervation hypersensitivity.

Sympathetic denervation hypersensitivity

Individual pupil responses to 0.5% phenylephrine hydrochloride in the PAF patients and control subjects are shown in Fig. 1. 0.5% phenylephrine ('0.5% P') ratios were significantly higher in patients with PAF (1.75 ± 0.36) than the control group (1.19 ± 0.19) ($p < 0.001$)(Table 2). An example of dilation of the left pupil resulting from 0.5% phenylephrine stimulation of the dilator pupillae in a patient with PAF is shown in Fig. 3.

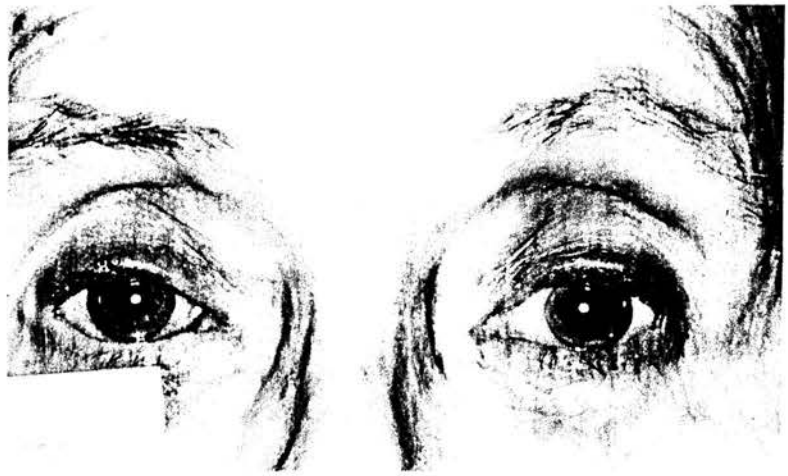


Fig. 3. Pupil dilation in the left eye of a patient with PAF following application of 0.5% phenylephrine, demonstrating sympathetic denervation hypersensitivity.

Age-adjusted normal tolerance intervals have been established for the 0.5% phenylephrine ratio [13]; the normal tolerance limit was arbitrarily set at the upper 95th percentile, and applied to the PAF results. By this criterion, ocular sympathetic neuropathy was present in 3 of the 4 patients (subjects 1, 3 and 4).

Conjunctival response to 2.5% methacholine

In addition to pupillary constriction following topical application of 2.5% methacholine chloride, there was also prominent conjunctival chemosis and hyperaemia in the PAF patients, a response one would anticipate in the presence of increased cholinergic receptors on conjunctival blood vessels subsequent to denervation hypersensitivity. This reaction occurred approximately 3–4 minutes following application of methacholine, and therefore before intraocular absorption had produced much pupil constriction. The response only occurred in the PAF patients and, to our knowledge, has not previously been reported.

Anterior chamber volume

The volume of the anterior chamber in all eyes of patients with PAF (Table 2) was compared with standard age-adjusted normal values [9]. All were within normal tolerance limits and there was no significant difference between the mean anterior chamber volume in patients with PAF ($149.4 \pm 26.3\,\mu l$) compared with the mean value of normal subjects aged 67 years ($159 \pm 38\,\mu l$)[9].

Angle assessment

Assessment of the peripheral irido-corneal angle demonstrated wide angles in all eyes of PAF patients (Table 2).

Intraocular pressure

Intraocular pressures were normal in all eyes of PAF patients (14.4 ± 2.06 mm Hg)(Table 2).

Discussion

The results of this study have confirmed the presence of ocular autonomic dysfunction in PAF by a series of neuropharmacological manipulations to

localise defective autonomic innervation of iris musculature. Observations of increased pupil responses to topical cholinergic and adrenergic agonists resulted in the treatise on denervation hypersensitivity by Cannon and Rosenbluth in 1949 [1]. The 'Mecholyl' test has been used subsequently to detect anterior segment parasympathetic denervation [14, 15]. Phenylephrine is a direct-acting sympathomimetic amine, a specific alpha-agonist which effects mydriasis by alpha-adrenoceptor stimulation of the sympathetically-innervated dilator pupillae [16] and is therefore eminently suited, in low concentration, to the assessment of sympathetic denervation hypersensitivity in the anterior segment of the eye. The standard concentration of phenylephrine used in clinical practice to cause mydriasis is 10%. Our test employed a concentration of 0.5%, which is 1/20th of the normal concentration used. Enhanced pupillary responses to topical phenylephrine have been demonstrated in Horner's syndrome [17].

Progressive autonomic failure occurs as a secondary complication, particularly in diabetes mellitus, but the primary disorder is extremely rare. Examination of such subjects with PAF provides an opportunity to assess and quantify ocular autonomic function in this disorder. We have shown marked abnormalities of ocular autonomic function, and evidence of denervation hypersensitivity in the eye in our subjects. Although increased pupillary responses to specific diagnostic agents have been described in PAF [18], accurate quantification of these pupil responses has not been defined. The presence of supersensitive pupil responses to topically-applied autonomic agonists has previously been described in patients with acute autonomic neuropathy [19, 20, 21], and familial dysautonomia (the Riley-Day syndrome)[15, 22, 23], but without detailed quantification of pupil responses.

Where abnormality is suspected, comparison with age-adjusted normal tolerance intervals is essential, as the effects of topical 2.5% methacholine and 0.5% phenylephrine are significantly dependent upon age [12, 13]. Methods to assess pupil size have included the use of a transparent millimeter rule, with measurements corrected to ± 0.5 mm [22, 23]. Our pupillometric technique is accurate to within ± 0.03 mm, thereby increasing accuracy by a factor of approximately seventeen. Determination of a grossly abnormal pupillary response is relatively simple. Of more importance is accurate definition of 'normal' pupil reactions to specific autonomic drugs, so that normal tolerance limits for each test may be clearly delineated and thereafter used to identify early stages of ocular autonomic dysfunction. To our knowledge, this is the first paper to quantify and define accurately ocular autonomic responses to specific parasympathetic and sympathetic agonists in the denervation hypersensitivity of PAF.

Our results suggest that significant autonomic neuropathy in the eye is not restricted to the iris. Cholinergic innervation of human conjunctival blood vessels has recently been confirmed [24]. This is supported by the parasympathetic denervation hypersensitivity tests in our PAF patients as significant conjunctival chemosis and hyperaemia occurred in response to 2.5% methacholine. If increased numbers of cholinergic receptors had not been present on the conjunctival blood vessels of PAF patients, no reaction would have occurred in response to a specific cholinergic agonist. This reaction to methacholine was not present in normal subjects and no conjunctival response occurred to placebo drops in the other eye of any of our subjects. Parasympathetic denervation hypersensitivity of both external and internal ocular structures is suggested, implying a more extensive lesion than the involvement of any single parasympathetic nerve or ganglion. The parasympathetic nerve supply to the iris travels with the IIIrd nerve and synapses in the ciliary ganglion. However, the parasympathetic nerve supply to the conjunctiva is postulated to originate in the facial nerve, synapsing in the pterygopalatine ganglion [24].

Recent studies have established that pupil cycle time is particularly affected by dysfunction in the parasympathetic efferent limb of the pupillary reflex arc [6]. Abnormalities in the pupil reflex, with impairment or absence of sustained pupil cycling in response to light, was present in all patients with progressive autonomic failure. This may represent the earliest sign of ocular autonomic dysfunction, on the basis that assessment of iris function over prolonged periods of active stimulation may reveal earlier impairment before specific anatomical alterations of the iris can be demonstrated by tests of denervation hypersensitivity.

Assessment of sympathetic denervation hypersensitivity of the iris may have a more specific practical application. Cardiovascular tests of sympathetic nerve function are not as sensitive as assessment of parasympathetic function [25]. In diabetes generalised sympathetic nerve damage is usually present before any clinical features of sympathetic neuropathy appear. Demonstration of sympathetic neuropathy of the iris by a simple, easily-performed test might therefore provide a sensitive indicator of early sympathetic nerve dysfunction. This is supported by the results of a recent study which showed impairment of iris sympathetic nerve function in diabetic subjects with no other evidence of autonomic neuropathy [26].

This study has demonstrated and quantified ocular autonomic nerve function in patients with progressive autonomic failure (PAF). Our results suggest that assessment of autonomic function in the anterior segment of the eye provides a sensitive indicator of early autonomic dysfunction.

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SECTION III

MISCELLANEOUS

PAPER 33

Decreased Skin Wrinkling in Diabetes Mellitus

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Skin wrinkling, which may be dependent on peripheral autonomic innervation, was assessed in 100 normal and 42 diabetic subjects using a temperature-controlled water bath with immersion of the hands for 30 min. Markedly decreased skin wrinkling was observed in diabetic subjects and in manual workers, but did not correlate with cardiovascular tests of autonomic function, control, type or duration of diabetes, or diabetic complications. The cause of decreased skin wrinkling in people with diabetes is unexplained. DIABETES CARE 7: 224–227, MAY–JUNE 1984.

Wrinkling of the skin of the finger pulps following immersion in warm water, a phenomenon reported in 1936 by Lewis and Pickering,[1] was proposed as a test of the integrity of peripheral innervation by Buncke[2] and O'Riain.[3] Subsequent reports have suggested that this change might also be of use in evaluating autonomic neuropathy.[4,5] We have therefore assessed the skin wrinkling responses in normal and diabetic subjects, and compared the results with cardiovascular tests of autonomic nerve function.

PATIENT AND METHODS

A control group of 100 normal subjects (43 men and 57 women) aged 18–83 yr (mean 41 yr), comprising hospital staff and patients awaiting minor elective surgical procedures, was examined. Subjects having had a previous surgical sympathectomy and those taking any medication known to influence the autonomic nervous system were excluded, as were 5 subjects with excessive preexisting wrinkling. Occupation and the dominant hand were noted. The subjects were grouped according to their work as "manual" or "nonmanual." A manual worker was defined as one whose occupation necessitated physical labor, and in whom working with the hands constituted a major integral part of that occupation.

The 42 diabetic patients (30 men and 12 women) were aged 29–71 yr (mean 46 yr). The duration of diabetes was 1–35 yr (mean 14 yr); 36 were treated with insulin and 6 with oral hypoglycemic agents. Control of diabetes was assessed by the mean blood glucose values of the previous four clinic visits: 10.0 mmol/L or less was defined as "good," 10.1–15.0 mmol/L as "fair," and 15.1 mmol/L or more as "bad." Control was good in 22%, fair in 45%, and bad in 33%. The eyes were examined by a diabetologist not involved in the study and the urine tested at the subject's routine clinic attendance. For the purpose of this analysis, "background," "exudative," and "proliferative" retinopathy have been pooled together and classed simply as "retinopathy." Proteinuria was defined as present when more than 30 mg/dl was found [+, ++, or +++ on testing with Albustix (Ames Division, Miles Laboratories, Elkhart, Indiana)]. Retinopathy was present in 60%, symptoms of peripheral or autonomic neuropathy in 45%, and proteinuria in 31%. (Incomplete details were available in 8 diabetic subjects.) Twelve diabetic subjects had normal tests of cardiovascular autonomic function, 14 had parasympathetic dysfunction, and 16 had both parasympathetic and sympathetic abnormalities as we have previously defined.[6] We used five tests: the heart rate responses to the Valsalva maneuver, and standing up and deep breathing are measures of cardiovascular parasympathetic integrity, while the blood pressure responses to standing up and sustained handgrip are abnormal only with more widespread sympathetic damage.[7] The autonomic nerve function test results were unknown to the observers evaluating the skin wrinkling. None of the diabetic subjects studied were using finger-prick assessment of blood glucose, which might have affected skin thickness.

In each subject, both hands were completely immersed in warm water for 30 min. Our procedure was based on that proposed by O'Riain,[3] with the following minor modifications: (1) a thermostatically controlled water bath at 41°C was used, rather than a "basin of warm water" at 40°C, and (2) the arms were immersed to the midforearm level.

The degree of wrinkling in each fingertip after 30 min of

TABLE 1
Skin wrinkling in 100 normal and 42 diabetic subjects (group mean values ± SD and range)

	Numbers of fingers wrinkled	Score		
		Right hand	Left hand	Combined
Maximum	10	20	20	40
Normal subjects (N = 100)	9.1 ± 1.6 (5–10)	13.3 ± 5.0 (5–20)	13.9 ± 4.7 (4–20)	27.2 ± 9.1 (11–40)
Diabetic subjects				
All (N = 42)	6.8 ± 3.1* (1–10)	6.6 ± 4.3* (0–17)	6.9 ± 4.7* (0–15)	13.5 ± 8.6* (2–34)
Normal cardiovascular reflexes (N = 12)	7.0 ± 3.7 (2–10)	7.7 ± 4.6 (0–13)	7.3 ± 5.0 (0–13)	14.9 ± 9.0 (2–26)
Parasympathetic dysfunction (N = 14)	6.7 ± 2.6 (1–10)	5.3 ± 3.1 (2–13)	5.9 ± 4.2 (0–15)	11.1 ± 6.9 (2–28)
Parasympathetic and sympathetic abnormalities (N = 16)	6.7 ± 3.2 (1–10)	6.9 ± 5.0 (0–17)	7.6 ± 5.0 (2–15)	14.5 ± 9.6 (2–34)

*Significantly different from normal subjects ($P < 0.001$).

immersion was independently graded by two observers, who had previously agreed on the following arbitrary scale: (0) absent: no evidence of any skin wrinkling, fingertip smooth; (1) very slight: wrinkling that was just present, so that the fingertip was not completely smooth; (2) slight: obvious wrinkling with 2 or less lines of wrinkling on the fingertip; (3) moderate: obvious wrinkling with 3 or more lines of wrinkling on the fingertip; and (4) marked: wrinkling completely distorting the pulp of the fingertip.

This gave a numerical score of 0–4 for each finger. The average score of the two observers was recorded and thus a maximum score of 20 was possible for each hand. In practice grading of the fingers using this scale was simple, and the assessments of the two observers agreed to ±1 point for each hand (i.e., a maximum difference of 1 in 20, or 5%, between the two observers). The results were tabulated as numbers of fingers wrinkled, scores for right and left hands separately, and combined score for both hands.

Fifteen randomly selected normal subjects were examined after 10, 20, and 30 min of immersion to assess how soon maximal wrinkling occurred. Ten normal and 10 diabetic subjects were retested within 3 mo of the initial procedure to evaluate reproducibility of the technique.

Fifteen other normal subjects were tested in the same manner for wrinkling of the toes after immersion in warm water for 30 min. However, extremely variable responses were found,

TABLE 2
Skin wrinkling in manual and nonmanual normal and diabetic subjects (group mean values ± SD)

	Numbers of fingers wrinkled	Score				
		Right hand	Left hand	Combined	Dominant hand	Nondominant hand
Maximum	10	20	20	40	20	20
Normal subjects						
Manual (N = 12)	6.7 ± 2.0	8.2 ± 5.0	8.7 ± 4.5	16.9 ± 9.1	8.6 ± 4.7	8.3 ± 4.8
Nonmanual (N = 88)	9.4 ± 1.2*	14.0 ± 4.6*	14.7 ± 4.3*	28.6 ± 8.3*	14.0 ± 4.5*	14.6 ± 4.4*
Diabetic subjects						
Manual (N = 17)	6.9 ± 3.2	6.7 ± 4.6	7.4 ± 4.8	14.1 ± 8.6	6.7 ± 4.6	7.4 ± 4.8
Nonmanual (N = 25)	6.7 ± 3.1†	6.5 ± 4.2†	6.6 ± 5.0†	13.1 ± 8.7†	6.5 ± 4.2†	6.6 ± 5.0†

Significantly different from normal manual workers ($P < 0.001$).
Significantly different from normal nonmanual workers ($P < 0.001$).

such that no consistent pattern of normal toe wrinkling emerged. No diabetic subjects were therefore tested for toe wrinkling.

Student's paired and unpaired *t* tests were used to assess statistical significance.

RESULTS

Normal subjects. The results of the normal subjects (N = 100) are shown in Table 1. There were no differences in individual scores between the dominant and the nondominant hands, nor between men and women. The degree of skin wrinkling was not correlated with age. Significantly less wrinkling was noted in the manual workers (N = 12) (Table 2, all columns $P < 0.001$). In the 10 subjects subsequently retested there were no significant differences between any of the initial or repeat scores. In the 15 subjects who were assessed after 10, 20, and 30 min of immersion, the skin wrinkling scores increased significantly with increasing duration. The mean combined score (±SD) after 10 min was 18.6 ± 5.8, after 20 min 25.0 ± 5.6, and after 30 min 29.4 ± 6.3 (significance 10 versus 20 and 30 min, and 20 versus 30 min, all $P < 0.001$).

Diabetic subjects. The mean scores for finger wrinkling in the 42 diabetic subjects were significantly lower than in the control group (Table 1, all columns $P < 0.001$), but there were no significant differences between diabetic subjects with different degrees of autonomic dysfunction. The degree of skin wrinkling and both the duration and control of diabetes were not significantly related, nor were significant differences found between diabetic subjects treated with insulin and those on oral hypoglycemic agents. There were no significant differences in mean scores between those with and without retinopathy, proteinuria, and symptoms of neuopathy, except for a smaller number of fingers wrinkled in those with symptoms of neuropathy (5.0 ± 3.5 versus 7.6 ± 2.7, $P < 0.02$). Manual and nonmanual working diabetic subjects had similar scores. There were no significant differences between manual-working diabetic and manual-working normal subjects, but the differences in scores between the nonmanual group of diabetic workers and the nonmanual group of normal subjects were highly significant (Table 2, all columns $P < 0.001$). In the 10 diabetic subjects subsequently retested, the repeat scores were not significantly different from the initial scores.

DISCUSSION

The diabetic subjects as a group showed significantly less wrinkling than the normal subjects. This new observation could not be explained on the basis of autonomic neuropathy as assessed by the cardiovascular reflex tests, nor was there any relationship with the duration or control of diabetes, or the type of therapy. Diminished skin wrinkling was also not obviously related to diabetic complications. The influence of autonomic neuropathy, however, cannot be discounted entirely, since the cardiovascular tests may not reflect early changes in the peripheral nerves.

The mean numbers of fingers exhibiting skin wrinkling among normal subjects was at least 9 out of 10, thus showing that skin wrinkling occurs in most normal subjects following immersion of the hands in warm water (41°C) for 30 min. The results were reproducible in normal and diabetic individuals. Like O'Riain[3] we found that the degree of skin wrinkling is related to the duration of continuous immersion. Whether even longer immersion increases skin wrinkling further is not established, but the prolonged nature of such a test would limit its practical application. Wrinkling of the skin was clearly related to occupation in the normal subjects, with manual workers showing markedly lower scores than nonmanual workers; therefore, the test is of limited value in manual workers. Interestingly, in a recent study of 54 normal subjects, some of whom were manual workers, one-third did not wrinkle.[8,9]

The physiologic basis of skin wrinkling in normal subjects is unexplained, but possible contributory factors include swelling of the stratum corneum, which may itself be related to the pH difference between the epidermal keratin and the fluid of the water bath.[10] Also, an increase in the water temperature itself results in increased epidermal swelling.[11] The decreased skin wrinkling in manual workers may be explained by the increased depth of keratin in the epidermis, thereby masking any changes in the underlying layers of skin.

Apart from neuropathy, possible mechanisms for decreased skin wrinkling in diabetic subjects could be changes in epidermal keratin, increased deep tissue turgor, or alterations in blood flow. Alterations in epidermal structural proteins, possibly related to glycosylation, or changes in other properties of keratin might explain the decrease in skin wrinkling. Alternatively, an increase in tissue turgor, which leads to decreased wrinkling, might follow damage to sympathetic nerves giving rise to vasodilatation in the finger pulps, such as has been shown to occur in sympathectomied nondiabetic subjects.[4]

We have therefore demonstrated that skin wrinkling of the finger pulps following immersion in warm water is markedly decreased in diabetic subjects when compared with normal subjects. Despite the indisputable nature of this association, the reasons for it are unexplained.

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PAPER 34

HISTOPATHOLOGY OF EXTRAOCULAR MUSCLE IN UNILATERAL CONGENITAL THIRD NERVE PALSY

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ABSTRACT

A case of congenital, unilateral third nerve palsy is described, with particular reference to the histopathology of the extraocular musculature. The literature of this uncommon diagnosis is briefly reviewed, and the implications of the present case are considered in the context of currently-proposed hypotheses in the pathogenesis of congenital third nerve palsy.

INTRODUCTION

Unilateral third nerve palsy is an infrequent diagnosis in children (1). Although approximately 43% of cases are estimated to be congenital in origin (2), the diagnosis is appropriately placed in perspective when one considers that only 16 cases were recorded in a major ophthalmological referral centre over a period of 21 consecutive years (1). It has been suggested that the primary lesion in congenital, unilateral third nerve palsy is located in the peripheral nerve (1), however it has also been proposed that involvement of the brain-stem may be a significant feature in many cases (3). For obvious reasons, there is virtually no pathological evidence to support either pathogenetic mechanism. This paper reports the clinical management of a patient with congenital, unilateral third nerve palsy, and describes the histopathology of the resected extraocular muscle.

CASE REPORT

A 29-year-old Caucasian female patient with left congenital third nerve palsy was admitted to Moorfields Eye Hospital in November 1986 for left strabismus surgery. Previous ophthalmic history was as follows: age 3 years, left medial rectus resection 8 mm and left lateral rectus recession 8 mm; age 3.5 years, Gruyden Friedenwald procedure to left upper lid to partially correct ptosis; age 11 years, left medial rectus resection 5.5 mm and advancement of insertion by 3 mm. Past history revealed no other significant features of note; the birth history was a normal delivery of a full-term infant, without perinatal complications. Specifically, there was no history of birth trauma or forceps delivery, and no neurological or physical abnormality at birth. Family history was similarly unremarkable.

On admission, the only ophthalmic symptom was of unacceptable cosmetic appearance. The patient was not on systemic medication and there were no known allergies. General systematic examination was unremarkable. The results of ocular examination are described in the table. Pre-operative orthoptic assessment was as follows. A moderate left exotropia with left hypertropia was present to an equivalent degree for both near and distance vision. Left fixation was impaired, and the angle of deviation increased on attempted left fixation. The angle of deviation (near and distance) was 65 prism dioptres by prism reflection test, with left/right of 10 prism dioptres; these measurements were unchanged by full optical correction. Ocular movements: in the right eye, normal extraocular movements were present; in the left eye, marked restiction of

adduction, elevation and depression was noted, with no adduction beyond the midline. Left suppression was present, and although the post-operative diplopia test revealed that diplopia was appreciated at 75 prism dioptres of exotropia, this was subjectively easily ignored. There was no evidence of aberrant regeneration.

On 27-11-86, left strabismus surgery was performed under general anaesthesia:

i. Disinsertion of left lateral rectus muscle

ii. Resection of left medial rectus muscle (6mm)
Muscle re-inserted at 5 mm from limbus

iii. Lid traction sutures.

At operation, the macroscopic appearance of the medial rectus muscle was normal. The 6 mm x 10 mm resected segment of medial rectus muscle was forwarded in Croker's transport medium for histopathological examination.

Lid traction sutures were removed on the fifth post-operative day. On that date, a consecutive left convergent squint of 18 prism dioptres was present, however two weeks later this had resolved to an exotropia of approximately 10 prism dioptres, with unchanged left/right of 10 prism dioptres. There was marked limitation of the left eye in adduction and abduction, with unaltered vertical eye movements. The cosmetic appearance showed considerable improvement, and the patient was satisfied with the final result. Unfortunately, the absence of Bell's phenomenon in the left eye precluded surgical correction of ptosis, as it was considered this procedure may adversely compromise the integrity of the cornea.

HISTOPATHOLOGY

When received in the laboratory after immersion in Croker's fixative, it was noted that the biopsy floated, an unusual finding, suggesting that heavy muscle fibres might be absent. On microscopy this was found to be so, almost the entire biopsy consisting of fine fibrous tissue; this is clearly demonstrated in the photomicrograph of the biopsy. Only five small groups of muscle fibres, with an enzymatically normal distribution of Type I:Type II fibres, were found. These fibres showed some central vacuolation and adjacent macrophages contained granular debris of similar density to muscle fibres. A few internalised nuclei, occasionally in some disarray, were present, suggesting little, if any, regeneration had occurred.

DISCUSSION

Although congenital third nerve palsy has been recognised for over 80 years (4), the rarity of the disorder has resulted in a relative paucity of literature on this subject (1,2,3,4,5,6). Reviewing 16 consecutive cases over a 21-year period, Victor (1976) suggested the location of the lesion in congenital third nerve palsy to be the peripheral nerve, proposing that absence of ocular movement - to any stimulus - and absence of other features associated with concomitant brain-stem pathology effectively eliminated a possible supranuclear aetiology (1). In contrast, a study of 10 patients with congenital third nerve palsy by Balkan and Hoyt (1984) recorded isolated third nerve palsy (without associated neurological abnormality) in only three patients; they concluded that although the lesion may be situated in the peripheral nerve, brain-stem involvement is an important feature in a signifcant proportion of cases, and must therefore be excluded by careful neurological examination (3). The demonstration of aberrant regeneration does not necessarily differentiate a peripheral from a central lesion. Although one would expect aberrant regeneration to be associated with a peripheral nerve lesion, this may also occur secondary to partial nuclear injury in the neonatal period; nuclear aplasia has been demonstrated following brain-stem ischaemia in utero (7,8,9), and may therefore be a significant predisposing factor to the subsequent development of congenital third nerve palsy. The only possible conclusions from this data is that no single aetiology may be proposed as a definitive cause of congenital, unilateral third nerve palsy, and, similarly, the location of the lesion may be either central or peripheral, with no obvious preponderance of either anatomical site.

The present case demonstrates an incomplete unilateral third nerve palsy, without evidence of aberrant regeneration - although this sign may be masked by the effects of previous surgical procedures. The most significant feature of this case is the unusual histopathology of the excised medial rectus muscle, with virtually complete replacement of muscle by fibrous tissue. It is probable that this appearance may be related, in part, to the previous surgery, however it seems unlikely that this represents the only explanation. At operation, the medial rectus muscle was easily exposed, post-operative adhesions were not unduly excessive, and surgical dissection of the muscle was relatively uncomplicated. As previously described, the macroscopic appearance of the medial rectus was apparently normal, with no obvious suggestion of almost-total replacement by fibrous tissue. In this context, it is unlikely that 6 mm of medial rectus muscle fibres would be virtually completely replaced by fibrous tissue as an exclusively secondary phenomenon to previous muscle resection. An alternative explanation is that the medial rectus muscle may not have fully developed from birth, possibly associated with the congenital denervation, or, if muscular development was present at birth, that disuse muscle atrophy may have occurred as a secondary phenomenon. As a corollary, one would not expect evidence of aberrant regeneration in patients with impairment of muscle function; aberrant regeneration can obviously only be demonstrated in patients with intact extraocular musculature. The absence of clinical signs of aberrant regeneration in the present case may therefore be ascribed to either absence of aberrant regeneration, or disordered muscle physiology. It is well-recognised that striated muscle atrophy occurs following interruption of muscle innervation (10); the consequences of

congenital denervation on extraocular muscle anatomy, which is significantly different from striated muscle (11), is not established. Accurate description of extraocular muscle pathology in congenital third nerve palsy has not been defined. Biopsy of the medial rectus muscle, performed in two of the sixteen cases described by Victor (1976), showed no evidence of denervation or myopathy (1). As these cases of congenital, unilateral third nerve palsy were specifically ascribed to a lesion of the peripheral nerve, with clinical evidence of aberrant regeneration, it is perhaps surprising that no pathological evidence of a denervative pattern was present.

A review of the literature on unilateral third nerve palsy in childhood confirms the potentially multifactorial aetiology of this condition. Approximately 43% of cases are congenital, although trauma (20%), inflammation (13%), neoplasm (10%) and aneurysm (7%) are major predisposing factors which require preliminary exclusion (2). The anatomical location of the lesion in congenital unilateral third nerve palsy may be either peripheral (1), central (3), or may involve both brain-stem and peripheral nerve (3). This paper describes the histopathological appearance of resected medial rectus muscle from a case of congenital unilateral third nerve palsy, and suggests that fibrous replacement of extraocular muscle may be associated with congenital denervation in some cases of third nerve palsy. This may explain the absence of evidence of aberrant regeneration in apparently peripheral nerve lesions, as this neurological sign requires the presence of an intact effector ie the extraocular musculature. The only definite conclusion which may be drawn from the evidence available to date, including the present case, is that congenital unilateral third nerve palsy is a diagnosis

of exclusion; the location of the neurological lesion may be central or peripheral, and the anatomy of the denervated musculature may be grossly abnormal.

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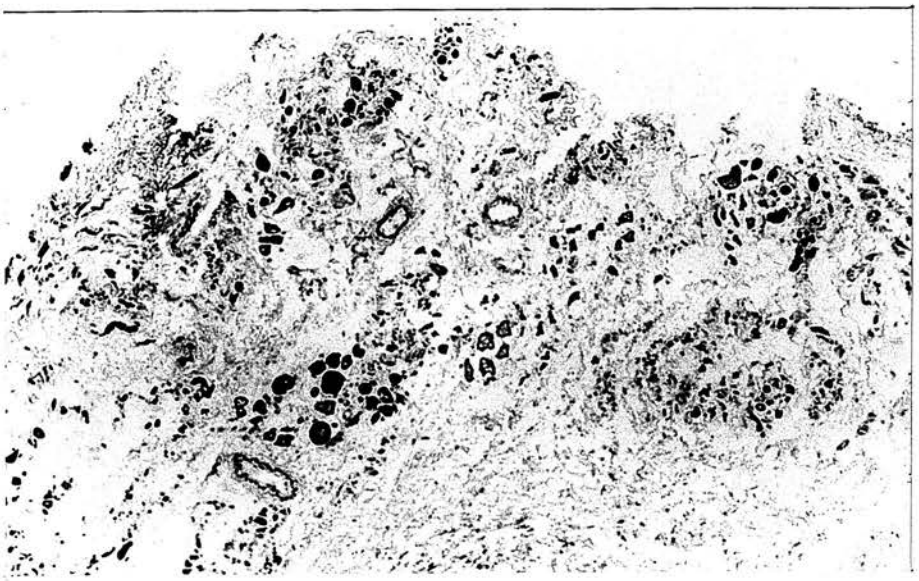
Table

PRE-OPERATIVE OCULAR ASSESSMENT OF A 29-YEAR-OLD FEMALE PATIENT WITH LEFT CONGENITAL THIRD NERVE PALSY

	Right eye	Left eye
Corrected visual acuity	6/6	6/18
Intraocular pressure	18 mm Hg	18 mm Hg
Pupil assessment		
i. Afferent pupillary defect	Absent	Absent
ii. Efferent pupillary defect	Absent	Present
Posterior segment	Normal	Normal
Lid examination		
i. Ptosis	Absent	Present
ii. Palpebral aperture	10 mm	5 mm
iii. Levator function	17 mm	5 mm
iv. Skin crease	6 mm	4 mm

LEGEND TO PHOTOMICROGRAPH

Photomicrograph showing biopsy composed largely of fibrous tissue and a few scattered muscle fibres of varying size and shape, with vacuolation of muscle fibres. (haematoxylin and eosin, x180)



PAPER 35

CARCINOID TUMOUR IN THE LACRIMAL GLAND

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CARCINOID TUMOUR IN THE LACRIMAL GLAND

SUMMARY

A case of carcinoid tumour in the lacrimal gland is described, thought to be a metastasis from a known primary lesion in the mediastinum. The results of light and transmission electron microscopic examination are presented, and the possibility of the lesion representing a second primary tumour discussed.

Keywords: lacrimal gland, carcinoid tumour.

INTRODUCTION

Carcinoid tumours were first described in detail in 1888 (1). This important group of neoplasms is no longer a pathological enigma; the tumours take their origin from APUD (amine precursor uptake and decarboxylation) cells in various sites and they were first shown to have the ability to produce peptide hormones in 1953 (2). In certain circumstances this is associated with the carcinoid syndrome, recognised as a defined clinical entity in 1954 (3). The carcinoid tumour often has banal histological appearance but can show malignant behaviour: their ability to metastasise was reported as early as 1890 (4).

The carcinoid tumour accounts for no more than 2% of all neoplasms; malignant lesions most often metastasise to the liver or lungs. Carcinoid tumours metastatic to the eye and orbit are rare. In a clinicopathologic study of fifteen such cases of metastasising tumours it was found that the most common primary site was the bronchus, followed by the ileum (5); orbital metastasis from a mediastinal carcinoid tumour has been reported on one previous occasion (6). A carcinoid metastasis in the lacrimal fossa from an ileal primary has also been documented (7).

This case report describes the clinical and morphological features of a carcinoid metastasis in the lacrimal gland from a presumed mediastinal primary.

CASE REPORT

An 82 year old male was admitted to hospital with a two year history of progressive swelling of the right upper eyelid, associated with pain and diplopia for six weeks prior to admission. A carcinoid tumour of 15 x 12 x 6 cm had been resected from the anterior mediastinum seven years previously, at which time there had been no evidence of metastatic spread. The patient denied symptoms suggestive of the carcinoid syndrome, and 24-hour urinary excretion of 5-hydroxy-3-indole-acetic acid (5-HIAA) was normal at 24 μmol.

Incisional biopsy of the right lacrimal gland was performed; one month later the swelling and diplopia had resolved. At the time of writing the patient remains well, with no overt evidence of tumour recurrence or of metastasis elsewhere.

PATHOLOGICAL FINDINGS

The specimen was a firm, lobulated mass of brownish tissue measuring approximately two centimetres in maximum dimension; on sectioning, the tumour had a variegated appearance, comprising areas of firm whitish-yellow tissue admixed with dark haemorrhagic foci.

Formalin-fixed, paraffin-embedded sections of the tumour were stained with Haematoxylin and Eosin, Congo Red, Periodic Acid Schiff and Grimelius stains. Tissue from the formalin-fixed material was post-fixed in glutaraldehyde and used to prepare

grids for transmission electron microscopy (Jeol JEM-100S microscope).

Microscopically, the lesion consisted of uniform small cells often with distinctly granular eosinophilic cytoplasm and round or oval faintly basophilic nuclei showing mild pleomorphism and occasionally prominent and multiple nucleoli. Mitotic figures occurred at an average frequency of 2-3 per high power field. The tumour cells were arranged in well-defined packets or as cords and ribbons in a vascular stroma; no rosettes or acini were noted, and amyloid was not present in the stroma. The tumour thus presented the "mixed" morphological pattern described by Soga and Tazawa (8). These appearances were identical to those of the mediastinal carcinoid tumour resected seven years previously. (Figures 1,2 and 3). The lesion extended to the margins of the biopsy specimen indicating incomplete resection.

Argyrophil staining using the Grimelius technique showed numerous positively staining cytoplasmic granules (Figure 4); many cells, however, failed to stain.

Transmission electron microscopy demonstrated abundant intracytoplasmic membrane-bound, dense-core (neuro-secretory type) granules measuring between 120 and 150 nm in diameter, and many mitochondria (Figure 5). This tumour thus presented the features of a carcinoid APUDoma.

DISCUSSION

Orbital disease - of any nature - is a rare phenomenon in the United Kingdom; only 4 to 5 patients per million per annum require orbital surgery (9). Metastasis to the lacrimal gland from any source is extremely unusual; a review of 227 cases of metastatic tumours involving the eye and orbit reported isolated involvement of the orbit in only 12.3%, with no reported involvement of the lacrimal gland (10).

Metastasis from carcinoid tumours (most frequently to sites such as liver, skeleton and skin) has been said to be related to both the site and the size of the primary tumour, although some authors maintain that size alone is the most important factor (11). It has been reported that metastasis is unlikely from carcinoid tumours of less than 1 cm diameter, while those that usually metastasise are greater than 2 cm (12,13). Considering site alone, ileal carcinoid tumours show the highest frequency of metastasis. In our case, the dimensions of the original tumour (15 x 12 x 6 cm) would indicate definite metastatic potential.

The time interval between diagnosis of the original carcinoid tumour and the development of symptoms related to the lacrimal gland metastasis (5 years) is in keeping with the series studied by Riddle, Font and Zimmerman (5), in which the mean duration between primary diagnosis and the detection of the orbital or ocular metastasis was 3 years (range 1-9 years).

Carcinoid tumours arising in the anterior mediastinum have been previously reported (14,15) , the thymus gland being the suspected site of origin; the tumour is thought to originate from Kulchitsky (enterochromaffin) cells normally found in the thymus (14, 16, 17, 18). The entity is distinct from a thymoma (15, 16). In our case, origin of the primary tumour from thymic tissue seems possible, with metastasis via the bloodstream to the lacrimal gland. In such a primary site, tumour growth may be considerable before giving rise to symptoms or signs, and its removal may be technically difficult. It is thus surprising that metastasis has been confined (as far as we know) to the lacrimal gland.

An alternative (and less likely) hypothesis that this may represent a second primary carcinoid tumour is worthy of consideration. Although argyrophil cells have not been described in the normal lacrimal gland, and we have been unable to identify neurosecretory granules in normal tissue examined by electron microscopy, the possibility of divergent differentiation of ductal stem cells resulting in a carcinoid neoplasm cannot be completely rejected. Such a concept has been previously considered in connection with gastric carcinoma (19), cervical adenocarcinoma (20) and neoplasms of the breast (21) and parotid gland (22), and offers an explanation for carcinoid tumour formation without the need to invoke the diffuse endocrine system of Feyrter (23) or the APUD system of Pearse (24).

A final point deserving discussion is the apparent disappearance of the lacrimal mass following an incisional biopsy, with incomplete excision. Debulking the tumour has presumably resulted in considerable regression of tumour-associated inflammation and oedema.

In summary, a case is presented of carcinoid tumour in the lacrimal gland. Although we cannot offer proof of the pathogenesis of this lacrimal gland carcinoid APUDoma, the similarity of its morphology and staining characteristics support its development as a metastasis from the previous mediastinal carcinoid tumour.

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ILLUSTRATIONS

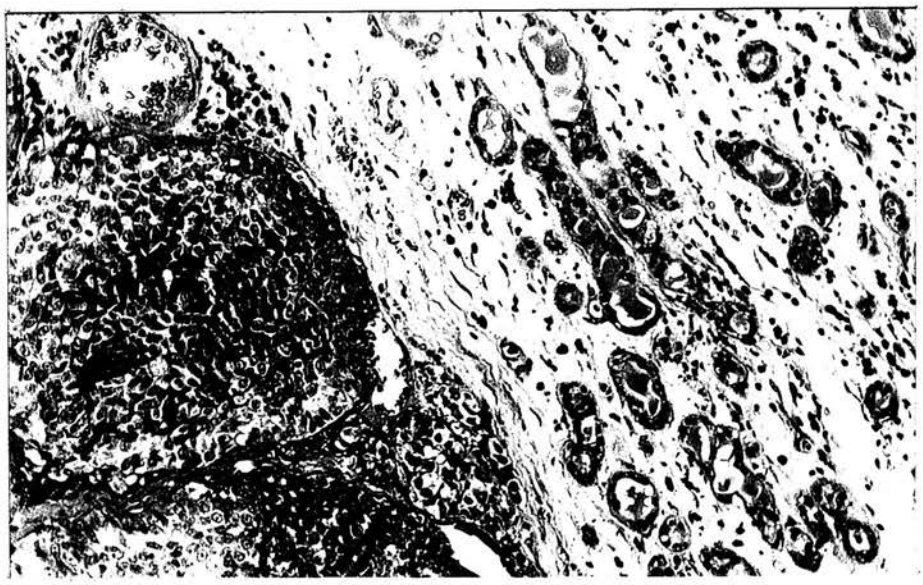
FIG 1 Photomicrograph showing carcinoid tumour (below) distorting native lacrimal gland elements. (H&E x 160)

FIG 2 The carcinoid tumour is composed of packets of uniform ovoid cells with intervening fibrovascular stroma (H&E x 125)

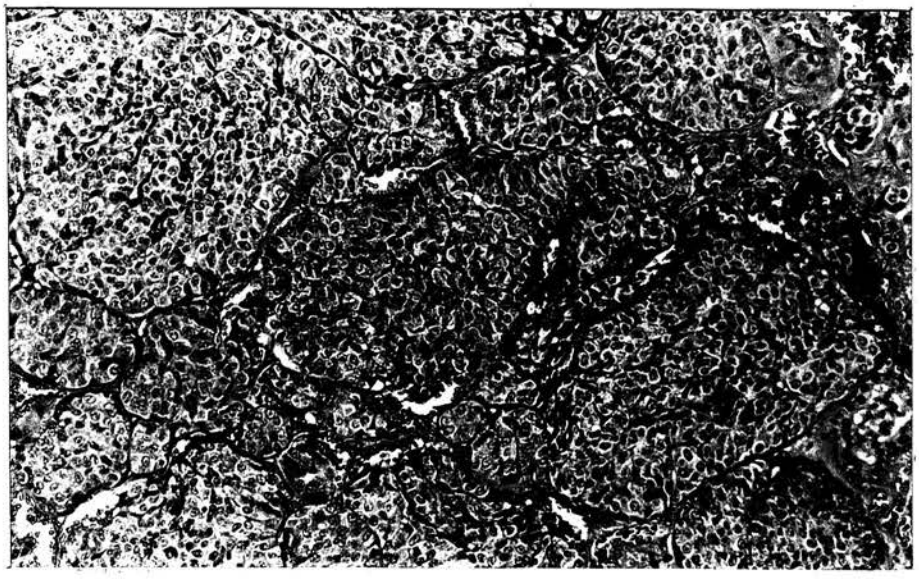
FIG 3 Histology of the original carcinoid tumour resected from anterior mediastinum (H&E x 160)
Note the similarity to the lacrimal gland lesion.

FIG 4 High power detail showing in several cells positive cytoplasmic silver staining by the argyrophil method (arrows). (Grimelius x 500).

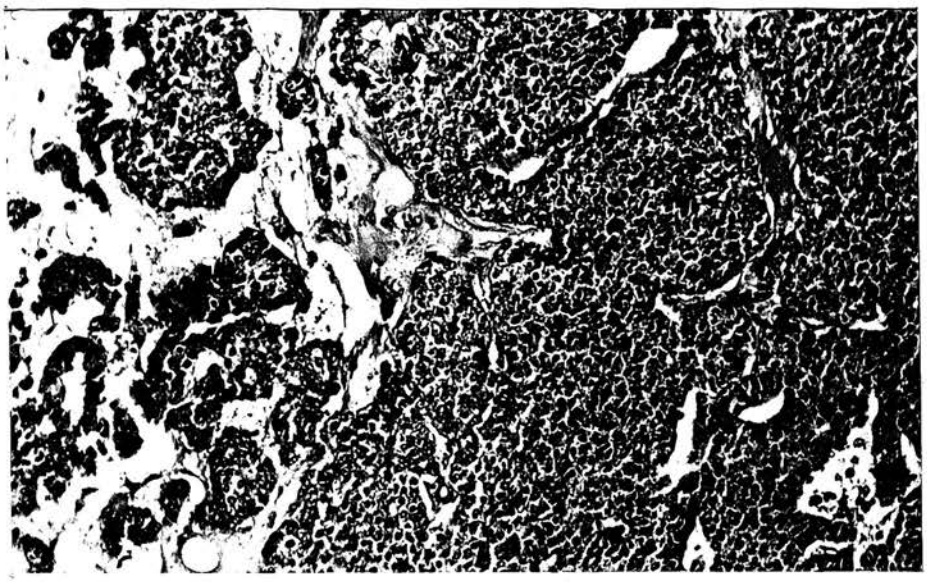
FIG 5 Electron micrograph revealing intracytoplasmic electron-dense membrane-bound neurosecretory granules (x 6000).



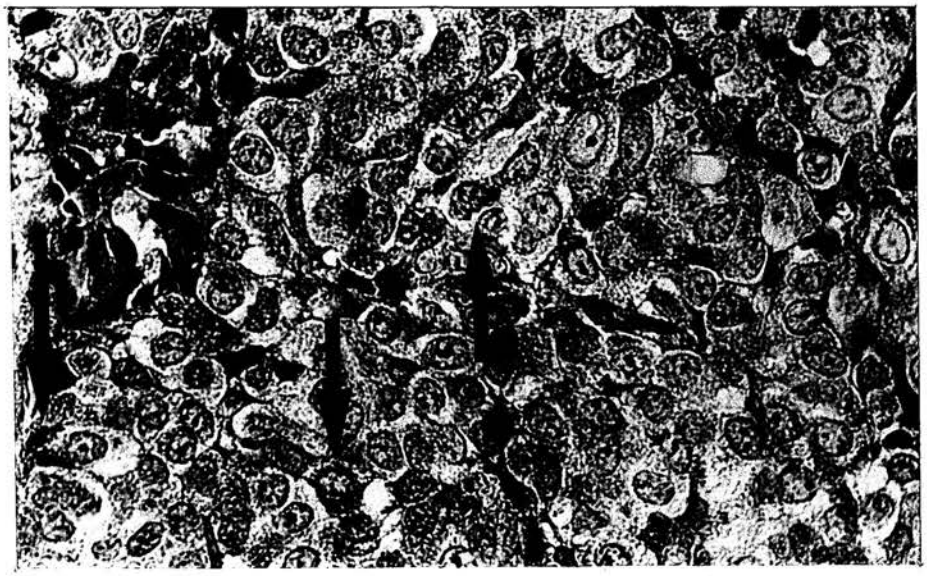
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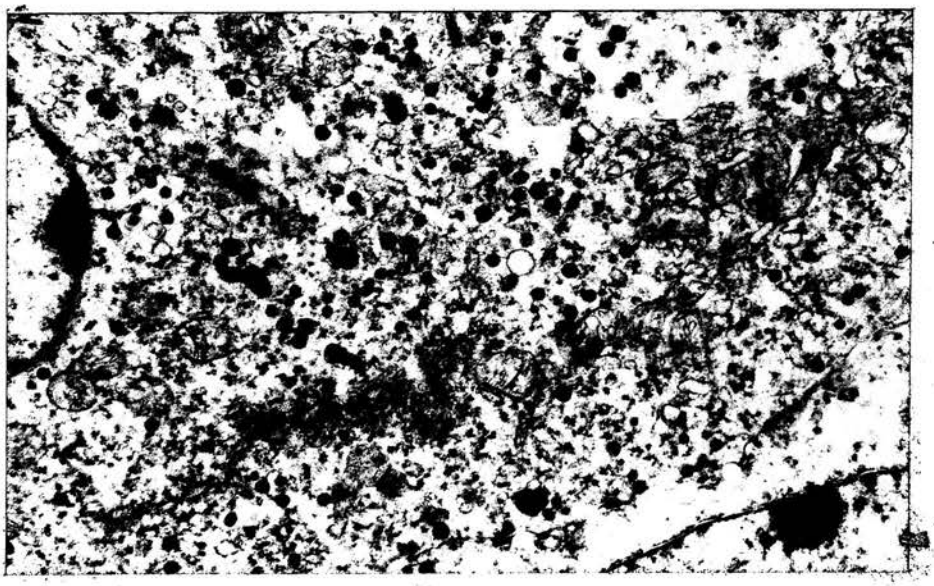
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