

AUTONOMIC NERVE FUNCTION IN THE PRIMARY GLAUCOMAS

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DECLARATION

In accordance with the statutes of the University of Edinburgh, I declare that all research described in this thesis was performed by the author, except where the contribution of others is acknowledged. The manuscript was composed entirely by the author, including preparation of diagrams and photographic plates.

Charles V. Clark

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ABSTRACT

The propensity of the anterior chamber to show rapid and transient fluctuations in dimension has been demonstrated. Closure of the irido-corneal angle is a dynamic process, which may be precipitated by either anterior translational movement of the iris-lens diaphragm or peripheral iris bombé. The basic aetiological factor common to both mechanisms is pupil block, a manifestation of relative autonomic activity in the anterior segment. Changes in autonomic activity in the anterior chamber, with particular emphasis on parasympathetic tone, have consequently been proposed as the ultimate effector of angle-closure. The rationale of this thesis was therefore to determine the efficacy of autonomic nerve function, at both systemic and ocular level, in a representative sample of patients from each of the major categories within the heterogeneous group comprising the primary glaucomas: closed-angle glaucoma, ocular hypertension, and open-angle glaucoma. Sub-classification of each category was performed depending upon angular configuration, into narrow-angles and wide-angles.

Generalised autonomic status was determined by a series of tests based upon cardiovascular reflexes, permitting accurate, reproducible quantification of nerve function. Ocular autonomic status was determined by two complementary methods: assessment of the pupillary reflex arc, as an indivisible unit, by the duration of the pupillary cycle in response to light; and application of the principle of denervation hypersensitivity, using topical autonomic agonists, to parasympathetic and sympathetic nervous systems in the anterior segment. In addition, the prevalence of diabetes mellitus in each of the primary glaucomas was established; this was essential, both to clarify inconsistencies and omissions in the present literature on this subject, and also as diabetes mellitus is the commonest cause of autonomic neuropathy in this country. All assessments were concurrently performed on a comparable, age- and sex-matched control group.

The results showed systemic parasympathetic dysfunction to be prevalent in each category of the primary glaucomas, with

significantly-increased prevalence in patients with narrow-angles. Parasympathetic neuropathy of the anterior segment was similarly present in all categories; sympathetic neuropathy of the anterior segment was present in all categories except OAGWA. The duration of the pupillary reflex cycle was significantly prolonged only in narrow-angle primary glaucomas: CAG and OHNA.

The association between diabetes mellitus and glaucoma was restricted to NIDDM and IGT - the prevalence of IDDM in the primary glaucomas was comparable to the control group - and was shown to be significantly-increased in narrow-angle categories only, in marked contrast to previous descriptions.

The results are discussed, and are shown to explain currently-proposed mechanisms of angle-closure. Although autonomic dysfunction in the primary glaucomas may be secondary to diabetes mellitus in a minority of cases, no predisposing cause is present in the remainder; primary glaucoma may therefore represent a manifestation of primary autonomic failure. As autonomic dysfunction is common to each of the primary glaucomas, an association between closed-angle glaucoma, ocular hypertension, and open-angle glaucoma is proposed, in close agreement with previous experimental observations.

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SECTION I

INTRODUCTION

CHAPTER 1

HISTORICAL REVIEW

1.1 Introduction

Glaucoma is derived from the Greek "glaukoma", an opacity of the crystalline lens. The term is first mentioned as a diagnosis by Hippocrates in "Aphorisms", and was applied to a change in colour within the pupillary area, inevitably leading to blindness. There was thus no definitive distinction between glaucoma and cataract until the anatomical site of the latter was established by Brisseau in 1707. The failure of lens extraction (Daniel, 1752) to improve vision in certain areas precipitated a search for the primary site of glaucoma in other structures.

Glaucoma has been defined as: "an eye disease in which the complete clinical picture is characterised by increased intraocular pressure, excavation and degeneration of the optic disc, and typical nerve fibre bundle damage, producing defects in the field of vision. Any or all of these signs may be present at a given examination." (1)

The historical association of these features in the diagnosis of the primary glaucomas is documented in the following paragraphs.

1.2 The glaucomatous disc

The invention of the ophthalmoscope by Helmholtz in 1854 ensured rapid advances in research directed towards the pathogenesis of glaucoma, a disorder characterised by optic disc changes. Edward Jaeger first described the glaucomatous disc (2), however the lack of stereopsis inherent within monocular ophthalmoscopy precipitated the conclusion that the glaucomatous disc represented a swelling of the papillary tissues, a description subsequently confirmed by von Graefe (3). In fact, the papillary tissues are excavated. Misinterpretation of relative depth assessment resulting from monocular indirect ophthalmoscopy was revealed by the investigations of Weber (4), and disc excavation confirmed by pathological examination. The correct mechanism of papillary

excavation was first suggested by Schnabel, who described initially-selective nerve fibre breakdown without involvement of supporting tissues as the basic anatomical factor leading to cavernous optic atrophy (5). However he mistakenly considered the optic nerve disease to result from absorption of pathological fluid from the vitreous. Remarkable perspicacity was shown by Priestly Smith in 1879, with this view of optic atrophy associated with glaucoma: "The glaucomatous cup is not a purely mechanical result of exalted pressure, but is in part at least, an atrophic condition which, though primarily due to pressure, includes vascular changes and impaired nutrition in the area of the disc and around its margin." (6)

1.3 Elevation of intraocular pressure

The second major feature which may be associated with glaucoma is raised intraocular pressure. This association was first suggested by At-Tabarie, an Arab, in the 10th century (7). The sign was initially documented in the English literature by Banister (1626), and subsequently by Platner, Professor of Anatomy at the University of Leipzig (1738). "Hardness of the eye" was repeatedly recognised in the early years of the 19th century as a characteristic feature of the disease (8,9,10); the presence of elevated intraocular pressure in glaucoma was finally established by Mackenzie in 1835 (11). Raised intraocular pressure, considered by von Graefe in the late 1850's as the "essence" of glaucoma, was ascribed to hypersecretion of intraocular fluid. This view was subsequently disproved by detailed investigation of ocular outflow fluid dynamics. The conclusion by Schwalbe that the anterior chamber directly communicated with anterior ciliary veins (12) was disputed by Leber, who recognised the intercession of a trabecular meshwork between the anterior chamber and the anterior ciliary veins, and realised the importance of this structure in determining subsequent outflow (13). Leber reasoned that aqueous dynamics depended upon a balance between production of aqueous humour by filtration, and elimination via the outflow route (14). He discovered increased resistance to outflow in enucleated glaucomatous eyes; this factor in the pathogenesis of glaucoma correlated with the association of changes in the configuration

of the anterior chamber angle (specifically peripheral anterior synechiae) with glaucoma by Knies in 1876 (15). The Leber-Knies theory, that elevation of intraocular pressure resulted principally from disorders of aqueous outflow, remains a cornerstone of glaucoma therapeutics.

Digital tonometry in the estimation of ocular tension had been performed for centuries. This was still considered the preferred method of intraocular pressure assessment by some observers until the early years of this century (16). The initial application of impression tonometry on human eyes is attributed to Donders in 1863. Inherent sources of inaccuracy by this technique were recognised by Weber in 1867; indentation by its very nature displaces intraocular fluid and thus alters the intraocular pressure. This stimulated Weber to produce the first applanation tonometer; regrettably, the necessity of minimal fluid displacement during assessment of intraocular tension was not widely accepted and this instrument fell into disuse. The theory was pursued by Maklakoff, who introduced an applanation tonometer with which the size of the applied surface is determined for a given force - the Maklakoff principle (17). This tonometer remains in clinical practice in certain areas of the USSR.

The development of an accurate impression tonometer by Schiötz (18) enabled widespread quantification of intraocular pressure for the first time, and delayed further advances in applanation tonometry for half a century. Inherent flaws in impression tonometry, expressed by Weber fifty years earlier, were reiterated by Schiötz in 1920: "I cannot imagine any method available for living eyes by which errors due to variations of the envelope could be eliminated."

The conception of an alternative type of applanation tonometer by Goldmann (1955) revolutionised estimation of intraocular pressure (19); image division inside the pressure body fixes the value of the applanation circle diameter. The theory of applanation tonometry is derived from the Imbert-Fick law, which states:

"If a plane surface is applied with force F to a thin, spherical membrane within which a pressure P_t exists, for an equilibrium condition the expression $P_t = F/A$ is valid, where A is the area of the applied surface." (20)

The physiological properties of the cornea and its wetting liquid necessitate the addition of two terms to the equation:

$$P_t = F/A - P_m + P_n$$

P_m = pressure caused by characteristic rigidity of the eye

P_n = pressure dependent upon:

- i. surface tension of liquid
- ii. wetting properties of cornea
- iii. flattening surface of pressure body (21,22)

The cornea resists "flattening", and attraction caused by the tear meniscus tends to draw the tonometer towards the cornea; these opposing forces cancel one another when the applanation circle diameter equals 3.06mm, the diameter employed in the Goldmann applanation tonometer. At this applanation area, $P_o = 0.98P_t$ where P_o = real intraocular pressure. A minimal amount of fluid (approximately 500nl) is displaced from the eye during Goldmann tonometry.

Other forms of applanation tonometer based upon similar principles are the hand-held Draeger (23,24) and Perkins models (25).

Tonometers employing other principles have been developed, such as the Mackay-Marg (26) and non-contact tonometer (27), although neither are as widely-used nor as accurate as the Goldmann applanation tonometer.

1.4 The visual field

Visual field defects in glaucoma correspond to damage of the nerve fibre bundles. This was described by Landesberg in 1869 (28), and subsequently confirmed by Bjerrum in 1889 (29). Characteristic defects of the visual field in open-angle glaucoma result from damage to individual nerve fibre bundles at the optic nerve head (30). Essentially, two alternative (or additive) hypotheses have been proposed to explain the aetiology of nerve fibre bundle defects:

- i. the vasogenic hypothesis (31,32)
- ii. the mechanical hypothesis (33,34,35)

The visual field is: "that portion of space in which objects are simultaneously visible to the steadily fixating eye." (36)

Thus visual field assessment examines the function of the visual system in this whole field, not merely determining the limits of the field (37).

von Graefe described paracentral field defects in chronic glaucoma in 1856, using a primitive campimeter (38). The first perimeter was introduced by Foerster in 1857; the introduction by Bjerrum of the 2-meter screen in 1889 permitted the discovery of scotomas encircling fixation and associated with the blind spot, a characteristic feature of glaucomatous damage. Another significant observation was made by Peter in 1927, with the detection of small scotomas, 12-20° from fixation, as an early sign of glaucomatous nerve fibre bundle damage (39). The relationship between intraocular pressure and visual fields was further reinforced by Samojloff (40). The earliest defects of the nerve fibre bundle in open-angle glaucoma are paracentral, associated with arcuate nerve fibres (41,42), however other characteristic defects associated with damage to these fibres include nasal steps (43), arcuate scotomas, and sector defects in the visual field.

1.5 Historical differentiation of the primary glaucomas

The aforementioned features apply equally to both open- and closed-angle glaucoma, however the latter entity has several distinct characteristics resulting from its intrinsically-acute onset. Probably due to the silent progression of open-angle glaucoma, the closed-angle form comprised approximately 70% of known cases in the mid-19th century. In fact, von Graefe did not include the open-angle form, which he termed "amaurosis with excavation of the optic nerve" (44) within the glaucomas until 1861 (45). This observation was credited to Donders who suggested the term "glaucoma simplex" for this disease entity after noting the "palpable tension" to be raised in those eyes. von Graefe had, however, classified acute glaucoma into two categories which now correspond to the acute and subacute (or intermittent) forms. He considered the disease to be inflammatory, resulting from either a choroiditis or iridochoroiditis. Elevated intraocular pressure was presumed

to be a natural consequence of inflammatory exudation effecting a relative increase in intraocular volume.

Thus a considerable proportion of the early research into glaucoma concentrated on the closed-angle, or "inflammatory", form. The mechanism of angle-closure was not fully recognised until the late 1920's, however earlier individual astute observations ensured ultimate accuracy in the description of events. A predisposition to glaucoma in certain individuals was predicted in 1890 (46); growth of the lens in eyes with a small cornea effected a progressive decrease in circumlental volume. Shallowing of the anterior chamber in the fellow eyes of patients with closed-angle glaucoma was noted by Czermak in 1893 (47). The importance of pupil block in the aetiology of angle-closure was independently reported by Seidel (48) and Curran (49). The latter stated that:

"Normally the aqueous passes through the pupil from the posterior to the anterior chamber, but it is here contended that in glaucoma this passage is impeded on account of the iris hugging the lens over too great a surface extent. Some of the aqueous gets through while some passes back, forcing the lens and the iris still more forward."

Based on his own hypothesis that angle-closure was dependent upon shallowing of the anterior chamber secondary to pupil block, Curran reasoned that the manufacture of an alternative route of aqueous flow from the posterior to the anterior chamber (an iridectomy) would prevent forward movement of the iris-lens diaphragm. This therapeutic measure was successfully employed and followed over a ten-year period (50). Barkan classified glaucoma into open- and closed-angle on the basis of gonioscopic appearances in 1938 (51), however the credit for recognising the essential importance of pupil block in the pathogenesis of angle-closure, with subsequent anterior translational movement of the iris-lens diaphragm, lies with Curran:

"In many cases of chronic glaucoma, including acute attacks, the chief mechanical factor is lack of proper drainage from the posterior to the anterior chamber, on account of the iris being too closely adapted to the lens over a great surface extent, thus impeding the flow of aqueous through the pupil, and allowing some to permeate the vitreous and help to push the lens and iris forward, making the anterior chamber shallow, and partly occluding the filtering angle as a secondary effect." (50)

Integral component forces which summate to effect pupil block were

finally elucidated by Mapstone in 1968 (52,53); changes in the dimensions of the anterior segment subsequent to displacement of the iris-lens diaphragm were confirmed by slit-image Polaroid photography in 1984 (54).

CHAPTER 2

CLASSIFICATION OF THE GLAUCOMAS

The glaucomas are a heterogeneous group of ocular diseases with common characteristics, defined in the introductory text. The non-specificity inherent within the definition indicates a relative lack of knowledge regarding pathogenetic mechanisms. Definitive classification of a heterogeneous group is obviously impossible. The following classification is not comprehensive, but seeks to place the various forms of glaucoma in an orderly and acceptable sequence, from which the rationale of this research may be more easily understood. Primary glaucomas are those not associated with known concomitant or antecedent disease. 70% of all glaucomas are primary.

2.1 Ocular hypertension

Ocular hypertension is defined as: "statistically significant elevated ocular pressures occurring in the presence of an open angle without characteristic field loss and without changes in the optic disc that are characteristic of glaucoma." (55)

By this definition, ocular hypertension is not a glaucoma per se, however it may be a premonitory indicator of subsequent glaucoma in certain instances and consequently is an essential integral part of this classification. Established classifications have included ocular hypertension within the primary glaucomas (56); this principle will be observed. Obviously as ocular hypertension is asymptomatic, the diagnosis is determined by specific assessment of the intraocular pressure, generally during a routine eye examination.

Ocular hypertension may be subdivided on the basis of the anatomical configuration of the anterior chamber angle into:

- i. ocular hypertension with wide irido-corneal angles
- ii. ocular hypertension with narrow irido-corneal angles

Angle assessment is explained in detail later.

2.2 Congenital glaucoma

This category of glaucoma results from defects developing at birth or during gestation. Sub-classification is usually on the basis of genetic or non-genetic aetiologies (57,58).

2.3 Open-angle glaucoma

Open-angle glaucoma is defined as: "statistically significant elevated ocular pressures occurring in the presence of an open angle associated with a characteristic field loss and changes in the optic nerve head." (55)

- i. Primary open-angle glaucoma
 - a. with wide irido-corneal angles
 - b. with narrow irido-corneal angles
- ii. Open-angle glaucoma with low tension
- iii. Open-angle glaucoma in association with the following conditions:
 - a. Pigment dispersion syndrome and pigmentary glaucoma
 - b. Exfoliation glaucoma
 - c. Retinitis pigmentosa
 - d. Fuchs' endothelial dystrophy
 - e. Retinal vein occlusion
 - f. High myopia
 - g. Retinal detachment
 - h. Diabetes mellitus
- iv. Secondary open-angle glaucoma
Glaucomatous damage developing as a result of known pre-existing or concomitant ocular or systemic disease.
 - a. Trauma
 - b. Lens-induced
 - c. Ocular inflammation
 - d. Neovascular glaucoma
 - e. Drug-induced
 - f. Intra-ocular lens-induced
 - g. Associated with alpha-chymotrypsin
 - h. Associated with tumours

2.4 Closed-angle glaucoma

This form of glaucoma occurs as a result of "obstruction to outflow... brought about solely by closure of the angle with apposition of the peripheral iris to the trabecular meshwork." (59)

Diagnostic features associated with closed-angle glaucoma include:

- i. Symptoms
 - a. Pain: either unilaterally localised to the eye, orbit, or frontal bone, or present as a generalised headache.
 - b. Depression of visual acuity: this feature has variable expression, from moderate, with blurred vision or "haloes" as a result of corneal oedema, to severe, with almost total loss of vision.
 - c. Nausea/vomiting

- ii. Signs
 - a. Elevated intraocular pressure - frequently > 50 mmHg
 - b. Circumcorneal ciliary injection
 - c. Corneal oedema
 - d. Pupil mid-dilated and non-reactive
 - e. Cells in the aqueous humour without keratic precipitates
 - f. Iris atrophy
 - g. Anterior chamber angle-closure - either gonioscopically or by the slit-lamp method of Van Herick (60,61)
 - h. Glaukomflecken of Vogt: grey spots appearing immediately posterior to the anterior lens capsule in the pupillary zone, representing focal necrosis of lens epithelium
 - i. Posterior synechiae
 - j. Optic atrophy

Individual features are associated with specific periods in the progression of acute glaucoma; for example, optic atrophy is a later feature resulting from a prolonged episode.

2.41 Primary closed-angle glaucoma

- i. With pupil block
 - a. Acute: closure of the anterior chamber angle occurs

- rapidly, producing acute and frequently dramatic symptoms.
- b. Subacute: intermittent, usually short-lived attacks occur which spontaneously resolve; the angle may not completely close.
 - c. Chronic: this is frequently asymptomatic, with gradual, silent closure of the anterior chamber angle.
- ii. Without pupil block
- Angle-closure may occur secondary to the mechanical effect of iris "bunching" during mydriasis, in the presence of anterior displacement of the iris root - the plateau iris.

2.42 Secondary closed-angle glaucoma

Glaucomatous damage developing as a result of known pre-existing or concomitant ocular or systemic disease.

- a. Trauma
- b. Neovascular glaucoma (rubeosis iridis)
- c. Miotic-induced
- d. Lens-induced
- e. Posterior synechiae
- f. Retrolental fibroplasia
- g. Nanophthalmos
- h. Associated with ocular procedures: scleral buckling, panretinal photocoagulation
- i. Malignant glaucoma from ciliary block
- j. Associated with iris tumours/cysts
- k. Anterior segment inflammation, with secondary peripheral anterior synechiae
- l. Posterior polymorphous dystrophy
- m. Iridocorneal endothelial dystrophy

2.5 Combined mechanisms

In this category, features of both open- and closed-angle glaucoma are present, but the relative contribution of each is not fully determined.

2.6 Anterior chamber angle assessment

This classification differs from those of previous authors in one important respect; in addition to all accepted features inherent within classifications of the glaucomas, ocular hypertension and primary open-angle glaucoma have been subdivided on the basis of the anatomical configuration of the anterior chamber angle.

The angle was first seen by Trantas in 1907 using an ophthalmoscope with a strong convex lens inset (62), however examination of the angle was not practical until the advent of the contact glass. This shifts the refracting surface of the corneal system to the convex surface of the glass, thus diminishing the degree of total internal reflection. In 1920, Koeppe used a binocular, stationary microscope and slit-lamp to study the condition of the trabeculae covering Schlemm's canal (63). A monocular instrument termed the "gonioscope" was devised by Tronosco in 1925, the origin of gonioscopy (64). The importance of detailed observation of the anterior chamber angle was placed in perspective by Barkan (51,65). Two methods of gonioscopy are principally used today; the direct and the indirect method. The direct method employs a Koeppe lens; the patient rests supine, the lens is positioned on the eye and the examiner uses a microscope (a gonioscope) and separately hand-held illuminator to observe the chamber angle. The indirect method requires the patient to be seated at a slit-lamp. A contact lens is placed on the eye, and the chamber angle is viewed via a mirror built into the contact lens. The two principle lenses in use are the Goldmann 1-mirror and the Zeiss 4-mirror gonioprism; the latter instrument was used in angle assessment during the present study. (Plate 1)

Estimation of the width of the anterior chamber angle is necessary for a number of reasons: decisions regarding the safety of mydriasis; interpretation of symptoms suggestive of angle-closure; determining the necessity of follow-up evaluation if the angle width is noted to change. Present systems of grading the anterior chamber angle have undoubtedly improved diagnostic accuracy - with consequent increased efficacy of treatment.

There are currently four methods of angle assessment in general use:

- i. Gonioscopic angle-grading - Scheie system, Shaffer system
Spaeth system
- ii. Slit-lamp angle-grading - Van Herick system.

2.61 Scheie system

This was based on the amount of angle recess which could be visualised (66).

Grade	Structures visible gonioscopically
0	Entire angle visible
1	Final roll of iris obscures part of ciliary body
2	Trabecular meshwork is the most posterior structure visible
3	Posterior part of trabecular meshwork not visible
4	No structures visible posterior to Schwalbe's line

2.62 Shaffer system

The angle is graded by the angular width of the recess (67).

Grade	Description	Angle width
0	Closed	0°
1	Extremely narrow	10°
2	Narrow	20°
3	Wide open	20-35°
4	Wide open	35-45°

In this grading system, a line tangential to the inner surface of the trabecular meshwork is used as a reference point. Angle width is estimated by drawing a tangent to the anterior iris surface, approximately one third of the distance from the angle recess, and the angle measured as the intersection of both tangents. This technique assesses the approach to the angle recess, but does not define the angularity of the recess.

2.63 Spaeth system

This is based on a semi-quantitative appraisal of three separate variables (68).

- i. Location of attachment of the iris to the ciliary body or the corneal endothelium.

Symbols are used to indicate the most anterior point of contact between the anterior iris surface and the inner lining of the globe.

Symbol	Point of iris contact
A	Iris touching corneal endothelium anterior to the trabecular meshwork
B	Point of iris contact posterior to Schwalbe's line
C	Iris root at the level of the scleral spur
D	Deep angle recess; ciliary body visible

- ii. Configuration of the peripheral iris

Symbol	Configuration
q	Concave curvature
r	Regular or relatively flat
s	Steep or convex curvature

- iii. Width of angle separation between trabecular endothelial surface and anterior iris

This is assessed by the same criteria as the Shaffer system, however the actual angle in 10-degree intervals is given, rather than a numerical classification.

2.64 Van Herick system

Gonioscopic angle-grading correlates closely with the technique of van Herick, in which the anterior chamber angle recess is assessed by slit-lamp biomicroscopy (60,61). The slit-beam is aligned vertically and positioned immediately on the corneal aspect of the corneoscleral limbus. The angle opening is viewed at an angle of 60° from the light beam. A narrow slit-beam is essential, and peripheral corneal width is used as the reference measurement

for estimation of the depth of the anterior chamber angle. The measurement is taken immediately prior to the point of disappearance of the corneal-iris space at the periphery.

Distance between posterior cornea and peripheral surface of iris / corneal section width	Equivalent angle grade Shaffer system	Angle description
0	0	Closed
< 0.25	1	Very narrow
0.25	2	Narrow
0.5	3	Wide open
> 0.5	4	Wide open

Slit-lamp grading of the anterior chamber angle was performed in all four quadrants. The anterior chamber angle is known to be narrowest in the superior quadrant (69), where most goniosynechia are found (70). The remainder of the angle is reasonably uniform.

Angle assessment during this study was performed by the slit-lamp technique of van Herick, coupled with Shaffer gonioscopic classification using a Zeiss 4-mirror gonioprism. Angles in the nasal and temporal quadrants were used to define angular configuration, thus providing an acceptable average of the total angle width.

This research thesis is restricted to investigation of the primary glaucomas: ocular hypertension, primary closed-angle glaucoma, and primary open-angle glaucoma. In the latter category, patients with low tension, pigmentary, and exfoliation glaucoma, discrete entities within the open-angle group, are specifically excluded from investigation.

CHAPTER 3

EPIDEMIOLOGY OF THE GLAUCOMAS

Epidemiology may be defined as the determination of the natural history of disease by a study of population samples; the elucidation of predisposing factors governing its distribution; and attempted determination of the borderline between normal and abnormal. This chapter has been included to place the primary glaucomas in perspective in relation to the general population.

Open-angle glaucoma is primarily a disease of the aged. Several population studies have been documented, with reasonably comparable results. Hollows and Graham examined a defined population in the Rhondda valley in 1963 (71). They defined open-angle glaucoma as:

- a. Glaucomatous cupping of the optic disc
- b. Visual field defects of the following types
 - i. Siedel
 - ii. Bjerrum
 - iii. Roenne's nasal step
 - iv. Tubular
- c. Pressure \geq 21 mmHg
- d. An anterior chamber free of abnormal mesoderm and unobstructed by the root of the iris."

With this definition, a prevalence rate of 0.28% was established. The inclusion of low tension glaucomas increased the prevalence to 0.43%. Similar surveys by Luntz in Oxford (72) and Walker in Birmingham (73) reported prevalences of 0.9% and 0.31% respectively. The Bedford glaucoma survey of 1968 showed a prevalence of 0.76% for open-angle glaucoma, increasing to 0.82% if patients with low tension glaucoma were included (74). This compares favourably with the study by Bengtsson in Dalby (1981), where the prevalence of open-angle glaucoma was 0.86% (75).

Ocular hypertension was defined by Hollows and Graham on the following basis:

- a. Intraocular pressure \geq 21 mmHg in one or both eyes by indentation and/or applanation tonometry
- b. No glaucomatous field defects
- c. Anterior chamber angle of the eye or eyes with a pressure of \geq 21 mmHg was free of abnormal mesoderm
- d. No history suggestive of angle-closure." (71)

Using this definition, a prevalence of 8.61% was recorded for ocular hypertension. The prevalence of ocular hypertension in the general population has been variously reported as 1% (72), 1.3% (73), 3% (74), 5% (76), and 8.9% (77).

The frequency distribution of intraocular pressure is positively skewed, with a tail in the upper pressure range (71,74,78,79,80). This skewed distribution occurs even if subjects with glaucomatous field loss are excluded (81,82). Two possible models are suggested. In the first, the distribution is assumed to reflect a continuous curve that is logarithmic normal, the upper range corresponding to subjects with ocular hypertension and glaucoma. The skewed frequency distribution is interpreted in the second model as representing the distributions of two separate populations: normal and ocular hypertension/glaucoma. With the latter interpretation, the normal Gaussian curve has a mean of 16 mmHg (± 3 mmHg). Accordingly, 97.5% of the normal population will have an intraocular pressure of ≤ 21 mmHg. There is evidence suggesting two populations; it has been shown by Hollows and Graham (71), and Armaly (82), that the distribution is symmetrical in young subjects, with gradually increasing asymmetry from age 40 years onwards.

Ocular hypertension is believed by some authors to be a preglaucomatous condition (83,84). The risk of patients with ocular hypertension subsequently developing glaucoma has been reported between 0.5-20%: Armaly and Graham, 0.5% (85,86); Linner and Stromberg, 2% (87); Perkins, 3.23% (88); David, 10.2% (89); Walker, 20% (73). Although there is no doubt that patients with ocular hypertension have a higher risk of developing glaucoma than the remainder of the population, the factors determining this increased risk remain mostly unknown. Heredity is certainly one factor. Several patterns of inheritance have been described in glaucoma (90); the most common mode of inheritance in open-angle glaucoma seems to be autosomal recessive with variable penetrance (91). Studies of both children and siblings of glaucoma patients have confirmed increased prevalence of glaucoma (92,93,94,95,96). The risk of developing glaucoma is also related to the initial level of intraocular pressure (89), acute blood loss (97), a history of

diabetes mellitus (78,97,98), and central retinal artery diastolic pressure (99).

In contrast, open-angle glaucoma is shown to be four times as common as closed-angle glaucoma; the latter has reported prevalences of 0.08% (71), 0.09% (100), 0.10% (75), and 0.17% (74). Narrow irido-corneal angles are considered to be a significant predisposing factor in the pathogenesis of closed-angle glaucoma. This anatomical configuration is relatively-rare: in van Herick's series, the prevalence of angles of grades 1 and 2 was only 0.64% and 1% respectively (60,61); in the study by Spaeth, only 1.1% of all angles were so narrow that the iris was in contact with the endothelial surface of the trabecular meshwork, although significant narrowing of the anterior chamber angle was noted in 6% of cases (68). However these studies have also shown a marked tendency for the anterior chamber angle to become narrow with increasing age. Francois (1948) reported 31% of cases to have a "small" angle recess (101). Although the overall prevalence of narrow angles was only 1.1% in Spaeth's study, narrow angles were present in 10% of those aged 88-92 years, 5% in the age category 83-87 years, and 4% in the age group 78-82 years.

Clearly there is a marked discrepancy between the prevalence of closed-angle glaucoma and that of narrow angles. The importance of narrow angles alone in the pathogenesis of closed-angle glaucoma was investigated by Mapstone in 1979; in 50 eyes from 27 patients with narrow irido-corneal angles (and no other features associated with angle-closure) subjected to a pilocarpine 2%/phenylephrine 10% provocative test, only one developed angle-closure (102). This suggested that although narrow angles may facilitate the subsequent development of closed-angle glaucoma, in isolation they are of little diagnostic value; other predisposing factors are essential. The hypothesis that narrow angles were not an essential prerequisite for angle-closure was confirmed by the demonstration of closed-angle glaucoma in eyes with non-shallow anterior chambers following autonomic provocation (103).

DIABETES MELLITUS AND THE PRIMARY GLAUCOMAS

4.1 Introduction

Prior to detailing the known associations between diabetes mellitus and the primary glaucomas, it is necessary to discuss the prevalence of diabetes within the general population, in order that viable comparisons can be made. In excess of thirty prevalence surveys have been performed in several affluent countries over the past thirty-five years. Male prevalence rates range from 1.18% in Edinburgh (104) to 10.67% in a retirement community of Southern California (105,106). Similarly, female prevalence rates vary from 0.84% (107) to 7.8% (108). Variations in the method of diagnosis, plus differences in the mean age of subjects, lead to difficulties in comparison.

The associations between diabetes mellitus and the primary glaucomas are examined in detail as follows:

- 4.2 Open-angle glaucoma in patients with diabetes mellitus
- 4.3 Ocular hypertension in patients with diabetes mellitus
- 4.4 Diabetes mellitus in patients with open-angle glaucoma
- 4.5 Diabetes mellitus in patients with ocular hypertension
- 4.6 Closed-angle glaucoma in patients with diabetes mellitus
- 4.7 Diabetes mellitus in patients with closed-angle glaucoma
- 4.8 Mechanisms to explain the association between diabetes mellitus and the primary glaucomas

4.2 Open-angle glaucoma in patients with diabetes mellitus

The earliest association between diabetes mellitus and open-angle glaucoma was made by Grafe in 1924 with the observation that proliferative retinopathy was less common in cases of diabetes with raised tension (109). In 1935, Waite and Beetham studied the visual system of 2,002 diabetics by central and peripheral field assessment, fundoscopy, refraction, and Schiötz tonometry (110). They concluded that 0.5% of diabetics had "clinical glaucoma" ie primary or secondary glaucoma. Re-examination of the data shows that 6% of patients had intraocular pressures > 25 mmHg.

Twenty years later, Palomar published a literature review encompassing 416 diabetics, in which he concluded that glaucoma did not occur more frequently in patients with diabetes mellitus (111).

The association was placed in perspective by Armstrong et al in 1960; of 393 diabetic patients studied, the prevalence of primary open-angle glaucoma was 4.1%, whilst the prevalence of all primary glaucomas was 4.8% (112). Using the same criteria in the diagnosis of glaucoma, 280 patients from the general medicine out-patient department were assessed to obtain control values; in this group the prevalence of open-angle glaucoma was 1.4%.

In any form of medical investigation relating to diabetes mellitus, the population under consideration must be subdivided according to type of diabetes; type 1 (IDDM) or type 2 (NIDDM). The only study to differentiate the prevalence of glaucoma within diabetics according to type of diabetes was performed by Nielsen in 1983 (113). This epidemiological study of 533 diabetics (166 type 1, 367 type 2) on the island of Falster, Denmark, showed the prevalence rate of primary open-angle glaucoma to be 6.0%; primary open-angle glaucoma occurred in 8.2% of patients with type 2 diabetes compared with 1.2% of patients with type 1 diabetes ($p < 0.01$). Neovascular glaucoma occurred in 2.1% of all diabetics, with no significant difference in prevalence between type 1 and type 2.

4.3 Ocular hypertension in patients with diabetes mellitus

The presence of higher intraocular pressures in diabetics compared with non-diabetics has been confirmed by several reports (114, 115, 116, 117). In contrast, Bouzas et al (1971) concluded that the mean intraocular pressure in diabetics did not significantly differ from the normal population (118); this is difficult to interpret in view of the overwhelming evidence suggesting the opposite conclusion. In 1962, de Rose and Becker noted increased intraocular pressure in 20% of adult diabetics without known glaucoma (119). Of 78 newly-diagnosed diabetics, initial applanation pressures of > 20 mmHg were recorded in 21%, and > 23 mmHg in 5%,

by Kolker in 1963 (119). A study by Hetherington and Shaffer in 1964 recorded intraocular pressures of > 20 mmHg in 18% and > 23 mmHg in 12% of diabetics (120). These figures compare favourably with those of Oates, Kolker and Becker (1965); of 78 diabetics, pressures of > 20 mmHg occurred in 18% of patients and > 23 mmHg in 6% (119). If patients with proliferative retinopathy were excluded from this sample (for reasons which will be explained later), of the 43 remaining patients, 26% had intraocular pressures > 20 mmHg and 12% > 23 mmHg.

In juvenile diabetic patients similar increased applanation intraocular pressures have been recorded. Safir et al (1964) examined 64 diabetic children aged 8-19 years; 30% were shown to have intraocular pressures in excess of 20 mmHg, and 15% greater than 23 mmHg (121). In 61 diabetic children aged 4-16 years studied by Morton and Becker (1964), 28% had applanation pressures > 20 mmHg, and 15% > 15 mmHg (119). A similar study by Sears (1965) involving 34 juvenile diabetics showed intraocular pressures > 20 mmHg in 21% and > 23 mmHg in 6% (119).

The exact relationship between intraocular pressure and retinopathy in patients with diabetes mellitus remains unclear; higher intraocular pressures have been reported in diabetics without retinopathy than in those with retinopathy (117,122,123). Proliferative retinopathy is rare in diabetics with primary open-angle glaucoma (109,124,125), and has significantly increased prevalence in diabetics with lower intraocular pressures, compared with those in the higher pressure category (117,126).

The study of 533 diabetic patients by Nielsen in 1983 reported the prevalence of ocular hypertension to be 3.0%, with significantly higher prevalence in patients with NIDDM ($p < 0.05$); 4.1% in type 2 diabetics compared with 0.6% in type 1 diabetics (113).

4.4 Diabetes mellitus in patients with open-angle glaucoma

In addition to establishing the prevalence of glaucoma in diabetics,

to which reference has already been made, Armstrong et al (1960) also investigated the prevalence of diabetes mellitus in patients with open-angle glaucoma. Known diabetes mellitus was present in 12.6% of patients, with a further 5.7% diagnosed by oral glucose tolerance test; the total prevalence of diabetes in patients with open-angle glaucoma was thus 18.3% (112). The prevalence of known diabetes in patients with open-angle glaucoma has been variously reported between 6-18% (112,125,127,128). Several studies have also confirmed the increased prevalence of asymptomatic diabetes in the glaucoma population (129,130). Lieb et al (1967) investigated 533 patients with open-angle glaucoma; diabetes mellitus was present in 8% of this group compared with 1% in the general population (131). The prevalence of diabetes in 490 patients with open-angle glaucoma attending King's College, London (1980) was 7.6% (122). The discrepancy between the figure quoted by Armstrong et al and those of the latter two studies is easily explained; Armstrong specifically tested for asymptomatic and previously-undiagnosed diabetes mellitus.

Becker (1971) examined a series of 100 consecutive patients with primary open-angle glaucoma but without known diabetes mellitus (132). A positive oral glucose tolerance test was obtained in 20% of the patients, which is comparable to the 18.3% determined by Armstrong et al. These patients were then subclassified on the basis of intraocular pressure control; the prevalence of diabetes was 14% if the mean intraocular pressure was > 25 mmHg, 25% in those with a mean < 25 mmHg, and 36% if the mean was < 20 mmHg. On the basis of these results, he postulated that either open-angle glaucoma patients with a positive OGTT were easier to control with medical therapy, or those patients spontaneously had lower intraocular pressures. Conclusions from this study are summarised as follows:

- i. Primary open-angle glaucoma in patients with diabetes mellitus

Primary open-angle glaucoma, raised intraocular pressure, increased intraocular pressure response to topical corticosteroid provocation, and cup:disc ratios of > 0.3 are significantly more prevalent in patients with diabetes mellitus. Becker's study also showed increased frequency of clinical glaucoma relative to ocular hypertension in diabetes, which prompted the hypothesis of greater susceptibility of progression to glaucoma, apart from any direct

association between diabetes and ocular hypertension.

ii. Diabetes mellitus in patients with primary open-angle glaucoma
Diabetes mellitus is more prevalent in patients with primary open-angle glaucoma (20%) and in patients with positive corticosteroid provocative tests.

As a considerable proportion of the patients in this study were asymptomatic, with diagnoses confirmed by OGTT, one may assume (although this is not stated) that the majority were type 2 diabetics. The distinction between type 1 and type 2 diabetes mellitus in relation to the primary glaucomas is essential, as will be explained in the final conclusions of the thesis.

4.5 Diabetes mellitus in patients with ocular hypertension

The prevalence of diabetes mellitus in patients with ocular hypertension is not well documented. Morgan and Drance (1975) considered diabetes to be less frequent in ocular hypertensives than in normal controls (97). In contrast, diabetes mellitus has been shown to be significantly associated with progression of ocular hypertension to overt glaucoma; in the Des Moines study by Armaly (1969), the only four cases of ocular hypertension which progressed to glaucoma were subsequently shown to have a positive oral glucose tolerance test (133). In a similar study by Wilensky et al (1974), of 15 ocular hypertensives who later developed field loss necessitating the diagnosis of glaucoma, 33% had a positive oral glucose tolerance test (98).

There are no accurate estimates of diabetes prevalence in patients with ocular hypertension.

4.6 Closed-angle glaucoma in patients with diabetes mellitus

Angle-closure is assumed to occur in diabetics as a consequence of the iritis and rubeosis which may be associated with this disease (122) - despite the fact that the prevalence of iritis in diabetics

is reported to be not significantly different from that in a non-diabetic population (110). There is no doubt that rubeotic vessels in the anterior chamber angle may lead to secondary angle-closure; equally, there is no evidence to suggest that this is the only mechanism for the genesis of angle-closure in diabetes mellitus. Other reports have stated that angle-closure appears to be no more frequent in diabetics than in the general population (119,134). The only epidemiological study of this subject, to date, is that by Nielsen (1983), which quoted the prevalence of closed-angle glaucoma in diabetics to be 0.18% (113) - similar to the prevalence in the general population (71,74,75,100).

4.7 Diabetes mellitus in patients with closed-angle glaucoma

There are no accurate estimates detailing the prevalence of diabetes mellitus in patients with a history of closed-angle glaucoma. This is remarkable when one considers that the prevalence of open-angle glaucoma in diabetics is quoted as 4.1% (112), whereas the prevalence of diabetes mellitus in patients with open-angle glaucoma is reported between 6-20% (125,127,128,132).

4.8 Mechanisms to explain the association between diabetes mellitus and the primary glaucomas

It has been proposed that the association may be due to a common genetic background; studies have suggested a relationship, particularly in juvenile diabetics, between glucose tolerance, suppression of plasma cortisol, and dexamethasone provocation (135,136,137). Topical corticosteroids are known to increase intraocular pressure in certain human eyes (138,139,140,141), a response genetically transmitted (142,143), and closely related to primary open-angle glaucoma (144).

The increased intraocular pressure in diabetics has also been attributed to increased aqueous flow (123,145), and decreased outflow facility (146). The definitive mechanism is not known.

CHAPTER 5

AUTONOMIC NERVE FUNCTION IN GLAUCOMA

5.1 Introduction

Angle-closure may occur as a direct result of relative parasympathetic and sympathetic activity in the anterior segment of the eye. The basis of this contention is discussed in the first section of this chapter. The next logical step was to assess whether the change in autonomic activity, or presence of autonomic neuropathy, was localised to the eye, or part of a generalised systemic dysfunction. This problem was resolved by investigating the integrity of autonomic nervous function using two separate, though complementary, approaches:

- a. Assessment of systemic autonomic nerve function
- b. Assessment of ocular autonomic nerve function

The association between autonomic nerve function and glaucoma is divided into three sections:

- 5.2 Autonomic effects in the pathogenesis of angle-closure
- 5.3 Systemic autonomic nerve function
- 5.4 Ocular autonomic nerve function

5.2 Autonomic effects in the pathogenesis of angle-closure

Curran (1931) suggested that angle-closure occurred as a secondary event, following anterior translational movement of the iris-lens diaphragm (50). The essential event precipitating shallowing of the anterior chamber is pupil block. The importance of pupil block in angle-closure is well-established (147,148,149,150), however the mechanism remained little known until comparatively recently. In 1968, Mapstone resolved pupil block force into three separate components (52):

- i. Force due to contraction of the sphincter muscle
- ii. Force due to contraction of the dilator muscle
- iii. Modulus of elasticity of the iris stroma

(Figure 1)

Pupil block is thus a posteriorly-directed vector, the direction and magnitude determined by the relative contribution of the three contributing elements. The importance lies in the relative contribution of each separate force. Independent component forces are not of equal potential magnitude; the sphincter muscle, when maximally-stimulated, may develop twice the power of the maximally-stimulated dilator muscle (151).

Sequential changes in the dimensions of the anterior chamber are impossible to quantify during the genesis of a spontaneous attack of closed-angle glaucoma. Deductions regarding pathogenesis must therefore be derived from models of experimental angle-closure - the rationale of provocative testing (152). Autonomic provocation, involving both sympathetic and parasympathetic nervous systems simultaneously, was developed during the 1970's based upon the theoretical principles of pupil block (152,153,154). Determination of the individual component forces of pupil block thus permits their isolation and manipulation. Contraction of the pupil may be resolved into a series of complementary forces, effecting increased iris-lens apposition (155). Summation of diametrically-opposed forces subsequent to isometric contraction of the iris musculature produces the posteriorly-directed vector of pupil block. The magnitude of pupil block force has been calculated, and shown to be maximal at a pupillary diameter between 3.8 mm and 4.2 mm (156). The only known method of maintaining pupillary diameter at mid-dilatation is to maximally stimulate both parasympathetic and sympathetic nervous systems in the anterior segment, the basis of the pilocarpine-phenylephrine provocative test (152,157). The position of the iris-lens diaphragm at equilibrium is determined by the production of aqueous humour, the facility of outflow, and the degree of pupil block. (Figure 2) Assuming a constant production of aqueous, both pupil block and facility of outflow are increased during the pilocarpine-phenylephrine provocative test. As aqueous flow from the posterior to anterior chamber is less than the rate of outflow from the anterior chamber (158), the resultant pressure differential effects forward movement of the iris-lens diaphragm and consequently shallowing of the anterior chamber (159,160). (Figure 3) The first stage of angle-closure

(ie irido-corneal contact) is thus facilitated (161,162). The pilocarpine-phenylephrine provocative test, with a sensitivity of 93% and a predictive value of 94%, has the added advantage of immediate reversal; guttae 0.5% thymoxamine, a sympathetic alpha-blocking agent (163), rapidly negates the alpha-sympathomimetic actions of guttae 10% phenylephrine, permitting elimination of antagonistic contraction of iris musculature with consequent decline in pupil block force (164).

It can be seen that manipulation of the autonomic nervous system in the anterior segment of the eye produces an effective model of angle-closure. Mapstone postulated in 1981 that the events preceding irido-corneal contact occurred as a direct result of autonomic activity, with specific emphasis on parasympathetic tone (155). Methods of autonomic function assessment are detailed in the following sections.

5.3 Systemic autonomic nerve function

5.31 Introduction

"I propose the term 'autonomic nervous system' for the sympathetic system and the allied nervous system of the cranial and sacral nerves and for the local nervous system of the gut." (165)

John Langley was responsible for the classification of the sympathetic nervous system into pre- and post-ganglionic nerves in 1895, and the introduction of the concept of a parasympathetic nervous system in 1905.

Autonomic failure is relatively rare. Bradbury and Eggleston first described "idiopathic orthostatic hypotension" - now known as progressive autonomic failure - in 1925 (166,167). Progressive autonomic failure with multiple system atrophy was documented by Shy and Drager in 1960 (168), and progressive autonomic failure with Parkinson's disease by Fichet in 1965 (169). As the essential pathogenetic mechanisms have not been identified, autonomic failure is generally categorised by a diagnostic classification. On this basis, these three disorders constitute the category of primary

autonomic failure. Other aetiological factors, principally within the general categories of secondary and drug-related autonomic failure, are listed in table 1.

The commonest cause of autonomic neuropathy is diabetes mellitus (170). Cardiac denervation was noted in a patient with diabetes mellitus by Eichhorst in 1892 (171), and features subsequently attributed to diabetic autonomic neuropathy were observed in ensuing years; neurogenic bladder disturbance (172), vasomotor instability (173,174,175), loss of testicular sensation (176), abnormal sweating (177), and impotence (178,179). The association between autonomic nerve function and diabetes is attributed to the observations of Jordan in 1936 (180) and Rundles in 1945 (181). Significant contributions were subsequently made in 1959 by Keen (182), and in 1960 by Sharpey-Shafer and Taylor (183), however major advances in both diagnosis and prognostic evaluation have been restricted to the past fifteen years.

Pathological studies of autonomic nerves are relatively infrequent. As a result of the rarity of autonomic neuropathy outwith the diabetic population, many reports are confined to descriptions of one or two cases. In primary autonomic neuropathy, severe loss of intermediolateral cells in the spinal cord has been evident (184,185,186), supporting the clinical diagnosis of sympathetic neuropathy. With regard to the parasympathetic nervous system, loss of Edinger-Westphal and dorsal vagal nuclei were reported by Shy and Drager in 1960 (168). In diabetic autonomic neuropathy, pathological evidence is more extensive, however pathogenetic mechanisms remain inconclusive. Segmental demyelination and axonal degeneration in the rami communicantes (187,188,189,190), vacuolated neurones in sympathetic ganglia, and marked loss of myelinated fibres in both sympathetic trunks and the vagus nerve has been demonstrated (191).

The pathogenesis of autonomic neuropathy in diabetes mellitus is not known, although the mechanism is considered to be similar to that in somatic neuropathy (192). Two hypotheses have been postulated; these may be complementary rather than mutually exclusive.

i. Vascular hypothesis

Small vessel disease has been proposed as the mechanism of diabetic retinopathy, nephropathy, and neuropathy (193,194). The importance of these changes in relation to autonomic nerve function is not known; microvascular pathology in autonomic ganglia has been described as both significantly present (195), and significantly absent (196).

ii. Metabolic hypothesis

Metabolic disturbances known to be associated with diabetes mellitus include myoinositol deficiency (197), increased glycosylation of structural protein (198), accumulation of sorbitol (199,200), and defects in lipid metabolism (201). Although these factors may affect nerve function, their importance in autonomic neuropathy is not known.

Autonomic insufficiency has an insidious onset, which is impossible to identify accurately due to the compensatory mechanisms inherent within the autonomic nervous system. Until comparatively recently, tests of autonomic nerve function have been difficult to interpret and virtually impossible to quantify. A series of autonomic function tests are described in table 2 (202,203 204,205,206,207,208, 209,210,211,212,213,214,215). The invasive and complex nature of these tests not only severely limits any subsequent clinical application, but also defies definitive interpretation in all but the most severe cases of autonomic neuropathy.

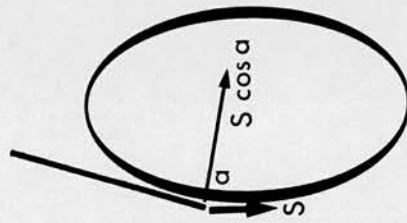
The recent development of non-invasive autonomic assessment, based upon cardiovascular reflexes, permits accurate, objective and reproducible measurement of autonomic function. As in all reflex assessments, this depends upon a standardised input stimulus, coupled with measurement of the subject's output reaction. Results are based on blood pressure and heart-rate responses to a variety of stimuli. A necessary corollary is that detected abnormalities cannot be localised to either the afferent, internuncial, or efferent components of the reflex. There is considerable evidence to sustain the conclusion that autonomic assessment by cardiovascular reflex responses correlates well with generalised autonomic function (216,217). In addition, the demonstration of significant systemic

postganglionic axonal lesions by chemical assay of noradrenergic responses to edrophonium stimulation, in diabetic patients with sympathetic neuropathy manifesting marked postural fall in systolic blood pressure, provides biochemical evidence in support of this contention (218).

Six autonomic function tests, based upon cardiovascular reflexes, were used to assess systemic autonomic status. Although each test has been used individually to give a measure of autonomic function, assessment based upon a series of complementary tests has been advocated by Ewing et al (1982) to preclude misleading deductions from single tests (219). This provides a comprehensive assessment of both sympathetic and parasympathetic function. The series of tests in this assessment are shown in table 3.

Diagnostic criteria for cardiovascular autonomic function tests are summarised in table 4 at the end of the chapter.

Figure 1



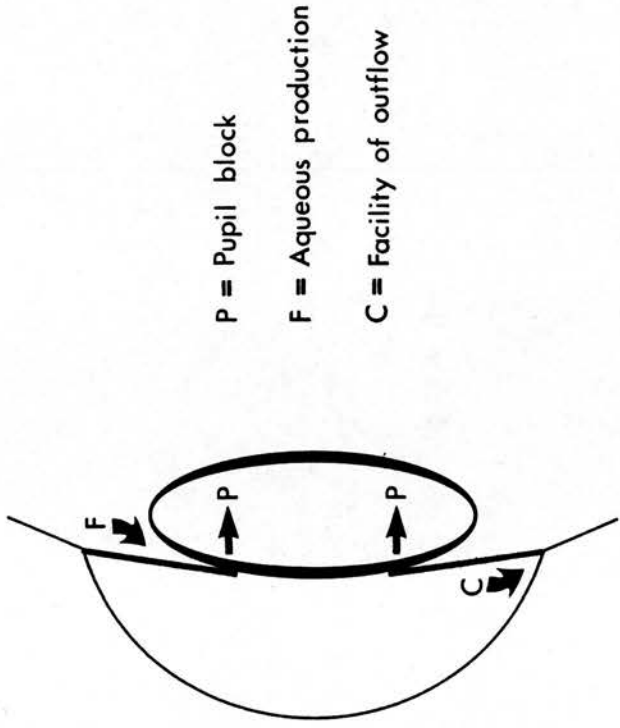
S = Sphincter contraction
force

$\cos \alpha = \text{cosine angle } \alpha$

PUPIL BLOCK

Figure 2

FORCES DETERMINING THE POSITION OF THE IRIS/LENS DIAPHRAGM



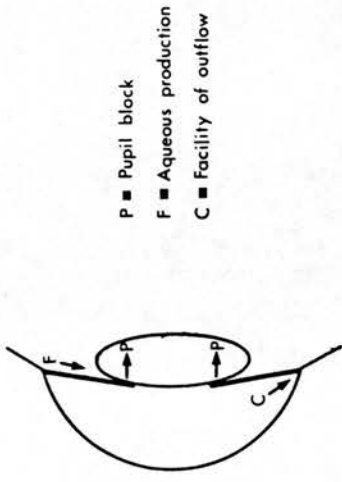
P = Pupil block

F = Aqueous production

C = Facility of outflow

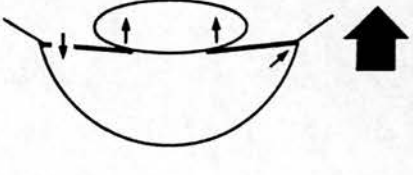
Figure 3

VARIATION IN ANTERIOR CHAMBER VOLUME DURING THE
PILOCARPINE - PHENYLEPHRINE PROVOCATIVE TEST



(a) Forces determining the position of the iris-lens diaphragm

Guttae pilocarpine-phenylephrine instilled



Movement of iris-lens diaphragm

(b) Eyes without a peripheral iridectomy

(c) Eyes with a peripheral iridectomy

Table 1

CLASSIFICATION OF AUTONOMIC FAILURE

- i. Primary
 - a. Progressive autonomic failure
 - b. Progressive autonomic failure with multiple system atrophy
 - c. Progressive autonomic failure with Parkinson's disease

- ii. Secondary
 - a. Diabetes mellitus
 - b. Amyloidosis
 - c. Neurological infection: syphilis, Chagas' disease
 - d. Autoimmune diseases: myasthenia gravis, rheumatoid arthritis, Guillain-Barré syndrome
 - e. Carcinoma
 - f. Alcoholism
 - g. Familial dysautonomia
 - h. Metabolic diseases: Fabry's disease, porphyria
 - i. Central nervous system lesions: especially involving the hypothalamus or the spinal cord
 - j. Renal disease
 - k. Hereditary sensory neuropathies
 - l. Drug effects:
 - i. Adrenergic neurone blocking drugs: eg guanethidine
 - ii. α -adrenergic blocking drugs: eg phenoxybenzamine
 - iii. β -adrenergic blocking drugs: eg propranolol
 - iv. Ganglion blocking drugs: eg mecamylamine
 - v. Vasodilator anti-hypertensive drugs: eg prazosin
 - vi. Centrally-acting anti-hypertensive drugs: eg methyldopa
 - vii. Tranquillisers: eg phenothiazine derivatives
 - viii. Antidepressant drugs: eg tricyclics
 - ix. Angiotensin converting enzyme inhibitors: eg captopril

Table 2

AUTONOMIC FUNCTION TESTS

Test	Reference
i. Amyl nitrite inhalation	202,203
ii. Tilt-table	204
iii. Phenylephrine hydrochloride (50-100 μ g) iv injection Angiotensin II (0.25-2.0 μ g) iv injection	205
iv. Hyperventilation	183
v. Cold pressor test	206
vi. Mental stress	207
vii. Quinizarin sweat test	208
viii. Direct sweat test	
a. intradermal injection of pilocarpine hydrochloride (5-15 mg)	209
b. electrophoresced acetylcholine	210
ix. Tyramine (250-6000 μ g) iv injection	211,212,213
x. Atropine sulphate (0.02 mg/kg) iv injection	214
xi. Norepinephrine bitartrate (0.05 μ g/kg) iv injection	215

Table 3

CARDIOVASCULAR AUTONOMIC FUNCTION TESTS

Parasympathetic_nerve_function

- i. Valsalva manoeuvre
- ii. Heart-rate variation during deep breathing
- iii. Immediate heart-rate response to standing
- iv. Immediate heart-rate response to lying

Sympathetic_nerve_function

- i. Systolic blood pressure response to standing
- ii. Diastolic blood pressure response to sustained isometric muscular contraction

5.32 Parasympathetic nerve function

5.321 Valsalva ratio

This test is based upon the circulatory response to forced, controlled respiration against resistance. The responses are differentiated into four separate phases, defined by Hamilton et al in 1936 (220). The heart-rate decreases and blood pressure increases for 2-3 seconds (Plate 2) due to the initial rise in intrathoracic pressure (phase 1). This is followed by a gradual increase in heart-rate in response to the reduction in venous return (Plate 3); subsequent decreased cardiac output leads to a fall in blood pressure (phase 2). The release of intrathoracic pressure in phase 3 results in a marked rise in the potential capacitance of the pulmonary venous plexus; a further decrease in cardiac output necessarily follows, with inevitably decreased systemic blood pressure and increased heart-rate (Plate 4). Phase 4 is characterised by increased cardiac output, rebound hypertension resulting from persistence of raised peripheral vascular resistance, and bradycardia as a vagal efferent response to increased baroreceptor afferent impulses (Plate 5).

The Valsalva manoeuvre had been used as a test of cardiac function during the 1950's (221,222), however the "Valsalva ratio", described by Levin in 1966 (223), enabled the heart-rate changes inherent within the technique to be placed on a measurable and comparable basis. The Valsalva ratio is calculated as the maximum R-R interval after the test (representing the bradycardia in phase 4) to the minimum R-R interval during the test (representing the tachycardia in phase 2). Systemic administration of intravenous atropine abolishes the heart-rate response (224), whilst cardiac sympathetic blockade has no effect (225). Rebound hypertension in phase 4, as a result of increased cardiac output during a period of raised peripheral vascular resistance, requires both parasympathetic and sympathetic blockade to be effectively negated (226,227). Pharmacological blockade thus confirms the importance of the parasympathetic nervous system in control of heart-rate changes during this reflex.

There are basically two forms of abnormal response to the test. The first may occur in patients with congestive heart failure, who exhibit a "square-wave" response (217); the blood pressure in this case directly reflects changes in intrathoracic pressure, and heart-rate remains constant. The second type of abnormal response is related to autonomic neuropathy. Damage to the cardiovascular reflex arc results in decreasing blood pressure during strain (as venous return is inhibited by increased intrathoracic pressure) followed by a gradual increase in blood pressure upon discontinuing the Valsalva manoeuvre; there is no rebound hypertension and no significant change in heart-rate.

Although other indices have been derived from the Valsalva manoeuvre, such as the post-strain bradycardia ratio (228) and the tachycardia ratio, these are significantly correlated with the Valsalva ratio (229,230), which thus remains a well-established, effective and reproducible measure of parasympathetic autonomic function. Clinically, a ratio of less than 1.10 is defined as abnormal, 1.11-1.20 as borderline, and 1.21 upwards as normal (219); the ratio is known to decrease minimally with age (223,231). Cardiac parasympathetic function has been assessed by this technique in other disorders; alcoholism (232), renal disease (233), and rheumatoid arthritis (234). Valsalva ratios agree closely with other clinical parameters of autonomic dysfunction (235,236).

5.322 Heart-rate variation during deep breathing

The association between heart-rate and respiration was first described by Ludwig in 1847 (237). Clynes (1960) suggested that respiratory sinus arrhythmia was not primarily determined by haemodynamic or central influences; control was effected by two separate reflexes, the first related to inspiration and the second to expiration, with unidirectional and biphasic characteristics (238). Stretch receptors in the chest initiate afferent impulses, mediating reflex inhibition of the cardioinhibitory centre in the medulla oblongata. The phasic relationship between heart-rate and respiration is explained by a differential equation relating

vagal inhibition and heart-rate.

$$\frac{1}{4\pi^2 r_0^2 - (V_0 + \Delta V)} \frac{d^2y}{dt^2} + y = 0$$

r_0 = heart-rate with absence of vagal inhibition

V_0 = normal vagal tone

ΔV = change in vagal tone by respiration

y = output of the pacemaker

This explanation was modified by Davies and Neilson (1967), by considering heart-rate and resting respiration to be related exclusively by a biphasic inspiratory heart-rate transient with an amplitude of 16 beats per minute; expiration was assumed to exert little effect on heart-rate (239). They also suggested that although stretch receptors are involved, most of the afferent impulses emanate from vasoreceptors on the left side of the heart responding to the alterations in blood flow which parallel respiratory cycles.

Beat-to-beat variation in heart-rate was first used to assess the integrity of the autonomic nervous system by Wheeler and Watkins in 1973 (240). Pharmacological autonomic blockade confirmed that the reflex was mediated by the parasympathetic nervous system; intravenous atropine abolishes the response, however intravenous propranolol has no effect (240). Several different techniques of assessing cardiac vagal function have employed the reflex evoked by respiratory sinus arrhythmia. The relative merits of each method are discussed:

i. Mean-square successive difference

Gundersen and Neubauer (1977) proposed this technique as an estimate of autonomic function (241). Heart-rate is recorded by electrocardiography with the subject at rest, and the mean of the squares of the differences between successive intervals of 150 consecutive beats is calculated. This method may also be measuring the intrinsic rhythmicity of the sino-atrial node, independent of parasympathetic control, and consequently is not an accurate estimate of vagal function.

ii. Standard deviation of resting heart-rate

In this method, a continuous electrocardiogram is recorded on magnetic tape for approximately five minutes with the subject at rest. The electrocardiogram is then analysed, with the exclusion of arrhythmias by an arrhythmia computer, and the mean and standard deviation of the R-R interval calculated. The standard deviation is then used as a measure of R-R interval variation (242). This technique has been used by several authors (243,244,245,246,247), however the standard deviation only assesses beat-to-beat variation if the heart-rate remains unaltered, and thus a dependence upon resting heart-rate is implicit. Subsequent studies have shown poor reproducibility and a wide range of "normal" values (248).

iii. Variation of heart-rate in response to a single deep breath
It has been suggested that the variation in heart-rate is maximal in response to a single deep breath (228). This assertion awaits detailed analysis.

iv. Heart-rate variation during deep breathing

Maximum and minimum heart-rates are measured during deep breathing at rest, and the differences expressed as beats per minute. The original method was advocated by Wheeler and Watkins in 1973, using an instantaneous heart-rate meter (240). The protocol was modified slightly by Hilsted and Jensen (1979); heart-rate was recorded by electrocardiography, and maximum/minimum levels calculated directly from the trace using an ECG ruler (249). This had the advantage of providing a documentary record of the reflex. Differences between maximum/minimum heart-rates of 10 beats per minute or less are indicative of autonomic dysfunction (250).

Sundkvist (1979) modified the method to calculate the ratio of the maximum R-R interval to the minimum R-R interval in each respiratory cycle - the E:I ratio (251); this is merely another form of expressing the maximum/minimum heart-rate variation. Methods of assessing parasympathetic function by respiratory sinus arrhythmia have been compared in several recent publications (252,253,254); each independently concluded that the best technique

was measurement of the difference between maximum and minimum heart-rates in beats per minute, using continuous electrocardiography.

Heart-rate variation during deep breathing diminishes with age (241,245,246,255,256,257,258,259), however the minimum "normal" difference in heart-rate between inspiration and expiration is 11 beats per minute at age 65 years (260). Thus the clinical diagnosis of abnormality when the heart-rate variation is ≤ 10 beats per minute, advocated by Ewing and Clarke in 1982 (219), is equally applicable to older age groups.

5.323 Immediate heart-rate response to standing

Alteration in posture from lying to standing evokes reflex heart-rate and blood pressure responses (261). The immediate heart-rate response to standing was first described by Page and Watkins in 1977 (262), and subsequently investigated as a method of autonomic nerve function assessment by Ewing et al in 1978 (263). In the original technique, the subject rested supine for approximately three minutes then was requested to stand unaided, whilst a continuous electrocardiogram was recorded on magnetic tape. Subsequent analysis showed a consistent response during the manoeuvre, with relative tachycardia occurring at approximately beats 10-15 after standing, and relative bradycardia maximal at beats 25-35. The response is reproducible in normal control subjects, but absent in diabetics with autonomic neuropathy. This computerised technique was compared with a simplified method for routine clinical application. The same rationale was employed, however the continuous cardiac trace was recorded by a direct-writing electrocardiograph, and the longest and shortest R-R intervals (corresponding to the bradycardia at beat 30 and tachycardia around beat 15) manually measured using a ruler; the longest R-R interval is divided by the shortest R-R interval to obtain the 30:15 ratio. The method correlated closely with the computerised technique, and thus obviated the necessity of complicated equipment.

Autonomic mechanisms responsible for this response were initially

proposed to be dependent upon a vagal reflex, a hypothesis examined by Ewing et al in 1980, subdividing the reflex into several component effects by selective pharmacological blockade (264). Although responses to change in posture from the supine to the standing position had been previously studied (265,266,267,268,269), the circulatory reflexes actually during the movement - rather than merely before and after change in posture - had received scant attention. On separate days, the manoeuvres were repeated under conditions of selective pharmacological blockade:

- i. The parasympathetic nervous system was blocked by intravenous atropine: 1.8 mg followed by a booster dose of 1.2 mg.
- ii. The sympathetic nervous system was blocked by intravenous propranolol: 10 mg.
- iii. Total cardiac autonomic blockade by both intravenous atropine and propranolol.

In addition, comparisons between active standing, and passive postural change (using a tilt table) were performed.

The results clearly indicated that the immediate heart-rate response to standing was dependent upon immediate decrease in cardiac vagal inhibition followed by vagal reactivation. Intravenous atropine reduced the relative tachycardia at beats 10-15, and totally eliminated the rebound bradycardia. The manoeuvre was not affected by intravenous propranolol, however if the vagus was blocked, increased sympathetic discharge effected a minor cardiac acceleration between beats 15-30 (264). The rebound bradycardia is dependent entirely upon the effect of muscular exercise; initial tachycardia occurred during both active and passive change in posture, however bradycardia was abolished by passive tilt.

Modifications of this reflex have been proposed as clinical tests: the maximum-minimum ratio, the relative heart-rate increase (250), and the acceleration and brake indices (270). There is no evidence to suggest any advantages conferred by these methods in comparison to the 30:15 ratio, and considerable evidence to the contrary. The 30:15 ratio is only slightly reduced in older subjects (260), and is not related to resting heart-rate (248). Consequently, it provides an excellent cardiovascular measure of systemic

parasympathetic nerve function.

5.324 Immediate heart-rate response to lying

This is the most recent of the cardiovascular autonomic function tests, the underlying mechanism having been elucidated in 1982 (271). Short episodes of muscular exercise were known to be associated with an immediate decrease in vagal tone, followed by vagal reactivation (264,272,273,274). Similar techniques employed in the investigation of the immediate heart-rate response to standing were used to determine the mechanisms in this response: selective pharmacological blockade by intravenous atropine and propranolol; comparisons between active and passive change in posture from the standing to the lying position; reproducibility studies; and assessment of the relationship with age.

Patients maintained a relaxed, erect posture for approximately three minutes then were requested to lie down unaided. Continuous electrocardiograms were recorded, both on magnetic tape for computerised analysis, and by direct-writing electrocardiography. The heart-rate changes during the manoeuvre were then compared by differences between successive R-R intervals. An initial relative tachycardia occurs, represented by decrease in R-R interval at about the 3rd-4th beat after commencing the manoeuvre, followed by a relative bradycardia, maximal at about the 25th-30th beat. Selective pharmacological blockade confirmed that the reflex was mediated by initial vagal withdrawal, followed by vagal reactivation; intravenous atropine abolished the reflex, whilst propranolol alone effected no change in the early stages of the response. Comparisons between active and passive postural change established the importance of muscular exertion in evoking the response.

Although similarities are evident between the lying/standing and standing/lying manoeuvres, there are separate cardiovascular reflexes in operation. Whereas vagal withdrawal occurs within 3-4 beats in the standing/lying manoeuvre, the minimum R-R interval

difference is not present until beats 10-15 during lying/standing. Immediate vagal reactivation does not occur during the 30:15 test, presumably as muscular activity - the stimulus to vagal withdrawal - continues to exert an effect. The sympathetic nervous system is known to exert effects only in the later stages of the immediate heart-rate response to lying. Sympathetic efferent discharge is increased during standing at rest, and decreased in the supine position (271). Examination of R-R intervals 50-60 beats after the manoeuvre during pharmacological blockade procedures suggests that the sympathetic nervous system effects this stage of the reflex response, with decreased sympathetic activity causing lengthening of the R-R interval.

Investigation of patients with various stages of diabetic autonomic neuropathy has confirmed the value of this test as a sensitive measure of early parasympathetic dysfunction (271,275). In normal subjects, the measure is reproducible, and not significantly affected by age (271,275).

5.33 Sympathetic nerve function

5.331 Systolic blood pressure response to standing

Change in posture from the supine to the erect position evokes characteristic cardiovascular reflexes. Gravitational pooling of blood in the venous capacitance vessels of the lower extremities precipitates an immediate fall in venous return and consequently cardiac output. Homeostasis in the maintenance of systemic blood pressure is effected by increased sympathetic efferent impulses; the resultant raised peripheral vascular resistance reverses the decrease in venous return, and in conjunction with direct chronotropic cardio-accelerator effects, preserves adequate cardiac output (268,276,277). Aortic and carotid sinus baroreceptors mediate these reflexes (278), with contributions from cardiopulmonary low-pressure receptors (279). Upon standing, parasympathetic activity decreases and sympathetic activity increases. The role of autonomic failure in the development of orthostatic

hypotension was recognised by Bradbury and Eggleston in 1925 (166), and orthostatic hypotension has subsequently been associated with several neurological disorders (280).

The assessment is performed according to the following technique. After resting supine for approximately three minutes, the subject is requested to stand unaided. Systolic blood pressure is recorded at rest, using a cuff sphygmomanometer, and immediately on standing. Splanchnic vasoconstriction is an integral component of the reflex; its importance in blood pressure control has been clearly demonstrated (281,282). Impaired constriction of splanchnic vessels is present in diabetics with postural hypotension (283).

The evidence regarding postural hypotension in relation to age is conflicting. Although decreased efficacy of postural reflexes is present in some elderly subjects (248,266), there is currently considerable debate regarding the exact degree of age-related postural fall in blood pressure. Some authors suggest that postural hypotension is directly age-related (266,284); others refute this contention, whilst accepting that postural fall in blood pressure is more prevalent in an elderly population (285). Similarly, the degree of postural change varies; using a level of 20 mmHg decrease in systolic blood pressure between lying and standing, the prevalence of postural hypotension in older "normal" subjects is variously reported as 24% (286), 22% (285), and 0% (287) - although the latter figure was obtained from a marginally younger sample. If a level of 40 mmHg difference in systolic blood pressure is accepted as diagnostic, 5% of the population aged > 65 years are affected (286).

Apart from differences in diagnostic criteria and patient populations, the prevalence of postural hypotension is affected by other factors; during change in posture, activation of the renin-angiotensin system may assist in the maintenance of blood pressure, and symptoms of postural hypotension are also limited by autoregulation of cerebral blood flow, possibly via myogenic and metabolic mechanisms (288). Thus compensatory mechanisms remain effective to various degrees in patients with sympathetic nerve dysfunction.

The exclusion of other known causes of orthostatic hypotension (eg significant decrease in circulating blood volume, drug effects, and neurological disease) is an essential prerequisite. Assuming this precaution is taken, postural changes in systolic blood pressure provide an effective cardiovascular assessment of sympathetic nerve function.

5.332 Diastolic blood pressure response to sustained isometric muscular contraction

Changes in the immediate metabolic environment of exercising, ischaemic muscle provide the afferent stimulation for subsequent reflex increases in heart-rate and blood pressure (289). The vagus and sympathetic nerves mediate the efferent component of this reflex, effecting increased cardiac output by both inotropic and chronotropic actions and peripheral vasoconstriction.

Isotonic or dynamic exercise produces relatively moderate increases in mean arterial pressure with marked tachycardia (290), however isometric exercise produces opposite effects: marked increase in mean arterial blood pressure with minimally-increased heart-rate (291). Lind et al (1968) has shown that cardiovascular responses occur during sustained isometric exercise providing 30% of maximum voluntary contraction is maintained; the response is not dependent upon the mass of muscle under examination (289). Sustained contraction of the forearm muscles is utilised as the basic experimental model, as this is both reproducible and well-documented in normal subjects (292). 30% of maximum voluntary handgrip has been equated with carrying a 10 kg weight (293). The rise in blood pressure results from two discrete autonomic effects: peripheral vasoconstriction via sympathetically-stimulated alpha-adrenoceptors (294), and heart-rate dependent increased cardiac output (289). After a period of sustained isometric muscular contraction at the requisite level, blood pressure rises significantly if the reflex pathway is intact (248). The distinction between normal and abnormal responses is most evident using diastolic blood pressure as the diagnostic index (295). The "normal"

tolerance intervals were established by Ewing et al in 1974 (295); in a normal population (age range 25-54 years), the mean increase in diastolic pressure was 33 ± 9 mmHg in males, and 24 ± 9 mmHg in females. The test is easily performed; after establishing the maximal voluntary contraction using a handgrip dynamometer, 30% of maximal voluntary contraction is maintained for as long as possible whilst blood pressure recordings are taken at one-minute intervals from the non-exercising arm.

Several studies have confirmed the value of this technique in the assessment of sympathetic autonomic function (235,248,296). Apart from obstruction to outflow secondary to mitral stenosis, the presence of cardiac pathology does not negate the reflex rise in diastolic blood pressure; the latter is only diminished or abolished by significant sympathetic autonomic neuropathy, thereby preventing peripheral vasoconstriction. The technique is safe, with reversion to normal levels of blood pressure within sixty seconds of discontinuing the manoeuvre (295). Heart-rate changes during this test have been documented, however the normal responses are variable, and are thus unsuitable for diagnostic application (295,297).

The rise in diastolic blood pressure precipitated by sustained forearm muscular contraction provides a reproducible, non-invasive and effective cardiovascular measure of sympathetic nerve function.

5.4 Ocular autonomic nerve function

Autonomic nerve function in the anterior segment of the eye was assessed by two complementary techniques: the pupil cycle time; and utilisation of the principles of denervation hypersensitivity, a phenomenon which may develop subsequent to neuropathy.

5.41 Pupil cycle time

Regular pupillary oscillations are induced by illumination of

the pupil margin with a thin beam of light. There are basically three types of pupillary movement in controlled lighting conditions:

i. Hippus

A physiological pupillary unrest in stable lighting conditions. Characteristic features include irregularity, continuous duration, and variable amplitude (298,299,300).

ii. Pupillary oscillation in drowsy subjects

This is characterised by persistence in the absence of light, episodic regularity, variable amplitude, and extended cycle time (301,302,303).

iii. Edge-light pupil cycle time

Although this phenomenon was first recorded by Lambert in 1760 using focussed candle-light (298), the edge-light pupil cycle time was placed in a clinical perspective by Stern in 1944 (304):

"If a fine pinpoint of light is projected on the eye with the slit lamp in such a way that it just enters the pupil near the margin of the iris, a light reaction follows, the pupil contracts. That means that the iris margin moves towards the centre of the pupil and prevents the light pencil from entering the pupil. As no light now reaches the retina the stimulus for the contraction of the pupil is no longer present, the pupil dilates - and thus again allows the light pencil to reach the retina."

Stark and Cornsweet (1958) applied a servoanalytic hypothesis to explain the pupil oscillations occurring with edge illumination, concluding that the phenomenon was a manifestation of the pupillary reflex arc (305,306). Localisation of autonomic nerve function assessment to the eye necessitates a brief description of the ocular anatomy of the autonomic nervous system, with particular reference to the pupil reflex; this response is initiated by receptors in the rods and cones, afferent impulses travelling via the optic nerve - probably in non-myelinated peripheral fibres. Following nasal decussation at the optic chiasm, the afferent limb continues via the optic tract without synapse through the superior brachium and thence to the pretectal nucleus. Synapse occurs at this point with decussation, and the interneurone passes to the Edinger-Westphal nuclei of both oculomotor nerves, via the posterior longitudinal bundle. The Edinger-Westphal nucleus is situated in the midbrain, at the postero-superior part of

the oculomotor nuclear complex. The efferent parasympathetic pupillomotor fibres travel with the oculomotor nerve, synapse at the ciliary ganglion, and innervate the sphincter pupillae via the short ciliary nerves (307). Postganglionic fibres in the short ciliary nerves pass to the ciliary, choroidal and iridic plexuses. Parasympathetic fibres from the Edinger-Westphal nucleus to the iridic plexus are both crossed and uncrossed, explaining the consensual pupillary response to light (308). Parasympathetic fibres controlling accommodation (ie innervating the ciliary muscle), and mediating the pupillary constriction which accompanies accommodation, may synapse in the episcleral ciliary ganglia (309), however this view has been questioned (310).

The first neurone in the sympathetic relay passes from the hypothalamus via the ipsilateral intermediolateral columns of the spinal cord to the cilio-spinal centre of Budge, situated at the root of the first thoracic nerve (311). Preganglionic nerves (the second neurone) leave the spinal cord at the level of the first two thoracic nerves, traverse the inferior and middle cervical ganglion, and synapse at the superior cervical ganglion. The postganglionic fibres (the third neurone) pass via the internal carotid plexus to the superior orbital fissure. At this point the sympathetic nerves pass into the orbit with the ophthalmic division of the trigeminal nerve, and enter the eye with the long ciliary branches of the nasociliary nerve to subsequently innervate the dilator pupillae.

Several different applications of the basic principle underlying the pupil oscillation occurring with edge illumination have been used to assess the pupillary reflex arc. Early observers employed a vertical slit-beam to measure the pupil cycle time (312,313). Sakuma (1965) obtained similar results with a technique which omitted light from the central portion of the pupil (314). The technique was placed on a clinical basis by Miller and Thompson in 1978 (315). They explained the mechanism underlying edge-light pupil cycle time as an overcompensation of the pupil in response to marginal illumination, resulting in a "high-gain" state; when the pupil is in this state, there is instability of the pupillary

reflex feedback loop and spontaneous pupillary oscillations occur. The technique is simply performed by seating the subject at the slit-lamp in a dimly-illuminated room, directing a thin, horizontal slit-beam to just overlap the inferior pupillary margin, and timing the resultant pupillary oscillations.

"Normal" pupil cycle time has a mean of 822 ± 69 ms in a population aged less than 50 years, with the 95th percentile set at 954 ms (315). The pupil cycle time is known to increase slightly with age; in the age group 50-79 years, the mean pupil cycle time was 872 ± 83 ms (316). The pupil cycle time is reproducible, and accurate to within $\pm 3\%$ (317); it is not dependent upon light adaptation, accommodation, amplitude of oscillation, initial pupil size, visual acuity, iris colour, refraction, or degree of pupillary unrest. Variation in stimulus intensity only alters the pupil cycle time to a maximum of $\pm 0.3\%$.

Pupil cycle time is actually measuring the time required for a single pupillary reflex ie initial constriction of the pupil followed by dilation. This involves a series of independent components summing to effect the reflex: afferent impulses transmitted by the optic nerve; synapses and interneurons within the midbrain; efferent discharge via parasympathetic outflow with the third nerve; and the mechanical efficacy of iris stroma and musculature. Prolongation of the pupil cycle time cannot be localised to any single constituent of the pupillary reflex pathway without additional information. It does, however, localise the defect to one or more of the constituents of the reflex.

Previous authors have used the pupillary reflex arc to assess the afferent limb of the reflex, by infrared videopupillography (318), and measurement of the relative afferent pupillary defect (319,320,321). In attempting to localise the defect, central and peripheral visual field assessments in patients with glaucoma are essential, as afferent pupillary defects are known to occur in glaucoma (322), and have been attributed to defects in the anterior visual pathway usually proximal to the optic chiasm (323).



The pupil cycle time has been used as an objective measure of the pupillary reflex arc in patients with lesions of the afferent limb of the reflex; it is significantly prolonged in patients with optic nerve compression from chiasmal tumours or Grave's disease (324), optic neuritis (313,325,326), and retrobulbar neuritis (327,328). This study is, to my knowledge, the first to use the technique in the investigation of disorders involving the efferent parasympathetic limb of the reflex.

5.42 Denervation hypersensitivity

5.421 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. Adrenergic and cholinergic supersensitivity of autonomically-denervated irides has been known since the early years of the 20th century; in 1904, Meltzer described paradoxical pupil dilatation with adrenaline (329), and in 1905 Markus (330) and Anderson (331) separately noted undue sensitivity of the denervated pupil to cholinergic drugs. Sensitisation of the denervated pupillary sphincter to acetylcholine was confirmed by Shen and Cannon in 1936 (332). Scheie (1940) subsequently observed enhanced cholinergic sensitivity of the tonic pupil (333); topically-applied 2.5% methacholine was shown to constrict tonic pupils, whilst normal pupils were unaffected by concentrations up to 15%. Adie's tonic pupil was considered to be specifically associated with a postganglionic parasympathetic defect, following the experimental demonstration of marked cholinergic sensitivity subsequent to removal of the ciliary ganglion or short ciliary nerves by Scheie and Adler in 1940 (334).

The law of denervation was placed in perspective by Cannon in 1939: "When in a series of efferent neurones, a unit is destroyed, an increased irritability to chemical agents develops in the isolated structure or structures, the effects being maximal in the part directly innervated." (335)

This stressed that supersensitivity of a tissue was maximal if the innervating neurone was damaged, compared with preceding neurones in the relay. The description was expanded in the definitive law of denervation supersensitivity by Cannon and Rosenbluth in 1949:

"When in a functional chain of neurones one of the elements is severed, the ensuing total or partial denervation of some of the subsequent elements in the chain causes a supersensitivity of all the distal elements, including those not denervated, and effectors if present, to the excitatory or inhibitory action of chemical agents and nerve impulses; the supersensitivity is greatest for the links which immediately follow the cut neurones and decreases progressively for more distant elements." (336)

Thus although sensitivity is greatest when the innervating neurone is affected, increased sensitivity occurs even if the lesion is more proximally situated in the synaptic chain.

In the presence of significant neuropathy, one may logically assume an intact effector tissue to exhibit hypersensitive responses to topically-applied chemical neurotransmitters. It is appropriate to briefly review the applications of the principle of denervation hypersensitivity to ocular structures innervated by the parasympathetic and sympathetic nervous systems.

5.422 Parasympathetic nervous system

Denervation hypersensitivity of the parasympathetic nervous system in the anterior segment of the eye is present in Adie's tonic pupil (337,338), and familial dysautonomia - the Riley-Day syndrome (339,340). Parasympathetic denervation of the iris in Adie's syndrome has been shown to occur following degeneration of the ciliary ganglion (310,341), resulting in a segmental palsy of the iris sphincter (342). Hypersensitivity to methacholine in this disorder may persist for at least 25 years (343).

Recent reports have reviewed the efficacy of methacholine in the diagnosis of denervation supersensitivity. These have concluded that methacholine elicits cholinergic hypersensitivity in 63% of Adie's pupils, whereas 0.125% pilocarpine increases diagnostic

sensitivity to 69% (344,345,346). Methacholine demonstrates high specificity with moderate sensitivity for cholinergic denervation.

5.423 Sympathetic nervous system

Interruption of the sympathetic innervation to the eye results in Horner's syndrome (347), with ipsilateral ptosis, miosis, and facial anhidrosis (348). The physiological properties of adrenaline were described by Langley in 1901 (349). Topical ocular application of the drug was noted to produce effects equivalent to those resulting from cervical sympathetic nerve stimulation; paradoxically, the efficacy of topical adrenaline was enhanced by sympathetic nerve section (350). These observations were explained by Cannon's law of denervation hypersensitivity. This principle enabled the development of topical drug combinations to indirectly determine the site of damage within the sympathetic three-neurone efferent chain: the central neurone, from the hypothalamus to the cilio-spinal centre of Budge; the preganglionic neurone, from the cervical/thoracic cord to the superior cervical ganglion; and the postganglionic neurone, from the superior cervical ganglion via the carotid plexus and long ciliary nerves to the iris. The drugs used, with presumed mechanisms of action, are shown in table 5.

Thus two mutually-exclusive drug effects may be utilised in the assessment of sympathetic innervation to the dilator pupillae:

i. Indirectly-acting sympathomimetics, which effect mydriasis by either releasing noradrenaline from presynaptic nerve stores (1% hydroxyamphetamine), or preventing re-uptake of noradrenaline (cocaine).

ii. Directly-acting sympathomimetics, which exert direct effects on adrenoceptors of the dilator pupillae (0.1% adrenaline, 1% phenylephrine).

Impaired efficacy of the dilator pupillae is clinically manifest as delayed pupillary dilatation in darkness (351). Denervation hypersensitivity has been assessed with 0.1% adrenaline,

10% phenylephrine, and 1% phenylephrine. Although 0.1% adrenaline is reported to elicit "maximal dilatation of the pupil" (352), subsequent studies have shown inconclusive results due to poor corneal penetration and variation in individual sensitivity (353). 10% phenylephrine produces marked dilatation of the denervated pupil, however similar effects are seen in normal pupils (353). 1% phenylephrine proved a more sensitive determinant of hypersensitivity; once again, mydriatic effects on the normal pupil await accurate definition (353,354,355). Postganglionic lesions exhibit markedly greater sensitivity to these topical drugs than central or preganglionic defects (356,357), and therefore a postganglionic lesion may be easier to identify (358).

Present indications suggest that hypersensitivity is not a transient phenomenon. Sympathetic denervation hypersensitivity to 0.1% adrenaline may persist for over 20 years (358); parasympathetic denervation hypersensitivity has been documented on repeated testing over a 25 year period (343).

The presence of sympathetic and parasympathetic autonomic neuropathy may thus be accurately assessed in the anterior segment of the eye by clinical application of the laws of denervation hypersensitivity.

Table 4

DIAGNOSTIC CRITERIA IN CARDIOVASCULAR AUTONOMIC FUNCTION TESTS

	Abnormal	Borderline	Normal
<u>Parasympathetic_nerve_function</u>			
i. Valsalva ratio	≤ 1.10	1.11-1.20	≥ 1.21
ii. Heart-rate variation during deep breathing (beats/minute)	≤ 10.0	10.1-14.9	≥ 15.0
iii. Immediate heart-rate response to standing (30:15 ratio)	≤ 1.00	1.01-1.03	≥ 1.04
iv. Immediate heart-rate response to lying (S:L ratio)	≤ 1.07	1.08-1.24	≥ 1.25
<u>Sympathetic_nerve_function</u>			
i. Decrease in systolic blood pressure on standing (mmHg)	≥ 30.0	29.9-10.1	≤ 10.0
ii. Increase in diastolic blood pressure in response to sustained handgrip (mmHg)	≤ 10.0	10.1-15.9	≥ 16.0

Table 5

TOPICAL DRUG TESTS TO DETERMINE THE SITE OF DAMAGE WITHIN THE OCULAR SYMPATHETIC EFFERENT OUTFLOW

	Pupil response			
	Normal	Central neurone lesion	Preganglionic neurone lesion	Postganglionic neurone lesion
<u>Indirect-acting sympathomimetic drugs</u>				
i. 2% cocaine	moderate dilation	slight dilation	no dilation	no dilation
ii. 1% hydroxyamphetamine	moderate dilation	moderate dilation	moderate dilation	no dilation
<u>Direct-acting sympathomimetic drugs</u>				
i. 0.1% adrenaline	no dilation	no dilation	moderate dilation	extreme dilation
ii. 1% phenylephrine	slight dilation	slight dilation	moderate dilation	extreme dilation
iii. 10% phenylephrine	extreme dilation	extreme dilation	extreme dilation	extreme dilation

RATIONALE OF STUDY

Autonomic integrity in the primary glaucomas was investigated. Several studies were performed on patients with primary open-angle glaucoma, primary closed-angle glaucoma, and ocular hypertension. The aims were:

- i. to define the relationship between diabetes mellitus, the commonest cause of autonomic neuropathy in this country, and the primary glaucomas.
- ii. to examine the efficacy of autonomic nerve function systemically, by a series of tests based upon cardiovascular reflexes, specifically designed to quantify parasympathetic and sympathetic nerve function.
- iii. to assess ocular autonomic nerve function by:
 - a. pupil cycle time - an accurate, reproducible measurement of the pupillary reflex arc.
 - b. denervation hypersensitivity - by definition, manifest only in the presence of significant neuropathy.

SECTION II

DIABETES MELLITUS IN THE PRIMARY GLAUCOMAS

CHAPTER 6

DIABETES MELLITUS IN THE PRIMARY GLAUCOMAS

6.1 Introduction

Known associations between diabetes mellitus and open-angle glaucoma have been detailed in the introductory text. The lack of controlled studies investigating the prevalence of diabetes mellitus in patients with closed-angle glaucoma and ocular hypertension prompted rectification of this omission. In addition, the relationship with open-angle glaucoma required further investigation; diabetic diagnostic criteria used in the United States of America 10-20 years ago are not applicable today, thus direct comparisons of prevalence rates are impossible. As the aim of this study was to assess autonomic function in the primary glaucomas, an estimate of the prevalence of diabetes mellitus - the commonest cause of autonomic neuropathy in this country - was an essential prerequisite.

Diabetes mellitus has been defined as a state of sustained hyperglycaemia (359). It is not the purpose of this introduction to examine the complexities of diabetes mellitus in detail, merely to clarify criteria employed in the diagnosis. Until comparatively recently, the diagnosis of diabetes mellitus was based upon differing diagnostic criteria applied to different forms of the oral glucose tolerance test (360), thereby confusing the determination of diabetes on an individual basis, and precluding comparisons of diabetic prevalence within discrete populations. Substantial variations in clinical diagnostic criteria are a subject of controversy; several studies have attempted to establish the relationship between the size of the glucose loading dose and subsequent plasma glucose levels (361,362). In North America, the 100 gram oral glucose load is widely used (363); in Europe, the test is performed with a 50 gram glucose loading dose (364). Recently, the National Diabetes Data Group and the World Health Organisation Expert Committee on Diabetes Mellitus have standardised the diagnosis of diabetes mellitus within specific criteria,

advocating oral glucose tolerance testing with a 75 gram glucose load (365,366). This has been well-received (367,368,369), and permits accurate comparisons of diabetic prevalence on a global basis for the first time.

The criteria proposed by the National Diabetes Data Group and the World Health Organisation subdivide the population into three categories:

- i. normal
- ii. impaired glucose tolerance
- iii. diabetes mellitus

Impaired glucose tolerance represents a region of diagnostic uncertainty, encompassing the following former diagnoses: chemical diabetes, asymptomatic diabetes, subclinical diabetes, borderline diabetes, latent diabetes. Studies have shown that between 2-4% per year will progress to overt diabetes mellitus (370).

Adequate categorisation of individual diabetic status requires information relating to the three specific characteristics of diabetes mellitus:

- i. type - subdivided by treatment; IDDM (type 1) and NIDDM (type 2)
- ii. duration of diabetes
- iii. control of diabetes

Several studies have indicated that glycosylated haemoglobin is a reliable index of diabetic control (371,372,373). Improved control has been correlated with a reduction in glycosylated haemoglobin (374,375). Providing care is taken in the experimental model to eliminate recent glycaemic fluctuations (376), this technique gives an accurate estimate of prevailing glucose concentrations throughout the life of the red blood cell (377); in consequence, glycosylated haemoglobin is a clinically-useful indicator of long-term diabetic control.

6.2 Patients and methods

Patients were included in the study on the basis of the following criteria:

- i. a history of closed-angle glaucoma
- ii. ocular hypertension
 - a. with narrow irido-corneal angles
 - b. with wide irido-corneal angles
- iii. open-angle glaucoma
 - a. with narrow irido-corneal angles
 - b. with wide irido-corneal angles

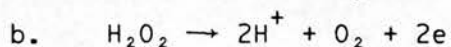
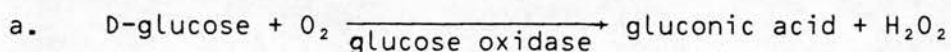
After informed consent had been obtained, diabetic status was assessed in 74 age- and sex-matched control subjects (mean age 66.2 ± 10.1 years) and 375 patients with primary glaucoma (mean age \pm SD): 123 CAG (66.5 ± 10.1 years), 90 OHNA (67.5 ± 9.2 years), 96 OHWA (64.8 ± 9.6 years), 24 OAGNA (71.5 ± 8.4 years), and 42 OAGWA (67.0 ± 8.2 years). The control group consisted of hospital staff, and patients attending the casualty department who were subsequently determined to have no detectable abnormality, or mild ocular changes within acceptable limits for an elderly population; this included early macular degenerative changes and refractive errors. Ocular examination included:

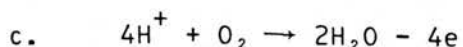
- i. Anterior segment assessment
 - a. slit-lamp examination of cornea, anterior chamber, iris integrity/dynamics, and lens
 - b. gonioscopy by Zeiss 4-mirror gonioprism to determine angle configuration and exclude angle abnormalities
 - c. intraocular pressure by Goldmann applanation tonometry
- ii. Posterior segment assessment
 - a. direct ophthalmoscopy of optic disc, cup:disc ratio, retinal vessels and macula
 - b. indirect ophthalmoscopy, after pupillary dilation, to assess posterior pole and retinal periphery
 - c. visual field examination: peripheral field by Goldmann perimetry; central field by either Goldmann perimeter or Friedmann II analyser

Subjects taking medication with known diabetogenic effects (365) were excluded from the study. Subjects with known diabetes mellitus were excluded from oral glucose tolerance test, but were included in the final results; exclusion of this group would have effected significant underestimation of the prevalence of diabetes mellitus. At the time of diabetic assessment, no subjects were influenced by extraneous factors known to bias the results of the oral glucose tolerance test.

The diagnosis of diabetes mellitus was made in accordance with National Diabetes Data Group and World Health Organisation criteria (365,366). The following protocol was observed:

- i. All tests were performed in the morning with the patients at rest, after at least three days of unrestricted diet (> 150 grams of carbohydrate per day) and physical activity.
- ii. The subject had fasted for at least ten hours, but not greater than sixteen hours.
- iii. The subject was not permitted to smoke during the test.
- iv. A fasting venous blood sample was taken, and transferred to a standard tube containing sodium fluoride.
- v. A 75-gram glucose load was administered over a 5 minute period, the glucose dose in a concentration of not greater than 25 g/dl.
- vi. The commencement of drinking the glucose solution was considered zero time, and 2.5 ml blood samples were collected at 1 hour intervals for 2 hours.
- vii. Blood samples were immediately centrifuged, and assessment of venous plasma was undertaken using a YSI Model 23AM glucose analyser (Yellow Springs Instrument Company, Ohio, USA). This technique permits direct readout of glucose concentrations, using an oxidase enzyme hydrogen peroxide sensor. The basis of this method of glucose determination is the conversion of glucose and oxygen to gluconic acid and hydrogen peroxide:





The current generated is directly proportional to the glucose level in the diluted sample (378,379). An accuracy of ± 0.05 mmol/L was obtained. Diagnostic criteria for diabetes mellitus and impaired glucose tolerance, in non-pregnant adults, are shown in table 6. If the fasting venous plasma glucose concentration was significantly elevated (> 7.8 mmol/L) on more than one occasion, OGTT was not required to establish the diagnosis of DM. The diagnosis of IGT or DM was made on the basis of 2 abnormal OGTT, or elevated fasting venous plasma glucose (> 7.8 mmol/L) on 2 occasions. Patients subsequently included within the classification of DM or IGT were assessed for long-term control of glucose metabolism by estimation of glycosylated haemoglobin. A micro-column chromatographic technique was used (Boehringer Mannheim, Lewes, England); by this method, the normal range of glycosylated haemoglobin is 5-8% (380,381). Factors known to produce falsely-elevated values of glycosylated haemoglobin include uraemia (382,383), lead poisoning (384), alcoholism (385), and high daily doses of aspirin (386); no subjects had a history of the aforementioned factors necessitating exclusion from the study.

Assessment of glycosylated haemoglobin was also performed on:

- i. the age- and sex-matched control group, to verify the normal range for this particular laboratory
- ii. a randomly-selected group of patients with CAG, OHNA, OHWA, OAGNA, and OAGWA, in whom the diabetic status was "normal" by OGTT, to determine the degree of long-term glycaemic control in these patients

The method of glycosylated haemoglobin assessment was as follows:

- i. A 5 ml sample of venous blood was taken, and transferred to a standard tube containing potassium EDTA.
- ii. Separation of labile HbA_1
In order to separate the labile fraction, the sample of venous blood was washed with glucose-free solutions. During this procedure the labile fraction dialyses out of the erythrocyte. 0.2 ml of EDTA venous blood was mixed with 2.0 ml of 0.9% sodium chloride solution; this

was allowed to stand for 2 hours with regular gentle mixing to prevent erythrocyte sedimentation. The mixture was then washed with 4 ml (EDTA-free) 0.9% sodium chloride solution, centrifuged for 3 minutes, and the supernatant removed with a suction pump; this procedure was performed 3 times.

iii. Haemolysis

0.05 ml of the washed sample of EDTA venous blood was pipetted into a test tube, to which was added 0.2 ml of the standard haemolysing reagent (0.1% detergent). This was permitted to stand for 10 minutes with occasional shaking.

iv. Measurement of HbA₁

All equipment, eluent solutions, and haemolysates to be tested were brought to a temperature of 23°C using a constant-temperature rack (HbA₁ rack no. 611948) before performing the assay. Chromatography was subsequently performed under strict temperature control ($\pm 0.25^\circ\text{C}$) in a water bath, as reproducibility depends upon maintenance of constant temperature within $\pm 1^\circ\text{C}$ (387). The collection tube was placed beneath the column, and the flow rate adjusted to 6 drops per minute. 0.05 ml of haemolysate and 0.20 ml of the phosphate buffer eluant for HbA₁ (40 mmol/l; pH 6.7) were pipetted directly onto the disc. The eluate was permitted to drain completely and discarded. A fresh collection tube was placed beneath the column and 4.00 ml of the phosphate buffer was added; the eluate was collected (over approximately 30 minutes) and mixed thoroughly. The absorbance of HbA₁ was measured within 10 minutes, according to the following conditions:

- a. assay temperature: 23°C
- b. wavelength: Hg 405 nm
- c. spectrophotometer: 415 nm
- d. cuvette: 1 cm light path
- e. measured against water

v. Measurement of "other haemoglobins"

A fresh collection tube was placed beneath the column. 4.00 ml of sodium chloride solution (400 mmol/L) was added to the column, and the eluate collected. This was diluted with 15.00 ml of redistilled water, mixed thoroughly, and the absorbance of HbA₂ measured within 5 minutes, following the conditions described above.

vi. Calculation of HbA₁ concentration

This was performed according to the following formula:

$$\% \text{HbA}_1 = \frac{A_1}{A_1 + (4.75 \times A_2)} \times 100$$

A₁ = absorbance of HbA₁-fraction

A₂ = absorbance of "other haemoglobins"

A₁ + (4.75 x A₂) = absorbance of total haemoglobin

Measurement of glycosylated haemoglobin was performed on a single-blind basis.

Table 6

DIAGNOSTIC CRITERIA FOR 75g ORAL GLUCOSE TOLERANCE TEST UNDER STANDARD CONDITIONS

	Venous plasma glucose concentration (mmol/L)	
	Fasting	2 hours after 75g oral glucose load
Normal	< 6.4	< 7.8
Impaired glucose tolerance	< 7.8	7.8-11.1
Diabetes mellitus	< 7.8	≥ 11.1

6.3 Results

The results of diabetic assessment in the primary glaucomas are described as follows:

- 6.31 The prevalence of diabetes mellitus in the primary glaucomas
- 6.32 The duration of diabetes mellitus in the primary glaucomas
- 6.33 Intraocular pressure variation in glaucoma by diabetic status
- 6.34 Glycosylated haemoglobin levels in the primary glaucomas
- 6.35 The prevalence of a familial history of diabetes mellitus in the primary glaucomas
- 6.36 Glaucoma treatment and diabetic status

6.31 The prevalence of diabetes mellitus in the primary glaucomas

The results of 75 gram OGTT in 123 patients with CAG, 186 patients with OH, 66 patients with OAG, and 74 age- and sex-matched control subjects are shown in table 7. The prevalence of diabetes mellitus in patients with CAG, OH, and OAG was compared by χ^2 test with that of the control group; Yates' correction was incorporated in all χ^2 analyses. In order to prevent inaccuracies resulting from multiple comparisons with a control, significant p values were multiplied by the number of comparisons made, thereby obtaining a corrected p value (p'). Ocular hypertension and open-angle glaucoma were then subdivided by irido-corneal angle assessment into narrow angles (NA) and wide angles (WA); the prevalence of diabetes mellitus within these sub-groups was similarly compared with the control group. Diabetes prevalence in the primary glaucomas is summarised in the following paragraphs; the actual p value plus the corrected p value (p') are quoted for significant results, to allow for multiple comparisons.

6.311 The prevalence of diabetes mellitus in patients with closed-angle glaucoma

Diabetes mellitus shows significantly-increased prevalence in patients with a history of closed-angle glaucoma compared with an age- and sex-matched control group:

- i. NIDDM: $p = 0.03$; $p' > 0.05$
- ii. IGT: $p = 0.03$; $p' > 0.05$
- iii. (NIDDM + IGT): $p = 0.003$; $p' < 0.05$

6.312 The prevalence of diabetes mellitus in patients with ocular hypertension

The prevalence of diabetes mellitus in patients with OH is not significantly different from an age- and sex-matched control group:

- i. NIDDM: $p > 0.05$
- ii. IGT: $p > 0.05$
- iii. (NIDDM + IGT): $p > 0.05$

Subdivision of ocular hypertension by angle measurement clarifies the association between OH and diabetes mellitus. The prevalence of diabetes mellitus in patients with OHNA and OHWA were separately compared with that of the age- and sex-matched control group:

a. OHNA

- i. NIDDM: $p > 0.05$
- ii. IGT: $p = 0.01$; $p' < 0.05$
- iii. (NIDDM + IGT): $p = 0.007$; $p' < 0.05$

b. OHWA

- i. NIDDM: $p > 0.05$
- ii. IGT: $p > 0.05$
- iii. (NIDDM + IGT): $p > 0.05$

The prevalence of impaired glucose tolerance and total abnormal glucose tolerance (NIDDM + IGT) is significantly increased in patients with OHNA, however no relationship is present between diabetes mellitus and OHWA.

6.313 The prevalence of diabetes mellitus in patients with open-angle glaucoma

The prevalence of diabetes mellitus in patients with open-angle glaucoma is not significantly different from the age- and sex-matched control group:

- i. NIDDM: $p > 0.05$
- ii. IGT: $p > 0.05$
- iii. (NIDDM + IGT): $p > 0.05$

No significant relationship is present between diabetes mellitus and OAGNA or OAGWA.

- a. OAGNA
 - i. NIDDM: $p > 0.05$
 - ii. IGT: $p > 0.05$
 - iii. (NIDDM + IGT): $p > 0.05$

- b. OAGWA
 - i. NIDDM: $p > 0.05$
 - ii. IGT: $p > 0.05$
 - iii. (NIDDM + IGT): $p > 0.05$

6.314 The prevalence of diabetes mellitus in the primary glaucomas by angle measurement

When the primary glaucomas are classified according to angle measurement, salient differences in the prevalence of diabetes mellitus are accentuated (table 8). The significance of comparing the prevalence of abnormal glucose tolerance (NIDDM + IGT) in CAG, narrow-angle patients (OHNA and OAGNA), and wide-angle patients (OHWA and OAGWA), with an age- and sex-matched control group are as follows: the prevalence of abnormal glucose tolerance was significantly increased in CAG ($p = 0.003$; $p' < 0.05$) and narrow-angle patients ($p = 0.003$; $p' < 0.05$), whilst the prevalence in subjects with wide-angles was not significantly different from the control group ($p > 0.05$).

6.32 The duration of diabetes mellitus in the primary glaucomas

Comparisons of mean duration of diabetes mellitus, between the control group and each of the primary glaucoma categories, were made by Student's unpaired t test. No significant differences in duration of diabetes ($p > 0.05$) were shown between any two groups.

Table 7

DIABETIC STATUS OF 123 CLOSED-ANGLE GLAUCOMA PATIENTS, 186 OCULAR HYPERTENSIVE PATIENTS, 66 OPEN-ANGLE GLAUCOMA PATIENTS, AND 74 AGE- AND SEX-MATCHED CONTROL SUBJECTS

	Normal	NIDDM	IDDM	IGT
Control subjects (n=74)	61	6 (0)	0	7
Closed-angle glaucoma patients (n=123)	75	22 (4)	1 (1)	25
Ocular hypertensives All (n=186)	131	24 (8)	0	31
OHNA (n=90)	56	13 (7)	0	21
OHWA (n=96)	75	11 (1)	0	10
Open-angle glaucoma patients All (n=66)	47	7 (2)	0	12
OAGNA (n=24)	15	3 (1)	0	6
OAGWA (n=42)	32	4 (1)	0	6

* The number in brackets equals known diabetics

Table 8

DIABETIC STATUS IN THE PRIMARY GLAUCOMAS BY ANGLE CONFIGURATION

	Normal	NIDDM	IDDM	IGT
Closed-angle glaucoma patients (n=123)	75	22	1	25
Narrow-angle subjects OHNA + OAGNA (n=114)	71	16	0	27
Wide-angle subjects OHWA + OAGWA (n=138)	107	15	0	16

6.33 Intraocular pressure variation in glaucoma by diabetic status

To determine the significance of diabetes mellitus on intraocular pressure, mean intraocular pressure in non-diabetic subjects was compared with mean intraocular pressure in diabetic subjects by Student's unpaired t test, within the control group and each of the primary glaucoma categories separately. Dunnett's multiple comparison procedure was applied to the levels of significance thus obtained, to obviate incorrect conclusions resulting from multiple comparisons with a control (388,389); the tables constructed by Dunnett for use in multiple comparisons with a control only extend to a maximum joint confidence coefficient of 99%, therefore every value of $p < 0.01$ will be included within this category. Three comparisons were performed:

- i. non-diabetic v NIDDM
- ii. non-diabetic v IGT
- iii. non-diabetic v (NIDDM + IGT)

No significant differences in intraocular pressures were shown between non-diabetic and diabetic subjects (NIDDM or IGT) in the control group, patients with ocular hypertension (OHNA and OHWA), and patients with open-angle glaucoma (OAGNA and OAGWA). However significant differences in intraocular pressures between non-diabetic and diabetic subjects were present in patients with closed-angle glaucoma; mean IOP \pm SEM is quoted in brackets.

- i. Subjects with NIDDM (19.64 ± 0.88) had significantly higher intraocular pressures than non-diabetics (17.58 ± 0.53), however this was non-significant when allowance was made for multiple comparisons: $p = 0.04$; $p' > 0.05$.
- ii. Subjects with IGT (20.24 ± 0.98) had significantly higher intraocular pressures than non-diabetics (17.58 ± 0.53): $p = 0.016$; $p' < 0.05$.
- iii. Subjects with NIDDM or IGT (19.96 ± 0.66) had significantly higher intraocular pressures than non-diabetics (17.58 ± 0.53): $p = 0.006$; $p' < 0.05$.

6.34 Glycosylated haemoglobin levels in the primary glaucomas

The results of glycosylated haemoglobin estimation in 60 patients with CAG, 78 patients with OH, 30 patients with OAG, and 31 age- and sex-matched control subjects are shown in table 9. Comparisons of glycosylated haemoglobin results were made between the control group and each of the primary glaucoma categories; significance was assessed by Student's unpaired t test, and corrected for multiple comparisons by Dunnett's procedure. Glycosylated haemoglobin levels were significantly higher than control values in CAG ($p = 0.001$; $p' < 0.01$), OH ($p < 0.001$; $p' < 0.01$), OHNA ($p < 0.001$; $p' < 0.01$), OAG ($p = 0.002$; $p' < 0.01$), and OAGNA ($p = 0.001$; $p' < 0.01$). Glycosylated haemoglobin levels were not significantly different from control values, when multiple comparisons were taken into account, in OAGWA ($p = 0.036$; $p' > 0.05$), or OHWA ($p > 0.05$).

The control group and primary glaucoma categories were then subdivided on the basis of diabetic status into diabetic and non-diabetic subjects; comparisons of glycosylated haemoglobin levels were made between the control group and each of the primary glaucoma categories for diabetic and non-diabetic subjects separately. Significantly-increased glycosylated haemoglobin levels in CAG, OH, OHNA, OAG, and OAGNA were only present in diabetic subjects; glycosylated haemoglobin levels were not significantly elevated in non-diabetic subjects.

Table 9

RESULTS OF GLYCOSYLATED HAEMOGLOBIN ESTIMATION IN 60 PATIENTS WITH
 CAG, 78 PATIENTS WITH OH, 30 PATIENTS WITH OAG, AND 31 AGE- AND
 SEX-MATCHED CONTROL SUBJECTS
 (Group mean values \pm SEM)

	Glycosylated haemoglobin (% age)
Control subjects (n=31)	5.70 \pm 0.14
Closed-angle glaucoma patients (n=60)	6.54 \pm 0.12
Ocular hypertensive patients All (n=78)	6.53 \pm 0.13
OHNA (n=43)	6.81 \pm 0.20
OHWA (n=35)	6.17 \pm 0.12
Open-angle glaucoma patients All (n=30)	6.63 \pm 0.22
OAGNA (n=13)	6.88 \pm 0.32
OAGWA (n=17)	6.44 \pm 0.30

6.35 The prevalence of a familial history of diabetes mellitus in the primary glaucomas

The prevalence of diabetes mellitus in the family history of 372 patients with primary glaucoma and 84 age- and sex-matched control subjects is shown in table 10. Comparisons were made between the prevalence of familial diabetes mellitus in the control group and each of the primary glaucoma categories; significance was assessed by χ^2 test with Yates' correction. To prevent inaccuracies from multiple comparisons with a control, significant p values were multiplied by the number of comparisons made to obtain a corrected p value (p'). There was a significantly-increased prevalence of familial diabetes mellitus in patients with OHWA (p = 0.04) compared with the age- and sex-matched control group, however this result was non-significant when multiple comparisons were taken into account (p' > 0.05). The prevalence of diabetes mellitus in the family history of patients with CAG, OHNA, OAGNA, and OAGWA was not significantly different from the control group (p > 0.05).

The prevalence of familial diabetes in the primary glaucomas and the control group was subdivided according to type of diabetes into type 1 (IDDM) and type 2 (NIDDM); results are shown in tables 11 and 12 respectively. Comparisons were made between the control group and each of the primary glaucoma categories for the prevalence of familial NIDDM and familial IDDM separately; significance was assessed by χ^2 test with Yates' correction, and adjusted for multiple comparisons as described above. This indicated that the increased prevalence of diabetes mellitus in the family history related exclusively to NIDDM; there was a significantly-increased prevalence of NIDDM in the family history of patients with CAG (p = 0.006; p' < 0.05) and OHWA (p = 0.003; p' < 0.05) compared with the control group. The prevalence of familial NIDDM in patients with OHNA, OAGNA and OAGWA was not significantly different from the control group (p > 0.05). The prevalence of familial IDDM in every category of the primary glaucomas was not significantly different from the control group (p > 0.05).

Table 10

THE PREVALENCE OF DIABETES MELLITUS IN THE FAMILY HISTORY OF 372
 PATIENTS WITH PRIMARY GLAUCOMA AND 84 AGE- AND SEX-MATCHED
 CONTROL SUBJECTS

	Patients without diabetes mellitus in the family history	Patients with diabetes mellitus in the family history
Control subjects (n=84)	75	9
Closed-angle glaucoma patients (n=122)	96	26
Ocular hypertensive patients ALL (n=185)	146	39
OHNA (n=90)	73	17
OHWA (n=95)	73	22
Open-angle glaucoma patients ALL (n=65)	55	10
OAGNA (n=24)	21	3
OAGWA (n=41)	34	7

Table 11

THE PREVALENCE OF INSULIN DEPENDENT DIABETES MELLITUS IN THE FAMILY HISTORY OF 372 PATIENTS WITH PRIMARY GLAUCOMA AND 84 AGE- AND SEX-MATCHED CONTROL SUBJECTS

	Patients without IDDM in the family history	Patients with IDDM in the family history
Control subjects (n=84)	79	5
Closed-angle glaucoma patients (n=122)	116	6
Ocular hypertensive patients ALL (n=185)	174	11
OHNA (n=90)	83	7
OHWA (n=95)	91	4
Open-angle glaucoma patients ALL (n=65)	64	1
OAGNA (n=24)	24	0
OAGWA (n=41)	40	1

Table 12

THE PREVALENCE OF NON-INSULIN DEPENDENT DIABETES MELLITUS IN THE FAMILY HISTORY OF 372 PATIENTS WITH PRIMARY GLAUCOMA AND 84 AGE- AND SEX-MATCHED CONTROL SUBJECTS

	Patients without NIDDM in the family history	Patients with NIDDM in the family history
Control subjects (n=84)	80	4
Closed-angle glaucoma patients (n=122)	99	23
Ocular hypertensive patients All (n=185)	153	32
OHNA (n=90)	78	12
OHWA (n=95)	75	20
Open-angle glaucoma patients All (n=65)	54	9
OAGNA (n=24)	21	3
OAGWA (n=41)	35	6

6.36 Glaucoma treatment and diabetic status

The only drug treatments for glaucoma taken by patients included in the study were guttae pilocarpine 2%/4% and/or guttae timolol maleate 0.25%/0.5%. The possible association between treatment and diabetic status was assessed by comparing the prevalence of diabetes mellitus (NIDDM, IGT, and NIDDM + IGT separately) in primary glaucoma patients on treatment with the prevalence in untreated primary glaucoma patients; this was performed for guttae pilocarpine 2%/4% and guttae timolol maleate 0.25%/0.5% separately, in each category of the primary glaucomas. Significance was assessed by χ^2 test with Yates' correction.

No significant differences ($p > 0.05$) in the prevalence of diabetes mellitus (NIDDM, IGT, or NIDDM + IGT) were present between primary glaucoma patients treated with guttae pilocarpine 2%/4% and/or guttae timolol maleate 0.25%/0.5% and primary glaucoma patients not on treatment.

6.4 Discussion

Deductions regarding the significance of the prevalence of diabetes mellitus in the primary glaucomas obviously depend upon comparison with a compatible control group. The prevalence of type 2 diabetes in the control group of the present study (8.1%) agrees with similar studies of diabetes prevalence in this age range (105,106,108). Thus the prevalence of diabetes mellitus in the control group provides an accurate standard for subsequent comparisons. Impaired glucose tolerance has been defined relatively recently (365,366); the prevalence of impaired glucose tolerance in the "normal" population is not established, and therefore the prevalence of impaired glucose tolerance in the present control group is impossible to verify with comparable studies.

Single-blind prospective studies to determine the prevalence of abnormal glucose tolerance (ie DM or IGT) have revealed definite associations between type 2 diabetes mellitus and primary glaucoma; no association was shown with type 1 diabetes mellitus. The prevalence of type 1 diabetes mellitus was 0.8% in patients with CAG, which parallels prevalence levels in the general population (104,107). In contrast, the prevalence of type 2 diabetes mellitus and impaired glucose tolerance was 17.2% and 20.5% respectively - significantly higher than in the control group. One may reasonably conclude that abnormal glucose tolerance is significantly associated with CAG. This conclusion is contrary to suggestions by previous authors (119,134), however - to my knowledge - detailed prospective studies have not been previously published establishing the prevalence of diabetes mellitus/impaired glucose tolerance in patients with CAG.

The prevalence of abnormal glucose tolerance is also significantly increased in patients with ocular hypertension (29.8%). Subdivision of this category on the basis of irido-corneal angle measurement localises the association exclusively to patients with narrow angles; the prevalence of abnormal glucose tolerance in patients with OHNA (37.8%) is significantly higher than in the control group ($p < 0.01$), whereas no significant differences are present between the prevalence of abnormal glucose tolerance in patients

with OHWA (21.9%). The prevalence of abnormal glucose tolerance is not significantly different from the control group in patients with OAG (28.2%).

When one considers the OH and OAG groups as a single entity, and classify on the basis of angle measurement into narrow-angle and wide-angle categories, differences in diabetic prevalence are accentuated. Significant differences in the prevalence of abnormal glucose tolerance are present between the control group and patients with narrow-angles ($p = 0.003$; $p' < 0.05$), between patients with narrow-angles and wide-angles ($p = 0.01$; $p' < 0.05$), and between patients with CAG and wide-angles ($p = 0.007$; $p' < 0.05$). No significant differences in the prevalence of abnormal glucose tolerance are present between the control group and patients with wide-angles, and between patients with CAG and narrow-angles.

Discrepancies between the prevalence of diabetes mellitus in glaucoma patients obtained in this study, and those quoted in the introductory text, are easily explained. One cannot equate the results of studies performed under different experimental criteria; studies of diabetic prevalence such as those of Armstrong (112) and Becker (132) utilised a 100 gram glucose loading dose, substantially larger than the present standard of 75 grams. Allowing for the problem of equivalent comparison, previously-quoted prevalences of diabetes mellitus (18.3% and 20% respectively) are similar to the prevalence of abnormal glucose tolerance (23.8%) in patients with OAGWA (the commonest form of OAG) in the present study. Unfortunately, previous studies omitted to concurrently examine a suitable control group for comparison. Diabetic prevalence levels of 18.3% and 20% seem conclusive until one determines the prevalence of abnormal glucose tolerance, using a lesser glucose loading dose, in an age- and sex-matched control group to be 17.5%. It must be stressed that abnormal glucose tolerance is not equivalent to diabetes mellitus - the prevalence of overt diabetes mellitus in the control group was 8.1% - however assessment with an increased glucose load, as in the aforementioned studies, is recognised to significantly increase subsequent plasma glucose levels. The problem of comparison between different diagnostic criteria

is thus insurmountable. To verify the true relationship between diabetes mellitus and glaucoma, if any, one must concurrently assess patients with primary glaucoma and suitable control subjects by a standard technique - as in the present investigation.

The family history of glaucoma patients provides further evidence of the association with type 2 diabetes mellitus; a significantly-increased prevalence of type 2 diabetes mellitus is present in the familial history of patients with CAG and OHWA.

Glycosylated haemoglobin provides a long-term measure of glycaemic control. Results of glycosylated haemoglobin assessment support the association between glaucoma and diabetes mellitus established by oral glucose tolerance testing. HbA_{1c} was significantly higher than control values in CAG ($p' < 0.01$), OH ($p' < 0.01$), and OAG ($p' < 0.01$). Subclassification on the basis of angle assessment restricted this association to narrow-angle categories, after correction for multiple comparisons with a control; OHNA ($p' < 0.01$) and OAGNA ($p' < 0.01$). These differences were related specifically to diabetic status; no significant differences in glycosylated haemoglobin levels were shown between non-diabetic subjects with primary glaucoma and the control group.

Several conclusions inevitably follow from the results:

- i. There is a significantly-increased prevalence of type 2 diabetes mellitus and impaired glucose tolerance in patients with CAG, and therefore an association between diabetes mellitus and CAG is probable. This association is supported by the relationship between IOP and abnormal glucose tolerance in patients with CAG; patients with abnormal glucose tolerance have significantly-higher IOP than non-diabetic patients.
- ii. The significantly-increased prevalence of abnormal glucose tolerance in patients with OH is restricted to narrow-angle subjects.
- iii. The prevalence of abnormal glucose tolerance is not significantly different from "normal" in patients with OAG.
- iv. The prevalence of abnormal glucose tolerance was similar

in patients with CAG (38.5%), OHNA (37.8%), and OAGNA (37.5%); prevalence in CAG and OHNA was significantly higher than normal, and in OAGNA just failed to reach significant levels after correction, however glycosylated haemoglobin levels were significantly higher ($p' < 0.01$) in all three groups than control values. The prevalence of abnormal glucose tolerance was not significantly different in OHWA (21.9%) and OAGWA (23.8%) from the control group (17.6%); glycosylated haemoglobin levels in OHWA and OAGWA were also not significantly different from control values. Results are therefore polarised, with abnormal results present only in narrow-angle forms of primary glaucoma.

- v. An association between the wide-angle form of OH and diabetes mellitus does exist, exemplified by the significantly-increased familial history of type 2 diabetes mellitus in patients with OHWA.

One may reasonably conclude that type 2 diabetes mellitus is associated with glaucoma, an association predominantly with narrow-angle primary glaucomas; no association was established between diabetes mellitus and OAGWA. The significance of the association between diabetes mellitus and primary glaucoma is investigated in subsequent chapters.

SECTION III

SYSTEMIC AUTONOMIC NERVE FUNCTION IN THE PRIMARY GLAUCOMAS

SYSTEMIC AUTONOMIC FUNCTION IN THE PRIMARY GLAUCOMAS

7.1 Introduction

The rationale for investigating systemic autonomic nerve function in the primary glaucomas has been explained in the introductory text. The development of autonomic function tests during the past twelve years has permitted accurate quantification of parasympathetic and sympathetic cardiovascular reflexes. These tests are objective, reproducible, non-invasive, and designed specifically for clinical application. Previously-quoted "normal" values are shown in table 4. As the "normal" values were determined in a population with a mean age significantly less than the present study, autonomic function assessments were performed on an age- and sex-matched control group to determine age-dependence by regression analysis. The data were fitted to mathematical models using the computer programme SPSSx on an IBM 4341 computer, to obtain "log normal" distributions from which tolerance intervals were derived for each of the autonomic function tests (390).

The close correlation between generalised autonomic function and assessment of cardiovascular reflexes permits reasonable inferences to be drawn regarding systemic autonomic integrity.

7.2 Patients and methods

Patients were included in the study on the basis of the following criteria:

- i. a history of closed-angle glaucoma
- ii. ocular hypertension
 - a. with narrow irido-corneal angles
 - b. with wide irido-corneal angles
- iii. open-angle glaucoma
 - a. with narrow irido-corneal angles
 - b. with wide irido-corneal angles

After informed consent had been obtained, cardiovascular autonomic function was assessed in 85 age- and sex-matched control subjects (mean age 66.1 ± 10.2 years) and 380 patients with primary glaucoma (mean age \pm SD): 124 CAG (66.5 ± 10.1 years), 90 OHNA (67.5 ± 9.2 years), 99 OHWA (64.8 ± 9.6 years), 24 OAGNA (71.5 ± 8.4 years), and 43 OAGWA (67.0 ± 8.2 years). The control group consisted of hospital staff, and patients attending the casualty department who were subsequently determined to have no detectable abnormality, or mild ocular changes within acceptable limits for an elderly population; this included early macular degenerative changes and refractive errors.

A comprehensive past, present and family history was obtained from each subject. The present history included details regarding presentation, current treatment, visual acuity, and visual symptoms. A detailed general systemic enquiry and drug history was also documented. If the patient was diabetic, the type, duration, and treatment were noted. Past medical history specifically recorded ophthalmic disease, cardiovascular or neurological disorders, diabetes mellitus and other predisposing factors to autonomic dysfunction. Family history included a general category, followed by specific details regarding ophthalmic disease (particularly glaucoma), neurological or cardiovascular disorders, and diabetes mellitus; if the latter was present, the type of diabetes and number in each category were noted.

Subjects known to have medical disorders predisposing to autonomic nerve dysfunction or taking medication with effects on the autonomic nervous system (table 1) were excluded from the control group. Diabetics attending casualty with no other abnormality were not excluded from the control group; exclusion of the "normal" acceptable proportion of subjects with diabetes mellitus in this age range would have markedly biased control values. Patients with CAG, OHNA, OHWA, OAGNA, and OAGWA on medication with autonomic effects, or with medical disorders predisposing to autonomic nerve dysfunction (apart from diabetes mellitus), were similarly excluded.

The history excluding the ophthalmic section was obtained on arrival. This expedient served two purposes: firstly, subjects were assessed

assessments were performed on a single-blind basis as the examiner was not aware of the subject's diagnosis during the test.

Systematic general medical examination was then performed, with emphasis on:

i. Cardiovascular system: to exclude subjects with cardiovascular pathology; valvular disorders, such as mitral stenosis, and congestive cardiac failure are particularly relevant, as these may adversely affect cardiovascular autonomic reflexes.

ii. Central and peripheral nervous system: with assessment of sensory and motor somatic nerves, and cerebellar function.

Subjects with significant cardiovascular or neurological abnormalities were excluded from autonomic function assessment; this included any form of "stroke" or cardiovascular accident, which may effectively negate cardiovascular reflexes.

Ocular examination was performed on all subjects, as follows:

i. Anterior segment assessment

a. slit-lamp examination of cornea, anterior chamber, iris integrity/dynamics, and lens

b. gonioscopy by Zeiss 4-mirror gonioscope to determine angle configuration and exclude angle abnormalities

c. intraocular pressure by Goldmann applanation tonometry

ii. Posterior segment assessment

a. direct ophthalmoscopy of optic disc, cup:disc ratio, retinal vessels and macula

b. indirect ophthalmoscopy, after pupillary dilation, to assess posterior pole and retinal periphery

c. visual field examination: peripheral field by Goldmann perimetry; central field by either Goldmann perimeter or Friedmann II analyser.

Six tests of autonomic integrity were employed (table 3). Determination of normal sinus rhythm is an essential prerequisite to cardiovascular autonomic nerve function assessment.

7.21 Parasympathetic nerve function

7.211 Valsalva manoeuvre

A standardised Valsalva manoeuvre was performed by the subject blowing into a mouthpiece connected to a modified sphygmomanometer and maintaining a pressure of 40 mmHg (5.3 kPa) for 15 seconds. A continuous electrocardiogram (Cambridge VS500B electrocardiograph, Picker International, England) was recorded, commencing 20 seconds prior to the manoeuvre and terminating 25 seconds after the manoeuvre. The patients were seated during the test, and rested for at least 2 minutes between consecutive assessments. The Valsalva ratio is the ratio of the longest R-R interval after the manoeuvre to the shortest R-R interval during the test. These were measured directly from the electrocardiogram, and the final ratio was the mean of 3 consecutive assessments.

The physiological principles underlying this reflex have been detailed in the introductory text. Normal heart-rate responses recorded by electrocardiography are shown in plates 2-5:

Phase 1: initial relative bradycardia for 2-3 seconds

Phase 2: gradually-increasing tachycardia

Phase 3: increased tachycardia for 2-3 seconds on release of intrathoracic pressure

Phase 4: bradycardia

The Valsalva ratio represents the ratio of the longest R-R interval in phase 4 divided by the shortest R-R interval in phase 2.

7.212 Heart-rate variation during deep breathing

The technique of Wheeler and Watkins (240), modified by Hilsted and Jensen (249), was used to evaluate parasympathetic nerve function by heart-rate variation during deep breathing. The subject was seated quietly at rest for 3 minutes prior to the test, then was requested to breathe deeply at a rate of approximately 6 breaths per minute (5 seconds inspiration, 5 seconds expiration) whilst

a continuous electrocardiogram was recorded. In normal subjects, a relative tachycardia occurs during inspiration, with a relative bradycardia in expiration. (Plate 6) Maximum and minimum R-R intervals were measured directly from the electrocardiogram using an ECG ruler, and expressed as beats per minute. Six consecutive measured cycles were calculated, and the final result was the mean of the differences between maximum and minimum heart-rates.

7.213 Immediate heart-rate response to standing

During alteration in posture from lying to standing a characteristic cardiovascular reflex response occurs under vagal control. This consists of an initial increase in heart-rate, maximal at about the 15th beat after standing (plate 7), followed by a relative bradycardia around the 30th beat (plate 8). The patient rested supine for approximately 3 minutes prior to the test, then was requested to stand unaided. An electrocardiogram was recorded from 20 beats prior to the manoeuvre until 40 beats after standing. The R-R intervals were measured to determine the shortest around the 15th beat and longest at about beat 30, and the result expressed as the 30:15 ratio.

7.214 Immediate heart-rate response to lying

Shortening of the R-R interval occurs during the 3rd-4th beat after commencing the manoeuvre, followed by a gradual increase in R-R interval. (Plate 9) Subjects were requested to stand for 2 minutes, then lie down unaided. Heart-rate was recorded by continuous electrocardiography from 20 beats prior to the manoeuvre until 40 beats after lying down. The results were calculated as the ratio of the longest R-R interval during the 5 beats before lying to the shortest R-R interval during the 10 beats after lying down, and the result expressed as the standing:lying ratio (S:L ratio).

7.22 Sympathetic nerve function

7.221 Systolic blood pressure response to standing

After 3 minutes lying supine on an examination couch, the patient's blood pressure was measured using a cuff sphygmomanometer, then again immediately after standing upright. The difference between the systolic pressure lying and standing is the postural change in blood pressure.

7.222 Diastolic blood pressure response to sustained handgrip

The physiological mechanisms involved in this cardiovascular reflex are detailed in the introduction. Equipment necessary to conduct the test includes a handgrip dynamometer (plate 10) and a cuff sphygmomanometer. The subject remained seated throughout the test; blood pressure was taken 3 times, and average resting systolic and diastolic pressures were recorded. The subject was then requested to grip the dynamometer maximally on 3 separate occasions; a linear displacement transducer converted mechanical to electrical energy, and a meter recorded maximal voluntary contraction (MVC) using this principle. (Plate 11) The handgrip dynamometer was set to a value of 33% MVC and the subject requested to grip continuously at this level for as long as possible, up to a maximum of 5 minutes; blood pressure was recorded from the non-exercising arm at 1-minute intervals. The test was terminated by either:

- i. the patient being unable to maintain sustained handgrip
- ii. a diastolic pressure increase of ≥ 16 mmHg
- iii. 5 minutes of continuous handgrip at 33% MVC

Following release of handgrip, blood pressure was recorded twice, and average systolic and diastolic pressures calculated.

Sustained handgrip was not found to be a suitable test of sympathetic nerve function in patients aged > 60 years; this age category is comprised of an ever-increasing female preponderance, who were shown to be unable, or unwilling, to maintain sustained isometric contraction. Consequently, the test was only performed on patients

less than 55 years of age, and the normal values established by Ewing et al (295), for a control group aged 25-54 years, were used as the diagnostic criteria. Essentially, a diastolic blood pressure increase of ≥ 16 mmHg is normal, ≤ 10 mmHg is abnormal, and the intermediate range designated "borderline".

7.3 Results

The results of cardiovascular autonomic function assessment in 380 patients with primary glaucoma and 85 age- and sex-matched control subjects are described as follows:

- 7.31 Age-adjusted normal tolerance intervals for cardiovascular autonomic function tests
- 7.32 Parasympathetic nerve function
- 7.33 Sympathetic nerve function
- 7.34 Significance of sex on systemic autonomic nerve function assessment
- 7.35 Diabetes mellitus and systemic autonomic nerve function
- 7.36 Cardiovascular autonomic nerve function in the primary glaucomas; comparisons with the age-adjusted normal tolerance limits
- 7.37 The prevalence of autonomic nerve dysfunction in the primary glaucomas
- 7.38 Glaucoma treatment and cardiovascular autonomic nerve function

7.31 Age-adjusted normal tolerance intervals for cardiovascular autonomic function tests

7.311 Log normal distribution and linear regression on age

The results of each test of cardiovascular autonomic nerve function in the age- and sex-matched control group were assessed for dependence on age by 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 after adjustment for age, thus permitting determination of the "normal" range. Logarithms of the data were used to derive a tolerance interval for the following reasons:

- i. Logarithms (after adjusting for age, where appropriate) are more nearly normally distributed than original readings, thus reducing skewness. Although this has only limited importance in statistical inferences about means (eg t-test), it is obviously of marked importance in the calculation of percentiles of the distribution - particularly at "extreme" values, the "tails" of the distribution.
- ii. Tolerance limits on $y = \log(x)$, after age-adjustment where appropriate, must always give positive values of x after transforming back to the original units, as e^y or 10^y are always positive.

Plots of the logarithmic values on age were linear, allowing the 2.5th, 5th, 95th, and 97.5th percentiles to be calculated by linear regression analysis:

$$E_{(y)} = a + bx$$

$$y = a + bx + \text{error}$$

$$\begin{aligned} \text{Residual (r)} &= y - \text{predicted value} \\ &= y - (a + bx) \end{aligned}$$

$$r_s = \frac{y - (a + bx)}{s_{y|x}}$$

r_s = standardised residual

$s_{y|x}$ = residual standard deviation

A "log normal" distribution was obtained for the results of each of the cardiovascular autonomic function tests, except systolic blood pressure response to standing; as postural changes in blood pressure may include negative values, a logarithmic distribution could not be described and thus a distribution based upon natural values was determined.

7.312 Determination of tolerance limits in cardiovascular autonomic function tests

Tolerance limits were calculated from the log normal distribution (after adjusting for age, if necessary) for all variables except systolic blood pressure response to standing; the latter was

calculated from natural values, as explained above.

i. In variables which did not depend significantly on age, approximate tolerance limits were obtained by:

mean \pm 1.67 SD (for 90% limits)

mean \pm 2.00 SD (for 95% limits)

The values 1.67 and 2.00 were obtained from percentiles of t at 60 degrees of freedom.

ii. In variables which depended significantly on age, approximate tolerance limits were obtained by:

a. Linear regression of $\log_{10}(\text{variable})$, or of systolic blood pressure response to standing untransformed, was performed on age to give:

$$\hat{y} = a + bx$$

$$\text{Residual} = y - \hat{y}$$

b. Tolerance limits from:

$$\hat{y} \pm 1.67 \times s_{y|x} \quad (\text{for 90\% limits})$$

$$\hat{y} \pm 2.00 \times s_{y|x} \quad (\text{for 95\% limits})$$

where $s_{y|x}$ is the residual standard deviation after regression.

Translating back from the transformation yields the "normal range" for each of the cardiovascular autonomic function tests.

7.313 Parasympathetic nerve function

i. Valsalva ratio

$\log_{10}(\text{Valsalva ratio})$ was shown to depend significantly on age. Linear regression analysis, permitting determination of 2.5th, 5th, 95th, and 97.5th percentiles, was calculated from:

$$\text{RLVR} = (\log_{10}\text{VR} - (0.43359 - 0.00409 \times \text{age}))/0.06960$$

assumed to have the standard Normal distribution.

$$\text{RLVR} = \text{standardised residual } \log_{10}(\text{VR})$$

Tolerance limits at age 65 years are shown in table 13, and the logarithms of the percentiles (allowing for regression

on age), translated back to natural values, with 2.5th, 5th, 95th and 97.5th percentiles in figure 4.

ii. Heart-rate variation during deep breathing

\log_{10} (heart-rate variation during deep breathing) was shown to depend significantly on age. Linear regression analysis, permitting determination of 2.5th, 5th, 95th, and 97.5th percentiles, was calculated from:

$$RLHRV = (\log_{10}HRV - (1.49598 - 0.00397 \times \text{age}))/0.16415$$

assumed to have the standard Normal distribution.

$$RLHRV = \text{standardised residual } \log_{10}(\text{HRV})$$

Tolerance limits at age 65 years are shown in table 13, and the logarithms of the percentiles (allowing for regression on age), translated back to natural values, with 2.5th, 5th, 95th, and 97.5th percentiles in figure 5.

iii. 30:15 ratio

\log_{10} (30:15 ratio) did not depend significantly on age. Linear regression analysis, permitting determination of 2.5th, 5th, 95th, and 97.5th percentiles, was calculated from:

$$RL30:15 \text{ ratio} = (\log_{10}30:15 \text{ ratio} - (0.08869 - 0.00031 \times \text{age}))/0.03751,$$

$$RL30:15 \text{ ratio} = \text{standardised residual } \log_{10}(30:15 \text{ ratio})$$

Tolerance limits at age 65 years are shown in table 13, and the logarithms of the percentiles (translated back to natural values) with 2.5th, 5th, 95th, and 97.5th percentiles in figure 6.

iv. S:L ratio

\log_{10} (S:L ratio) did not depend significantly on age. Linear regression analysis, permitting determination of 2.5th, 5th, 95th, and 97.5th percentiles, was calculated from:

$$RLS:L \text{ ratio} = (\log_{10}S:L \text{ ratio} - (0.08535 - 0.00008 \times \text{age}))/0.03191,$$

$$RLS:L \text{ ratio} = \text{standardised residual } \log_{10}(S:L \text{ ratio})$$

Tolerance limits at age 65 years are shown in table 13, and the logarithms of the percentiles (translated back to

natural values) with 2.5th, 5th, 95th, and 97.5th percentiles in figure 7.

7.314 Sympathetic nerve function

Systolic blood pressure response to standing

Systolic blood pressure response to standing was shown to depend significantly on age. Linear regression analysis, permitting determination of 2.5th, 5th, 95th, and 97.5th percentiles, was calculated from:

$$RPH = (PH - (-6.12798 + 0.21258 \times \text{age})) / 12.56912$$

assumed to have the standard Normal distribution.

PH = postural hypotension (or systolic blood pressure response to standing)

RPH = standardised residual (postural hypotension)

Tolerance limits at age 65 years are shown in table 13, and the percentiles (allowing for regression on age) with 2.5th, 5th, 95th, and 97.5th percentiles in figure 8.

Table 13

AGE-ADJUSTED NORMAL TOLERANCE LIMITS (AT AGE 65 YEARS), DEFINED BY THE LOWER 5th PERCENTILE, FOR CARDIOVASCULAR AUTONOMIC FUNCTION TESTS

	Abnormal
<u>Parasympathetic function</u>	
i. Valsalva ratio	< 1.13
ii. Heart-rate variation during deep breathing (beats/minute)	< 9.20
iii. 30:15 ratio	< 1.01
iv. S:L ratio	< 1.06
<u>Sympathetic function</u>	
Decrease in systolic blood pressure on standing (mmHg)	> 29.38

Figure 4
AGE-ADJUSTED NORMAL TOLERANCE INTERVALS FOR VALSALVA RATIO

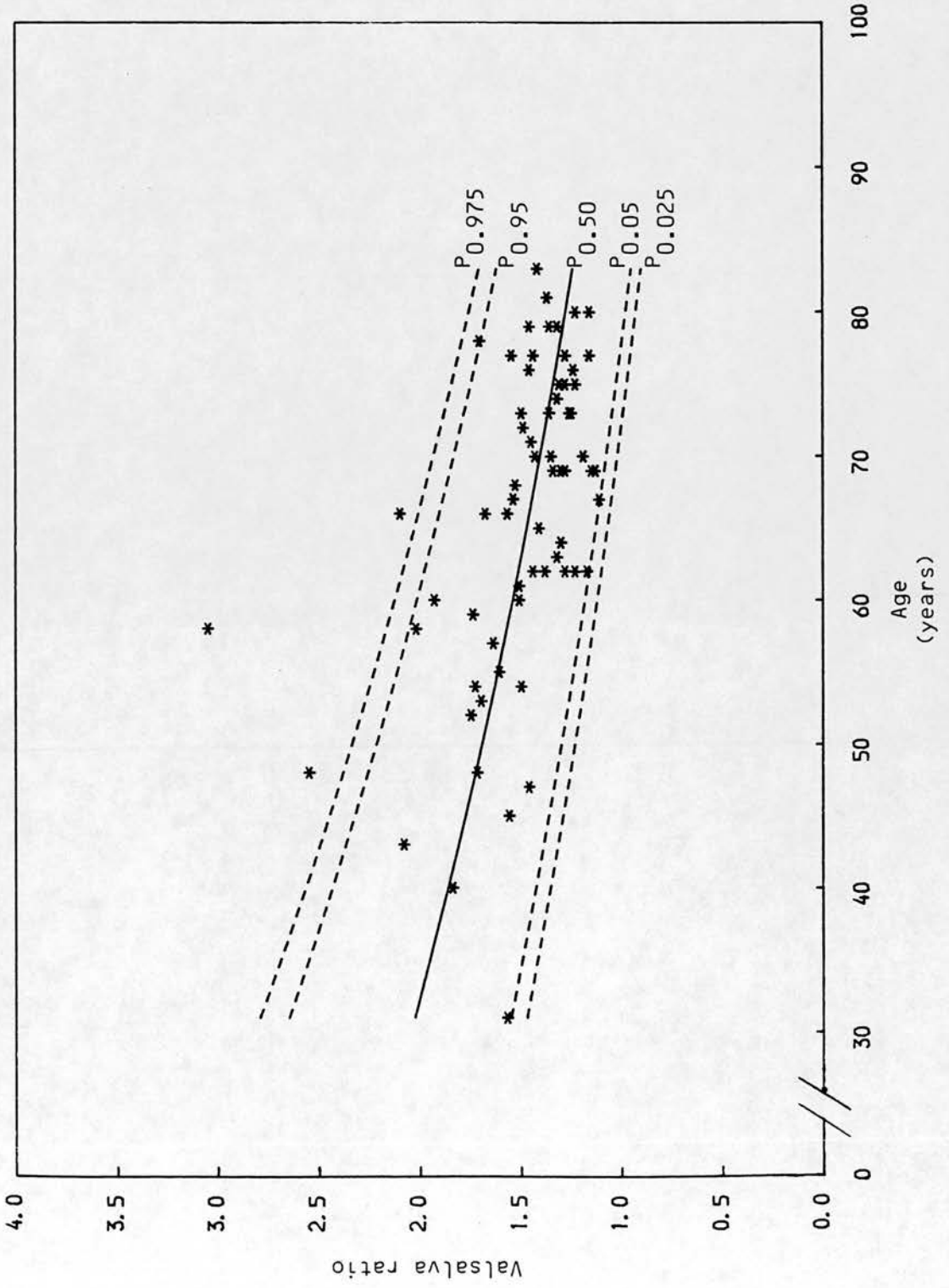


Figure 5

AGE-ADJUSTED NORMAL TOLERANCE INTERVALS FOR HEART-RATE VARIATION DURING DEEP BREATHING

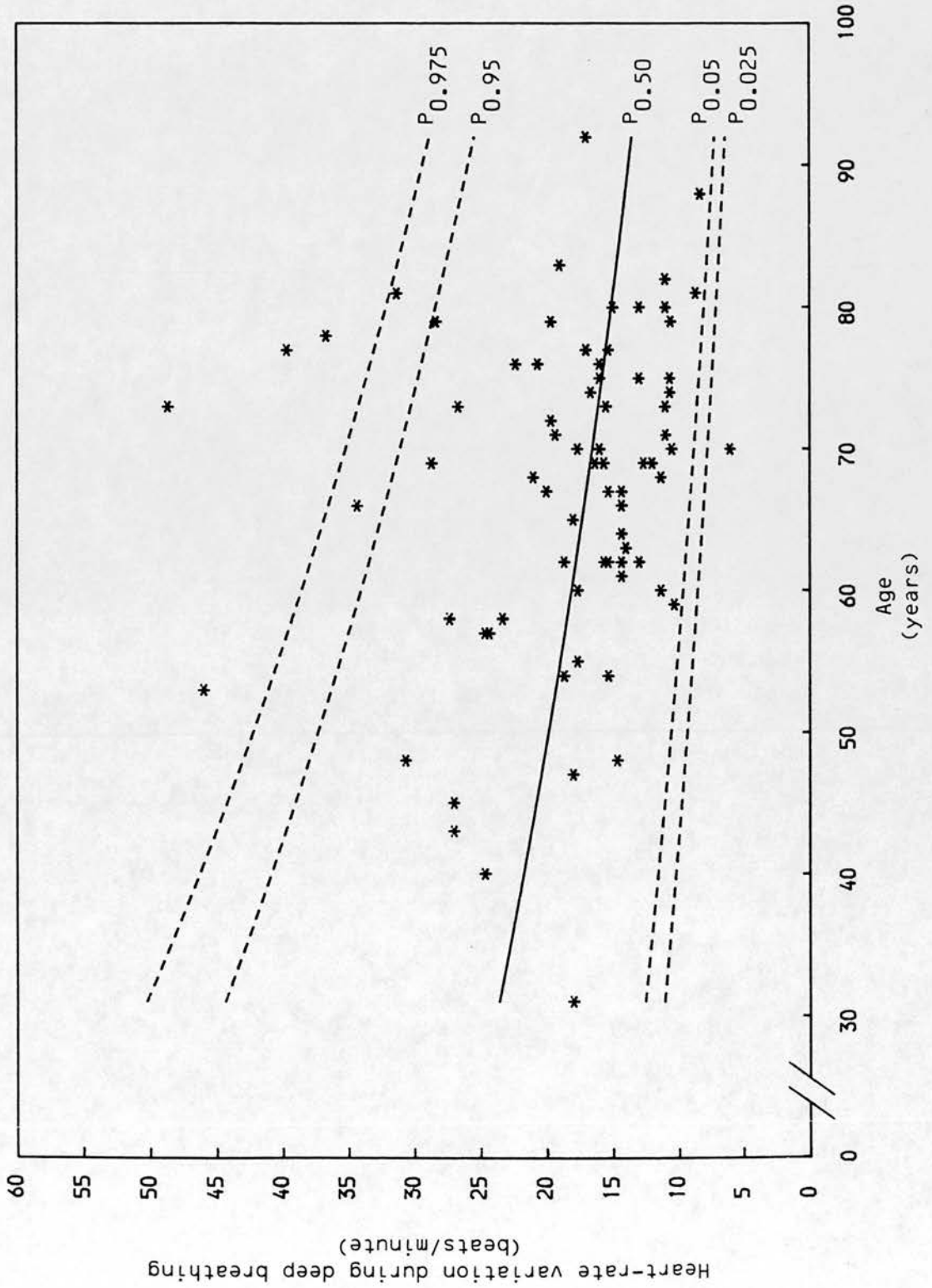


Figure 6
 AGE-ADJUSTED NORMAL TOLERANCE INTERVALS FOR 30:15 RATIO

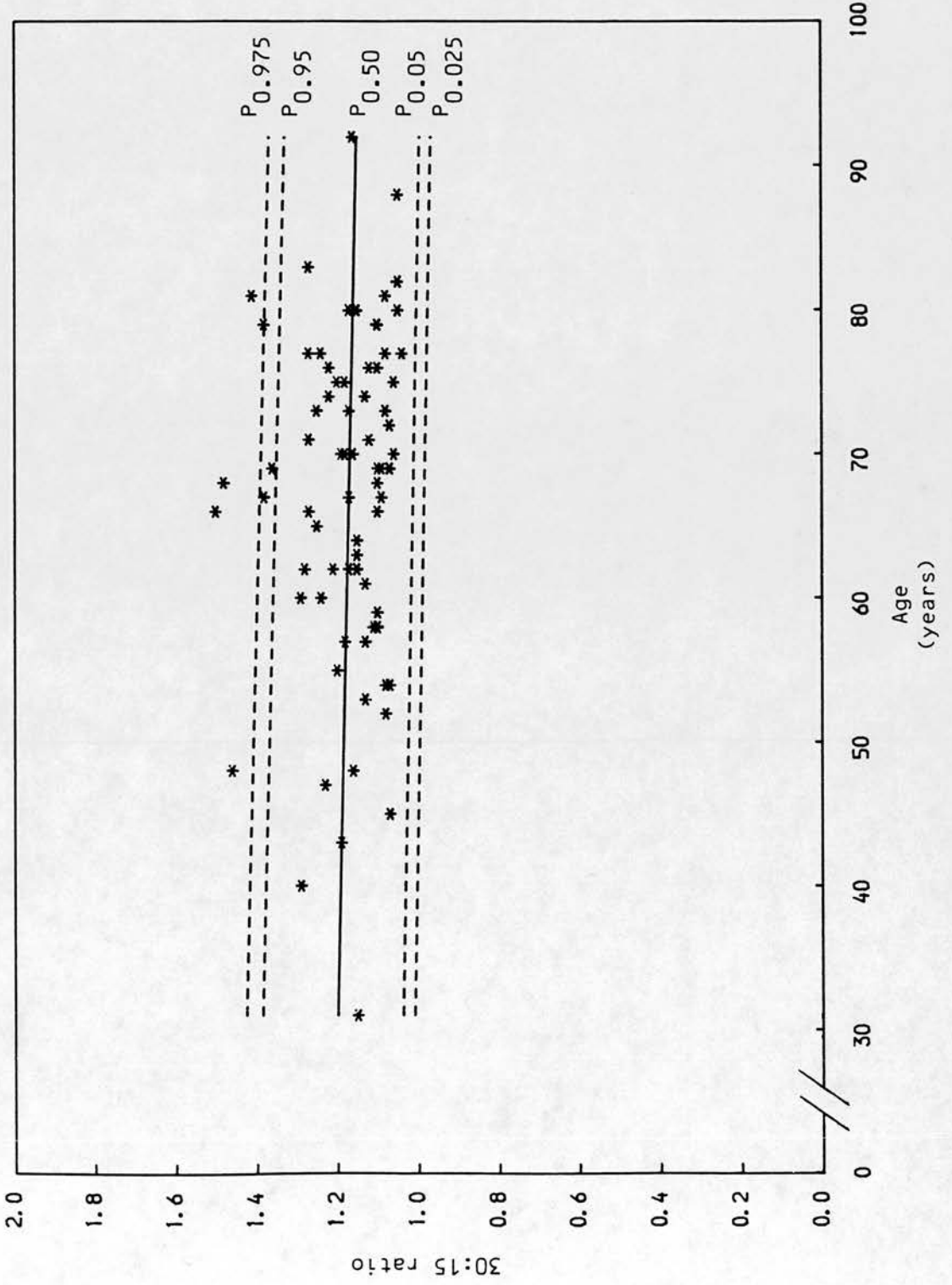


Figure 7

AGE-ADJUSTED NORMAL TOLERANCE INTERVALS FOR S:L RATIO

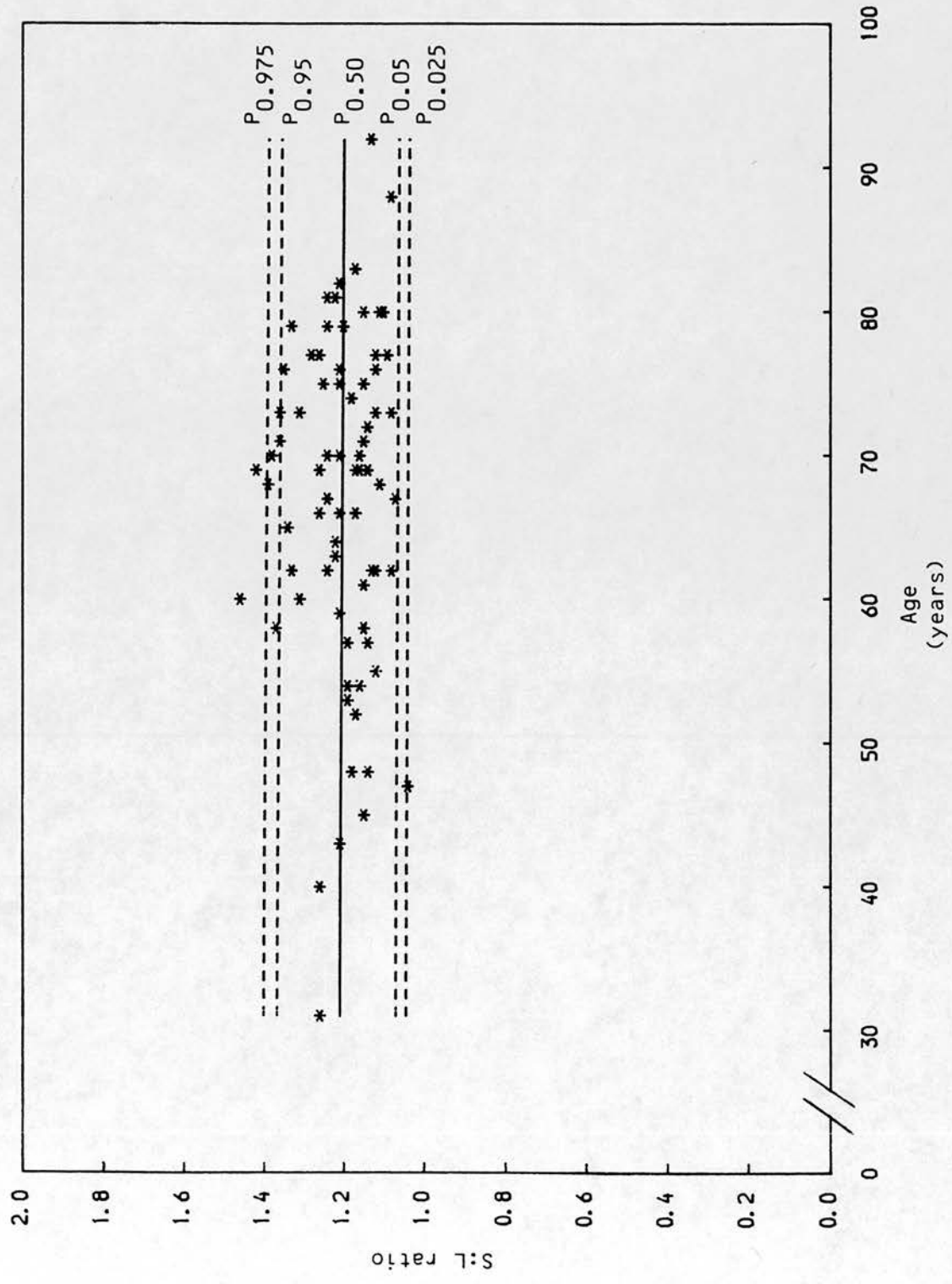
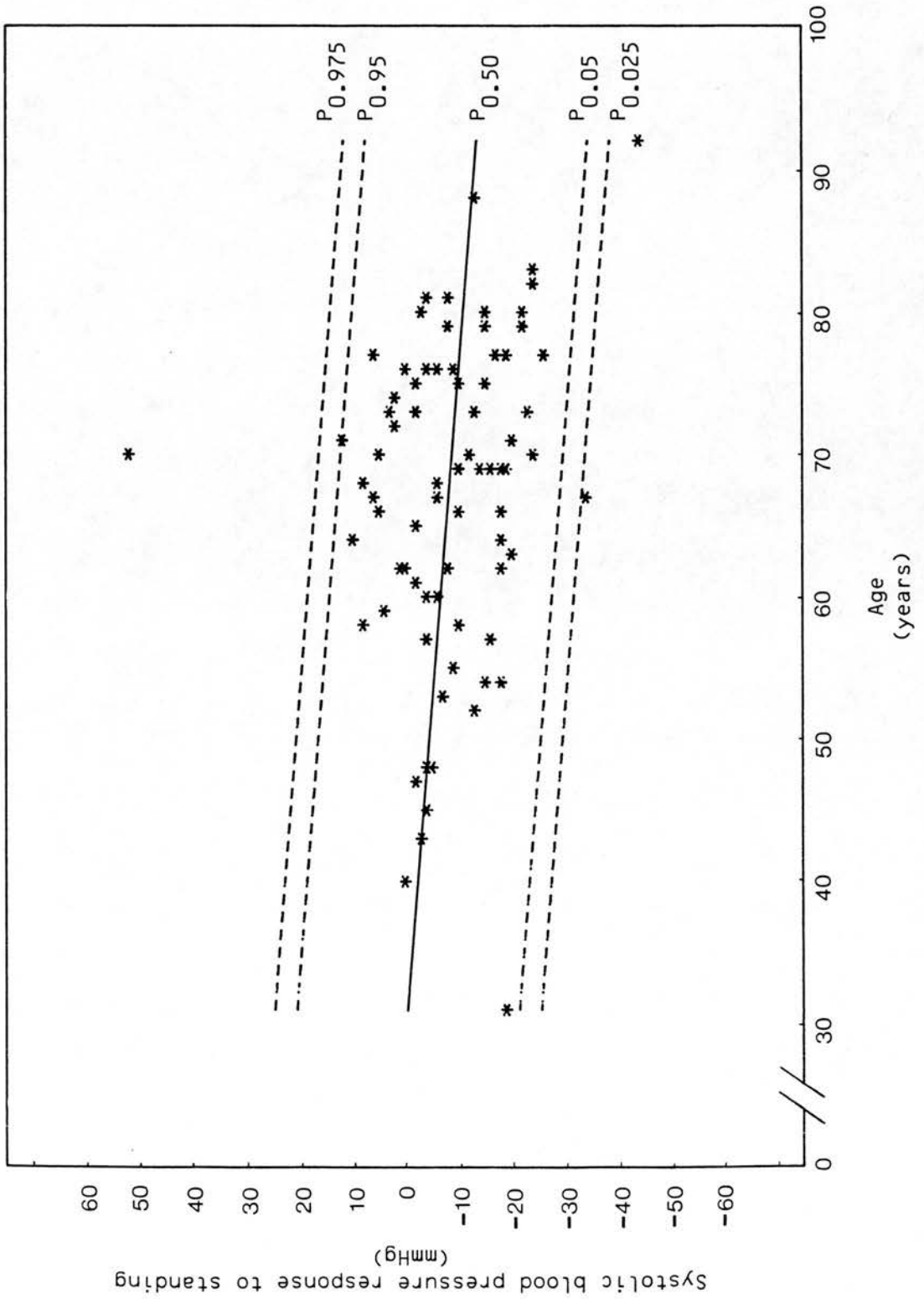


Figure 8
 AGE-ADJUSTED NORMAL TOLERANCE INTERVALS FOR SYSTOLIC BLOOD PRESSURE
 RESPONSE TO STANDING



7.32 Parasympathetic nerve function

The results of cardiovascular parasympathetic nerve function assessment in 380 patients with primary glaucoma and 85 age- and sex-matched control subjects is shown in table 14. In each test of autonomic function, comparisons were made between the results of the control group and each category of the primary glaucomas separately; significance was assessed by Student's unpaired t test. Dunnett's multiple comparison procedure was used to obtain a corrected p value (p'), thereby obviating inaccuracy from multiple comparisons with a control. The significance of the results of parasympathetic nerve function tests in 380 patients with primary glaucoma compared with results in 85 age- and sex-matched control subjects is summarised in the following paragraphs; the actual p value plus the corrected p value (p') are quoted for significant results.

7.321 Valsalva ratio

There were no significant differences ($p > 0.05$) between the control group and patients with CAG, OHWA, and OAGWA. Results of Valsalva ratio in the control group were significantly higher than in patients with OHNA ($p = 0.01$; $p' < 0.05$), and OAGNA ($p = 0.01$; $p' < 0.05$).

7.322 Heart-rate variation during deep breathing

Heart-rate variation during deep breathing was significantly higher in the control group than in patients with CAG ($p < 0.001$; $p' < 0.01$), OHNA ($p < 0.001$; $p' < 0.01$), OHWA ($p < 0.001$; $p' < 0.01$), OAGNA ($p = 0.002$; $p' < 0.05$), and OAGWA ($p < 0.001$; $p' < 0.01$).

7.323 30:15 ratio

30:15 ratio in the control group was significantly higher than in patients with CAG ($p < 0.001$; $p' < 0.01$), OHNA ($p < 0.001$; $p' < 0.01$), and OHWA ($p = 0.01$; $p' < 0.05$). No significant differences were noted between the control group and patients with OAGNA ($p = 0.016$; $p' > 0.05$) and OAGWA ($p > 0.05$), after allowing for multiple comparisons.

7.324 S:L ratio

S:L ratio in the control group was significantly higher than in patients with CAG ($p < 0.001$; $p' < 0.01$) and OHNA ($p = 0.02$; $p' > 0.05$), although only the former remained significant after allowing for multiple comparisons. No significant differences were noted between the control group and patients with OHWA ($p > 0.05$), OAGNA ($p > 0.05$), and OAGWA ($p > 0.05$).

Table 14

RESULTS OF CARDIOVASCULAR PARASYMPATHETIC FUNCTION ASSESSMENT IN 380 PATIENTS WITH PRIMARY GLAUCOMA
AND 85 AGE- AND SEX-MATCHED CONTROL SUBJECTS

(Group mean values \pm SEM)

	Valsalva ratio	Heart-rate variation during deep breathing	30:15 ratio	S:L ratio
Control group (n=85)	1.49 \pm 0.04	18.24 \pm 0.93	1.17 \pm 0.01	1.21 \pm 0.01
Closed-angle glaucoma patients (n=124)	1.42 \pm 0.03	12.05 \pm 0.67	1.11 \pm 0.01	1.15 \pm 0.01
Ocular hypertensive patients ALL (n=189)	1.41 \pm 0.03	13.13 \pm 0.55	1.13 \pm 0.01	1.19 \pm 0.01
OHNA (n=90)	1.36 \pm 0.03	11.57 \pm 0.74	1.12 \pm 0.01	1.17 \pm 0.01
OHWA (n=99)	1.45 \pm 0.03	14.54 \pm 0.77	1.13 \pm 0.01	1.20 \pm 0.01
Open-angle glaucoma patients ALL (n=67)	1.38 \pm 0.04	13.32 \pm 0.83	1.13 \pm 0.01	1.18 \pm 0.01
OAGNA (n=24)	1.31 \pm 0.05	13.03 \pm 1.49	1.12 \pm 0.02	1.19 \pm 0.03
OAGWA (n=43)	1.43 \pm 0.05	13.48 \pm 1.00	1.14 \pm 0.02	1.17 \pm 0.02

7.33 Sympathetic nerve function

The results of cardiovascular sympathetic nerve function assessment in 380 patients with primary glaucoma and 85 age- and sex-matched control subjects is shown in table 15. In each test of autonomic function, comparisons were made between the results of the control group and each category of the primary glaucomas separately; significance was assessed by Student's unpaired t test, corrected for multiple comparisons by Dunnett's procedure. The significance of the results of sympathetic nerve function tests in 380 patients with primary glaucoma compared with results in 85 age- and sex-matched control subjects is summarised in the following paragraphs.

7.331 Systolic blood pressure response to standing

Postural changes in systolic blood pressure in the control group were not significantly different ($p > 0.05$) from those in patients with CAG, OHNA, OHWA, OAGNA, and OAGWA.

7.332 Diastolic blood pressure response to sustained handgrip

Diastolic blood pressure responses to sustained handgrip in the control group were not significantly different ($p > 0.05$) from those in patients with CAG, OHNA, OHWA, and OAGWA. Increase in diastolic blood pressure following sustained handgrip was significantly higher in patients with OAGNA than the control group ($p < 0.001$; $p' < 0.01$).

Table 15

RESULTS OF CARDIOVASCULAR SYMPATHETIC FUNCTION ASSESSMENT IN 380 PATIENTS WITH PRIMARY GLAUCOMA
AND 85 AGE- AND SEX-MATCHED CONTROL SUBJECTS

(Group mean values \pm SEM)

	Decrease in systolic blood pressure on standing (mmHg)	Increase in diastolic blood pressure during sustained handgrip (mmHg)
Control group (n=85)	8.36 \pm 1.42	19.72 \pm 1.55
Closed-angle glaucoma patients (n=124)	10.72 \pm 1.18	22.41 \pm 1.61
Ocular hypertensive patients All (n=189)	11.14 \pm 0.98	21.69 \pm 1.11
OHNA (n=90)	11.53 \pm 1.43	24.18 \pm 2.07
OHWA (n=99)	10.77 \pm 1.36	20.30 \pm 1.25
Open-angle glaucoma patients All (n=67)	11.28 \pm 1.21	24.26 \pm 2.21
OAGNA (n=24)	13.25 \pm 1.73	34.19 \pm 3.76
OAGWA (n=43)	10.12 \pm 1.62	18.68 \pm 1.48

7.34 Significance of sex on systemic autonomic nerve function assessment

In each test of cardiovascular autonomic nerve function, comparisons were made between the results of males versus females, in the control group and each category of the primary glaucomas separately; significance was assessed by Student's unpaired t test. No significant differences were noted in any test between the results of male and female subjects.

7.35 Diabetes mellitus and systemic autonomic nerve function

The significance of diabetic status on cardiovascular autonomic function assessment was examined by comparing the results of non-diabetic patients with those of patients with NIDDM, IGT, and (NIDDM + IGT) separately. This was performed for each test of cardiovascular autonomic function individually, in the control group and each category of the primary glaucomas. Significance was determined by Student's unpaired t test, corrected by Dunnett's procedure.

7.351 Control group

No significant differences were noted in comparisons of autonomic function test results between non-diabetic subjects and those with NIDDM, IGT, or (NIDDM + IGT).

7.352 Closed-angle glaucoma

No significant differences were noted in comparisons of autonomic function test results between non-diabetic patients and those with NIDDM, IGT, or (NIDDM + IGT).

7.353 OHNA

No significant differences were noted in comparisons of autonomic function test results between non-diabetic patients and those with

NIDDM. Significant differences in autonomic function test results between non-diabetic patients and those with IGT or (NIDDM + IGT) are as follows; mean \pm SEM is quoted in brackets.

i. Non-diabetic v IGT

Patients with IGT (1.22 ± 0.04) had significantly lower Valsalva ratio than non-diabetics (1.42 ± 0.04): $p = 0.005$; $p' < 0.05$.

Patients with IGT (8.46 ± 1.36) had significantly lower heart-rate variation during deep breathing than non-diabetics (13.13 ± 0.95): $p = 0.009$; $p' < 0.05$.

ii. Non-diabetic v (NIDDM + IGT)

Patients with (NIDDM + IGT) (1.25 ± 0.04) had significantly lower Valsalva ratio than non-diabetics (1.42 ± 0.04): $p = 0.006$; $p' < 0.05$.

Patients with (NIDDM + IGT) (9.04 ± 1.05) had significantly lower heart-rate variation during deep breathing than non-diabetics (13.13 ± 0.95): $p = 0.006$; $p' < 0.05$.

7.354 OHWA

No significant differences were noted in comparisons of autonomic function test results between non-diabetic patients and those with IGT. The only significant difference in autonomic function test results between non-diabetic patients and those with NIDDM or (NIDDM + IGT) was in 30:15 ratio. This result was not significant after allowing for multiple comparisons; patients with NIDDM (1.07 ± 0.01) or (NIDDM + IGT) (1.09 ± 0.02) had significantly lower 30:15 ratio than non-diabetics (1.15 ± 0.01), before correction: $p = 0.02$; $p' > 0.05$.

7.355 OAGNA

No significant differences were noted in comparisons of autonomic function test results between non-diabetic patients and those with NIDDM, IGT, or (NIDDM + IGT).

No significant differences were noted in comparisons of autonomic function test results between non-diabetic patients and those with NIDDM, IGT, or (NIDDM + IGT).

7.36 Cardiovascular autonomic nerve function in the primary glaucomas; comparisons with the age-adjusted normal tolerance limits

The age-adjusted normal tolerance limits, at the lower 5th percentile, for each of the cardiovascular autonomic function tests (figures 4-8) were applied to the results obtained from patients with primary glaucoma, to determine the proportion of patients in each glaucoma category outwith the normal tolerance limits. Results of parasympathetic function tests are shown in table 16, and sympathetic function tests in table 17. Normal tolerance limits were not determined for diastolic blood pressure responses to sustained handgrip; this test was found to be impractical in patients aged > 60 years, as explained on page 89.

Table 16

THE PROPORTION OF PRIMARY GLAUCOMA PATIENTS AND CONTROL SUBJECTS WITH ABNORMAL RESULTS OF CARDIOVASCULAR PARASYMPATHETIC NERVE FUNCTION, DEFINED BY THE AGE-ADJUSTED NORMAL TOLERANCE LIMITS AT THE LOWER

5th PERCENTILE

	Valsalva ratio	Heart-rate variation during deep breathing	30:15 ratio	S:L ratio
Control subjects (n=85)	0%	1.3%	0%	1.3%
Closed-angle glaucoma patients (n=124)	14.3%	38.5%	7.9%	15.5%
Ocular hypertensive patients All (n=189)	8.8%	32.0%	11.0%	7.0%
OHNA (n=90)	7.3%	42.7%	15.7%	7.2%
OHWA (n=99)	10.0%	22.2%	6.7%	6.8%
Open-angle glaucoma patients All (n=67)	11.5%	23.9%	4.8%	14.5%
OAGNA (n=24)	5.0%	25.0%	8.7%	13.0%
OAGWA (n=43)	15.7%	23.2%	2.6%	15.4%

Table 17

THE PROPORTION OF PRIMARY GLAUCOMA PATIENTS AND CONTROL SUBJECTS WITH ABNORMAL RESULTS OF CARDIOVASCULAR SYMPATHETIC NERVE FUNCTION, DEFINED BY THE AGE-ADJUSTED NORMAL TOLERANCE LIMIT AT THE LOWER 5th PERCENTILE

	Systolic blood pressure response to standing
Control group (n=85)	2.6%
Closed-angle glaucoma patients (n=124)	11.5%
Ocular hypertensive patients All (n=189)	8.8%
OHNA (n=90)	9.0%
OHWA (n=99)	8.5%
Open-angle glaucoma patients All (n=67)	3.0%
OAGNA (n=24)	4.2%
OAGWA (n=43)	2.4%

7.37 The prevalence of autonomic nerve dysfunction in the primary glaucomas

Values outwith the normal tolerance limits for each of the cardiovascular autonomic function tests (figures 4-8) were designated "abnormal". Autonomic nerve dysfunction was defined by the presence of at least one abnormal result of cardiovascular autonomic function tests. The proportions of patients with evidence of parasympathetic nerve dysfunction, sympathetic nerve dysfunction, and combined parasympathetic plus sympathetic nerve dysfunction by cardiovascular autonomic function assessment in 380 patients with primary glaucoma, and 85 age- and sex-matched control subjects, are shown in table 18.

7.38 Glaucoma treatment and cardiovascular autonomic nerve function

As stated previously, the only drug treatments for glaucoma taken by patients included in the study were guttae pilocarpine 2%/4% and/or guttae timolol maleate 0.25%/0.5%. The possible association between treatment and cardiovascular autonomic nerve function was assessed by comparing the results of each test of cardiovascular autonomic function separately (ie Valsalva ratio, heart-rate variation during deep breathing, 30:15 ratio, S:L ratio, systolic blood pressure response to standing, and diastolic blood pressure response to sustained handgrip) in primary glaucoma patients on treatment with the results in untreated primary glaucoma patients; this was performed for guttae pilocarpine 2%/4% and guttae timolol maleate 0.25%/0.5% separately, in each category of the primary glaucomas. Significance was assessed by Student's unpaired t test.

No significant differences ($p > 0.05$) in the results of any cardiovascular autonomic function test were present between primary glaucoma patients treated with guttae pilocarpine 2%/4% and/or guttae timolol maleate 0.25%/0.5% and primary glaucoma patients not on treatment.

Table 18

THE PREVALENCE OF AUTONOMIC NERVE DYSFUNCTION IN 380 PATIENTS WITH PRIMARY GLAUCOMA
AND 85 AGE- AND SEX-MATCHED CONTROL SUBJECTS

	Parasympathetic dysfunction	Sympathetic dysfunction	Parasympathetic plus sympathetic dysfunction
Control subjects (n=85)	2.6%	2.6%	0%
Closed-angle glaucoma patients (n=124)	50.0%	12.9%	8.9%
Ocular hypertensive patients All (n=189)	36.7%	14.8%	7.9%
OHNA (n=90)	50.0%	13.3%	11.1%
OHWA (n=99)	34.3%	16.2%	5.1%
Open-angle glaucoma patients All (n=67)	37.3%	6.0%	0%
OAGNA (n=24)	41.7%	4.2%	0%
OAGWA (n=43)	34.9%	7.0%	0%

7.4 Discussion

The utilisation of cardiovascular reflexes to define systemic autonomic function provides an accurate, reproducible, and quantifiable measurement of a nervous system previously virtually impossible to isolate. Heart-rate is determined at all times by the balance between sympathetic and parasympathetic influences (391,392,393). The relative importance of each component during characteristic cardiovascular reflexes has been determined by selective pharmacological autonomic blockade.

All assessments were performed on a single-blind basis. Evaluation of appropriate control subjects was necessary, not only to ensure objectivity and negate subjective bias, but also to provide a series of standard "normal" values in autonomic function assessment for this age category. Although heart-rate variation during deep breathing has been examined in the elderly by Smith (244) and Wieling et al (260) (forced respiratory sinus arrhythmia in relation to age, shown in plate 12), reference values for other parameters of autonomic nerve function are applicable to a younger age range. Tolerance limits for the results of each of the 5 tests of cardiovascular autonomic function in the control group are shown in table 13. Valsalva ratio, heart-rate variation during deep breathing, and systolic blood pressure response to standing were significantly correlated with age; consequently, tolerance limits based on the lower 5th percentile are shown in those categories relative to the mean age of the control group. If the tolerance limits of autonomic function assessment for this control group are compared with those advocated for younger age categories (table 4), remarkable similarities are apparent. In the following list, the tolerance limits of the present control group are compared with established diagnostic criteria of abnormality in a younger age range: Valsalva ratio, 1.13 cf 1.10; heart-rate variation during deep breathing (beats/minute), 9.2 cf 10.0; 30:15 ratio, 1.01 cf 1.00; S:L ratio, 1.06 cf 1.07; decrease in systolic blood pressure on standing (mmHg), 29.38 cf 30.00.

The assessment of cardiovascular autonomic nerve function is thus equally possible in an elderly population; verification of tolerance limits, based on the lower 5th percentile, permits reasonable inferences from subsequent comparisons with "normal" values. Primary glaucoma, with few exceptions, is prevalent in older age-groups; it was therefore essential to establish effective tolerance limits of cardiovascular autonomic nerve function directly applicable to this population.

Comparisons between the primary glaucomas and the control group show significant differences in the results of every test of parasympathetic function:

i. Valsalva ratio

The mean Valsalva ratio was significantly higher in the control group than in patients with OHNA and OAGNA.

ii. Heart-rate variation during deep breathing

Heart-rate variation during deep breathing was significantly diminished in every category of the primary glaucomas: CAG, OHNA, OHWA, OAGNA, and OAGWA.

iii. 30:15 ratio

The mean 30:15 ratio was significantly higher in the control group than in patients with CAG, OHNA, OHWA, and OAGNA.

iv. S:L ratio

The mean S:L ratio was significantly higher in the control group than in patients with CAG and OHNA, although only the former remained significant after allowing for multiple comparisons.

Results of cardiovascular parasympathetic function assessment were significantly lower than reference values in each sub-group of the primary glaucomas; the decreased efficacy of parasympathetic cardiovascular reflexes was particularly prominent in patients with CAG and OHNA. This association of impaired parasympathetic function in patients with narrow irido-corneal angles is similar to the association between diabetes mellitus and primary glaucoma.

No significant differences were noted in comparisons of sympathetic function results between the primary glaucomas and the control group. Systolic blood pressure responses to standing were similar in all categories, and the mean diastolic blood pressure increase during sustained handgrip was significantly higher in patients with OAGNA than the control group. There are two possible explanations for these results. The first is that cardiovascular tests of sympathetic nerve function based upon blood pressure responses, although highly specific, are recognised to be less sensitive than cardiovascular assessments of parasympathetic nerve function (219); this is an important consideration and cannot be disregarded. The second explanation is that cardiovascular sympathetic dysfunction is not present to any significant degree in patients with primary glaucoma. One may only conclude that, within the limits imposed by decreased sensitivity, cardiovascular sympathetic nerve dysfunction is not associated with the primary glaucomas.

Diabetes mellitus is associated with parasympathetic nerve function in the primary glaucomas. Although the results of autonomic function tests were not significantly influenced by diabetic status in the control group, definitive conclusions are restricted by the limited number of subjects with diabetes mellitus in this category. In OHNA and OHWA, results of parasympathetic function tests were significantly lower in diabetic than non-diabetic patients, although only OHNA remained significant after multiple comparisons were taken into account. It is note-worthy that in patients with OHNA, results of parasympathetic function tests were also significantly lower in patients with IGT than non-diabetics. As approximately 2-4% of patients with IGT progress to overt diabetes mellitus per year (370), the association with autonomic nerve dysfunction - an important indicator in the prognosis of diabetes mellitus (229) - in these patients is significant.

No association was present between diabetes mellitus and sympathetic nerve function in the primary glaucomas.

Individual tests of cardiovascular autonomic function have different

degrees of sensitivity, manifesting abnormality at different stages in the evolution of autonomic nerve dysfunction. This is clearly shown by the proportions of "abnormal" results for each of the autonomic function tests in the primary glaucomas (tables 16 and 17). Previous descriptions of diabetic autonomic neuropathy have commented on the predictable progression of impaired autonomic efficacy (228,229); parasympathetic dysfunction occurs initially, with involvement of the sympathetic nervous system at a later stage in the pathogenesis of autonomic neuropathy. Heart-rate variation during deep breathing tends to be the first parasympathetic test to exhibit abnormality (235), with Valsalva ratio the last affected (216). The established course of events in diabetic autonomic neuropathy is closely mirrored by results obtained in the primary glaucomas, with most abnormal results in response to forced deep breathing and least following Valsalva manoeuvre. A similar gradual, stepwise progression of autonomic dysfunction is thus present in the primary glaucoma population as shown by patients with diabetes mellitus.

S:L ratio is a relatively-recent measure of parasympathetic integrity. Initial results have suggested that this test may be a sensitive indicator of parasympathetic nerve function, with abnormal values occurring at an early stage in the disease process (275); this is supported by the present results of parasympathetic evaluation in the primary glaucomas. The view that a series of tests to accurately determine autonomic nerve function is preferable to any single measure (219,235) - which may give misleading results - is also supported by this study. Prevalence statistics from parameters of nerve function with different levels of sensitivity would obviously have given conflicting results; a series of established tests more-accurately defines the degree of autonomic function in the individual patient.

The most significant observation to be highlighted by these results is the marked prevalence of systemic parasympathetic dysfunction in the primary glaucomas, particularly in patients with narrow irido-corneal angles (table 18). The proportion of patients with impaired sympathetic reflexes was significantly lower.

Thus there is a significant association between systemic autonomic dysfunction and primary glaucoma; this is principally an abnormality of the parasympathetic nervous system, although combined dysfunction of both parasympathetic and sympathetic cardiovascular reflexes is present to a lesser degree in patients with CAG, OHNA and OHWA. Parasympathetic dysfunction is an integral feature of all sub-groups of the primary glaucomas studied, with significantly-increased prevalence in narrow-angle categories.

The definitive aetiology of autonomic dysfunction in patients with primary glaucoma is not known, however there is undoubtedly a close relationship between diabetic status and cardiovascular autonomic nerve function; in OHNA and OHWA, results of autonomic function tests were significantly lower in diabetic than non-diabetic patients. The distribution of systemic autonomic dysfunction parallels that of diabetes mellitus in the primary glaucomas, with maximal prevalence in narrow-angle categories. When one considers that diabetes mellitus is the commonest cause of autonomic neuropathy in this country (170), and that 20-40% of diabetics have evidence of autonomic dysfunction (183,249,295,394), these results concur with established features of autonomic neuropathy.

One may conclude that systemic dysfunction of the autonomic nervous system is associated with CAG, OAG, and OH, with increased prevalence in patients with narrow irido-corneal angles. As autonomic nerve assessment by cardiovascular reflex responses correlates closely with generalised autonomic function (192,216), one may reasonably assume that the autonomic dysfunction associated with primary glaucoma is not restricted to the cardiovascular system. The relevance of diminished efficacy of the autonomic nervous system, particularly the parasympathetic nervous system, in the pathogenesis of glaucoma will be examined in the final discussion.

SECTION IV

OCULAR AUTONOMIC NERVE FUNCTION IN THE PRIMARY GLAUCOMAS

PUPIL CYCLE TIME

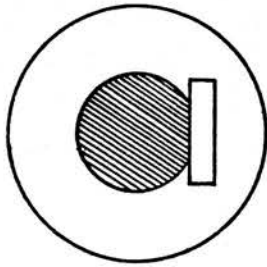
8.1 Introduction

Measurement of the pupil cycle time permits simple, non-invasive, objective assessment of the pupillary reflex arc. Providing the ultimate effector, the iris musculature, is structurally normal with intact innervation, the pupil cycle time is dependent upon velocity of nerve conduction, synaptic delay, and the number of nerve impulses transmitted. A thin slit-beam focussed at the pupillary margin produces pupil oscillations which can be accurately timed with a stop-watch. The underlying mechanism is described in figure 9. The beam is positioned to just overlap the pupillary margin; light stimulating the retinal receptors initiates the pupil reflex, with subsequent constriction of the pupil. Elimination of afferent stimulation results in reflex dilation of the pupil, light re-enters the eye and a second cycle is initiated.

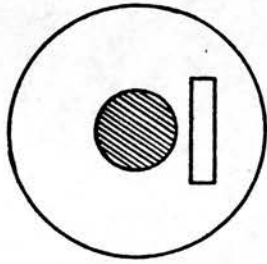
The diagnostic criteria for ocular hypertension, open-angle glaucoma and closed-angle glaucoma, stipulated on pages 9-11, were strictly applied; central and peripheral visual field assessment of all eyes of patients with ocular hypertension, and fellow eyes of patients with closed-angle glaucoma, were normal, whereas visual field defects were present in patients with open-angle glaucoma. This is particularly relevant in the assessment of the pupillary reflex arc, as the pupil cycle time is intrinsically dependent upon both afferent and efferent components of the reflex pathway. Afferent pupillary defects have been described in open-angle glaucoma (322), and attributed to defects in the anterior visual pathway usually proximal to the optic chiasm (323). In this study, pupil cycle time was not assessed in patients with open-angle glaucoma, as definitive interpretation of results would not have been possible; the presence of established lesions in the afferent limb of the reflex arc (ie visual field defects) negates conclusions regarding the efferent limb from a test which assesses the reflex pathway as an indivisible unit.

Figure 9

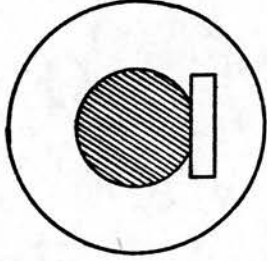
PUPIL CYCLE TIME



i. Slit-beam overlapping pupillary margin



ii. Pupil constricts



iii. Pupil dilates, cycle re-commences

8.2 Patients and methods

Patients were included in the study on the basis of the following criteria:

- i. a history of closed-angle glaucoma
- ii. ocular hypertension
 - a. with narrow irido-corneal angles
 - b. with wide irido-corneal angles

After informed consent had been obtained, pupil cycle time was measured in 70 age- and sex-matched control subjects (mean age 66.2 ± 10.1 years) and 313 patients with primary glaucoma (mean age \pm SD): 124 CAG (66.5 ± 10.1 years), 90 OHNA (67.5 ± 9.2 years), and 99 OHWA (64.8 ± 9.6 years). The control group consisted of hospital staff, and patients attending the casualty department who were subsequently determined to have no detectable abnormality, or mild ocular changes within acceptable limits for an elderly population; this included early macular degenerative changes and minimal refractive errors. All subjects had a corrected visual acuity of $\geq 6/9$ in each eye.

A comprehensive past, present and family history was obtained from each subject, followed by systematic general medical examination and ocular examination according to the protocol described on pages 85-86. Pupil cycle time was measured 4 weeks after the initial ocular examination. Eyes were excluded from assessment if there was a history of:

- i. eye operations - either intra- or extra-ocular. Only unoperated eyes were included in the study. In patients with closed-angle glaucoma, assessments were performed only in unoperated fellow eyes; eyes which had sustained closed-angle glaucoma were specifically excluded.
- ii. eye trauma
- iii. current ophthalmic drug treatment: only eyes not on treatment were included in the study.

Subjects were also excluded from assessment on the basis of the following criteria:

- i. on treatment with autonomic or somatic neurological effects
- ii. a past history of:
 - a. medical disorders predisposing to autonomic nerve dysfunction - except diabetes mellitus
 - b. neurological disease - including cardiovascular accidents
 - c. eye disease: the only exception to this stipulation was the presence of ocular hypertension or closed-angle glaucoma in those particular categories under examination

After applying the exclusion criteria, pupil cycle time was measured in:

- i. Control group: 122 eyes in 70 subjects
- ii. CAG: 118 eyes in 118 patients
- iii. OHNA: 165 eyes in 90 patients
- iv. OHWA: 183 eyes in 99 patients

Edge-light pupil cycle time was measured according to the following method. The patient was seated at the slit-lamp (Zeiss 30SL, Carl Zeiss, Welwyn Garden City, England) in a room with very low, constant background illumination (20 lux), and requested to fixate straight ahead on an object at 6 metres. A 0.5 x 6 mm slit-beam was positioned horizontally and elevated until it just overlapped the margin of the pupil, then focussed on the iris-lens border. The pupil constricted, then reflexly dilated in response to iris blocking the light and thus removing afferent stimulation. 30 consecutive pupillary cycles were timed using a stop-watch; the result divided by 30 is the duration of a single cycle of the pupillary reflex arc. Infrequent, rapid "blinks" during measurement do not significantly affect the pupil cycle time; prolonged "blinks" necessitate repeat assessment. The pupil cycle time was measured 4 times for each eye separately (ie 4 x 30-cycles) and the result averaged. The measurement of 30 cycles with subsequent division to determine the duration of a single cycle reduces subjective error in manual timing.

All assessments were performed on a single-blind basis.

8.3 Results

The results of pupil cycle time assessment in 313 patients with primary glaucoma and 70 age- and sex-matched control subjects are described as follows:

- 8.31 Age-adjusted normal tolerance intervals for pupil cycle time
- 8.32 Pupil cycle time in the primary glaucomas
- 8.33 Absent pupillary oscillation in the primary glaucomas and the age- and sex-matched control group
- 8.34 Pupil cycle time in the primary glaucomas; comparisons with the age-adjusted normal tolerance limit
- 8.35 Significance of sex on pupil cycle time
- 8.36 Diabetes mellitus and pupil cycle time
- 8.37 Glaucoma treatment and pupil cycle time

Comparisons were made between the results of pupil cycle time in right eyes versus left eyes, in the control group and each category of the primary glaucomas separately; significance was assessed by Student's unpaired t test. No significant differences ($p > 0.05$) were present, in any category, between pupil cycle times in right and left eyes.

8.31 Age-adjusted normal tolerance intervals for pupil cycle time

The results of pupil cycle time measurement in the age- and sex-matched control group were assessed for dependence on age by 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 after adjustment for age, thus permitting determination of tolerance intervals. Logarithms of the data were used to derive tolerance intervals for the reasons stated on page 91. A log normal distribution was obtained; tolerance intervals were calculated from the log normal distribution (after adjusting for age) by the method described on pages 91-92. $\text{Log}_{10}(\text{PCT})$ did not depend significantly on age. Linear regression analysis, permitting determination of 2.5th, 5th, 95th, and 97.5th percentiles, was calculated from:

$$\text{RLPCT} = (\log_{10}\text{PCT} - (-0.02883 + 0.00019 \times \text{age}))/0.05469$$

assumed to have the standard Normal distribution.

$$\text{RLPCT} = \text{standardised residual } \log_{10}(\text{PCT})$$

The logarithms of the percentiles (allowing for regression on age), transformed back to natural values, with 2.5th, 5th, 95th, and 97.5th percentiles, are shown in figure 10. The upper tolerance limit for pupil cycle time at age 65 years, based on the 95th percentile of the log normal distribution after regression for age, is 1190 ms.

8.32 Pupil cycle time in the primary glaucomas

The results of pupil cycle time assessment in 313 patients with primary glaucoma and 70 age- and sex-matched control subjects are shown in table 19. Sustained pupillary oscillation in response to edge-light stimulation was absent in a significant percentage of eyes examined; consequently, measurement of pupil cycle time was possible in a proportionately decreased number of eyes:

- i. Control group: 106 eyes in 61 subjects
- ii. CAG: 64 eyes in 64 patients
- iii. OHNA: 107 eyes in 63 patients
- iv. OHWA: 151 eyes in 78 patients

Comparisons of mean PCT were made between the control group and each category of the primary glaucomas separately; significance was assessed by Student's unpaired t test. Dunnett's multiple comparison procedure was used to obtain a corrected p value (p'). The significance of the results of PCT assessment in 313 patients with primary glaucoma compared with results in 70 age- and sex-matched control subjects are as follows; the actual p value plus the corrected p value are quoted for significant results. PCT in patients with CAG ($p < 0.001$; $p' < 0.01$) and OHNA ($p < 0.001$; $p' < 0.01$) were significantly higher than the control group. PCT in patients with OHWA ($p = 0.02$; $p' > 0.05$) was also significantly higher than the control group, however this was non-significant after allowing for multiple comparisons.

Figure 10
AGE-ADJUSTED NORMAL TOLERANCE INTERVALS FOR PUPIL CYCLE TIME

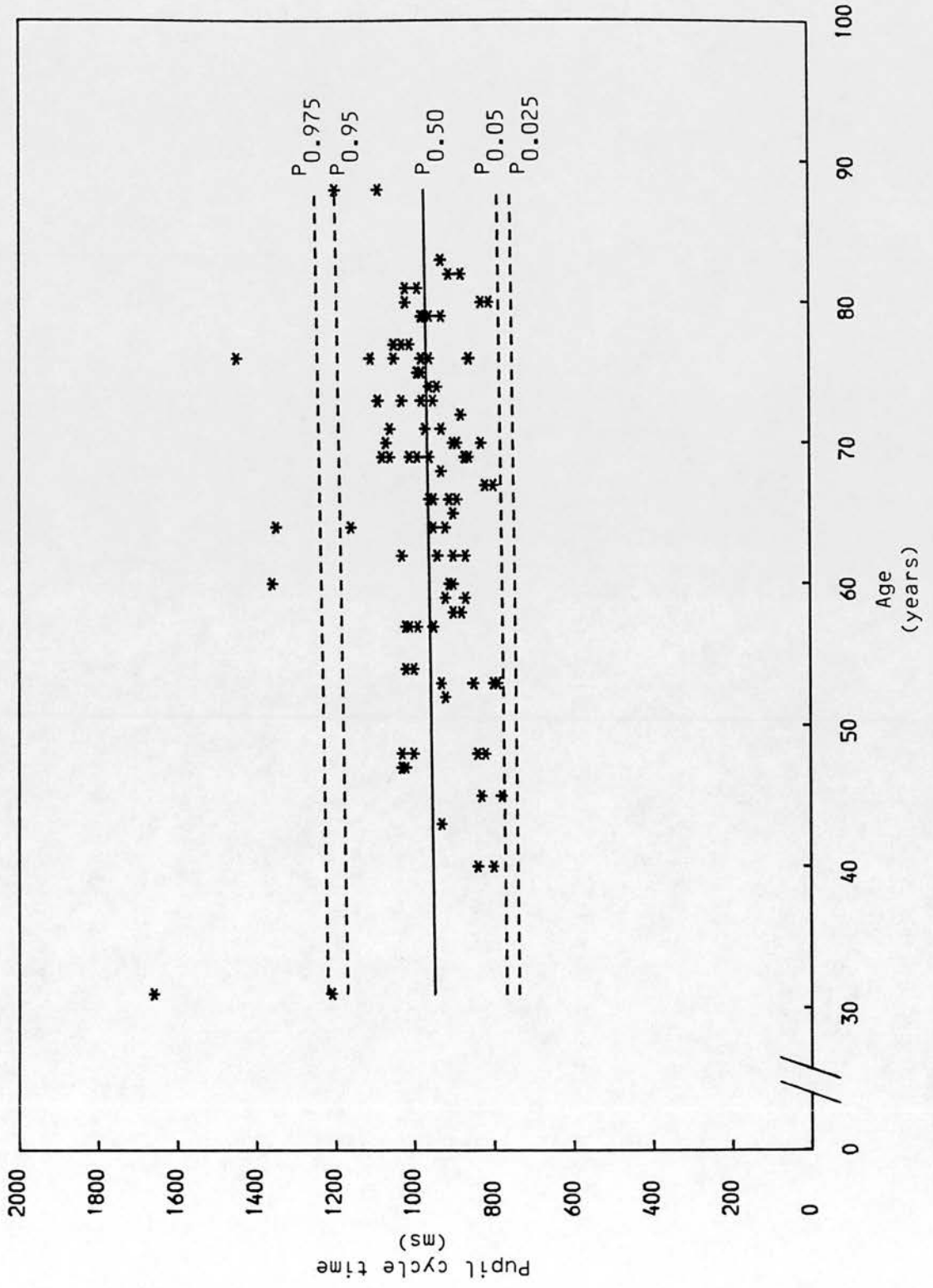


Table 19

RESULTS OF PUPIL CYCLE TIME ASSESSMENT IN 313 PATIENTS WITH PRIMARY
 GLAUCOMA AND 70 AGE- AND SEX-MATCHED CONTROL SUBJECTS
 (Group mean values \pm SEM)

	Pupil cycle time (s)
Control subjects (n=70)	0.97 \pm 0.01
Closed-angle glaucoma patients (n=124)	1.07 \pm 0.02
Ocular hypertensive patients ALL (n=189)	1.03 \pm 0.01
OHNA (n=90)	1.05 \pm 0.01
OHWA (n=99)	1.01 \pm 0.01

8.33 Absent pupillary oscillation in the primary glaucomas and the age- and sex-matched control group

Pupillary oscillation was absent in a significant proportion of eyes examined (table 20):

- i. Control group: 16 eyes in 11 subjects
- ii. CAG: 54 eyes in 54 patients
- iii. OHNA: 58 eyes in 38 patients
- iv. OHWA: 32 eyes in 19 patients

Comparisons were made between the prevalence of absent pupillary oscillation in the control group and each category of the primary glaucomas separately; significance was assessed by χ^2 test with Yates' correction. To allow for multiple comparisons with a control, significant p values were multiplied by the number of comparisons made to obtain a corrected p value (p'); the actual p value plus the corrected p value are quoted for significant results.

The prevalence of absent pupillary oscillation was significantly higher in patients with CAG ($p < 0.001$; $p' < 0.001$) and OHNA ($p < 0.001$; $p' < 0.001$) than the control group. No significant differences were present between the prevalence of absent pupillary oscillation in the control group and patients with OHWA ($p > 0.05$).

8.34 Pupil cycle time in the primary glaucomas; comparisons with the age-adjusted normal tolerance limit

The age-adjusted normal tolerance limit, ie the upper 95th percentile of the log normal distribution after regression for age, was applied to the results obtained from patients with primary glaucoma, to determine the proportion of eyes in each glaucoma category outwith this limit; results are shown in table 21. This refers only to eyes where measurement of pupil cycle time was possible; eyes with absent pupillary oscillation are considered separately in section 8.33.

Table 20

THE PREVALENCE OF ABSENT PUPILLARY OSCILLATION IN EYES FROM
 313 PATIENTS WITH PRIMARY GLAUCOMA AND 70 AGE- AND SEX-MATCHED
 CONTROL SUBJECTS

	Prevalence of absent pupillary oscillation
Control subjects (n=70)	14.3%
Closed-angle glaucoma patients (n=124)	45.6%
Ocular hypertensive patients ALL (n=189)	25.6%
OHNA (n=90)	35.6%
OHWA (n=99)	16.6%

Table 21

THE PREVALENCE OF ABNORMAL PUPIL CYCLE TIME, DEFINED BY THE AGE-ADJUSTED NORMAL TOLERANCE LIMIT AT THE UPPER 95th PERCENTILE, IN EYES FROM 313 PATIENTS WITH PRIMARY GLAUCOMA AND 70 AGE- AND SEX-MATCHED CONTROL SUBJECTS

	Prevalence of abnormal PCT
Control subjects (n=70)	4.6%
Closed-angle glaucoma patients (n=124)	18.8%
Ocular hypertensive patients ALL (n=189)	12.5%
OHNA (n=90)	17.1%
OHWA (n=99)	9.3%

8.35 Significance of sex on pupil cycle time

Comparisons were made between the results of pupil cycle time measurement in males versus females, in the control group and each category of the primary glaucomas separately; significance was assessed by Student's unpaired t test. No significant differences were present between the results of male and female subjects.

8.36 Diabetes mellitus and pupil cycle time

The possible association between diabetes mellitus and pupil cycle time was assessed by comparing the results of pupil cycle time measurement in diabetic subjects (NIDDM, IGT, and NIDDM + IGT separately) with results in non-diabetic subjects, in the control group and each category of the primary glaucomas separately. Significance was assessed by Student's unpaired t test.

No significant differences in results of pupil cycle time measurement were present between diabetic and non-diabetic subjects in any category.

8.37 Glaucoma treatment and pupil cycle time

The only drug treatments for glaucoma taken by patients included in the study were guttae pilocarpine 2%/4% and/or guttae timolol maleate 0.25%/0.5%. (NB Pupil cycle time was only measured in eyes not on drug treatment, thus the purpose of this comparison was to assess whether drug treatment to the contralateral eye affected pupil cycle time in the untreated eye.) The possible association between treatment and pupil cycle time was assessed by comparing the results of pupil cycle time measurement in primary glaucoma patients on treatment with the results in untreated primary glaucoma patients; this was performed for guttae pilocarpine 2%/4% and guttae timolol maleate 0.25%/0.5% separately, in each category of the primary glaucomas. Significance was assessed by Student's

unpaired t test. No significant differences ($p > 0.05$) in the results of pupil cycle time measurement were present between primary glaucoma patients treated with guttae pilocarpine 2%/4% and/or guttae timolol maleate 0.25%/0.5% and primary glaucoma patients not on treatment.

8.4 Discussion

Pupil cycle time enables the integrity of the pupillary reflex arc to be accurately and objectively evaluated by a simple, non-invasive, clinically-orientated technique. The duration of the cycle is prolonged in older subjects; a mean pupil cycle time of 822 ± 69 ms has been proposed for subjects aged < 50 years (315), and a mean of 872 ± 83 ms in those aged 50-79 years (316). In the present study, pupil cycle time was assessed in a control group with mean age 67.1 ± 11.5 years (range 30-91 years); although the reflex was shown to be only minimally age-dependent, mean pupil cycle time (ie 968 ± 131 ms) was significantly higher than previous descriptions. Edge-light pupil cycle time, as originally described by Miller and Thompson in the younger age group (315), represented the average of 5 consecutive 30-cycle measurements (150 cycles). When the pupil cycle time was determined in older subjects (316), the final result represented the average of only 3 x 30-cycle measurements (90 cycles). In the present study, a compromise figure of 4 x 30-cycle measurements (120 cycles) were employed in assessment. Prolongation of pupil cycle time in older control subjects, indicated by present results, may represent a manifestation of fatigue within the reflex, which is precipitated by the increased number of consecutive cycles, whilst not revealed by less-exacting assessments.

14.3% of eyes in control subjects were characterised by an absence of sustained pupillary oscillation in response to edge-light stimulation; consequently, definitive measurement of pupil cycle time was not possible. This phenomenon has not been reported in previous descriptions of pupil cycle time. The following 3 sub-groups are included within this category:

- i. initial pupil oscillation, rapidly diminishing in amplitude and velocity, with eventual pupil immobility prior to the completion of 120 cycles
- ii. irregular pupillary unrest or hippus, with minimal pupillary "fibrillating" oscillations which were impossible to quantify
- iii. relative pupillary immobility, interrupted at irregular intervals by segmental contractions of the sphincter pupillae

Age-adjusted normal tolerance intervals for pupil cycle time may only be defined in subjects where measurement of pupil cycle time is possible; appropriate consideration must be given to absence of pupillary oscillation, however it is obviously impossible to assign an absolute numerical value in this circumstance. An effective compromise was obtained by enumerating subjects with absent pupillary oscillation separately. In an elderly population, pupil cycle time is not always a directly-measurable parameter, however using this definition tolerance limits may be established - with qualification. Whether the lack of quantifiable movement results from subclinical sphincter innervational defects (316), increased conduction time in anterior optic pathways (316), or arteriosclerosis and deposition of hyaline in the iris stroma and muscles (395), remains speculative, however it is present in a significant proportion of control subjects.

In comparisons with the control group, pupil cycle time was significantly prolonged in patients with CAG and OHNA; pupil cycle time was also significantly prolonged in patients with OHWA, however this failed to remain significant after allowing for multiple comparisons. The prevalence of abnormal pupil cycle time, defined by the 95th percentile of the log normal distribution after regression on age, was significantly increased in patients with CAG and OHNA. Thus impairment of pupil cycle time is specifically associated with narrow-angle primary glaucomas: CAG and OHNA. The prevalence of absent pupillary oscillation in response to edge-light stimulation was also significantly increased in patients with CAG and OHNA; the prevalence in patients with OHWA was not significantly different from the control group. Pupil cycle time was not significantly associated with diabetic status.

Pupil cycle time assesses the integrity of the pupillary reflex arc as a discrete and indivisible unit; inferences to localise any defect within the reflex pathway revealed by prolongation of the pupil cycle time may only be made by supplementing the data with additional information relating to individual components of the pathway. There was no evidence of retinal nerve fibre bundle defects, following meticulous peripheral and central visual field assessment, in all eyes of patients with ocular hypertension and fellow eyes of patients with closed-angle glaucoma. Nerve fibre bundle defects characteristic of glaucoma may affect the afferent limb of the reflex arc; when afferent defects are not revealed by standard techniques, prolonged pupil cycle time may be attributed to defects in either the internuncial or efferent component of the arc by reasonable implication. In the presence of generalised cardiovascular parasympathetic dysfunction, one may reasonably postulate co-existent parasympathetic dysfunction in the anterior segment of the eye. However as localisation in this test is not possible, one may equally postulate that pupil cycle time is prolonged as a result of early defects in the afferent limb, undetected by standard techniques. The only definite conclusion from these results is that the duration of the pupillary reflex, represented by the pupil cycle time, is prolonged in patients with closed-angle glaucoma and the narrow-angle form of ocular hypertension, implying impairment of the pupillary reflex arc: the aetiology is not known.

Irrespective of the site of the lesion, the duration of the pupillary reflex represents a simple and reproducible measure of ocular nerve function - both afferent (sensory,somatic) and efferent (motor,autonomic) - in patients with glaucoma. Thus pupil cycle time can be effectively utilised in the objective evaluation of ocular nerve function in the primary glaucomas.

DENERVATION HYPERSENSITIVITY

9.1 Introduction

The principles of denervation hypersensitivity are explained in the introductory text. Adrenoceptor density, determined by radioligand binding, is significantly increased at the postsynaptic receptor site in autonomic denervation hypersensitivity (396,397). This mechanism may be applied to the anterior segment of the eye, where pupillary diameter is directly controlled by the autonomic nervous system; parasympathetic nerves effect pupillary constriction via the sphincter pupillae, and sympathetic nerves effect pupillary dilation via the dilator pupillae. Pupillary diameter therefore represents an external manifestation of relative autonomic activity in the anterior segment of the eye. It has been shown that 2.5% methacholine is an effective determinant of parasympathetic denervation hypersensitivity; topical guttae 2.5% methacholine constricts Adie's tonic pupils, whilst normal pupils may not be affected by concentrations up to 15% (333). 2.5% methacholine was therefore used in the present study to determine the degree of parasympathetic denervation hypersensitivity in the anterior segment of patients with primary glaucoma.

Assessment of sympathetic denervation hypersensitivity in the anterior segment has not been accurately defined. Various authors have reported using 0.1% adrenaline (352), and 1% or 2% phenylephrine, however 0.1% adrenaline has given conflicting results, and 1%/2% phenylephrine effect a degree of mydriasis in the normal pupil (353,354,355); detailed statistics of mydriatic efficacy at these concentrations have not been established. It was therefore decided to use low concentrations (0.1% and 0.5%) of topically-applied guttae phenylephrine hydrochloride, a direct-acting alpha-adrenoceptor agonist effecting pupillary dilation, to determine whether anterior segment sympathetic denervation was a feature of the primary glaucomas. Identical studies were concurrently performed on an age- and sex-matched control group to determine "normal" responses.

9.2 Patients and methods

Patients were included in the study on the basis of the following criteria:

- i. a history of closed-angle glaucoma
- ii. ocular hypertension
 - a. with narrow irido-corneal angles
 - b. with wide irido-corneal angles
- iii. open-angle glaucoma
 - a. with narrow irido-corneal angles
 - b. with wide irido-corneal angles

After informed consent had been obtained, pupillary responses to guttae 2.5% methacholine, 0.1% phenylephrine and 0.5% phenylephrine were assessed in 63 age- and sex-matched control subjects (mean age 60.3 ± 19.1 years) and 117 patients with primary glaucoma (mean age \pm SD): 45 CAG (66.3 ± 10.1 years), 26 OHNA (67.1 ± 9.4 years), 26 OHWA (64.2 ± 9.8 years), 7 OAGNA (68.6 ± 8.8 years), and 9 OAGWA (66.4 ± 8.5 years). The control group consisted of hospital staff, and patients attending the casualty department who were subsequently determined to have no detectable abnormality, or mild ocular changes within acceptable limits for an elderly population; this included early macular degenerative changes and minimal refractive errors. All subjects had a corrected visual acuity of $\geq 6/9$ in each eye.

A comprehensive past, present and family history was obtained from each subject, followed by systematic general medical examination and ocular examination according to the protocol described on pages 85-86. Assessment of pupillary denervation hypersensitivity was performed at least 4 weeks after the initial ocular examination.

Eyes were excluded from assessment if there was a history of:

- i. eye operations - either intra- or extra-ocular. Only unoperated eyes were included in the study. In patients with closed-angle glaucoma, assessments were performed only in unoperated fellow eyes; eyes which had sustained closed-angle glaucoma were specifically excluded.
- ii. eye trauma
- iii. current ophthalmic drug treatment: only eyes not on treatment were included in the study.

Subjects were also excluded from assessment on the basis of the following criteria:

- i. on treatment with autonomic or somatic neurological effects
- ii. a past history of:
 - a. medical disorders predisposing to autonomic nerve dysfunction - except diabetes mellitus
 - b. neurological disease - including cardiovascular accidents
 - c. eye disease: the only exception to this stipulation was the presence of ocular hypertension or closed-angle glaucoma in those particular categories under examination

9.21 Assessment of parasympathetic denervation hypersensitivity

Pupil diameters were recorded photographically. Subjects' eyes were photographed between 9-11am under standard lighting conditions; the subject faced a surface with measured luminance of 20 apostilbs, whilst constant background luminous flux of 1000 lumens was maintained in a white-walled room 2.5m x 3.5m. The subjects remained seated in the room for 5 minutes prior to assessment. They were then requested to sit at a small table on which was placed an ophthalmic head-rest (plate 13) against which they rested their foreheads, thereby effecting constant, reproducible positions for photography (398). A scale was positioned against the lower eyelid, in the perpendicular plane of the iris (plates 14 and 15). (The scale was constructed using Rotring engineering-drawing equipment, with lines of width 0.1 mm at 1 cm intervals.) The subjects fixated at 6 metres, then a photograph at x3 magnification was taken of both eyes together using a Nikon F camera with Medical Nikkor 120mm f4 lens (plate 16), and Kodak ektachrome professional film (ASA 200). The camera settings were fixed; focussing was effected by movement of the camera. The subject's head was tilted back, and one drop of 2.5% methacholine was placed in the conjunctival sac of the right eye; if the right eye had previously sustained closed-angle glaucoma, or had a history of eye trauma/eye operations/drug treatment, the left eye was assessed. The subjects remained in the room throughout the test. 45 minutes later, the subjects were repositioned on the head-rest and a second photograph taken in a similar manner.

The photographic slides were projected on to a white screen at

5 metres, producing a final magnification of x17. Horizontal pupillary diameters were measured to an accuracy of ± 0.5 mm, and corrected to actual values by comparison with relative magnification of the scale; this resulted in measurements of actual pupillary diameters to ± 0.03 mm. The horizontal pupillary diameter 45 minutes after installation of guttae in the tested eye was divided by the horizontal pupillary diameter before installation of guttae, and the result expressed as the 2.5% methacholine ratio:
$$2.5\% \text{ methacholine ratio} = \frac{\text{horiz. pupil diameter 45 min post-test}}{\text{horiz. pupil diameter pre-test}}$$

The fellow (non-tested) eyes could not be used as experimental controls for the following reasons:

- i. CAG: the non-tested eye in patients with CAG had sustained CAG, and thus was not an acceptable model for experimental control
- ii. OAG: the non-tested eye in patients with OAG had either a history of eye operation(s), or on treatment with topical glaucoma medication; in either case, these eyes were not acceptable as experimental controls.

The concurrent assessment of an age- and sex-matched control group provided effective standards for comparison. One eye was tested in each subject, and all assessments were performed on a single-blind basis.

The constituents of the 2.5% methacholine solution are detailed in appendix 1.

9.22 Assessment of sympathetic denervation hypersensitivity

The same technique was used to assess denervation of the sympathetic nervous system in the anterior segment of the eye, replacing 2.5% methacholine by 0.1% phenylephrine and 0.5% phenylephrine on separate occasions. Two weeks elapsed between each of the 3 ocular assessments of denervation: 2.5% methacholine, 0.1% phenylephrine, and 0.5% phenylephrine. All 3 assessments were performed on the same eye in each subject. Guttae 0.5% thymoxamine hydrochloride was instilled in the tested eye at the conclusion

of each 0.1% phenylephrine and 0.5% phenylephrine test, to reverse pupil dilation and negate possible complications of mydriasis (164).

The 0.1% phenylephrine and 0.5% phenylephrine ratios were separately calculated in the same manner as the 2.5% methacholine ratio ie horizontal pupillary diameter (in the tested eye) 45 minutes after installation of guttae divided by the horizontal pupillary diameter (in the tested eye) before installation of guttae.

The age- and sex-matched control group were concurrently assessed. One eye was tested in each subject, and all assessments were performed on a single-blind basis.

The constituents of the 0.1% phenylephrine and 0.5% phenylephrine solutions used in assessment are detailed in appendix 2.

9.3 Results

The results of pupillary responses to 2.5% methacholine, 0.1% phenylephrine, and 0.5% phenylephrine in 117 patients with primary glaucoma and 63 age- and sex-matched control subjects are described as follows:

- 9.31 Age-adjusted normal tolerance intervals for 2.5% methacholine ratio, 0.1% phenylephrine ratio, and 0.5% phenylephrine ratio
- 9.32 2.5% methacholine ratio, 0.1% phenylephrine ratio, and 0.5% phenylephrine ratio in the primary glaucomas
- 9.33 Significance of sex on denervation hypersensitivity
- 9.34 Diabetes mellitus and denervation hypersensitivity
- 9.35 Glaucoma treatment and denervation hypersensitivity

Comparisons were made, for 2.5% methacholine ratio/0.1% phenylephrine ratio/0.5% phenylephrine ratio separately, between the ratios in right eyes versus left eyes, in the control group and each category of the primary glaucomas separately; significance was assessed by Student's unpaired t test. No significant differences ($p > 0.05$) were present, in any category, between 2.5% methacholine/0.1% phenylephrine/0.5% phenylephrine ratios in right and left eyes.

9.31 Age-adjusted normal tolerance intervals for 2.5% methacholine ratio, 0.1% phenylephrine ratio, and 0.5% phenylephrine ratio

2.5% methacholine ratios, 0.1% phenylephrine ratios, and 0.5% phenylephrine ratios in the age- and sex-matched control group were separately assessed for dependence on age by 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. Logarithms of the data were used to derive tolerance intervals for the reasons stated on page 91. A log normal distribution was obtained for the results of each test; tolerance intervals were calculated from the log normal distribution (after adjusting for age) according to the method described on pages 91-92.

i. 2.5% methacholine ratio

\log_{10} (2.5% methacholine ratio) was shown to depend significantly on age. Linear regression analysis, permitting determination of 2.5th, 5th, 95th, and 97.5th percentiles, was calculated from:

$$RLM_{2.5} = (\log_{10}M_{2.5} - (0.01756 - 0.00076 \times \text{age}))/0.05241$$

assumed to have the standard Normal distribution.

$$RLM_{2.5} = \text{standardised residual } \log_{10}(2.5\% \text{ methacholine ratio})$$

The logarithms of the percentiles (allowing for regression on age), transformed back to natural values, with 2.5th, 5th, 95th, and 97.5th percentiles, are shown in figure 11. The lower tolerance limit for 2.5% methacholine ratio at age 65 years, based on the 5th percentile of the log normal distribution after regression on age, is 0.76.

ii. 0.1% phenylephrine ratio

\log_{10} (0.1% phenylephrine ratio) was shown to depend significantly on age. Linear regression analysis, permitting determination of 2.5th, 5th, 95th, and 97.5th percentiles, was calculated from:

$$RLP_{0.1} = (\log_{10}P_{0.1} - (-0.01766 + 0.00047 \times \text{age}))/0.04028$$

assumed to have the standard Normal distribution.

$RLP_{0.1}$ = standardised residual \log_{10} (0.1% phenylephrine ratio)

The logarithms of the percentiles (allowing for regression on age), transformed back to natural values, with 2.5th, 5th, 95th, and 97.5th percentiles, are shown in figure 12. The upper tolerance limit for 0.1% phenylephrine ratio at age 65 years, based on the 95th percentile of the log normal distribution after regression on age, is 1.20.

iii. 0.5% phenylephrine ratio

\log_{10} (0.5% phenylephrine ratio) was shown to depend significantly on age. Linear regression analysis, permitting determination of 2.5th, 5th, 95th, and 97.5th percentiles, was calculated from:

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

assumed to have the standard Normal distribution.

$RLP_{0.5}$ = standardised residual \log_{10} (0.5% phenylephrine ratio)

The logarithms of the percentiles (allowing for regression on age), transformed back to natural values, with 2.5th, 5th, 95th, and 97.5th percentiles, are shown in figure 13. The upper tolerance limit for 0.5% phenylephrine ratio at age 65 years, based on the 95th percentile of the log normal distribution after regression on age, is 1.50.

Figure 11

AGE-ADJUSTED NORMAL TOLERANCE INTERVALS FOR 2.5% METHACHOLINE RATIO

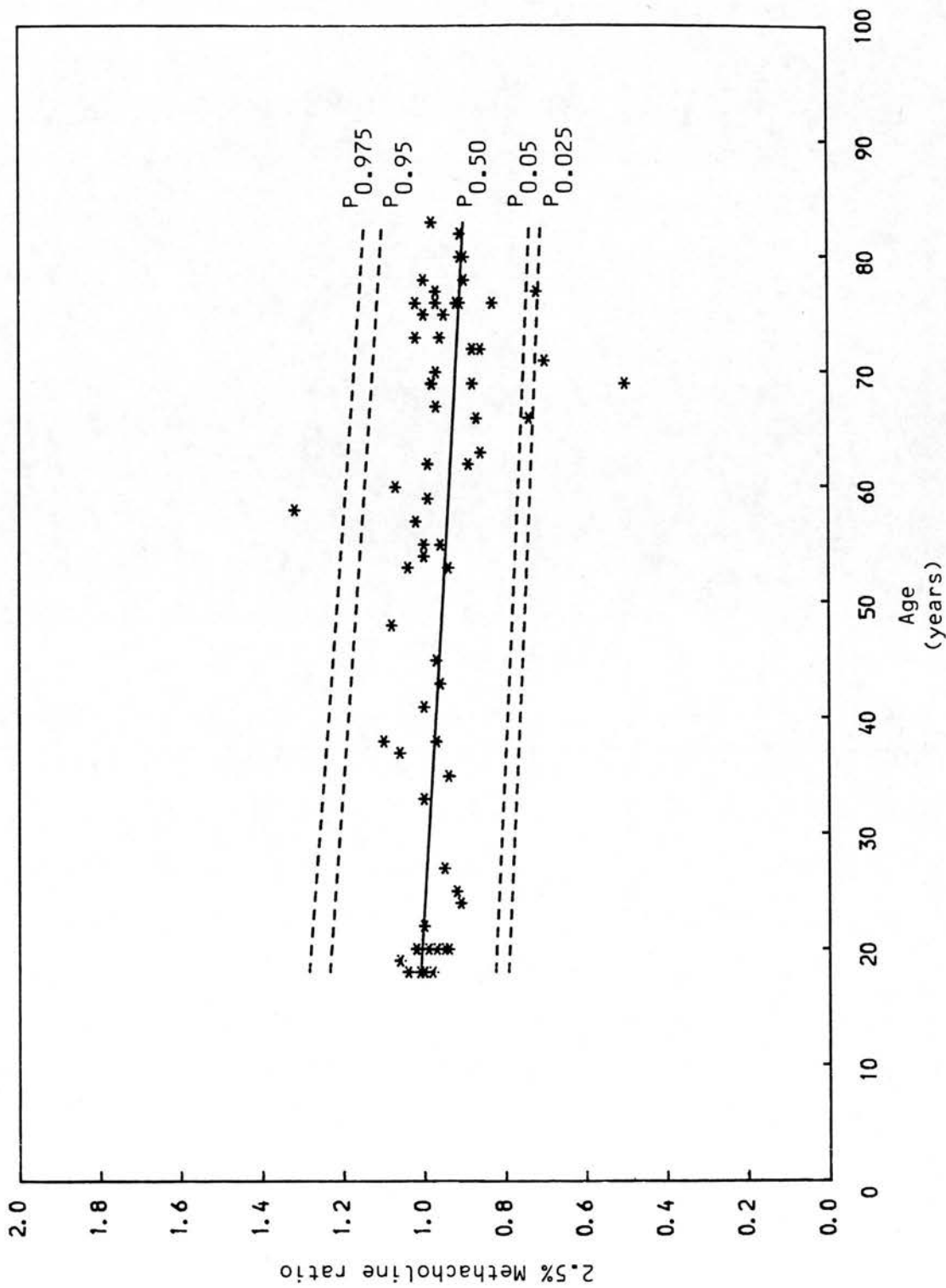


Figure 12

AGE-ADJUSTED NORMAL TOLERANCE INTERVALS FOR 0.1% PHENYLEPHRINE RATIO

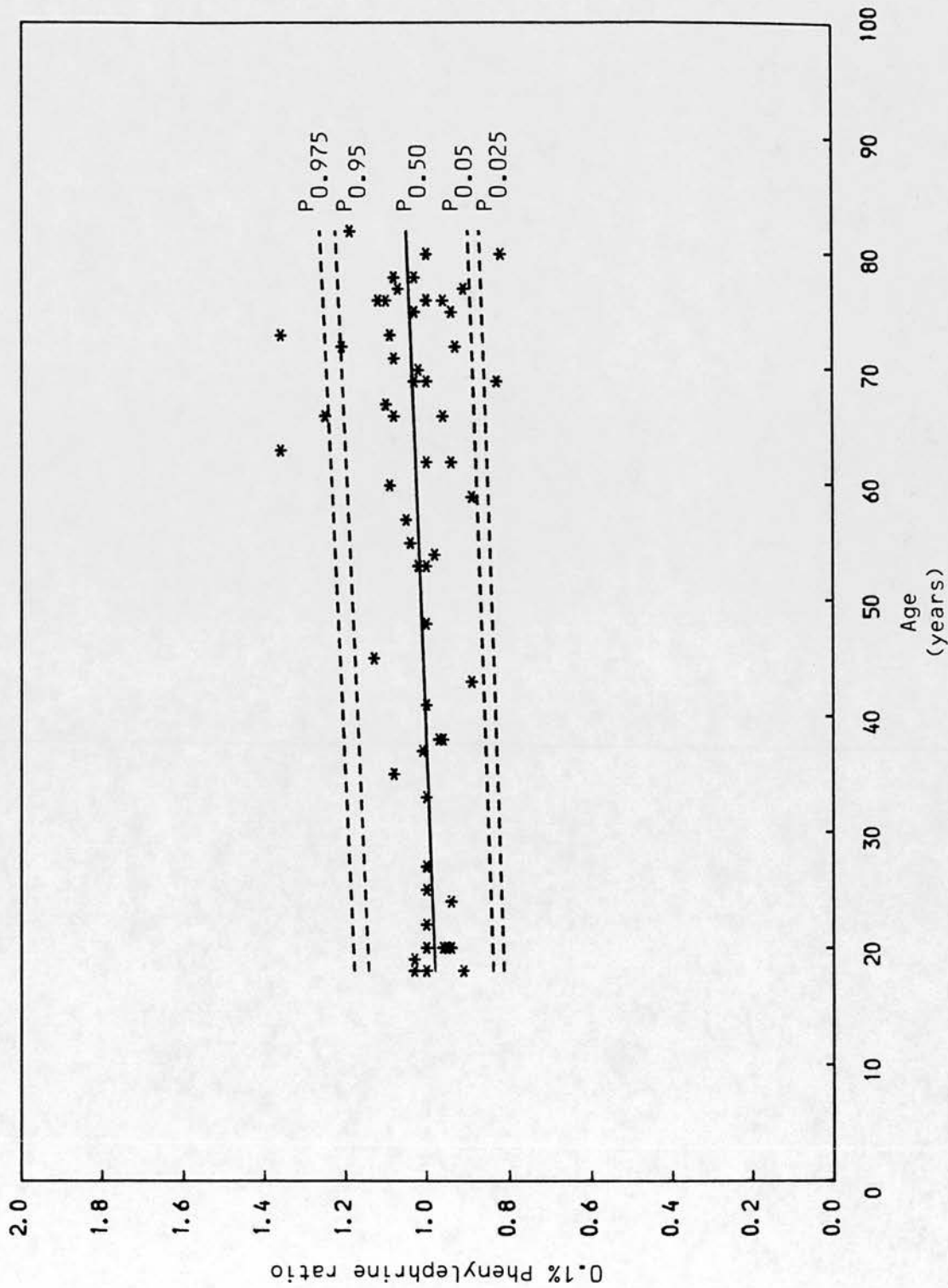
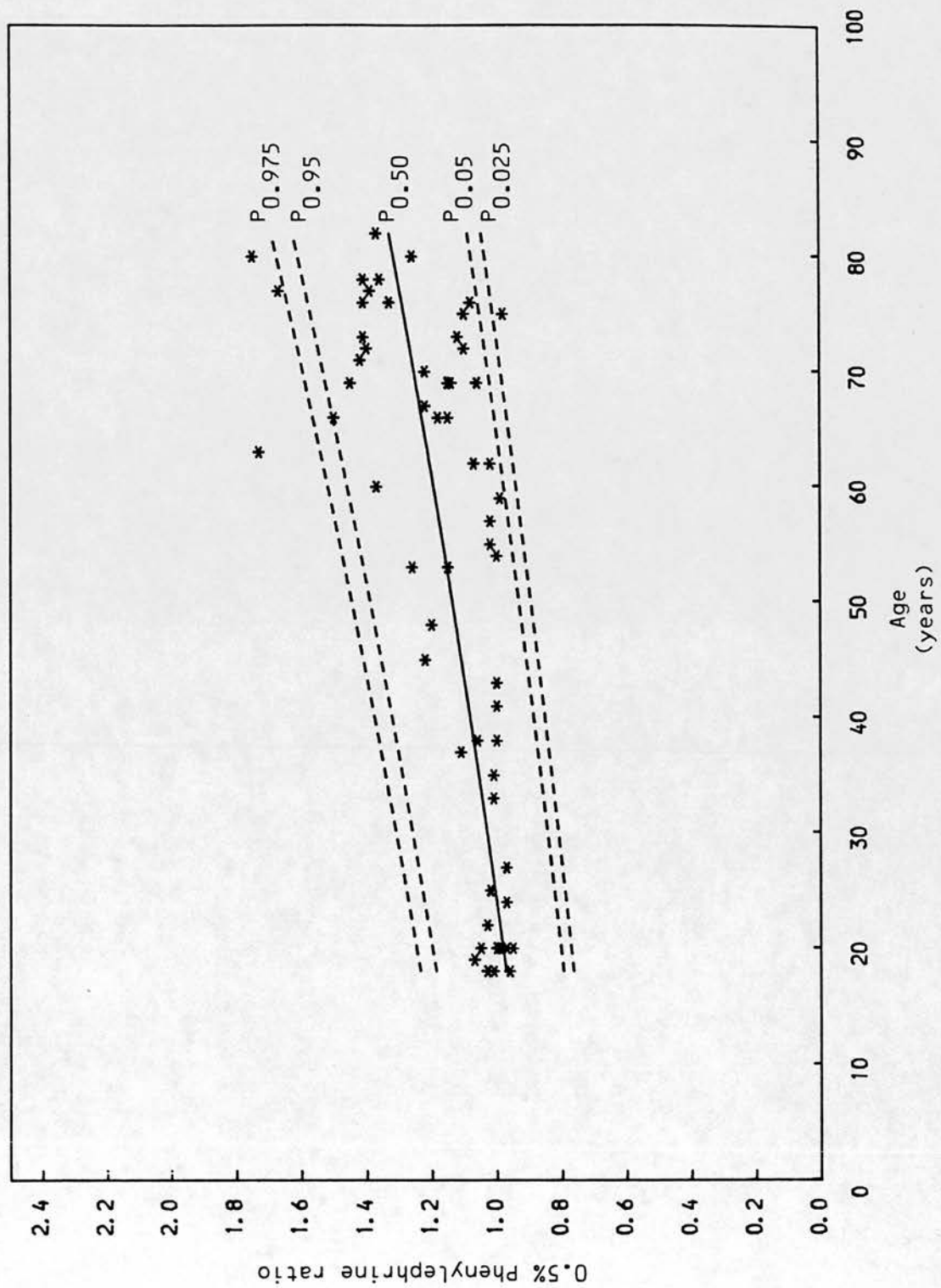


Figure 13

AGE-ADJUSTED NORMAL TOLERANCE INTERVALS FOR 0.5% PHENYLEPHRINE RATIO



9.32 2.5% methacholine ratio, 0.1% phenylephrine ratio, and 0.5% phenylephrine ratio in the primary glaucomas

The results of pupillary responses to 2.5% methacholine, 0.1% phenylephrine, and 0.5% phenylephrine in 117 patients with primary glaucoma and 63 age- and sex-matched control subjects are shown in table 22. In each test of denervation hypersensitivity, comparisons were made between the results of the control group and each category of the primary glaucomas separately; significance was assessed by Student's unpaired t test. Dunnett's multiple comparison procedure was used to obtain a corrected p value (p'), to prevent incorrect conclusions from multiple comparisons with a control. The significance of the results of pupillary responses to 2.5% methacholine, 0.1% phenylephrine, and 0.5% phenylephrine in 117 patients with primary glaucoma compared with results in 63 age- and sex-matched control subjects is summarised in the following paragraphs; the actual p value plus the corrected p value (p') are quoted for significant results.

9.321 2.5% methacholine ratio

2.5% methacholine ratios were significantly lower than the control group in patients with OHWA ($p < 0.001$; $p' < 0.01$), OAGNA ($p < 0.01$; $p' < 0.05$), and OAGWA ($p < 0.01$; $p' < 0.05$). 2.5% methacholine ratios were also significantly lower than the control group in patients with CAG ($p = 0.02$; $p' > 0.05$) and OHNA ($p = 0.02$; $p' > 0.05$), however these were not significant after allowing for multiple comparisons.

9.322 0.1% phenylephrine ratio

0.1% phenylephrine ratios were significantly higher than the control group in patients with OAGNA ($p < 0.001$; $p' < 0.01$) and OHWA ($p < 0.01$; $p' < 0.05$). 0.1% phenylephrine ratios were also significantly higher than the control group in patients with CAG ($p = 0.013$; $p' > 0.05$), however this just failed to retain significance after multiple comparisons were taken into account. 0.1% phenylephrine ratios were not significantly higher than the

control group in patients with OHNA ($p = 0.05$; $p' > 0.05$) and OAGWA ($p > 0.05$).

9.323 0.5% phenylephrine ratio

0.5% phenylephrine ratios were significantly higher than the control group in patients with CAG ($p < 0.001$; $p' < 0.01$), OHNA ($p < 0.002$; $p' < 0.01$), OHWA ($p < 0.002$; $p' < 0.01$), and OAGNA ($p < 0.001$; $p' < 0.01$). 0.5% phenylephrine ratios were not significantly different from the control group in patients with OAGWA ($p > 0.05$).

9.33 Significance of sex on denervation hypersensitivity

Comparisons were made, for 2.5% methacholine ratio/0.1% phenylephrine ratio/0.5% phenylephrine ratio separately, between the results in males versus females, in the control group and each category of the primary glaucomas separately; significance was assessed by Student's unpaired t test. No significant differences ($p > 0.05$) were present, in any category, between the 2.5% methacholine ratio/0.1% phenylephrine ratio/0.5% phenylephrine ratio in male and female subjects.

9.34 Diabetes mellitus and denervation hypersensitivity

The possible association between diabetes mellitus and denervation hypersensitivity was assessed by comparing the results of each test of denervation hypersensitivity (2.5% methacholine/0.1% phenylephrine/0.5% phenylephrine) in diabetic subjects (NIDDM, IGT, NIDDM + IGT separately) with results in non-diabetic subjects, in the control group and each category of the primary glaucomas separately. Significance was assessed by Student's unpaired t test.

No significant differences ($p > 0.05$) in results of denervation hypersensitivity tests were present between diabetic and non-diabetic subjects in any category.

Table 22

2.5% METHACHOLINE RATIO, 0.1% PHENYLEPHRINE RATIO, AND 0.5% PHENYLEPHRINE RATIO IN 117 PATIENTS WITH
PRIMARY GLAUCOMA AND 63 AGE- AND SEX-MATCHED CONTROL SUBJECTS

(Group mean values \pm SEM)

	2.5% Methacholine ratio	0.1% Phenylephrine ratio	0.5% Phenylephrine ratio
Control subjects (n=63)	0.95 \pm 0.01	1.02 \pm 0.01	1.17 \pm 0.03
Closed-angle glaucoma patients (n=45)	0.91 \pm 0.02	1.08 \pm 0.02	1.30 \pm 0.03
Ocular hypertensive patients ALL (n=56)	0.87 \pm 0.01	1.08 \pm 0.02	1.31 \pm 0.03
OHNA (n=26)	0.89 \pm 0.01	1.07 \pm 0.02	1.30 \pm 0.03
OHWA (n=30)	0.85 \pm 0.02	1.10 \pm 0.02	1.31 \pm 0.04
Open-angle glaucoma patients ALL (n=16)	0.84 \pm 0.04	1.13 \pm 0.05	1.30 \pm 0.08
OAGNA (n=7)	0.84 \pm 0.05	1.25 \pm 0.11	1.53 \pm 0.15
OAGWA (n=9)	0.84 \pm 0.07	1.06 \pm 0.03	1.16 \pm 0.07

9.35 Glaucoma treatment and denervation hypersensitivity

The only drug treatments for glaucoma taken by patients included in the study were guttae pilocarpine 2%/4% and/or guttae timolol maleate 0.25%/0.5%. (NB Denervation hypersensitivity was only assessed in eyes not on drug treatment, thus the purpose of this comparison was to assess whether drug treatment to the contralateral eye affected responses to 2.5% methacholine/0.1% phenylephrine/0.5% phenylephrine in the untreated eye.) The possible association between treatment and denervation hypersensitivity was assessed by comparing the results of each test of denervation hypersensitivity separately (2.5% methacholine ratio/0.1% phenylephrine ratio/0.5% phenylephrine ratio) in primary glaucoma patients on treatment with the results in untreated primary glaucoma patients; this was performed for guttae pilocarpine 2%/4% and guttae timolol maleate 0.25%/0.5% separately, in each category of the primary glaucomas. Significance was assessed by Student's unpaired t test.

No significant differences ($p > 0.05$) in the results of denervation hypersensitivity tests were present between primary glaucoma patients treated with guttae pilocarpine 2%/4% and/or guttae timolol maleate 0.25%/0.5% and primary glaucoma patients not on treatment.

9.4 Discussion

Neuropharmacological manipulations are effectively utilised in the localisation of defects in autonomic innervation of the iris musculature. Observations of hypersensitive reactions to topical cholinergic and adrenergic agonists in parasympathetic and sympathetic denervated irides (329,330,332) resulted in the use of dilute guttae methacholine as a pathognomonic test of pupillotonia (333,334), and subsequently to the definitive treatise on denervation hypersensitivity by Cannon and Rosenbluth in 1949 (336). The efficacy of the "Mecholyl test" in the detection of anterior segment parasympathetic denervation has been confirmed by several studies (399,400). Although various pharmacological agents have been used in the diagnosis of sympathetic neuropathy of the anterior segment (346,352), "normal" responses are not established; definitive conclusions are therefore not possible. Phenylephrine is a direct-acting sympathomimetic amine, a specific alpha-agonist which effects mydriasis by alpha-adrenoceptor stimulation of the sympathetically-innervated dilator pupillae (398). Studies in subjects with Horner's syndrome have confirmed that pupillary responses to phenylephrine are enhanced in the presence of sympathetic denervation (401). Guttae 1% and 2% phenylephrine have been utilised in this context, however mydriasis - to a variable degree - is a "normal" response (346), and thus the promotion of these concentrations as diagnostic in the determination of sympathetic denervation hypersensitivity in the anterior segment is open to reasonable doubt. Responses to guttae 0.1% phenylephrine and 0.5% phenylephrine have not, to my knowledge, been previously reported. Mydriatic ratios in an age-matched control group confirmed that responses to 0.1% phenylephrine and 0.5% phenylephrine are age-dependent, with mean \pm SD increase in post-phenylephrine pupil diameter in a ratio of 1.03 ± 0.04 mm (0.1% phenylephrine) and 1.22 ± 0.05 mm (0.5% phenylephrine) at age 65 years. Similarly, responses to guttae 2.5% methacholine are significantly related to age, with a post-methacholine pupil diameter ratio of 0.93 ± 0.05 mm in the control group at age 65 years. The determination of autonomic denervation in the anterior segment would therefore seem to be facilitated by neuropharmacological manipulation with these

concentrations of autonomic neurotransmitters.

Pupillary constriction following administration of topical 2.5% methacholine was significantly greater than the control group in each category of the primary glaucomas - CAG, OHNA, OHWA, OAGNA, OAGWA - although CAG and OHNA just failed to retain significance after allowing for multiple comparisons with a control. These results support the hypothesis of parasympathetic denervation of the iris in the primary glaucomas.

Assessment of ocular sympathetic integrity confirms that the pupil demonstrates increased sensitivity to low concentrations of phenylephrine in several of the primary glaucomas. Pupillary mydriasis following topical 0.1% phenylephrine was significantly greater than the control group in patients with CAG, OHWA, and OAGNA; mydriatic ratios in patients with OHNA just failed to reach statistical significance ($p = 0.05$). Pupillary mydriasis following topical 0.5% phenylephrine was significantly greater than the control group in patients with CAG, OHNA, OHWA, and OAGNA. Increased sensitivity to minute concentrations of sympathomimetic amines was not present in patients with OAGWA.

A high incidence of parasympathetic denervation of the iris has been reported in patients with diabetes mellitus (402); measurable miotic responses to 2% methacholine were noted in 81% of diabetic subjects compared with 8% of a comparable control group. The increased response to methacholine in subjects with diabetes mellitus was confirmed by Hayashi and Ishikawa (1979) who also demonstrated an increased mydriatic response to 1% adrenaline, suggesting concomitant sympathetic neuropathy (403). This is of particular relevance in view of the significant association between diabetes mellitus and the primary glaucomas.

In this study, the presence of autonomic denervation hypersensitivity was not significantly related to diabetic status. Thus although both diabetes mellitus and anterior segment autonomic neuropathy are significantly associated with primary glaucoma, autonomic denervation is apparently not directly associated with diabetes.

These results must be interpreted with caution, as autonomic neuropathy is unlikely to be an isolated complication of diabetes mellitus in the anterior segment; lesions of connective tissue, blood vessels and smooth muscle are frequently present in diabetic irides (404). Fujii, Ishikawa and Uga (1977) noted autonomic nerve endings to be virtually non-existent in diabetic irides compared with control subjects, confirming the presence of autonomic neuropathy; three pathological manifestations of myopathy were described in the iris muscles (dilator and sphincter pupillae) of diabetic patients: lamellar structures, vacuoles, and lipid droplets (404). Quantitative assessments of autonomic integrity are dependent upon an intact effector; restrictions of structural mobility may conceal concomitant denervation hypersensitivity by preventing responses to topical neurotransmitters.

Parasympathetic denervation hypersensitivity, manifest as significantly-increased pupillary responses to topical 2.5% methacholine, is associated with CAG, OH (NA and WA), and OAG (NA and WA). Sympathetic denervation hypersensitivity is associated with CAG, OH (NA and WA), and OAGNA; responses to concentrations of direct-acting sympathomimetic amines suggesting autonomic denervation were not significantly different from normal in patients with OAGWA. Tests based upon pupillary responses to topical agents, although apparently assessing the efficacy of individual components of the autonomic nervous system, do not definitively localise parasympathetic and sympathetic dysfunction, as pupillary diameter is determined by relative sympathetic and parasympathetic activity in the anterior segment; significantly-increased pupil constriction in response to 2.5% methacholine may be facilitated by relatively-decreased sympathetic efficacy in patients with primary glaucoma. Similarly, significantly-increased pupil mydriasis in response to 0.1% phenylephrine and 0.5% phenylephrine may be facilitated by relatively-decreased parasympathetic efficacy in patients with primary glaucoma. The most important conclusion from these results is that relative autonomic efficacy in the anterior segment of the eye is impaired in the primary glaucomas; inferences regarding individual components of the autonomic nervous system may only be made with allowance for possible facilitatory effects resulting

from impairment of the antagonist.

One may conclude that parasympathetic neuropathy of the iris is associated with the primary glaucomas. Although proximally-situated lesions in the inter-neurone autonomic efferent outflow effect lesser degrees of hypersensitive reactions, responses to diagnostic neuropharmacological tests of denervation are maximal in lesions of the postganglionic neurone (346); with responses of such a significant degree in primary glaucoma patients, one must assume the most likely site of autonomic neuropathy to be the postganglionic neurone, with consequent increase in either the number or activity of receptors on the effector (smooth muscle) surface. Similar considerations are applicable to the sympathetic innervation of the iris musculature, with the exception of patients with OAGWA; sympathetic denervation hypersensitivity is not a feature of these patients. Autonomic neuropathy is not characteristically a localised disorder; it is unlikely that significant autonomic neuropathy in the anterior segment of the eye is restricted to the iris.

The relevance of anterior segment autonomic neuropathy in the primary glaucomas will be examined within the context of previous results in the final discussion.

PLATES

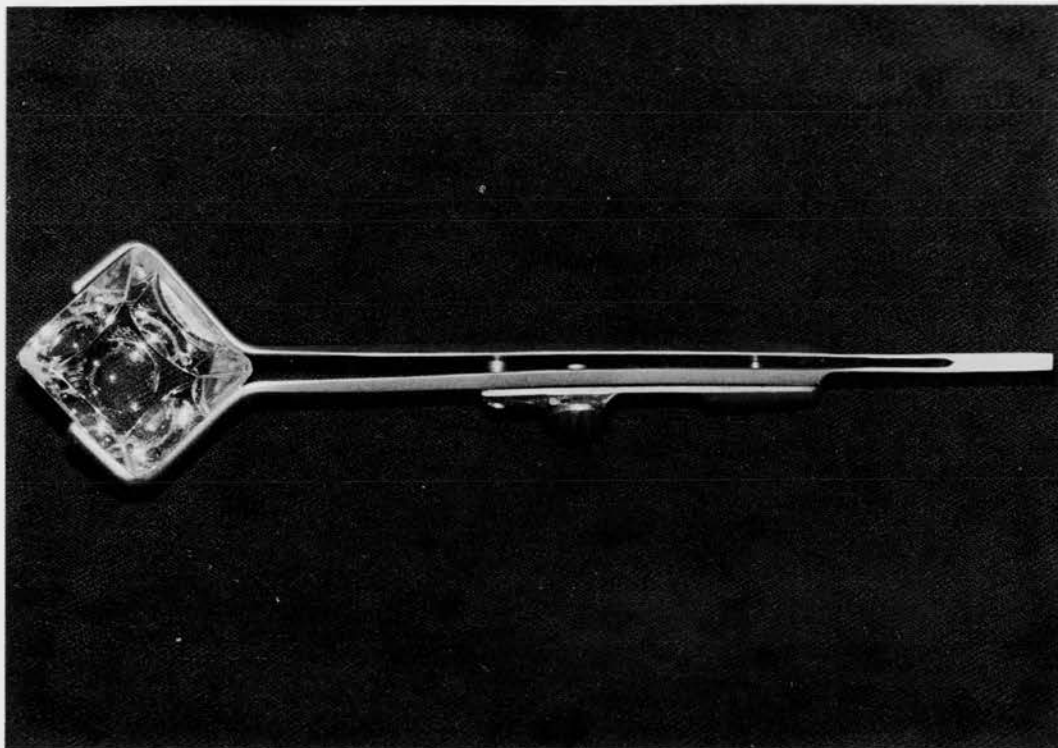


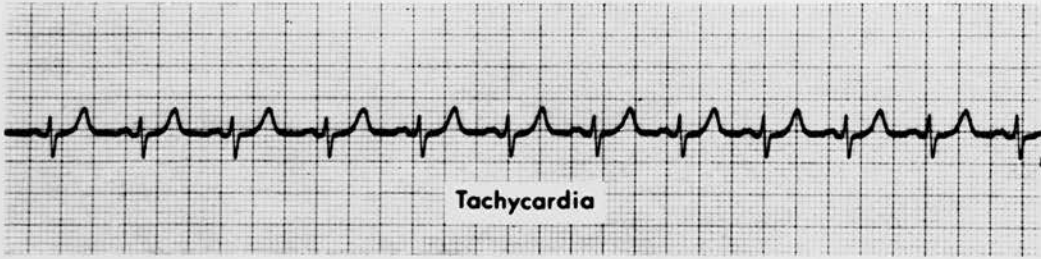
PLATE 1

Valsalva
commenced



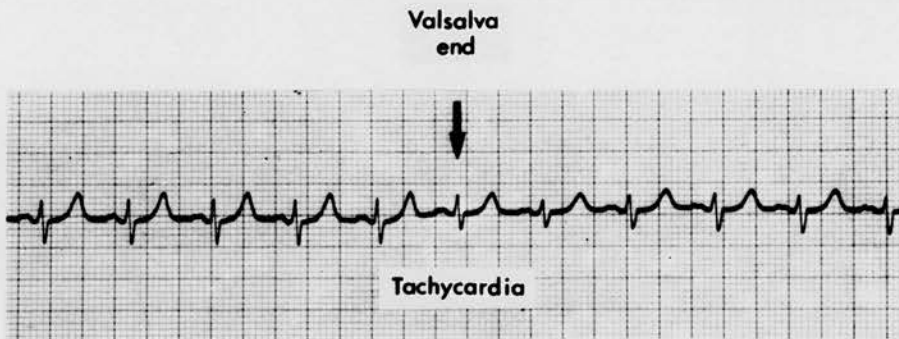
PHASE 1

PLATE 2



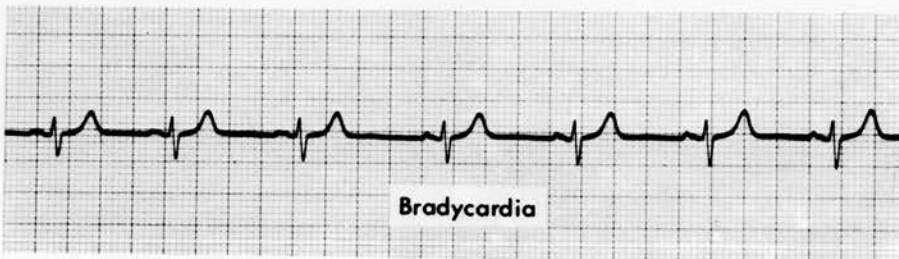
PHASE 2

PLATE 3



PHASE 3

PLATE 4



PHASE 4

PLATE 5

Inspiration

Expiration

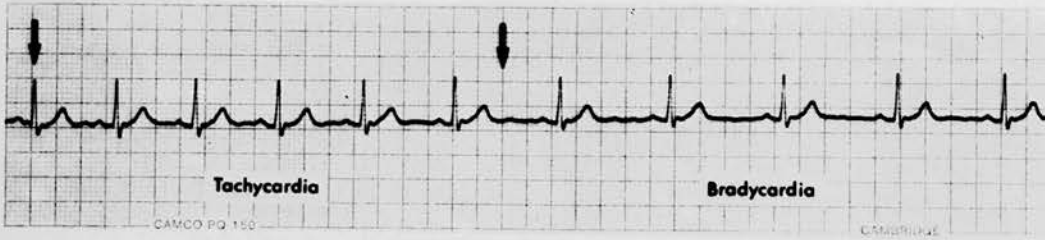


PLATE 6

15th beat

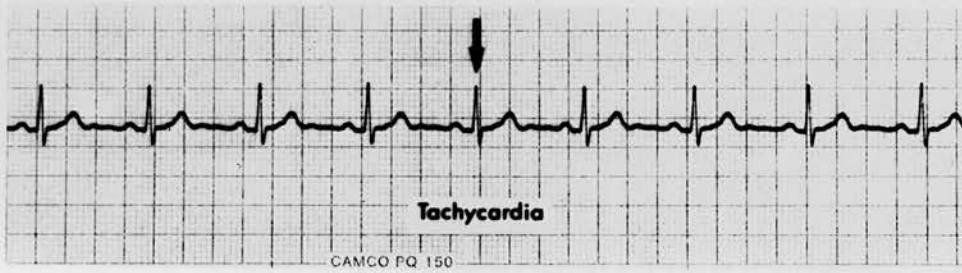


PLATE 7

30th beat

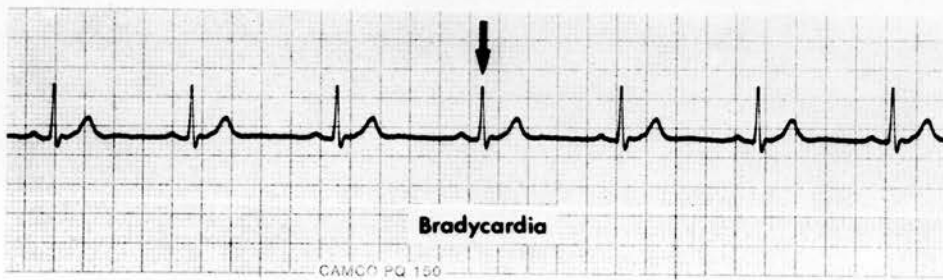


PLATE 8

Standing

Lying



PLATE 9

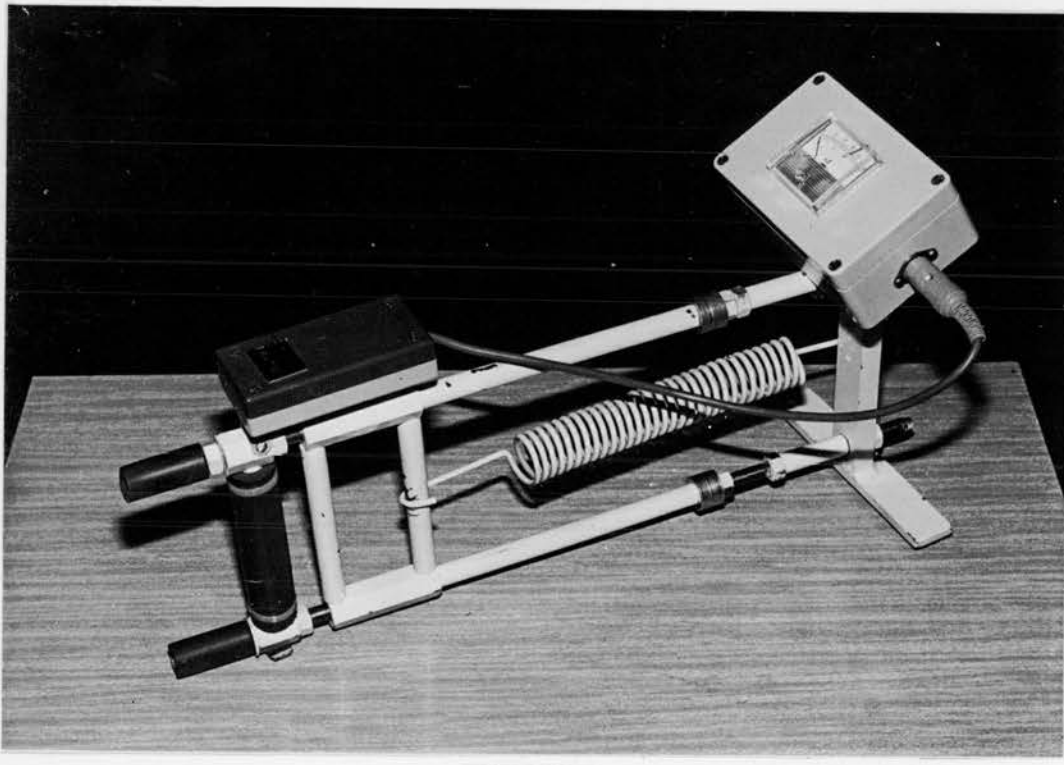


PLATE 10

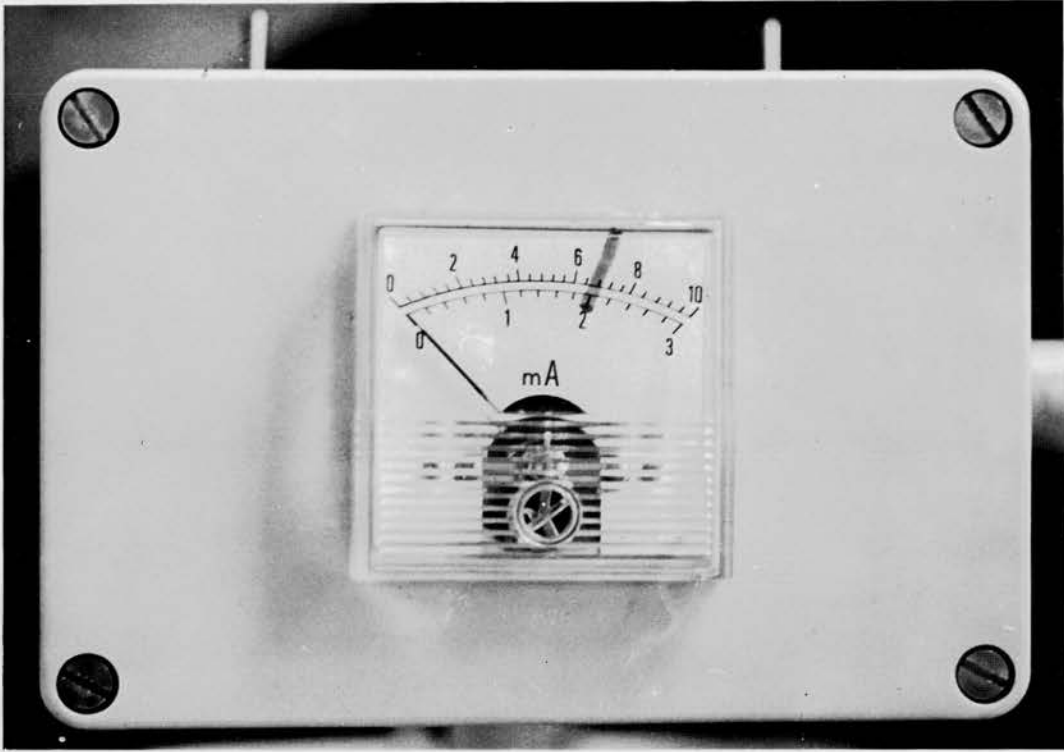


PLATE 11

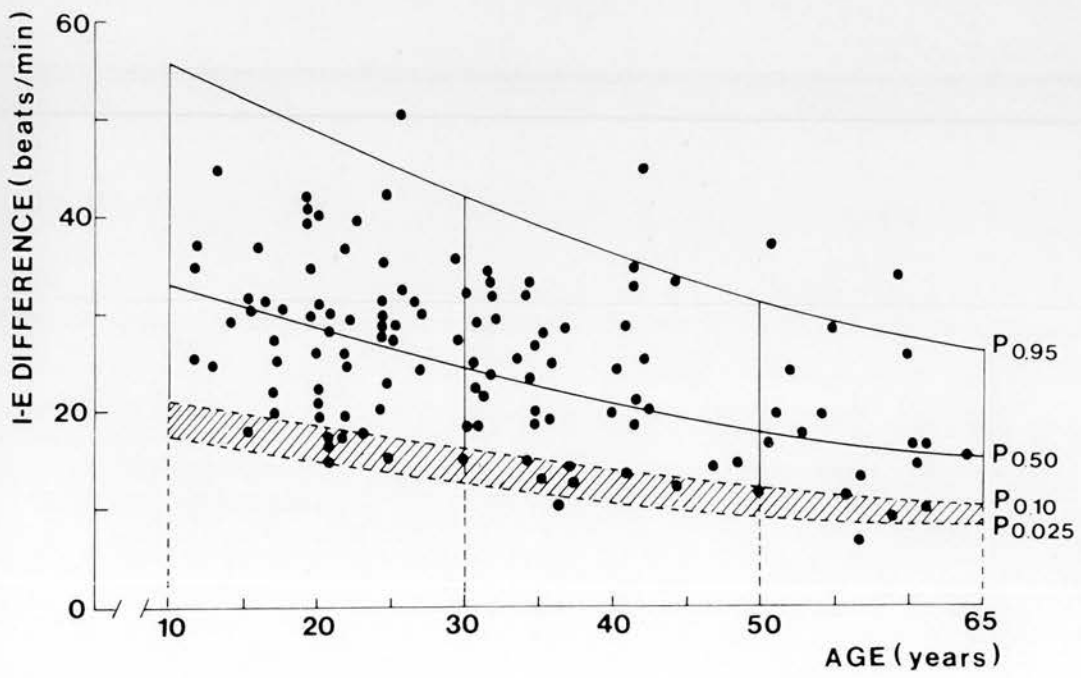


PLATE 12

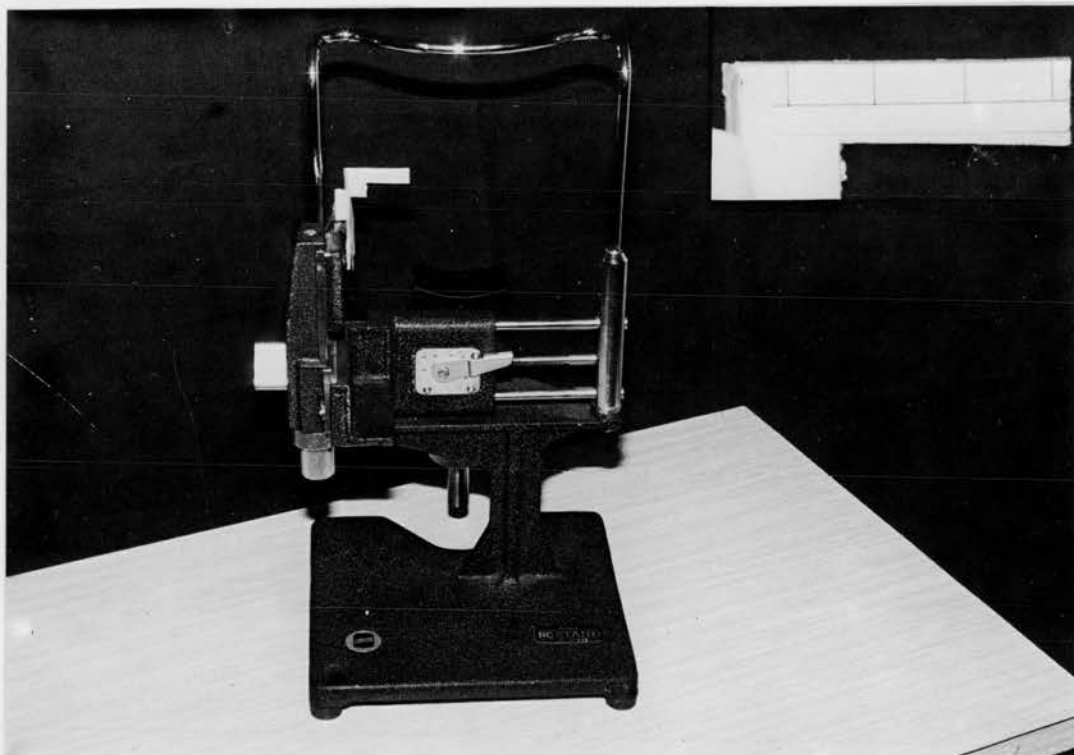


PLATE 13



PLATE 14



PLATE 15

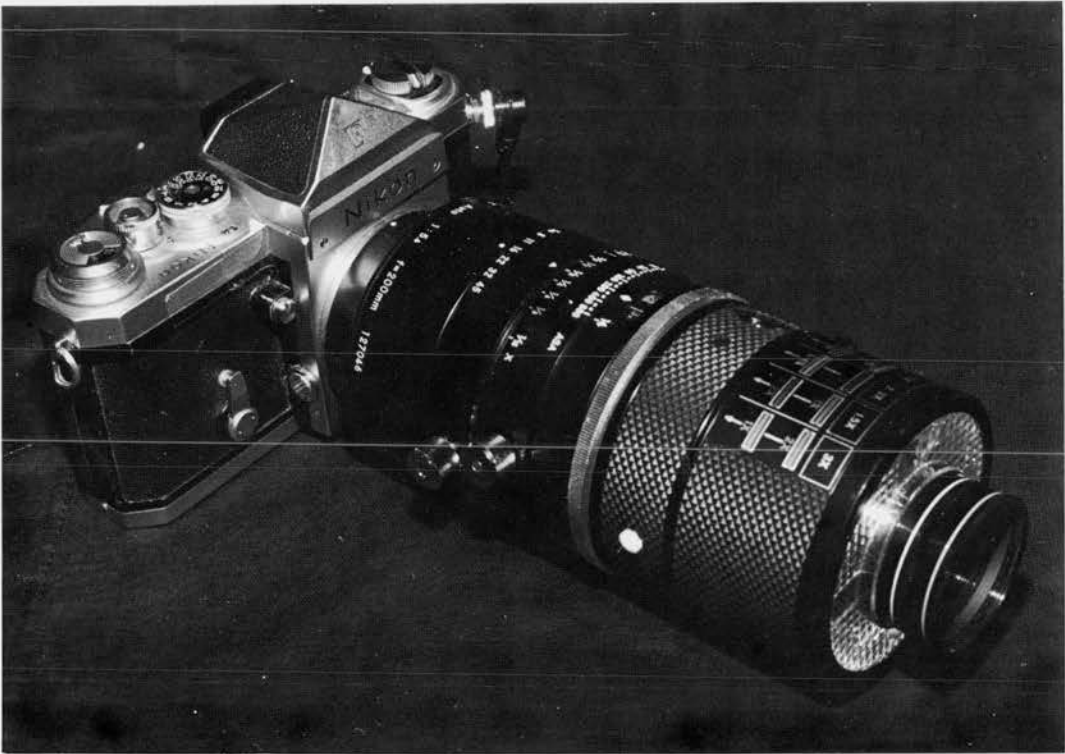


PLATE 16

SECTION V

DISCUSSION AND CONCLUSIONS

CHAPTER 10

DISCUSSION

The essential anterior segment event precipitating irido-corneal contact, and consequently angle-closure, is postulated to be a change in autonomic activity - with particular emphasis on parasympathetic tone (155). The depth of the anterior chamber is not a static dimension, and may show rapid and transient changes (159); alterations in depth are directly related to the pupil block force (52,103), itself a manifestation of autonomic activity in the anterior segment. The pathogenesis of angle-closure is described in the introductory text; the salient points are as follows. The vector force, representing the resolution of the constituent elements of pupil block, increases iris-lens apposition. In eyes without a peripheral iridectomy, aqueous flow from the site of production (ciliary processes) to that of escape (trabecular meshwork and uveoscleral route) can only occur via the pupil; increased pupillary block thus results in decreased aqueous flow from the posterior to the anterior chamber. As facility of outflow remains unaltered, impedance of aqueous flow via the pupil results in the development of a pressure differential across the iris-lens diaphragm, with increasing pressure in the posterior chamber (effected by continuing aqueous production with impaired outflow) and decreasing pressure in the anterior chamber (continuing outflow with impaired inflow). In a biological system capable of marked variation, changes in the dimensions of the anterior chamber necessarily follow; for example, the volume of the anterior chamber decreases by 16% in the fellow eye of a patient with closed-angle glaucoma during the course of a provocative test with pilocarpine and phenylephrine (54). Anterior translational movement of the iris-lens diaphragm facilitates irido-corneal contact and thus subsequent angle-closure: if the anterior chamber shallows by 0.2 mm, the distance between peripheral iris and cornea decreases by 0.08 mm; if the anterior chamber shallows by 0.33 mm, the irido-corneal gap narrows by 0.12 mm (103). As irido-corneal separation in eyes with narrow angles is only approximately 0.15 mm (155), relatively-minor anterior displacement of the iris-lens diaphragm may cause

disproportionate decreases in the irido-corneal interval, with eventual irido-corneal contact. The pilocarpine-phenylephrine provocative test, effecting maximal parasympathetic and sympathetic stimulation in the anterior segment, has a sensitivity of 93% and a predictive value of 94% (160). Selective pharmacological manipulation of the autonomic nervous system in the anterior segment of the eye thus satisfies the requirements of an effective model of angle-closure, supporting the hypothesis that events preceding irido-corneal apposition occur as a direct result of changes in autonomic activity.

Having established this fundamental principle, one must determine the predisposition in certain individuals to develop the necessary change in ocular autonomic activity precipitating angle-closure. Assessment of cardiovascular autonomic reflexes correlates closely with autonomic function in other systems (216,217), and thus effectively provides an objective, quantifiable, and reproducible measure of generalised autonomic function. On this basis, the present results indicate that systemic autonomic dysfunction is associated with the primary glaucomas, with significantly-increased prevalence in patients with narrow-angles. Impairment of systemic autonomic integrity is restricted to the parasympathetic component of the autonomic nervous system - although results of sympathetic assessment must be viewed within the context of the limited sensitivity of sympathetic cardiovascular reflex tests compared with those of the parasympathetic nervous system, the former only becoming positive during advanced stages of sympathetic neuropathy. The distribution of systemic parasympathetic dysfunction is similar to that of abnormal glucose tolerance in the primary glaucomas, with increased prevalence in narrow-angle categories. A salient difference between both features associated with primary glaucoma was that the prevalence of parasympathetic dysfunction was significantly increased relative to the control group in every category of the primary glaucomas studied, with particularly elevated levels in those with narrow angles, however the prevalence of abnormal glucose tolerance was only significantly increased in narrow-angle categories.

A similar pattern emerges from the results of pupil cycle time assessment in the primary glaucomas; pupil cycle time was significantly prolonged only in narrow-angle categories (CAG and OHNA). The duration of pupil cycle time in OHWA was not significantly different from the control group after allowing for multiple comparisons. As previously explained, the exact site of the lesion cannot be definitively localised in an assessment of the reflex arc as a single unit, however as concurrent detailed visual field assessment revealed no evidence of afferent defects in patients with CAG, OHNA and OHWA, one may reasonably postulate the lesion prolonging the duration of the pupillary reflex to be located within the efferent parasympathetic component, a hypothesis supported by the evidence of concomitant systemic parasympathetic dysfunction in these patients.

Impairment of autonomic integrity in the anterior segment of the eye was confirmed by the demonstration of denervation hypersensitivity in the irides of primary glaucoma patients. Hypersensitive responses to cholinergic agonists were present in each category of the primary glaucomas studied; hypersensitive responses to adrenergic agonists were present in CAG, OHNA, OHWA, and OAGNA, but significantly not present in patients with OAGWA. Although lesser degrees of increased response to concentrations of neurotransmitters indicative of denervation are associated with proximal nerve lesions of the efferent outflow, hypersensitive responses are maximal in lesions of the postganglionic neurone (335); the significant responses associated with glaucoma imply the probable site of degeneration to be the postganglionic neurone, although co-existent proximal lesions cannot be excluded.

The association between diabetes mellitus and the primary glaucomas has been accurately defined by specific determination of diabetes prevalence in each of the major categories of primary glaucoma. Concurrent examination of a comparable age-matched control group permits existing data to be viewed in perspective, whilst prevalence levels have been determined in categories where data was previously inconclusive or absent (ie OH and CAG respectively). The type of diabetes mellitus associated with primary glaucoma is specifically

type 2 diabetes (NIDDM) and impaired glucose tolerance; the prevalence of type 1 diabetes (IDDM) in the primary glaucomas is similar to that in the control group.

Significantly-increased prevalence of abnormal glucose tolerance is restricted to primary glaucomas with narrow irido-corneal angles: CAG and OHNA. Accurate comparisons with previous studies, employing different diagnostic criteria, is not possible, however several salient features are worthy of note. The prevalence of diabetes mellitus in patients with OAG has been reported as 18.3% (112) and 20% (132); unfortunately, control values were not concurrently determined. These statistics compare favourably with the presently-defined prevalence of abnormal glucose tolerance in patients with OAGWA (the commonest form of OAG), 23.8%, which is not significantly different from the prevalence of abnormal glucose tolerance in the comparable control group (17.5%). Similarly, the reported prevalence of diabetes mellitus in patients with OAG by studies not specifically determining diabetic status by OGTT was 8% (131) and 7.6% (122) - compared with diabetes prevalence in the present control group of 8.1%.

The association between diabetes mellitus and the primary glaucomas has been confirmed, although detailed investigation revealed the association to be different from previous assumptions. The prevalence of diabetes mellitus - specifically NIDDM and IGT - was only significantly increased in CAG and OHNA. Glycosylated haemoglobin levels were significantly increased only in narrow-angle primary glaucomas: CAG, OHNA, and OAGNA. Significant, though less direct, associations with wide-angle categories were present in familial history, with significantly increased prevalence of familial NIDDM in patients with CAG and OHWA. Of more importance is the obvious link connecting CAG, OH, and OAG; the common association with NIDDM suggests that these discrete categories within the heterogeneous primary glaucomas are unlikely to represent mutually-exclusive entities, with more than merely similar external manifestations in the form of raised intraocular pressure.

The role of diabetes mellitus in the pathogenesis of glaucoma

is probably multifactorial; diabetic effects on autonomic nerve function are likely to be prominent in this context. Systemic parasympathetic dysfunction is a characteristic feature - in variable proportions dependent upon category - of the primary glaucomas, and diabetes mellitus - also significantly associated with primary glaucoma - is the commonest cause of autonomic neuropathy in this country (170). The prevalence of abnormal cardiovascular parasympathetic function parallels that of diabetes mellitus in the primary glaucomas; parameters of parasympathetic function were significantly associated with diabetes mellitus in patients with OHNA and OHWA.

Severe systemic complications may occur secondary to diabetes mellitus, principally vasculopathy, nephropathy, retinopathy and neuropathy (somatic and autonomic), although almost every system can be affected. Adverse effects of diabetes mellitus are equally ubiquitous in the eye; anterior and posterior segments are frequently involved. Although complications in the posterior segment, with potentially-blinding retinopathy, are more comprehensively documented, anterior segment structures are also affected - with inevitable consequences in the pathogenesis of glaucoma. The lens in diabetic eyes is characteristically large (134), thus facilitating closure of the irido-corneal angle by mechanical shallowing of the anterior chamber. Rapid changes in lens dimensions, resulting in varying refraction, are associated with fluctuating levels of plasma glucose in diabetics (405). Parasympathetic neuropathy of the anterior segment, determined by pupillary responses to 2% methacholine, is reported to be present in 81% of unselected diabetic patients (402); denervation hypersensitivity, determined by pupillary responses to 0.1% adrenaline, implying significant neuropathy of the sympathetic nervous system, have an estimated prevalence of 28.9% in diabetics with background retinopathy, although only 2.4% in diabetics without retinopathy (403). Pupillary abnormalities are well-recognised complications of diabetes mellitus, particularly impaired reflex responses to light, and excessive miosis (406,407); these have been variously attributed to somatic neuropathy (408) and autonomic neuropathy (409). The efficacy of pupillary responses has been correlated with diabetic control; pupillographic studies

demonstrated a direct relationship between efficacy of pupil response and co-existent metabolic stability (410).

Diabetes mellitus has been implicated in the aetiology of iritis for over 100 years (411). Whittington and Lawrence (1951) described a rare albuminous iritis associated with diabetes mellitus, and characterised by dilated iris vessels which subsided with the termination of iritis, in marked contrast to the established features of rubeosis iridis (412). A recent study reported the presence of iritis in 30% of diabetics with autonomic neuropathy, postulating an association between diabetic autonomic neuropathy and iritis based on a common immunological aetiology from cross-reaction of insulin antibodies and nerve growth factor (413).

Iridopathy is a recognised complication of diabetes mellitus (414,415); in addition to glycogenous atrophy of the iris (122), lesions may be present in connective tissue (415), smooth muscle (404), and blood vessels (416,417). Subsequent impairment of iris mobility necessarily influences methods of ocular autonomic function assessment dependent upon measurement of end-organ responses to determine neurological integrity. This is particularly relevant in the assessment of denervation hypersensitivity; significant neuropathy may be present though not revealed on specific testing because of concomitant restrictive iridopathy.

Diabetes mellitus is significantly associated with the primary glaucomas; several aetiological mechanisms are implicated in the pathogenesis of glaucoma, resulting from the ubiquitous nature of diabetic complications in anterior segment structures, however present evidence suggests autonomic dysfunction to be the most significant.

Accurate determination of autonomic integrity in the primary glaucomas was obtained by the integration of separate, though complementary, assessment techniques. Several well-defined trends consistently emerged from the results of each section. Systemic autonomic dysfunction, specifically localised to the parasympathetic nervous system, was significantly associated with CAG, OH, and OAG,

with increased prevalence in narrow-angle categories.

Parasympathetic neuropathy of the anterior segment, by the diagnostic criterion of denervation hypersensitivity, was also established as a feature of each major category of the primary glaucomas; sympathetic neuropathy of the anterior segment, by the same criterion, was a significant feature of CAG, OHNA, OHWA, and OAGNA. The integrity of the pupillary reflex arc, assessed by pupil cycle time, was impaired only in narrow-angle primary glaucomas. Thus autonomic dysfunction, systemic and localised, is significantly associated with the primary glaucomas. Parasympathetic dysfunction is present, at both systemic and local level, in every category studied; sympathetic dysfunction is only demonstrable in ocular structures - and specifically not in OAGWA.

The prevalence of autonomic nerve dysfunction in each of the major categories of the heterogeneous group of disorders encompassed within the title of "primary glaucomas" confirms the existence of an association between these formerly-distinct entities, and inevitably suggests the presence of a common aetiological factor in pathogenesis. Marked similarities between these apparently-dissimilar constituents of the primary glaucoma population have been previously shown in responses to autonomic provocation, observations which are amenable to logical explanation on the basis of the present results. The role of autonomic dysfunction in the aetiology of CAG, OH, and OAG necessitates a brief reiteration of pathophysiological mechanisms in angle-closure. Closure of the irido-corneal angle in the anterior chamber is, by definition, a dynamic process. The relative contribution of anterior translational movement of the iris-lens diaphragm and peripheral iris bombé remains a source of debate, however the basic aetiological factor common to both mechanisms is pupil block, a direct result of relative parasympathetic and sympathetic activity in the anterior segment. Pupil block causes pressure inequalities between the posterior and anterior chambers, with consequent shallowing of the anterior chamber. It may be resolved into 3 separate components:

- i. force due to contraction of the sphincter muscle
- ii. force due to contraction of the dilator muscle
- iii. modulus of elasticity of the iris stroma

Contraction of antagonistic iris musculature is a dynamic variable; thus pupil block represents a constantly-changing vector, dependent upon the relative emphases of the 3 contributing elements. Sphincter and dilator pupillae (the effectors of parasympathetic and sympathetic nerve stimulation) though diametrically opposite in effects on pupil diameter, are not of equal potential magnitude; the maximally-stimulated sphincter muscle may develop twice the power of the maximally-stimulated dilator muscle (151). In consequence, parasympathetic tone has been proposed as the major determinant of pupil block (155). Impairment of parasympathetic nerve function effects a partial dilation of the pupil, thereby increasing pupil block. It has been shown that pupil block force is maximal at a pupil diameter of 3.8-4.2 mm, with exponential decrease in pupil block force as the pupil diameter either increases or decreases from this value. A concomitant denervation of sympathetic nerve function in the anterior segment would render the pupil relatively immobile; in the presence of a mid-dilated pupil, maximal pupil block would be sustained, with inevitable dimensional changes in the anterior chamber possibly terminating in overt angle-closure.

Angle-closure is not exclusively restricted to CAG; this principle is of fundamental importance in the determination of common aetiological factors in the pathogenesis of the primary glaucomas. In OH and OAG, autonomic provocation by simultaneous installation of guttae 2% pilocarpine and 10% phenylephrine produces significant partial closure of the anterior chamber angle, with consequent impairment of aqueous outflow. Responses to pilocarpine-phenylephrine provocation in patients with OAG were similar to "normal" eyes in only 22% of cases; positive provocative tests were significantly associated with narrow angles (418). In OH, 45% of subjects demonstrated closure of all or part of the irido-corneal angle following autonomic provocation; results were directly related to angular configuration, with angle-closure in 88% of patients with grade 1 angles (Shaffer angle-grading), 69% of patients with grade 2 angles, and 11% of patients with grade 3 angles (419). Therefore angle-closure is not an all-or-none phenomenon; it is only when the angle is virtually completely closed that intraocular

pressure rises precipitously, manifesting the characteristic signs of CAG. Intermittent partial angle-closure produces intermittently-raised intraocular pressure, with inevitable damage to outflow mechanisms; repeated episodes result in impaired facility of outflow, which also causes increased intraocular pressure, thus generating a self-perpetuating pathological cycle (419). Whilst field defects are not present, the condition is OH; when field defects occur, the diagnosis becomes OAG.

The configuration of the anterior chamber angle is of marked importance; a narrow irido-corneal angle facilitates angle-closure by permitting a lesser anterior translational movement of the iris-lens diaphragm to effect angle-closure. However it is essential to note that a narrow angle is facilitatory, not diagnostic. Angle-closure, both complete (103) and partial (418), may occur in patients with wide-angles, although not as frequently as in those with narrow-angles (419). If an eye has a narrow angle and no other features associated with glaucoma, the risk of subsequent angle-closure developing is minimal; of 50 eyes with narrow angles from "normal" subjects assessed by autonomic provocation using guttae 2% pilocarpine and 10% phenylephrine, only 1 (2%) developed acute CAG (102). However if features associated with glaucoma are present, the risk of angle-closure rises precipitously; in patients with CAG, OH, and OAG, the probability of angle-closure during pilocarpine-phenylephrine provocation was inversely related to the magnitude of the irido-corneal interval (161,418,419). This probability is closely paralleled by the proportionate distribution of systemic and ocular autonomic dysfunction and abnormal glucose tolerance in the primary glaucomas, with increased prevalence in narrow-angle categories. Thus the narrow angle in isolation is not necessarily important, however in conjunction with established features of glaucoma it assumes marked prognostic significance.

A sequence of events in the pathogenesis of glaucoma may be postulated; angle-closure, mediated by autonomic activity in the anterior segment, has been proposed as a common aetiological mechanism in the primary glaucomas, a hypothesis supported by

results of autonomic provocation in patients with CAG, OH, and OAG. Autonomic dysfunction, particularly of the parasympathetic nervous system, is significantly associated with the primary glaucomas, thereby providing a logical explanation for the aforementioned experimental observations. Autonomic dysfunction in the anterior segment would result in a mid-dilated, relatively-immobile pupil, with inevitable consequences: maximal pupil block and anterior translational movement of the iris-lens diaphragm facilitating angle-closure, particularly in patients with narrow angles.

In addition to effects on angle-closure, autonomic dysfunction may also affect the outflow mechanism directly. Parasympathetic nerve stimulation has been shown to increase facility of outflow by inducing ciliary muscle contraction on the scleral spur, thereby actively "opening" the trabecular meshwork. In the presence of parasympathetic dysfunction, as in primary glaucoma, one may assume this mechanism to be impaired. This has been suggested by the results of previous studies; in the early stages of autonomic provocation, facility of outflow may rise dramatically in response to parasympathetic stimulation by pilocarpine (158). Diminished outflow would exert inevitable effects on intraocular pressure, as previously described.

The aetiology of autonomic nerve dysfunction in the primary glaucomas is not known. Results of several autonomic function tests were significantly associated with diabetes mellitus, and autonomic dysfunction may be secondary to diabetes mellitus in these cases; as diabetes mellitus is the commonest cause of autonomic neuropathy in this country, this assumption is reasonable. However, in the majority of cases there was no obvious predisposing factor; recognised causes of secondary autonomic dysfunction were excluded (table 1). In the absence of evidence to the contrary, one may therefore postulate a primary autonomic dysfunction in these patients. The classification of "primary autonomic failure" encompasses patients in whom symptoms and signs of autonomic nerve dysfunction are present, without known predisposing pathology; primary glaucoma may be included in this category.

CHAPTER 11

CONCLUSIONS

Impairment of autonomic nerve function is a significant feature of the primary glaucomas. Assessment based upon cardiovascular autonomic reflexes, established as an effective index of generalised autonomic integrity, demonstrated the presence of significant systemic parasympathetic dysfunction in each category, with increased prevalence in narrow-angle primary glaucomas. In contrast, sympathetic nerve function was intact, within the limits of sensitivity for these tests. Ocular autonomic function was inferred from objective assessment of denervation hypersensitivity, and evaluation of the pupillary reflex arc by measurement of pupil cycle time. Determination of denervation hypersensitivity permitted assessment of localised parasympathetic and sympathetic nerve function: parasympathetic neuropathy of the anterior segment, by the diagnostic criterion of pupil responses to guttae 2.5% methacholine, was present in each category of the primary glaucomas; sympathetic neuropathy of the anterior segment, by the diagnostic criteria of pupil responses to guttae 0.1% phenylephrine and 0.5% phenylephrine, was present in all categories except OAGWA. Significant impairment of the pupillary reflex arc was restricted to narrow-angle primary glaucomas.

Anatomical configuration of the anterior chamber angle is considered of essential diagnostic importance in the pathogenesis of angle-closure; recently, this principle has been questioned, with the demonstration of angle-closure in patients with wide angles, and the relatively-insignificant proportion of patients (2%) with narrow angles alone (in the absence of any other feature associated with glaucoma) who exhibit positive results to autonomic provocative testing. The results of the present study link these seemingly-incompatible assertions. Currently-proposed mechanisms of angle-closure are based on relative autonomic activity in the anterior segment of the eye; the prevalence of autonomic dysfunction in the primary glaucomas provides a logical explanation for previous experimental observations of dynamic angle-closure during provocative

testing, the details of which are elaborated in the discussion. The possible effects of autonomic dysfunction are shown to concur with present concepts of pathogenetic mechanisms in glaucoma. Autonomic dysfunction, at both systemic and local level, is significantly associated with narrow angles in the primary glaucomas. It would appear that the narrow angle in glaucoma is facilitatory, permitting a lesser degree of anterior translational displacement of the iris-lens diaphragm to effect angle-closure. However narrow angles are probably not pathognomonic in isolation, the pathogenesis of angle-closure depending upon concomitant predisposing features - possibly involving changes in the autonomic integrity of the anterior segment. The prevalence of diabetes mellitus in narrow-angle primary glaucomas (CAG and OHNA) supports this assertion. Diabetes mellitus is a well-recognised cause of autonomic dysfunction, and present results of cardiovascular autonomic function are significantly related to diabetic status in several of the primary glaucomas; autonomic dysfunction may represent an effect of diabetes mellitus on the pathogenesis of glaucoma in these patients. Diabetic complications may also be present in other anterior segment structures - including blood vessels, connective tissue, and smooth muscle - with inevitable impairment of iris function. These alternative adverse effects of diabetes mellitus may be equally implicated in the subsequent development of glaucoma, as previously described.

The precise aetiology of disordered autonomic function in the primary glaucomas is not known. Although autonomic dysfunction may be secondary to diabetes mellitus in a proportion of cases, no predisposing cause is apparent in the majority of primary glaucoma patients. In the absence of evidence to the contrary, one may therefore presume primary glaucoma to represent a presentation of primary autonomic dysfunction. The angular configuration, irrespective of glaucoma category (CAG, OH, and OAG), is shown to be of central importance in the association between primary glaucoma and autonomic function, suggesting that, on the basis of pathogenetic mechanisms, effective classification of the primary glaucomas is enhanced by angle assessment. Probably the most significant conclusion of this research is that autonomic dysfunction is a

feature common to each of the primary glaucomas studied, thereby establishing an association between CAG, OH, and OAG.

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ABBREVIATIONS

ABBREVIATIONS

CAG	closed-angle glaucoma
cm	centimetre(s)
dl	decilitre(s)
EDTA	ethylenediaminetetraacetate
g	gram(s)
HbA _{1c}	glycosylated haemoglobin
Hg	mercury
IDDM	insulin dependent diabetes mellitus
IGT	impaired glucose tolerance
IOP	intraocular pressure
iv	intravenous
kg	kilogram(s)
kPa	kilopascal(s)
l	litre(s)
m	metre(s)
mg	milligram(s)
min	minute(s)
ml	millilitre(s)
mm	millimetre(s)
mmol	millimole(s)
ms	millisecond(s)
µg	microgram(s)
MVC	maximal voluntary contraction
NA	narrow angles
NIDDM	non-insulin dependent diabetes mellitus
nl	nanolitre(s)
nm	nanometre(s)
OAG	open-angle glaucoma
OAGNA	open-angle glaucoma (narrow angles)
OAGWA	open-angle glaucoma (wide angles)
OGTT	oral glucose tolerance test
OH	ocular hypertension
OHNA	ocular hypertension (narrow angles)
OHWA	ocular hypertension (wide angles)
PCT	pupil cycle time

s	second(s)
SD	standard deviation
SEM	standard error of the mean
SPSSx	statistical package for the social sciences (extended)
WA	wide angles
w/v	weight/volume

APPENDICES

APPENDIX 1

CONSTITUENTS OF 2.5% METHACHOLINE SOLUTION

Methacholine chloride	1.25 g
Phenylmercuric acetate solution 1:32,000	16.0 ml
Water for injection: to	50.0 ml

APPENDIX 2

CONSTITUENTS OF 0.1%/0.5% PHENYLEPHRINE SOLUTIONS

i. 0.1% Phenylephrine solution

Phenylephrine hydrochloride	100 mg
Sodium metabisulphite	500 mg
Sodium citrate	300 mg
Benzalkonium chloride 1:250 w/v	5.0 ml
Water for injection: to	100.0 ml

ii. 0.5% Phenylephrine solution

Phenylephrine hydrochloride	250 mg
Sodium metabisulphite	250 mg
Sodium citrate	150 mg
Benzalkonium chloride 1:250 w/v	2.5 ml
Water for injection: to	50.0 ml

Several points in the text require further clarification:

3.1 Definition of the primary glaucoma and control samples

i. Control subjects

The control group comprised 85 subjects (mean age 66.1 ± 10.2 years), 83 subjects attending the casualty department with no detectable abnormalities (mean age 66.5 ± 10.2 years) and 2 members of hospital staff (mean age 58.6 years). 74 subjects consented to undergo oral glucose tolerance test to assess diabetic status, 84 subjects were able to supply details regarding family history of diabetes, 70 subjects were eligible to be included in the assessment of pupil cycle time - after application of the exclusion criteria stipulated on p. 120 - and 63 subjects consented to assessment of anterior segment denervation hypersensitivity. Control subjects were attending for refraction, or had been referred by an optician for an ophthalmological opinion; no detectable abnormality was present in all subjects, following comprehensive ocular assessment. As attendance for routine refraction in an older age group, with progressively-increasing presbyopia, is normal, this represents a self-selected control sample with no subjective bias.

ii. Primary glaucoma patients

All patients studied were attending the Glaucoma Unit at St Paul's Eye Hospital, Liverpool. All patients diagnosed to have primary glaucoma in St Paul's Eye Hospital are referred to the Glaucoma Unit for initial assessment; the present sample is therefore a representative, consecutive series of well-documented cases from a single Teaching Health Authority. Detailed definition of the patients in each category are as follows:

- a. Closed-angle glaucoma: 124 consecutive cases of closed-angle glaucoma, presenting between 1975 and 1983.
- b. Ocular hypertension: 189 consecutive cases of ocular hypertension referred to the Glaucoma Unit between 1979 and 1982.
- c. Open-angle glaucoma: 67 consecutive cases of open-angle

glaucoma referred to the Glaucoma Unit between 1981 and 1982.

Patients with closed-angle glaucoma presented with characteristic symptoms of this condition (p. 11), necessitating hospital admission. Ocular hypertension is asymptomatic; all patients in this category were diagnosed by assessment of intraocular pressure during routine refraction by an optician, with subsequent referral to the Glaucoma Unit. Open-angle glaucoma is symptomatic when visual field loss is detected by the subject, however this is a late stage in the disease process and represents a very small proportion of patients. Almost all patients currently referred to St Paul's Eye Hospital are asymptomatic of glaucoma, diagnosis occurring during either routine optician refraction, or following ophthalmoscopy by a general practitioner in the investigation of an unrelated illness. It can be seen that both the control group and the OH/OAG categories were self-selected in a comparable manner ie following routine optician refraction, which is normally a biennial appointment in subjects of this age category. Primary glaucoma patients - excluding CAG - were thus detected at the asymptomatic stage in almost all cases. Patients with CAG were self-selected by the nature of this condition; this sample of 124 consecutive cases is the largest published, to date. Treatment, prior to study, of patients with CAG and OAG is shown in table 23.

3.2 Diabetes mellitus in the primary glaucomas

Diabetic status was determined in accordance with National Diabetes Data Group and World Health Organisation criteria (365,366). Oral glucose tolerance test is an invasive procedure, with concomitant risks of significant side-effects, which many patients consider unpleasant. It was therefore decided that one could not ethically subject patients to a repeat procedure of this nature unless the result would materially influence prognosis and treatment. As sustained elevated glucose concentration during the OGTT on more than one occasion is required to establish a diagnosis of diabetes mellitus or impaired glucose tolerance, if the initial test is normal, a diagnosis of DM/IGT could not be made irrespective of

the result of a second test. There was therefore considered to be no moral justification for submitting patients to this procedure a second time in this instance. If, however, the first test was abnormal, a second test is essential to substantiate a potentially serious diagnosis.

In determining the presence of diabetes mellitus in the family history, all subjects were questioned personally by myself on two occasions, with a one-month interval between visits. On the first visit, in addition to direct questioning, the patients were asked to specifically inquire into evidence of diabetes mellitus from all first-degree relatives, prior to the return visit.

Patients with narrow angles - principally CAG and OHNA - are at significant risk of developing CAG if maximal dilation is employed; dilation is therefore normally achieved by installation of guttae 10% phenylephrine, with immediate reversal by guttae 0.5% thymoxamine. Maximal dilation is not safe in these patients, and is only performed in exceptional circumstances. Unfortunately, these are the categories in which the prevalence of type 2 diabetes mellitus and impaired glucose tolerance are significantly elevated. However there is a relevant corollary which must be taken into consideration in this particular study; the Glaucoma Unit in St Paul's Eye Hospital is managed exclusively by Mr R Mapstone - and all patients are seen by either Mr Mapstone or myself. This point is of essential importance in the present circumstance. In all patients with glaucoma, examination of the posterior segment is mandatory on each clinic visit. As these patients have been followed-up by a Senior Consultant Ophthalmic Surgeon alone, specialising in glaucoma, for a period of up to eight years, it is inconceivable that significant retinopathy could have been overlooked - and therefore the absence of marked retinopathy in these patients assumes greater significance.

On the basis of the present results, primary glaucoma is not associated with type 1 diabetes mellitus, although these patients are frequently characterised by more severe autonomic neuropathy than in type 2 diabetes mellitus. There are a number of significant

facts which may explain this apparent paradox. Patients with type 1 diabetes mellitus are generally younger with more severe complications, particularly retinopathy; diabetic retinopathy is postulated to confer protection against the subsequent development of glaucoma (122). Proliferative retinopathy was first noted to be less common in cases of diabetes with raised tension by Grafe in 1924 (109). The exact relationship between intraocular pressure and retinopathy in patients with diabetes mellitus remains unclear; a detailed review of the literature on this subject is given on page 22.

There is evidence to suggest that autonomic neuropathy is a significant factor in the pathogenesis of primary glaucoma - but only in conjunction with several other features, none of which are pathognomonic in isolation. The anatomical configuration of the anterior chamber angle is of paramount importance in this respect. If the anterior segment of the eye is subjected to maximal autonomic provocation with guttae 2% pilocarpine and guttae 10% phenylephrine, the volume of the anterior chamber decreases by 16% (54), however a relatively-greater decrease occurs peripherally than centrally. In normal subjects, diurnal variation in the dimensions of the anterior chamber has been demonstrated; anterior chamber depth and volume measurements in the evening were significantly lower than morning values, with particular emphasis on the periphery of the anterior chamber: axial depth decreased by 2.1%, peripheral depth by 21.1%, and anterior chamber volume by 5.7%. (420) Thus a narrow angle, although not pathognomonic in isolation, permits lesser decreases in anterior chamber depth to produce angle-closure. Anterior translational movement of the iris-lens diaphragm facilitates irido-corneal contact and thus subsequent angle-closure: if the anterior chamber shallows by 0.2 mm, the distance between peripheral iris and cornea decreases by 0.08 mm; if the anterior chamber shallows by 0.33 mm, the irido-corneal gap narrows by 0.12 mm (103). As irido-corneal separation in eyes with narrow angles is only approximately 0.15 mm (155), relatively-minor anterior displacement of the iris-lens diaphragm may cause disproportionate decreases in the irido-corneal interval, with eventual irido-corneal contact. Although narrow angles are relatively-rare in the population (1.1%), the angle narrows with

age; narrow angles are present in 4% of those aged 78-82 years, 5% in the age category 83-87 years, and 10% in the age group 88-92 years (68). Type 1 diabetics have a significantly-lower life expectancy than type 2 diabetics, and therefore one would reasonably expect a far higher proportion of narrow angles in patients with type 2 diabetes mellitus.

The pathogenesis of glaucoma is based on the concept of pupil block, which represents an external manifestation of relative parasympathetic and sympathetic activity in the anterior segment. Pupil block is recognised to be maximal at mid-dilatation; as the sphincter pupillae may develop twice the power of the dilator pupillae, pupil dilation is facilitated by parasympathetic dysfunction - which is a significant feature of patients with primary glaucoma. Resting pupil diameter is diminished in patients with type 1 diabetes mellitus; assessment of topical denervation hypersensitivity in these patients with guttae 2% phenylephrine suggested the small pupil in diabetic patients to primarily result from neuropathy of sympathetic innervation (421). Thus it is possible that relatively-increased anterior segment sympathetic neuropathy of parasympathetic neuropathy in patients with type 1 diabetes may serve a protective function; pupillary constriction is therapeutically desirable in the treatment of primary glaucoma, the basis of current treatment with parasympathomimetics. The important factor in pupil block is relative autonomic activity; present results suggest that in predisposed individuals parasympathetic function is relatively more impaired than sympathetic function, precipitating maximal pupil block and therefore anterior translation of the iris-lens diaphragm, with ultimate angle-closure.

Studies recently completed in the Glaucoma Unit have shown that patients with type 1 diabetes mellitus, with either background or proliferative retinopathy, are characterised by deep anterior chambers with wide irido-corneal angles; of 50 consecutive patients examined, all had an angle width of ≥ 3 (Shaffer system). In this circumstance, even the presence of significant anterior segment neuropathy would not effect angle-closure in the presence of wide angles.

It can be seen that there are several reasons which may be reasonably forwarded to explain the association between exclusively type 2 diabetes mellitus and primary glaucoma. The most important point is that glaucoma is undoubtedly a multifactorial disease; isolated features are insufficient to precipitate primary glaucoma. It is well-established that the prevalence of closed-angle glaucoma in patients with narrow angles alone is comparable to that in the general population. Similarly, the presence of autonomic neuropathy in the absence of narrow angles does not result in primary glaucoma. Narrow angles are rare in the general population (1.1%); there is no evidence to suggest that the prevalence in type 1 diabetics differs from normal - and current evidence suggests the opposite conclusion. However it must be emphasised that narrow angles occur in 100% of patients with CAG and OHNA, and thus the eye is predisposed to angle-closure in the presence of concomitant autonomic neuropathy. Relative autonomic neuropathy in the anterior segment determines pupil block force, and therefore predisposition to angle-closure; patients with primary glaucoma have significant emphasis on parasympathetic neuropathy, however it may be that type 1 diabetics have a concomitant emphasis on anterior segment sympathetic neuropathy - as suggested by recent observations (421) - producing a small pupil which serves a protective function regarding intraocular pressure.

There is no published data regarding the prevalence of primary glaucoma in patients with primary autonomic failure, however considering that this is a very recent association - and the numerical limitations of the population - this is not surprising.

3.3 Anterior segment neuropathy

Ratios of relative pupil diameter before and after assessment of denervation hypersensitivity were used, rather than actual pupil measurement, for a number of reasons. Direct measurement of pupil diameter is only possible after allowing for magnification by the cornea; this may only be determined by assessment of corneal curvature by keratometry (representing approximately 43 dioptries in "normal"

eyes) plus accurate measurement of anterior chamber depth. In normal patients, keratometry is often assumed to be constant, however patients with CAG and OHNA are often significantly hypermetropic, with reduced corneal diameters, increased corneal curvature, and reduced anterior chamber depth - all summing to relatively increased corneal magnification of pupil diameter. Thus when ratios of pupil diameter are used, inaccuracies inherent in the determination of absolute measurements are eliminated, as the relative corneal magnification is obviously equivalent both before and after assessment in the same eye.

All provocative tests in the assessment of glaucoma are based on the responses of the anterior segment to maximal stress - usually autonomically mediated. Intermittent closed-angle glaucoma is characterised by a normal anterior chamber between episodes. There is thus no obvious benefit to be derived from determining pupil position, or any other anterior segment parameter at rest - probably because the "rest" position varies under different levels of illumination. Most episodes of closed-angle glaucoma occur in the evening. The rationale of all current predictive provocative tests is to determine anterior chamber responses in a dynamic situation; the most important aspect of pupil block is the length of time it is sustained. For example, the pupils of a normal subject dilate in darkness, and for a short period pupil block force is maximal, however pupillary mid-dilation is rapidly passed, and pupil block decreases accordingly. In patients with CAG, the period of pupil block force is sustained for a considerable length of time due to significant anterior segment autonomic neuropathy; if the subject has narrow angles, a potentially dangerous situation may rapidly develop. There is thus no advantage to be gained from assessing pupil size at rest, on the basis of current glaucoma concepts.

The age-adjusted normal tolerance limit, ie the lower 5th percentile in the case of 2.5% methacholine ratio and the upper 95th percentile of the log normal distribution after regression on age in the 0.1% and 0.5% phenylephrine ratios, was applied to the results obtained from patients with primary glaucoma, to determine the proportion

of eyes outwith this limit; results are shown in table 24.

Increased responses to topically-applied drugs may be due to increased reactions of the effector as a result of denervation hypersensitivity in primary glaucoma patients, however this assumes that the concentration of drug reaching the iris musculature is equivalent in both primary glaucoma and control subjects. Identical drug preparations were used in both groups. Corneal permeability is intrinsically dependent upon the integrity of the corneal epithelium and endothelium. Damage to corneal epithelium will markedly increase topical drug penetration into the anterior chamber; intraocular pressure was determined by applanation tonometry at least one month prior to assessment to preclude this possibility. Corneal epithelium is dependent upon both intact sensory innervation and an adequate pre-corneal tear film; there is no confirmed evidence of abnormality in either of these features in patients with primary glaucoma. Corneal endothelial damage causes increased hydration of the cornea; the cornea is normally maintained in a state of partial dehydration by an active transport mechanism, involving sodium/potassium pumps in the endothelium. There was no evidence of clinical endothelial impairment in the primary glaucoma patients included in this study. Acute elevation of intraocular pressure - as in acute closed-angle glaucoma - may result in corneal oedema, however the sudden increase in intraocular pressure in this situation characteristically precludes topical medication; current glaucoma teaching stipulates that topical therapy is ineffective due to poor corneal drug penetration in acute closed-angle glaucoma, and should only be employed after intraocular pressure has been lowered by either intravenous acetazolamide or mannitol. Thus acutely-raised intraocular pressure clinically impaires drug penetration. The final variable in the estimation of drug responses is the muscle effector, however in intermittent closed-angle glaucoma this is frequently damaged by segmental ischaemia. There is therefore no evidence to suggest extrinsic causes of increased drug responses in primary glaucoma patients - rather the opposite; as iris musculature may be damaged by ischaemia during intermittent closed-angle glaucoma, denervation hypersensitivity, determined by pupil responses to topical agents, is probably underestimated in these patients.

3.4 The association between primary autonomic failure and primary glaucoma

It has been shown that results of several autonomic function tests were significantly associated with diabetes mellitus, however in the majority of cases there was no obvious predisposing factor; the most important category in this respect was closed-angle glaucoma, in which the results of autonomic function tests were specifically not associated with diabetes mellitus. In the absence of evidence to the contrary, a primary autonomic dysfunction is postulated in these patients. This statement is obviously entirely dependent on the comparisons between the control group and the primary glaucoma sample. As previously explained, with the obvious exception of closed-angle glaucoma which is a disease characterised by acute symptomatology, both patients (OH and OAG) and control group were self-selected on an equivalent basis - almost exclusively following routine optician refraction which is perfectly normal in this presbyopic age category - and not selected due to the disease process. Thus the reason for self-selection was not the actual disease; this was an incidental finding on examination. One may therefore reasonably assume that the groups are self-selected on a similar basis, and are as evenly matched as possible.

On the basis of present results, there is undoubtedly a significant association - of some nature - between primary glaucoma and autonomic dysfunction. Substantial previous experimental evidence, detailed in the text, supports the concept of autonomic neuropathy as a primary aetiological factor in the pathogenesis of angle-closure:

- i. Autonomic provocation, the basis of diagnosis in the primary glaucomas, has a sensitivity of 93% and a predictive value of 94%. As there are few false negatives, a reasonable inference is that the event involved in the pathogenesis of the disease process is similar to those occurring during a positive provocative test. Normal provocative tests occur in only 22% of patients with open-angle glaucoma. Partial angle-closure is present in 45% of patients with ocular hypertension, with increased prevalence in narrow-angle categories.

- ii. Autonomic effects on aqueous dynamics are recognised to be of central importance in the ultimate determination of intraocular pressure. The most-frequently prescribed medications in the treatment of glaucoma - pilocarpine, timolol, guanethidine, adrenaline - exert primary actions via manipulation of the autonomic nervous system in the anterior segment. Response to treatment, assessed by lowering of intraocular pressure, is significantly greater in primary glaucoma patients with abnormal results of cardiovascular autonomic function tests.

To assume that the demonstration of significant autonomic dysfunction associated with primary glaucoma is unrelated to the disease process would require one to totally disregard all current concepts of the pathogenesis of glaucoma. Age-related changes in the autonomic nervous system are documented, however the nature of selection of controls and patients (not selected on the basis of disease process, but asymptomatic at the time of diagnosis) has eliminated this variable within the limits of experimental accuracy. As there is no evidence of bias in selection of either control group or patient sample, there is no reason to suppose that pre-selection has influenced the results. All ocular hypertensive patients, and most open-angle glaucoma patients, are completely asymptomatic at the time of discovery, therefore this primary glaucoma group represents a sample of patients which are randomly selected from the glaucoma population; open-angle glaucoma patients seldom present with symptoms directly attributable to the disease except in the late stages of the disease process. As both control and patient samples were self-selected on an identical basis, this represents the ultimate situation a researcher can obtain in precluding subjective bias of comparable experimental samples.

Primary autonomic failure encompasses patients in whom symptoms and signs of autonomic nerve dysfunction are present, without known predisposing cause; primary glaucoma may be included in this category - with the proviso that this may be amended with future research.

Table 23

MEDICAL TREATMENT OF 124 CLOSED-ANGLE GLAUCOMA PATIENTS AND 67 OPEN-ANGLE GLAUCOMA PATIENTS

	Peripheral Iridectomy	Trabeculectomy	Laser Trabeculoplasty	Guttae 0.5% Timolol	Guttae 2/4% Pilocarpine
Closed-angle glaucoma patients (n=124)	84	5	0	16	19
Open-angle glaucoma patients ALL (n=67)	11	6	11	16	23
OAGNA (n=24)	5	1	2	6	10
OAGWA (n=43)	6	5	9	10	13

Table 24

THE PREVALENCE OF DENERVATION HYPERSENSITIVITY, DEFINED BY THE AGE-ADJUSTED NORMAL TOLERANCE LIMIT AT THE LOWER 5th PERCENTILE FOR 2.5% METHACHOLINE RATIO AND THE UPPER 95th PERCENTILE FOR 0.1% AND 0.5% PHENYLEPHRINE RATIOS, IN EYES FROM 117 PATIENTS WITH PRIMARY GLAUCOMA AND 63 AGE- AND SEX-MATCHED CONTROL SUBJECTS

	Abnormal 2.5% Methacholine ratio	Abnormal 0.1% Phenylephrine ratio	Abnormal 0.5% Phenylephrine ratio
Control subjects (n=63)	4.7%	4.9%	5.0%
Closed-angle glaucoma patients (n=45)	17.8%	12.5%	17.1%
Ocular hypertensives ALL (n=56)	17.8%	15.1%	18.9%
OHNA (n=26)	15.4%	11.5%	15.4%
OHWA (n=30)	19.0%	18.5%	22.2%
Open-angle glaucoma patients ALL (n=16)	25.0%	15.4%	15.4%
OAGNA (n=7)	14.3%	40.0%	20.0%
OAGWA (n=9)	33.3%	0%	12.5%