EXAMINATION OF THE RETINAL NERVE FIBER LAYER IN THE RECOGNITION OF EARLY GLAUCOMA DAMAGE*

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INTRODUCTION

THIS THESIS ATTEMPTS TO PROVIDE INFORMATION PERTINENT TO THE EARLY DIAGnosis of glaucoma. It contains histological studies of monkey and human eyes with glaucomatous optic nerve damage. Direct correlations are made between retinal histology and the visible appearance of the retinal nerve fiber layer (NFL). An attempt is made to estimate the degree of damage to the NFL that must be present before it can be detected ophthalmoscopically under ideal conditions (monkeys) and under actual clinical observations. In the monkey eyes, serial observations of the development and evolution of NFL atrophy are described in chronic experimental glaucoma for the first time. The accumulated observations of more than 3000 sets of NFL photographs in human glaucoma suspects and patients with visual field loss are used to present a practical set of findings to guide the clinician in the use of NFL examination for glaucoma management.

THE NEED FOR BETTER METHODS IN GLAUCOMA DIAGNOSIS

During the last 20 years, the diagnosis and therapy of early open angle glaucoma have undergone dramatic changes. Prior to the advent of quantitative projection perimetry, glaucoma therapy consisted of using the few available medications in any eye with intraocular pressure (IOP) above somewhat arbitrary limits. When this failed to achieve an IOP lower than that desired, surgery was often carried out. Then, the ability to judge optic nerve damage improved by better recognition of glaucomatous optic disc injury[1] and by improved perimetry using the Goldmann instrument. Eyes with elevated IOP could be divided into those with defined damage

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of disc and field and those with statistically high IOP alone. While the definitely damaged eyes continue to undergo medical, laser and surgical therapy, the much larger group of eyes with high IOP were studied by a number of researchers using modern epidemiologic techniques.[2-5] These studies concluded that only a small percentage of those with elevated IOP would develop one of a defined set of visual field abnormalities in each year of untreated follow-up. Many such eyes began to be followed without IOP-lowering treatment under the assumption that the risk of rapid, serious damage was low and was not great enough to justify the side-effects of therapy.

To a large degree, this approach still dominates the management of early glaucoma. However, several lines of evidence now have pointed to the need for substantial improvements in the recognition of early glaucoma damage. First, perimeters have been developed with sophisticated strategies combining static testing and microprocessor control. These instruments demonstrate visual function loss in eyes previously thought to have normal visual fields by manual, combined static/kinetic methods on the Goldmann perimeter.[6-9] There is still need for clinical research to redefine which apparent defects on the newer instruments represents true glaucoma injury and which are nonspecific variations from normal. However, it is now clear that glaucomatous visual field loss begins prior to the stage at which it was being detected with the Goldmann perimeter.

A second body of evidence to suggest damage present in some eyes previously referred to as ocular hypertensives came from histologic study of the ocular nerves of human glaucoma patients.[10-13] When the number of fibers in the optic nerves were estimated and compared to the expected normal number, some eyes that had no detectable field defect on the Goldmann perimeter were seen to have lost between one-third and one-half of their fibers.

Stamper[14] recently presented new evidence that glaucoma damage is present in the central vision area, even at an early stage of the process. It had been assumed that foveal vision function was largely spared in glaucoma until late in the process. This *relative* sparing of some fibers from the foveal zone was confirmed by histologic study,[12] but there was loss of some of the fibers in the portions of the nerve subserving the central 5° of the field. This field zone is the active area for such a large proportion of the optic nerve cells that it is not possible to lose half the nerve without involving them, no matter how selective the process. It is obvious that visual acuity tested with the Snellen chart is not sensitive enough to detect damage to some macular functions. Certainly, projection perimetry with the Goldmann instrument largely ignored this area, since the

central 2° to 3° zone is occupied by its fixation-monitoring telescope.

It had been known for many years that the optic disc cup enlarged in many glaucoma eyes prior to detection of visual field defects.[15] In order to explain how this could occur without optic nerve fiber (since the field was thought to be normal), it was hypothesized that cup enlargement might occur by loss of astrocytic glial cells from the nerve head.[16,17] Subsequent histological and experimental data[10,11,18-20] showed that glial cells are insensitive to injury from elevated IOP and occupy so small a portion of the disc tissue that their loss would not account for increased cup/disc ratios in early glaucoma.

There was, however, an increased interest in signs of early glaucoma damage at the optic disc. Kirsch and Anderson[1] pointed to selective loss of the vertical disc rims as a specific sign. While vertically oval cup size enlargement is most often seen in glaucoma, Pederson and Anderson[21] later showed that progressive cup enlargement occurs by symmetrical rim thinning in many glaucoma eyes. Therefore, while vertically oval cup size increase had a high degree of specificity as a glaucoma finding, its frequency in glaucoma eyes was not as high as might have been hoped. Susanna et al[22] pointed to hemorrhages on or near the disc rim as a sign of progressive glaucoma damage, and showed that they are a prospective indicator of eyes that will develop new or worsening field loss. Recently, estimation methods for the amount of remaining neuroretinal rim area have shown some promise as additional indicators of the presence of nerve damage.[23]

These data show that management of the glaucoma suspect cannot be reduced to a rigid approach of treating only eyes with field loss. There are eyes with optic nerve damage whose field tests are normal. Yet, there is almost surely a much larger proportion of suspects without damage. And, in most eyes, damage occurs so slowly that careful quantitative observations alone may be preferable to combining these observations with IOP-lowering treatment. In order to decide which patients are already in a stage of early damage (and have a higher risk of vision loss) and which are undamaged, further improvements in our diagnostic methods are clearly needed. The ideal new method would have the following characteristics: (1) simple; (2) rapid; (3) objective; (4) uses equipment already in the ophthalmic office; (5) shows damage earlier than present methods; (6) information additive to present methods; (7) quantifiable; and (8) easily learned. The examination of the retinal NFL may not satisfy all of these criteria, but the information presented in this report will indicate how well it does so.

HISTORY OF RETINAL NERVE FIBER LAYER EXAMINATION

Vogt[24] may have been the first to note that the pattern of retinal nerve fibers is best seen when ophthalmoscopy is performed with red-free light. Potts,[25] Behrendt and Wilson,[26] and Mizuno and co-workers[27] published some of the first fundus photographs of the normal pattern of retinal ganglion cell fiber bundles using red-free (green) illumination. The nerve fiber bundles enter the optic disc in radial fashion nasally, but temporally the pattern arches over and under the fovea, with relatively strict division between those cells superior and inferior to a horizontal line through the fovea.[28] Hoyt et al[29] began the description of abnormalities in the visible NFL in glaucomatous eyes. They reported that defects in the normal NFL pattern occurred in some eyes that had no defects on tests using the Goldmann perimeter. In most of the eyes they studied, however, tangent screen defects could be elicited subjectively when the known areas of likely deficit were probed.

The subsequent phase of NFL study began with the report of Sommer and co-workers[30] on serial color disc photographs of eyes that were followed during the Collaborative Glaucoma Study. In a controlled evaluation of photographs taken once per year prior to development of visual field defects, each of 14 glaucoma suspect eyes demonstrated consistent abnormality of the NFL pattern in a masked reading, beginning as long as 5 years prior to field loss detection (mean, 1.5 years). However, the photographs available for this study were taken in white light on color transparencies, and in some eyes that NFL was difficult or impossible to see well. While the NFL can be seen in many eyes quite well with the direct ophthalmoscope or slit lamp/contact lens using green light, for the purposes of clinical research studies, improved photographic methods were required. This was particularly important in order to mask the observer reading the NFL to the appearance of the optic disc, whose cup size represents a substantial biasing element in attempts to mask a direct viewing protocol. Miller and George[31] presented such basic improvements in filters and photographic technique and Sommer and co-workers[32] later reported on preferred black and white film and developing methods.

Using the new photographic method, Quigley et al[33] surveyed the NFL appearance in 335 eyes of normal, glaucoma suspect, and field loss patients with the observer masked to history, disc appearance, and field status. The normals were correctly identified in 97% of eyes, and 84% of field loss eyes were detected as damaged. Those glaucoma eyes with NFL appearance read as normal had the lowest degree of perimetric abnormality accepted as damage (paracentral scotoma to I/2/e target in one half

field). The observer was quite accurate in predicting the half field in which the field defect was either present or was worse. Most important, 13% of eyes with elevated IP but no field loss on the Goldmann perimeter had abnormal NFL appearance. And, in eyes with normal fields whose fellow eye had field loss, the rate of NFL defect was 28%. Hence, the rate of NFL abnormality seemed to follow the expected risk of optic nerve damage already being present. Unfortunately, the NFL could not be seen well enough to comment upon its normality in some eyes, with an inability to obtain adequate photographs in as many as 15% of older persons with smaller pupils and cataract. This might have been more a limitation of a clinical study requiring photography than a problem in clinical management, since some of the eyes with poor photographs had acceptable NFL assessment by direct ophthalmoscopy.

In two additional reports, the NFL appearance was compared to optic disc evaluation as a predictor of which eyes would progress to the stage of field loss.[34,35] The sensitivity and specificity of NFL assessment were somewhat better than any of the disc features examined in this comparison. Other investigators[36-38] found that NFL examination is an accurate method for estimation of optic nerve damage. It is well-demonstrated that the larger the cup/disc ratio, the more likely field loss is to occur in untreated patients.[39] It therefore remained to be shown whether the NFL assessment would also predict prospectively which eyes were undergoing progressive optic nerve damage, and with what sensitivity.

Such a study has now been underway for 4 years. Over 1500 eyes with either elevated IOP and normal visual field (Goldmann), glaucomatous IOP and field, or normal IOP and field are being observed serially. Both masked clinical and photographic evaluations of disc cup, NFL, and field are included. In the first report from this study,[40] the high level of detection of glaucomatous field loss patients from their NFL appearance was again documented. Unexpectedly, single local dark slits in the NFL were found to have a much lower specificity for glaucoma-damaged eyes than originally reported. Two-thirds of those normal IOP and field eyes that were thought to have abnormal NFL appearance were classified as defective because of single slit-like defects in one eye. The data pointed to broader, diffuse decrease in the NFL pattern as a more reliable sign of glaucoma damage. This has been confirmed by Airaksinen et al.[37] In addition, two independent observers were shown to read 85% of the photographs in similar fashion.

The most important result of the prospective NFL study would be whether those eyes reaching the stage of field loss will have been detected to have NFL atrophy prior to their perimetric defect. As in other

similar studies, the data now indicates that approximately 1% of glaucoma suspects per year develop their first field defect by standard criteria using the Goldmann perimeter. Hence, even with a large pool of patients, not enough eyes have demonstrated first field loss to reach a statistically significant result. However, at this time, those that have progressed show a dramatically higher rate of NFL abnormality than those that have remained normal.

HISTOLOGY OF NERVE FIBER LAYER DEFECTS

In order to understand the clinical significance of the NFL appearances in normal and abnormal eyes, it is important to delineate the histologic basis for the observations. Unsold and Hoyt[41] used the clinical features of their patients as a means of interpreting the normal and abnormal appearances they described. On one occasion, a patient with a homonymous field loss and NFL atrophy in the corresponding zones was able to be studied histologically. While detailed study of the retina itself was not performed, at least the appearance of diffuse NFL atrophy could be seen to correspond to the appropriate areas of atrophy in an optic nerve cross-section.

Radius and Anderson[42] produced experimental loss of the normal NFL pattern by xenon arc photocoagulation of monkey eyes, performing light and electron microscopy on the retina. Their study showed that the light stripes seen are the neural bundles of axons and the dark separating stripes are made up of the end feet of Muller cell glia, whose processes divide one bundle from another. They found that the dark appearance assumed to represent NFL atrophy by Hoyt did indeed correspond to areas of thinned NFL.

Quigley and Addicks[43] produced experimental lesions in monkey optic nerves by lateral orbital approach. When changes in the photographic appearance of the NFL resulted, the histologic appearance was studied. This study provided an initial quantitative basis for the NFL examination. The thickness of the normal NFL is nearly 200 μ at the 12 and 6 o'clock positions of the disc rim. Toward the foveal and nasal directions, the NFL height is only 60 μ. The thickness decreases rapidly toward the peripheral retina, confirming that there is substantial convergence of fibers radially. By 2 disc diameters from the nerve head, the NFL thins to < 40 μ in every meridian. This confirms that the thickness of the NFL is directly proportional to the brightness of the reflexes seen in green light. The thickest zones at the upper and lower disc poles are invariably the areas where the NFL is most easily seen. And, as one looks peripherally from the disc in any direction, the brightness of the reflexes decreases. Hence, the brightness is a measure of the number of axons in the bundles and can

be used as a semiquantitative standard of atrophy. The investigators attempted to judge by actual comparison of photographs and the same areas in retinal sections how much loss of thickness it took to recognize atrophy as a change in NFL brightness and loss of pattern. When the actual thickness of NFL in the retina was measured in areas that had lost their striped NFL pattern in the photographs, it seemed most accurate to conclude that loss of 50% of the NFL thickness was detectable in any area. Furthermore, in any area in which NFL thickness had fallen to 25 μ or less, there was no detectable pattern.

In that study, however, the lesions were not produced by elevated IOP, but rather by one traumatic insult at one point in time. As a result, the change in NFL appearance happened rapidly and in only one step. Furthermore, the degree of atrophy was judged by comparison to a pooled set of control data, rather than to the same animal's fellow eye. The variety of degrees and positions of atrophy was also not extensive. The monkey data to be presented in the present study correct each of these potential weaknesses. In addition, for the first time human glaucoma eyes are presented in which clinical photographs of normal and atrophic NFL appearance can be compared to the histologic findings in the retina and optic nerve in the same eyes. This provides important correlative support for the data from monkey eyes.

MATERIALS AND METHODS

METHODS FOR MONKEY STUDIES

Only one eye of each animal was treated to produce glaucoma, the other eye remaining as a clinical and histological control. The treatment was performed on confluent fashion with 125 to 150 deliveries of argon blue-green energy directed at the upper half of the meshwork. Each delivery was 1 W power with 50 μ spot size, 0.5-second duration. IOP was measured with a calibrated pneumatonograph or an applanation tonometer every other day until stable, then weekly thereafter under ketamine sedation. Fundus photographs were taken prior to treatment and at intervals afterward to document important changes in disc and NFL status. A Nikon fundus camera was used with Kodak technical pan film 2415, developed in full strength D-11 developer for 4 minutes at 68°. Clinical observations of the disc and NFL were made with a direct ophthalmoscope and slit lamp/contact lens and the green illumination provided with this equipment.

The clinical photographs of each animal were then evaluated prior to histologic study. Each glaucoma eye was studied to determine if, in the

opinion of the observer, a change had occurred in the NFL appearance from the photographs prior to laser treatment.

Animals were sacrificed at different stages of NFL damage to produce a range of injury among the entire group. Nine of the animals were observed for serial changes in NFL clinical appearance, but were utilized in other experiments so that the NFL was not examined histologically. In the other 11 animals, both the normal and glaucoma eye were fixed by perfusion of 5% glutaraldehyde and 4% paraformaldehyde in cacodylate buffer retrograde through the abdominal aorta after opening the right heart. After division of the tissue to yield standard pieces of retina, optic nerve head, and optic nerve cross-sections, each piece was post-fixed in 1% osmium tetroxide, dehydrated in alcohol, and embedded in epoxy resin. One-micron thick sections were examined by light microscopy.

Sections of retina were examined to identify precisely the areas from which they had been removed by direct comparison to clinical photographs and the positions within them of retinal blood vessels. In each section, the thickness of the NFL was measured with an image analysis system as the distance from the internal limiting membrane to the ganglion cell bodies, every 250 μ along the retina. In most cases, the areas examined were within 2 disc diameters of the optic nerve head. Thicknesses were compared directly with the same area in the fellow eye, so that absolute and relative data could be obtained.

Optic nerve cross-sections were examined to determine the extent, if any, of fiber loss.[12] In every animal, the area of remaining nerve fiber bundles was measured. This neural area has been previously shown to be an accurate estimation of the relative number of nerve fibers remaining.[13] In some special cases, an estimation system for counting the number of fibers was used in which approximately 5% of the total number of axons was counted in a random sampling.

METHODS FOR STUDY OF HUMAN EYES

The eyes included in this study were those in which clinical photographs had been obtained prior to the acquisition of the tissues. Each eye also had to have adequate preservation of retina and optic nerve tissue for quantitative observations. Eyes were obtained by donation from Eye Banks, at autopsy, or after surgical enucleation due to intraocular tumor that had additionally caused secondary glaucoma damage. One eye was free of glaucoma or other intraocular disease, but was removed as part of an exenteration for sinus cancer involving the orbit. The remainder, 12 eyes, exhibited various degrees of glaucoma damage.

The fixation of surgically removed eyes was by immediate immersion in

combinations of glutaraldehyde and formaldehyde in buffer. In the autopsy or eye bank tissues, up to 12 hours elapsed between death and fixation. After tissue division similar to that of the monkey eyes above, portions of retina, nerve head, and optic nerve were post-fixed in osmium, embedded in epoxy resin, and 1-μ sections cut for light microscopy. The NFL thickness was measured in the retinal sections, again with constant reference to the clinical photographs available on each eye. Since there was no normal retina in each case for comparison, the data in the human eyes are controlled by reference to the one normal human eye and by comparing the upper to the lower NFL thickness in the same eye or the right to the left eye NFL thickness in pairs of eyes from the same person.

In the optic nerve cross-sections, counting of nerve fiber number was not feasible in every case due to inadequate preservation. However, the remaining area of neural bundle tissue in the optic nerve serves as a satisfactory estimate of the amount of optic nerve damage.[13] This was performed by outlining the neural bundle tissue of each nerve in an enlarged photograph on an image analysis system.[12]

RESULTS

MONKEY CLINICAL/PATHOLOGIC CORRELATION

The 20 monkey eyes in which serial photography was performed after induction of elevated IOP provide an overview of the development of NFL atrophy. A summary of all information on the 20 animals is provided in Table I. The group was divided into four groups by the severity of their photographic NFL findings.

No NFL Defect (4 Eyes)

In the first group of four eyes, serial observations lasting up to 20 months disclosed no clinically detectable damage. These were eyes that had relatively lower IOP levels and their cup-to-disc ratios had also not appreciably enlarged. Furthermore, the optic nerve neural area in the glaucoma eye was 90% or more of the normal fellow eye in each case. One member of this group had histologic measurements of the NFL thickness (M623, Table I). The superior NFL was only 9% thinner than the fellow eye on average, while the inferior NFL measured an average of 2% thicker than the normal eye. The image analysis system was proven to be capable of measuring to much smaller tolerances than these differences. However, within normal eye's NFL measurements, there were frequently variances as great as ± 5%. Therefore the differences seen in this

TABLE I: SUMMARY DATA ON MONKEY EYES STUDIED

NO.	MEAN IOP (mm Hg)	MONTHS IOP ELEVATED	FINAL C/D RATIO	NERVE FIBER LAYER FINDING	% NORMAL NERVE AREA	% NORMAL FIBERS	% NFL THICKNESS LOST		RANGE OF NFL LEFT (μM)	GREATEST LOSS NFL (μM)
							SUPERIOR	INFERIOR		
M575W45	30 + 8	8	0.4	No change	100					
M576W46	26 + 4	5	0.5	No change	99					
M566V29	30 + 8	20	0.5	No change	93					
M623W51	31 + 13	5	0.3	No change	89		−9	+2	134-220	25-50
M574W60	33 + 8	4	0.7	Super: no change Infer: wedge diffuse atrophy	93					
M616W67	26 + 5	12	0.6	Super: wedge diffuse atrophy Infer: no change	78	65	−30	−11	50-145	70-120
M624T65	28 + 8	16	0.3	Super: wedge diffuse atrophy Infer: no change	73	71	−28	+6	75-110	50
M570W47	34 + 11	8	0.7	Super: no change Infer: wedge diffuse atrophy	72					
M626W56	27 + 11	13	0.6	Super: wedge diffuse atrophy Infer: no change	70	46	−45	0	50-70	75
M571W5	24 + 6	8	0.8	Moderate broad diffuse atrophy	63					
M534V30	39 + 9	4	0.8	Moderate broad diffuse atrophy	69		−75	−75	25	NA*
M619X33	38 + 11	16	0.9	Moderate broad diffuse atrophy	†		−66	−47	50-100	100
M565W58	34 + 11	8	0.8	Moderate broad diffuse atrophy	50					

M564W62	48 + 8	8	1.0	Severe diffuse atrophy	20						
M563W64	47 + 7	8	1.0	Severe diffuse atrophy	20						
M549W59	34 + 13	4	1.0	Severe diffuse atrophy	12	3	−90	−92	12	160
M550W61	34 + 5	3	0.9	Severe diffuse atrophy	55		−85	−71	25-50	145
M620U45	43 + 18	11	0.9	Severe diffuse atrophy	18	8	−69	−65	36-50	158
M622X32	36 + 11	11	1.0	Severe diffuse atrophy	6		−82	−86	25	145
M533V4	43 + 17	10	1.0	Severe diffuse atrophy	9		−90	−90	12	NA*

*Computation not possible as eye compared only to pooled normals.
†Technical error prevented measurement of nerve area.
C/D = cup/disc ratio.

TABLE II: SUMMARY OF HUMAN DATA*

PATIENT	DIAGNOSIS	FIXATION	AGE,R,S	CUP/DISC	ACUITY	FIELD (GOLDMANN)	NEURAL FIBER		CLINICAL NFL STATE	NFL THICKNESS (μ)			
							AREA (mm^2)	NO.		DISK RIM: ARCUATE	FOVEAL	ARCUATE RETINA	FOVEAL BUNDLE
1	Normal	A	58 WF	0.3	20/20	Normal	5.55	922,000	Normal	400	275	150-200	—
2	Tumor sella	C	37 BF	0.4	20/30	Upper junctional scotoma	6.70	673,000	Normal	—	—	125-150	—
3 R	Optic neuritis	C	51 BM	0.7	20/50	Central scotoma	4.33	416,000	Atrophy foveal, arcuate normal	250	50	100-150	50
4 R	OAG	B	67 WF	0.6	20/25 CAT	Normal	4.00	—	Normal	—	—	125-250	—
4 L	OAG	B		0.7	20/25 CAT	Normal	3.75	—	Normal	—	—	150-220	—
5 R	OAG	B	65 BF	0.7	20/40 CAT	Inferior nasal step	—	—	Mod. superior diffuse atrophy	Sup: 175, Inf: 275	—	Sup: 50, Inf: 100	—

5 L	OAG	B		0.7	20/70 CAT	General contraction	—	—	Normal	—	—	Sup: 75, Inf: 75	—
6	OAG	B	79 BM	0.8	20/25	Dense upper arcuate defect	2.00	—	Infer>Super diffuse atrophy	Sup: 125, Inf: 65	—	Sup: 50, Inf: 40	—
7	Secondary glaucoma	A	30 WM	0.9	20/30	Mild upper depression	1.87	268,000	Infer>Super diffuse atrophy	Sup: 190, Inf: 50	—	Sup: 75, Inf: 50	—
8	Secondary glaucoma	A	62 WF	0.8	20/20	Moderate upper depression	3.00	164,000	Severe diffuse atrophy	Sup: 100, Inf: 50	—	Sup: 50, Inf: 50	50
9 R	OAG	C	88 BM	Anomal	20/200	Central scotoma	1.15	—	—	—	—	100-150	—
9 L	OAG	C		0.9	20/40 CAT	Dense upper arcuate defect	1.03	—	Severe diffuse atrophy	—	—	60-100	—
10 R	OAG	B	77 WM	0.95	20/60 CAT	Dense upper & lower defects	2.12	96,000	Severe diffuse atrophy	Sup: 110, Inf: 150	—	Sup: 100, Inf: 60	65
10 L	OAG	B		0.95	CF	Temporal island	1.41	75,000	Severe diffuse atrophy	Sup: 250, Inf: 25	25	Sup: 75, Inf: 25	25
3 L	OAG	C	51 BM	0.9	CF	Temporal island	0.75	—	Severe diffuse atrophy	Sup: 40, Inf: 150	150	Sup: 40, Inf: 50	—

*R = right; L = left; OAG = open-angle glaucoma; A = optimal, B = fair, C = some autolysis; R,S = race, sex; WM = white male; BF = black female; Anomal = anomalous disc; CAT = visual acuity affected by cataract; CF = counts fingers; Infer,Inf = inferior; Super,Sup = superior.

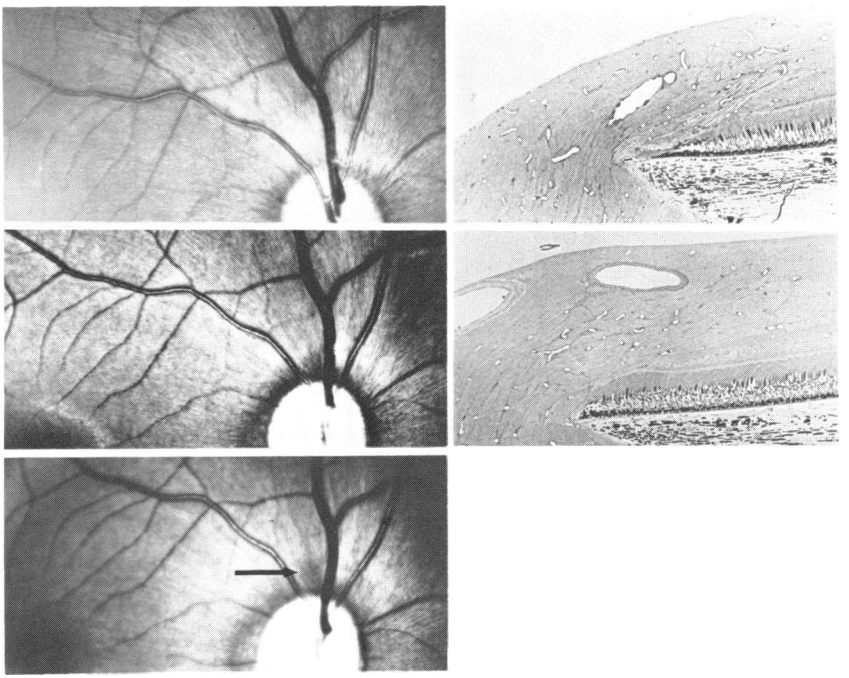


FIGURE 1

A: Serial photographs of monkey that developed subtle atrophy of NFL between arteriole and venule at 11 to 12 o'clock position (monkey M616W67). Top photo is taken prior to elevated IOP. Middle is after 7 months of elevation, without definite change, though striations of NFL between arteriole and venule begin to disappear. The greater loss of NFL striations near disc is more evident 15 months after IOP increase in bottom photo (*arrow*). B: Histological sections of the nerve head rim from the same eye as Fig 1A at the affected 12 o'clock position (above) and the normal 12 o'clock rim in the fellow eye (below). The glaucomatous superior rim measured an average of 30% narrower than the corresponding normal rim. Retinal pigment epithelium is seen as dark line from right to mid-photo in each picture, vitreous cavity is at top, entry to nerve head for nerve fibers is at left in each case (paraphenylenediamine, × 150).

first primate eye appear to be within the range of normal variation. It should be noted that in none of these eyes was an abnormality in the NFL suspected. The NFL therefore correlated well with the other measures of optic nerve integrity.

Mild Localized NFL Defects (5 Eyes)

The next group of five eyes exhibited changes in their NFL appearance, but the differences that developed were mild in extent. This appeared to correlate with the clinical disc examination, IOP history, and optic nerve

damage in cross-section. The eyes in this group ranged from cup/disc ratios of 0.3 to 0.7 (Table I). Four of the five showed some cup enlargement, but in none was the disc strikingly damaged. Mean IOP levels during 4 to 16 months of elevation ranged from mid-20s to low 30s in this group. The IOP increases were, then, higher and longer than the eyes in the first group that had no NFL damage detected. Finally, the neural area measured from 70% to 78% of normal in four of the five and was 93% of normal in the other. This suggests that there was at least a 25% decline in nerve fiber number in most of this group.

In each eye, an NFL abnormality was detected and was present only in one-half of the retina, in three eyes in the upper retina and in two eyes in the inferior NFL. These areas were invariably found in the zones known to contain the arcuate area ganglion cell axons. In a right eye, this is from 5 to 8 o'clock and from 10 to 1 o'clock. Contrary to some initial reports of NFL abnormalities in glaucoma, the nature of these changes was not sharply localized to small slit-like areas, but were changes in the normal pattern of NFL striation that included at least one hour of the clock. In some eyes, the change was subtle enough that careful observation of serial photographs was necessary (Fig 1). The indication of abnormality was a loss of brightness in the NFL pattern either near the disc or in the area 1 disc diameter distant (Fig 2). In the first two eyes shown, retinal histology was available. In both, the superior retina was approximately 30% thinner than in the normal eye. The inferior retina measured 11% less or 6% more than normal in each of these eyes and their inferior NFL photographs were read as normal.

In two other eyes in this mild NFL atrophy group, the gradual development of inferior NFL atrophy was observed (Fig 3 and 4). The features that allow the identification of this change are common to most of the other findings in this study. The brightness of the striations decreases and concomitantly the visibility of the retinal blood vessel walls increases. In the two eyes illustrated, this gradually occurred over a period of months. In another eye, the loss of striations developed prominently throughout the upper NFL, ending abruptly alongside an arteriole (Fig 5). Measurements of the NFL showed that the area with apparently intact NFL striations temporal to the arteriole was of equal thickness to the fellow eye in that area. Nasal to the vessel where the striations were deficient, the NFL was only 45% as thick as the normal eye. The inferior NFL in this same eye had no clinically detectable defect and was less than 1% different from normal thickness in histological section.

It is important to know whether the recognition of NFL atrophy can be related to the actual loss of NFL thickness. In the areas discussed thus far,

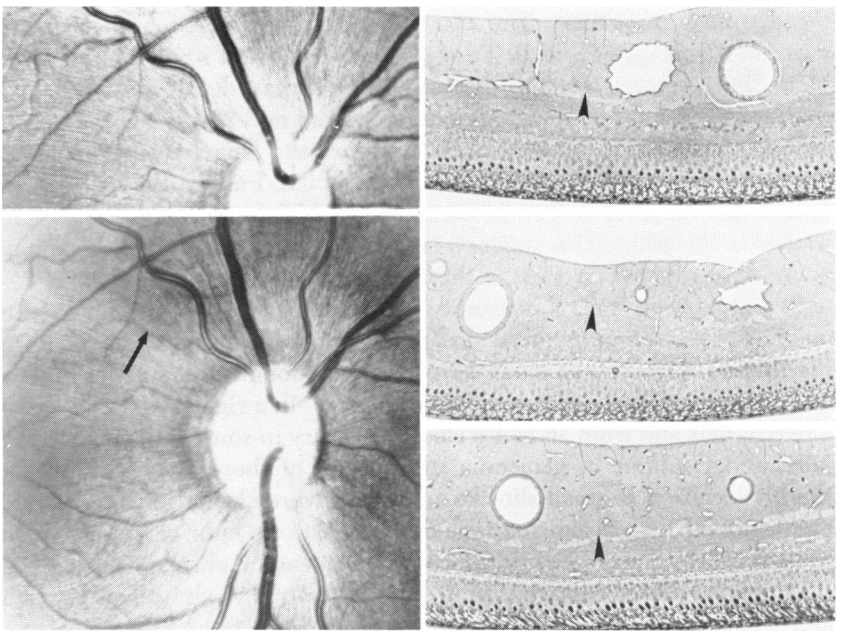
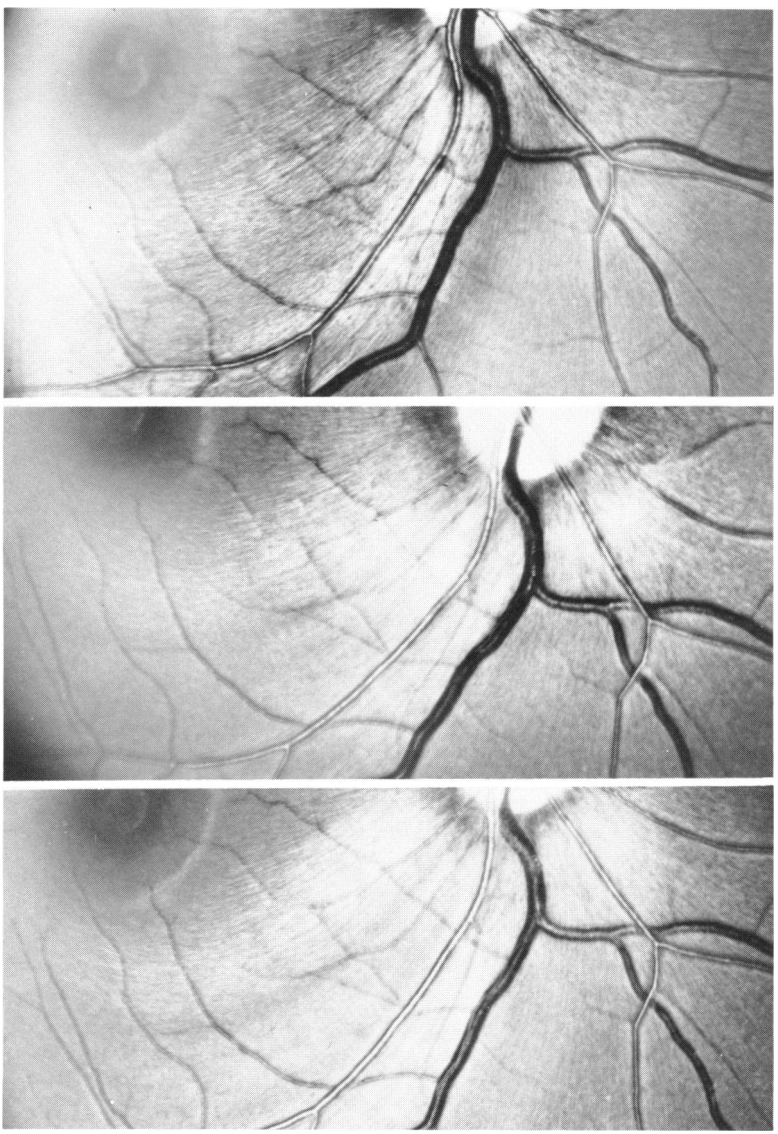


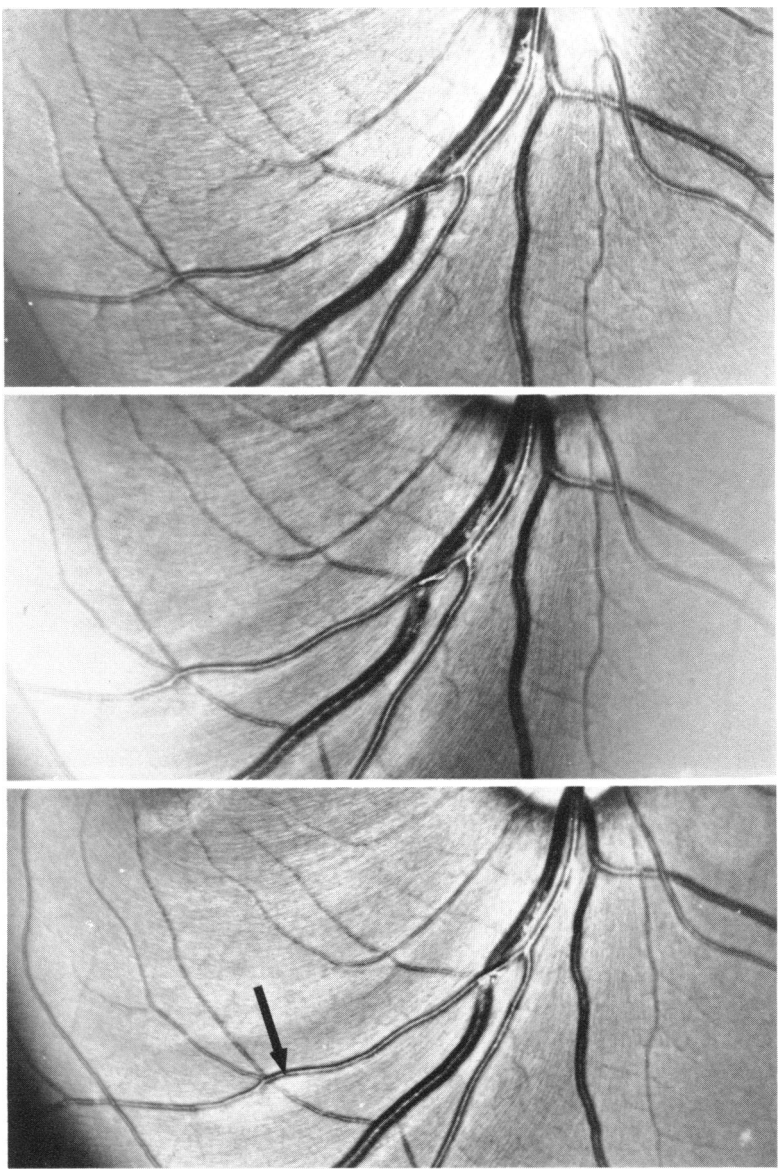
FIGURE 2

A: Over 13 months, monkey developed local zone of decreased NFL striation brightness between 10 and 11 o'clock. Normal appearance at top. Note atrophic area is most easily appreciated by recognizing that NFL should become brighter from 9 o'clock around to 12 (as in the normal picture), but in lower photo, an area inferotemporal to superior arteriole is darker than it should be (from the *arrow* to the arteriole) (M624T65). B: Three histologic sections of retina from monkey in 2A. In each, NFL thickness is from *arrowhead* up to clear space at vitreoretinal interface. Upper photomicrograph is normal left eye superior arcuate retina, and bottom is normal thickness NFL from inferior arcuate retina of glaucomatous right eye. Note that at least 50 μ of NFL overlies vessels causing some blurring of their walls in clinical view (compare bottom photomicrograph to its corresponding area inferior to disc in Fig 2A; larger arteriole is the vessel at 6 o'clock heading down and left). Middle photomicrograph is taken through the area shown to have mild localized atrophy in superior glaucomatous eye (2A). NFL was 28% thinner here than in normal eye shown above (paraphenylenediamine, × 150).

FIGURE 3

Clinical photographs show development of localized wedge of NFL atrophy inferiorly (monkey M574W60). Top is prior to elevated IOP, and middle and bottom are at 3 and 6 months after initiation of glaucoma. Note that NFL striation pattern becomes less distinct to left of arteriole. As a result, NFL pattern is seen better above and to left (toward fovea in upper left corner). In normal picture at top, NFL pattern is brightest in 6 o'clock position and becomes less distinct progressively toward upper left. A discontinuity in this normal progression of brighter at 6 o'clock (or 12 o'clock) and dimmer toward fovea is a key clue to early NFL atrophy. This is one of the asymmetries referred to in "Discussion" on systematic approach to NFL examination.





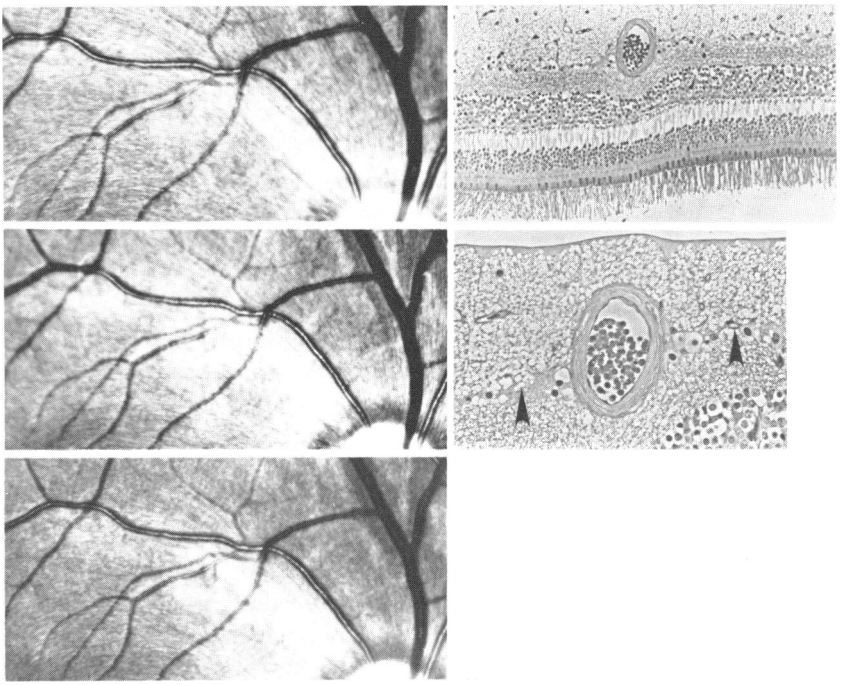


FIGURE 5

A: This monkey's photographs span nearly 8 months of progressive NFL loss in the upper zone between the arteriole at 11 o'clock and the venule at 12 o'clock. Note how the venular first-order branch crossing toward arteriole loses its overlying NFL striations. The second-order branches of vessels farther from the disc are still covered by remaining NFL thickness, however. B: These photomicrographs represent a low and a higher power view of area surrounding the superior arteriole at 11 o'clock in the monkey eye of Fig 5A. NFL is of normal thickness temporal to vessel (to left) and is thinned nasally, toward 12 o'clock venule (to right). Position of ganglion cell bodies, inferior limit of NFL, is marked by *arrowheads* on each side. Thinning to right represents loss of approximately one-half the normal NFL thickness (paraphenylenediamine, × 160 and 400).

FIGURE 4

Clinical photographs show development of localized wedge of NFL atrophy inferiorly in another mildly damaged monkey (M570W47). Normal picture is at top, and the two below are 3 and 6 months after induction of elevated IOP. Note that two major features help to identify the abnormality. First, the pattern of NFL is lost, though not completely, in the wedge between venule and *arrow*. Second, but actually part of the same process, the first-order branch vessels (one at end of *arrow*), become more distinctly visible (compare bottom, atrophic, to top, normal). This occurs when overlying NFL thins, allowing clearer view of vessel wall.

the thicknesses were given as percentage of the normal eye. If one examines the absolute thicknesses in microns in the atrophic areas and the absolute amount of NFL thickness lost, a consistent pattern seems to emerge. In the eyes that had NFL thicknesses within 11% of normal (M623W51, and the inferior NFL of M616W67, M624T65, and M626W56), two general rules were applicable. The total thickness of the NFL was > 100 μ. If one calculates the absolute loss of NFL in microns by subtracting the glaucoma eye from the normal eye, the greatest loss in each of the five normal zones of these four eyes was < 25 μ. By contrast, in the three measured zones that had declined by 28%, 30%, and 45% compared to normal thickness (Table I), the absolute NFL thickness dipped to 50 μ in two and was 75 μ in the third. These same zones had a greatest loss of NFL of between 50 and 120 μ when compared to the fellow eyes.

Moderate, Diffuse NFL Atrophy

The next group of four eyes each developed moderate diffuse NFL atrophy (Table I). The clinical findings other than NFL appearance were correspondingly more extreme than in the preceding groups. Cup/disc ratios were either 0.8 or 0.9 and had definitely changed from the pretreatment appearance in all eyes. Each would have been recognized as having glaucoma-type damage even if its normal, original appearance had not been known. The level of IOP after laser treatment was somewhat higher on average than the prior groups, as well, with three of four eyes averaging between 34 and 39 mm Hg for their several months of elevation. The neural area of optic nerve remaining was from 50% to 69% of normal, definitely lower than the preceding groups. Furthermore, inspection of neural bundles in detail in some of these eyes suggested that while neural area (amount of nerve bundles) was decreased only about one-half, the number of fibers within the bundles (density) was decreased substantially as well. While none of the eyes in this group had actual fiber number estimates, it is likely that the combination of lower density and lower neural area indicate that these eyes had between one-third and one-half the usual number of fibers compared to their fellow eyes.

In contrast to the preceding, milder group, NFL atrophy appeared clinically to involve 2 to 3 clock hours of the upper and the lower NFL centered on the disc poles. None of these eyes had isolated slit-like defects in the NFL pattern as their broader loss of NFL striation pattern occurred. The NFL loss was most easily appreciated in many areas by evaluating to what degree retinal blood vessels were covered by the NFL pattern (Figs 6 to 8). The decrease in NFL brightness was accompanied

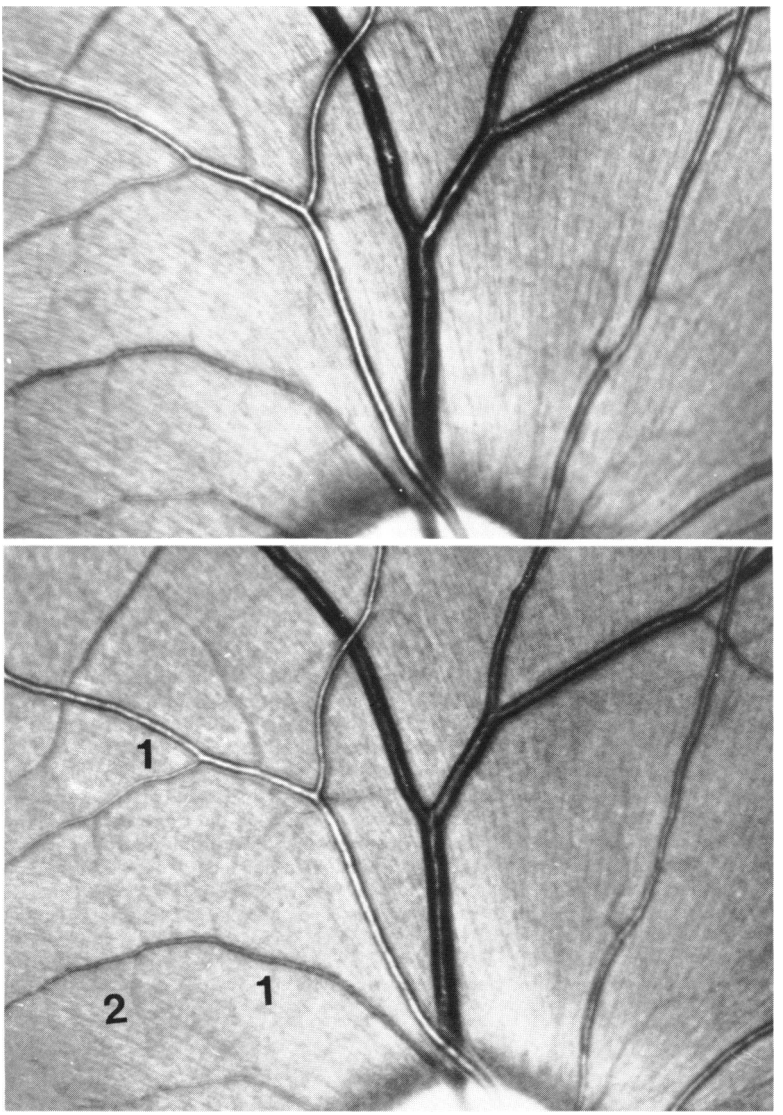


FIGURE 6

This pair of photographs show the important pattern of diffuse atrophy of NFL that developed in superior NFL of monkey M571W5 (bottom) compared to normal appearance of same eye 7 months earlier. Disc margin is at bottom of each photo. Note that NFL striated pattern is less distinct, and this is confirmed by paying particular attention to distinctness with which blood vessel walls are seen. In this case of moderate diffuse atrophy, the first-order branches are bare (two of these are marked with the number 1), but second-order branches are still blurred by overlying NFL (one of these is marked by the number 2).

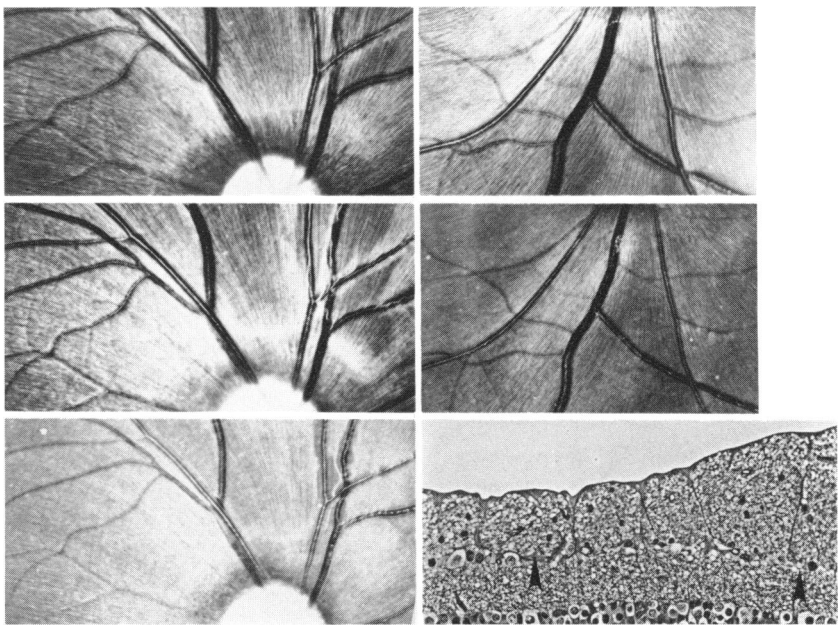


FIGURE 7

A: Ten months of elevated IOP resulted in moderate diffuse atrophy in bottom photograph (monkey M619X33). In this superior NFL, the greatest effect was centered at 12 o'clock, between the two pairs of vessels. NFL pattern is considerably dimmer than in the top, normal photo, and vessels are seen better, particularly the arteriole just to the right of 12 o'clock. Note that in affected area, remaining NFL pattern takes on a finely etched appearance, compared to the normal, brighter, but less organized striated pattern. Narrowing of retinal arterioles and venules is not a product of photography, but represents a real finding in all forms of optic atrophy. B: Inferior NFL of same eye had diffuse atrophy with same features as superiorly, but more careful inspection is needed to detect this loss. The first-order venular branch passing from middle to lower right shows loss of overlying reflexes and the pattern is more difficult to see there. Histologic photograph (bottom) is taken from area between venule and *arrow*. NFL in this area varied from loss of 25% of normal thickness (right side of picture) to loss of 65% of normal bundle height (left side). Position of ganglion cell bodies shown by *arrow*, vitreous cavity above (paraphenylenediamine, × 400).

by an increase in the ease of seeing retinal blood vessels. This improved view of vessels appeared to occur because of the lack of overlying NFL striations. In two of the illustrated eyes (Figs 6 and 7) main retinal arterioles or venules and their first order branches show the NFL atrophy by losing their overlying NFL striations. In the third eye (Fig 8), not only are main vessels and first order branches free of overlying NFL, but even second order branches appear much more easily seen.

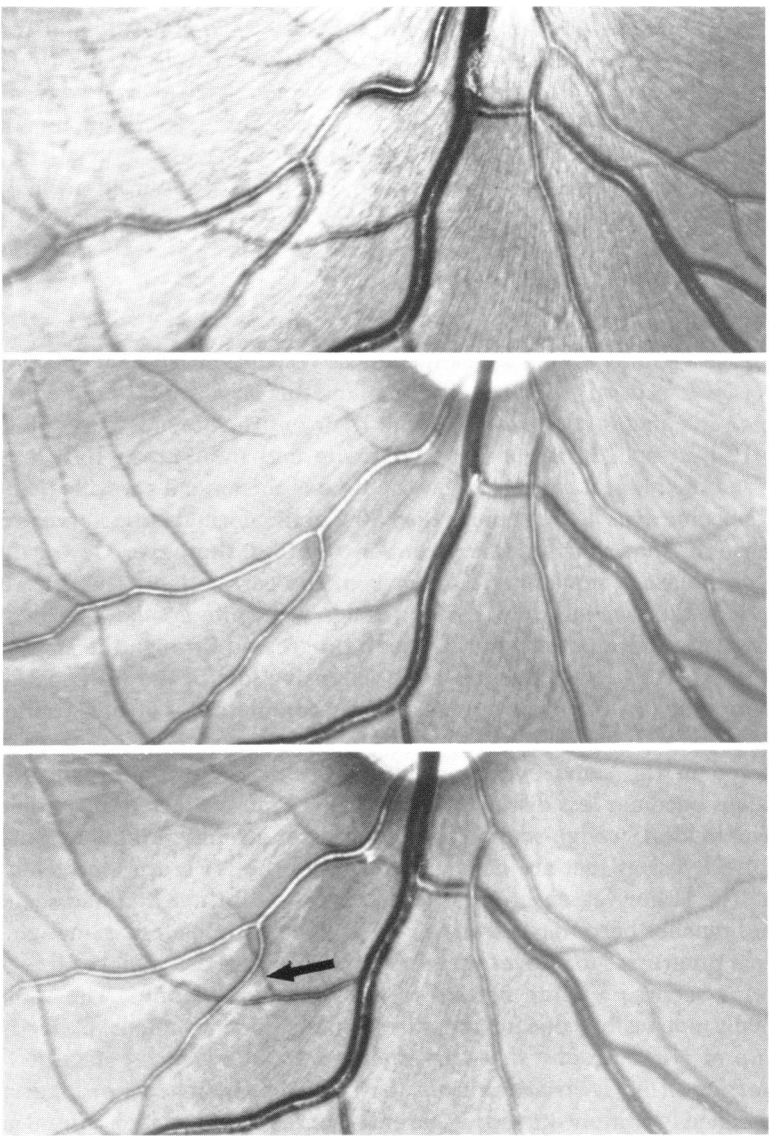


FIGURE 8

Compared to Figs 6 and 7, atrophy was more substantial in this monkey eye, whose inferior NFL progressive atrophy is shown from top to bottom, occurring over an 8-month period. Striations are seen to become much less bright. Blood vessels are seen more easily. This is nearly an example of severe-grade atrophy, since second-order vessels that could not be detected prior to atrophy have become more distinct (eg, venular branch passing under arteriole at *arrow*). Monkey M565W58.

Two of the eyes in this group were measured histologically and their NFL thickness had declined from normal by 47% to 75% (Table I). The average remaining NFL thickness was approximately 25 μ in one eye, and between 50 and 100 μ in the other. The absolute decreases in NFL thickness, then were measured as 100 μ in several areas.

In three of the four eyes in this group, the progression from normal to atrophic NFL could be observed in stages (Figs 6 to 8). In none of the three was there a stage of thin, local slit defects in the NFL without accompanying diffuse loss of the pattern.

Severe Diffuse Atrophy (7 Eyes)

The largest group of this series includes eyes that progressed to severe loss of NFL pattern. It was useful to follow this larger number of eyes to observe the stages of damage through their entire course. Nearly all the eyes had advanced loss of disc rim tissue and their mean IOP levels included the highest measured. At the time of the animal's sacrifice, all of the glaucoma eyes had lost more than 80% of the normal optic nerve area. In those that had detailed fiber number counting, there were fewer than 10% of the axons remaining. Once again, the correlation between NFL atrophy and estimated optic nerve damage was quite good.

The loss of NFL pattern in this group progressed through the stages of mild, local wedge loss through more extensive loss of the NFL pattern above and below, finally achieving a nearly complete loss of any striations in some eyes. In some eyes, several key features of NFL loss could be observed in the same eye over time (Fig 9). As the pattern of NFL striations becomes less distinct, some areas are more affected than others, leading to local, wedge-shaped zones of greater atrophy. Simultaneously, the blood vessels that are normally buried in the NFL are more clearly seen. The larger vessels are first to be bared, with their first and second order branches becoming bared to view if atrophy procedes far enough. If the loss progresses to a severe stage (as in this group) the actual wall of the vessel is seen as a white outline to the red blood column. The wall is normally not visible due to the surrounding NFL striations. A further feature of NFL atrophy that has proceded to an advanced stage is the conversion of NFL striations from their normal, bunched up, luxuriant appearance to a finely etched, apparently more organized look (Figs 9 and 10). This finely etched appearance is also visible in the eye shown in Fig 11. Here the upper NFL had lost 85% of its normal thickness and no striations at all are seen. Inferiorly, where the loss was somewhat less (71%), the fine lines of the bundles thinned to a thickness of 50 μ are seen. In areas of the retina in which the NFL thickness was 25 μ or less, no striated pattern at all was seen, even the finely etched one.

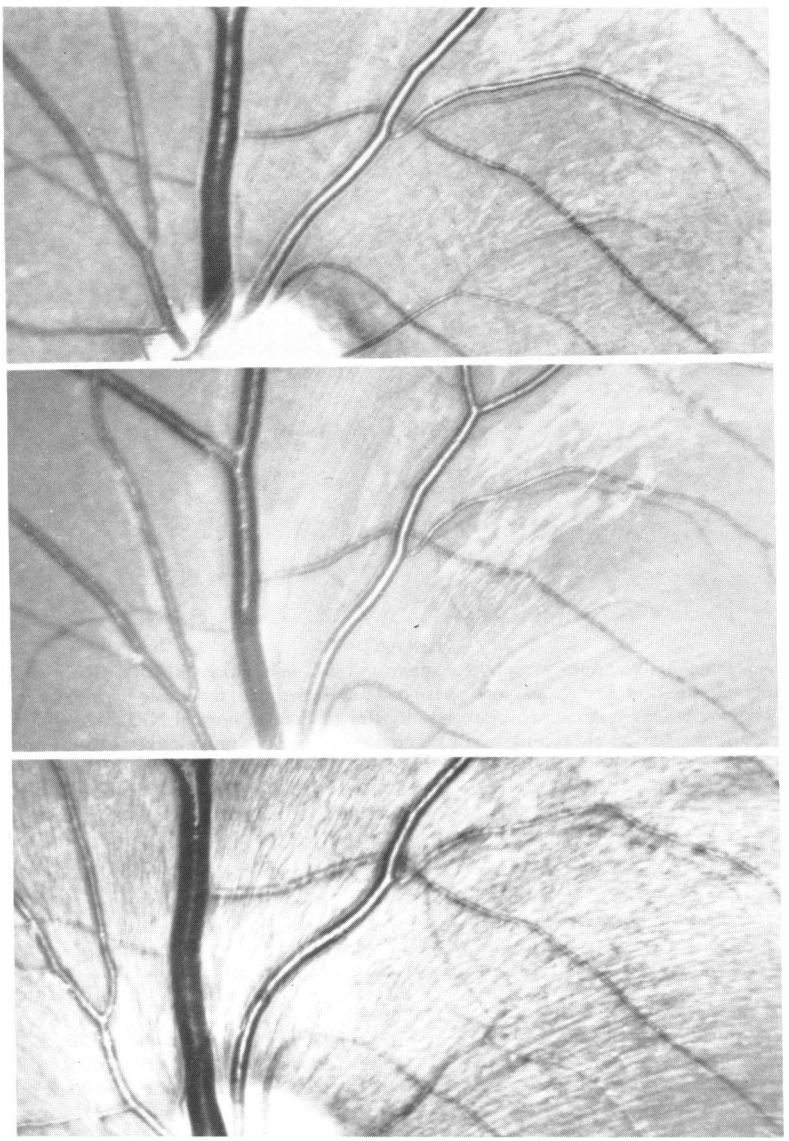


FIGURE 9

In a 6-month period, this monkey developed severe loss of NFL pattern. In middle photograph, one sees stage of decreasing striation brightness and increased linearity (finely etched appearance). At end-stage, bottom, there was no detectable pattern in zone at 6 o'clock. All vessels are seen clearly and wall of large venule is apparent as a white border for some distance away from disc on either side of red blood column. This wall is not seen normally (top) due to surrounding NFL reflexes (monkey 564W62).

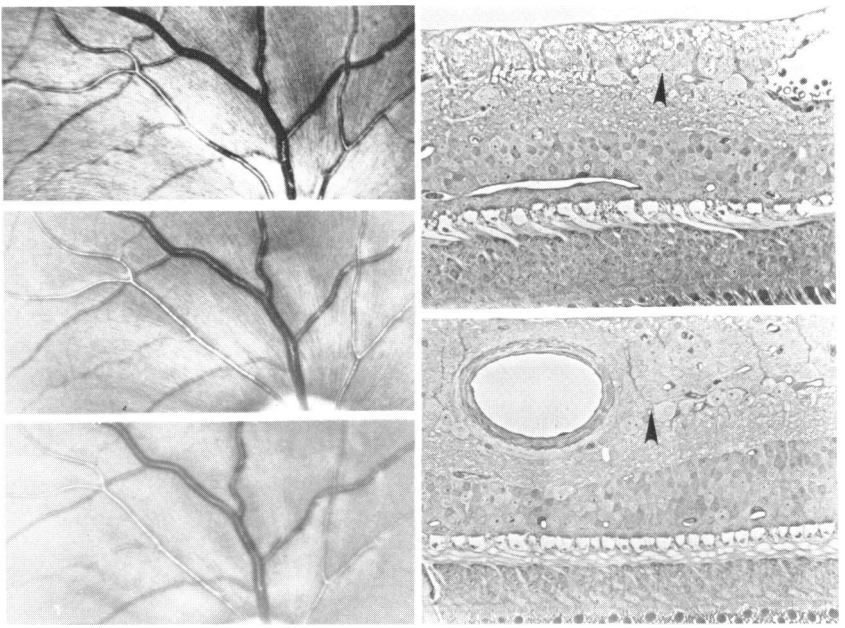
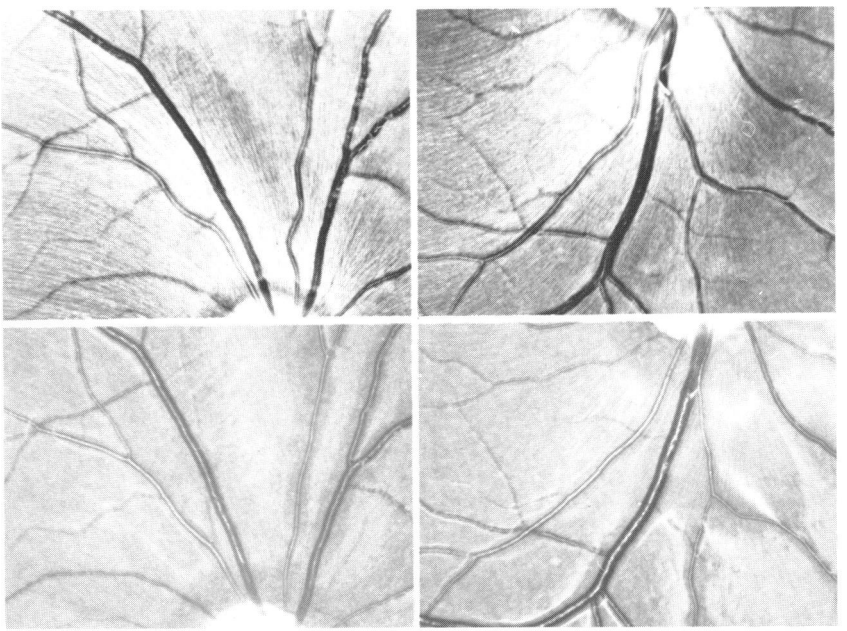


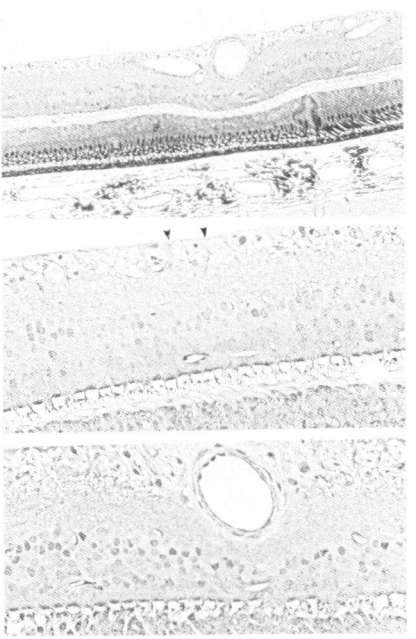
FIGURE 10

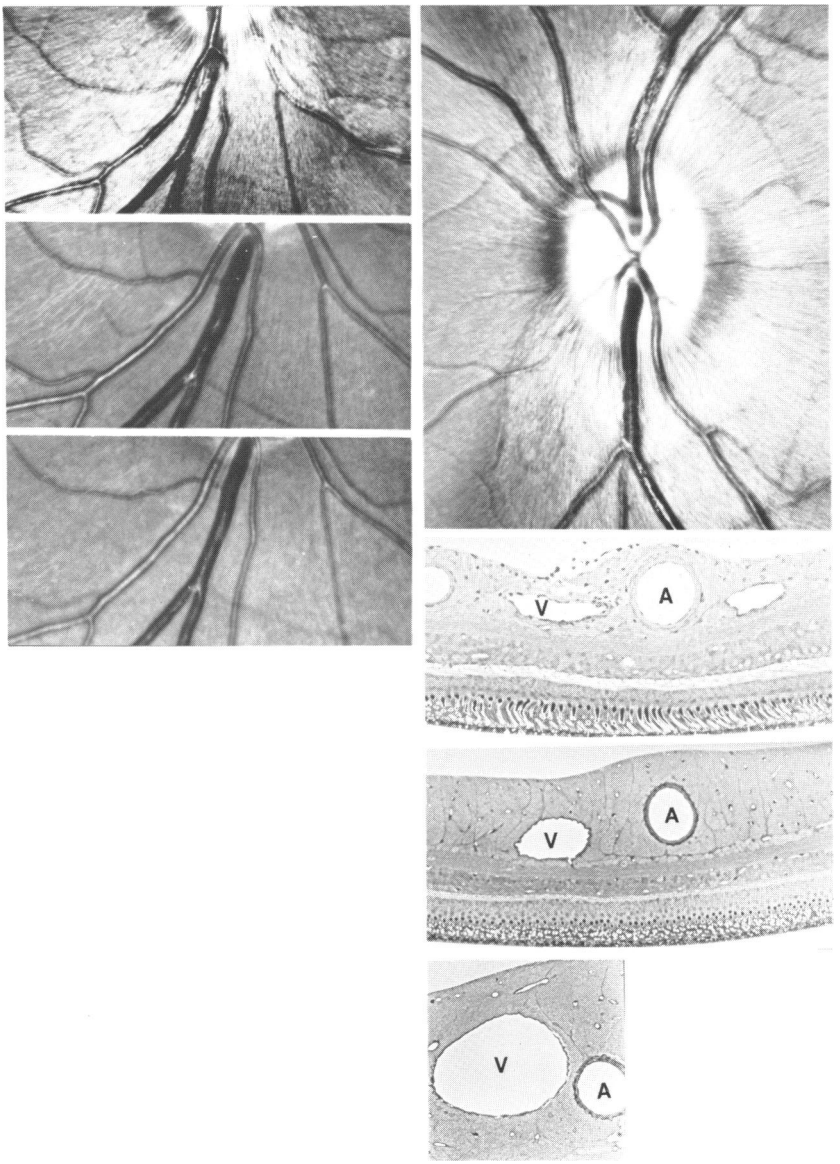
A: Development of diffuse atrophy in superior NFL of monkey M550W61. In middle photograph, finely etched appearance phase had developed with moderate baring of vessels. Note how striations seem much more cleanly drawn than in normal NFL above. By last photo, atrophy was nearly complete. B: Photomicrographs of same monkey eye in superior NFL (above) and inferiorly (below). Greater loss of NFL thickness superiorly, though both show thinning and little NFL cover for blood vessels that were clinically no longer blurred by overlying NFL. Remaining NFL is between inner retinal surface and *arrowhead* (paraphenylenediamine, × 400).

FIGURE 11

A: This monkey developed severe-grade NFL atrophy that was worse above, shown here, than in the inferior NFL, seen in (B) (monkey M549W59). Loss of striations was nearly complete in superior NFL and even second-order vessels were bared. C: Histologic micrographs, top, inferior NLF is seen to be so atrophic that no tissue overlies the vessels. Likewise, the arteriole from superior NFL seen in bottom micrograph has only 5 μ of NFL thickness over it, and was clinically bare. Middle micrograph shows inferior NFL and corresponds to area of finely etched striations in place of normal luxuriant NFL appearance at 6 o'clock. This appearance develops because NFL bundles thin so considerably that they are approximately same width as the Muller cell end feet (*arrowheads*) that make up dividing dark lines between bundles. This allows dark lines to stand out more sharply, producing a clearer definition of striations characteristics of this stage of atrophy (paraphenylenediamine, × 160, 400, and 400, respectively).



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The zone between 25 and 50 μ of NFL thickness appeared to be a critical one for visibility of any striated pattern overlying blood vessels, as well. For large arterioles and venules, there could be from 25 to 50 μ of NFL over a vessel without any blurring of the vessel wall (Fig 12). When there was more than this thickness, however, the vessel had a blurred margin or an overtly striated pattern overlying it. When the NFL on top of the vessel was in the range of 100 μ thick, the vessel wall was more difficult to identify clearly. In most of the eyes in this severely damaged group, not only first order, but also second order branches of retinal vessels had a "too distinct" vessel wall, indicative of near total NFL atrophy. The average amount of NFL left in the upper and lower zones that were surveyed was 12 and 50 μ. This represented from 65% to 95% loss of NFL thickness, or 150 μ of lost thickness in absolute terms at this point in the retina (Table I).

HUMAN CLINICAL/PATHOLOGIC CORRELATION

The human eyes that are presented must, by the nature of how they can be acquired, differ from the monkeys. Optimal conditions of photography were present in some, but in others, cataract or miotic pupils prevented perfect photographs. Whle we were able to generate a range of NFL atrophy, there was not the same ability to "produce" very many mildly damaged specimens. Finally, the fixation was good in surgically obtained eyes, but poorer in eye bank material. This final deficiency was counteracted to some degree by attempting to compare findings within different areas of the same eye. A summary of the human data is given in Table II.

Normal Eye

One normal eye was obtained at exenteration for sinus cancer involving an orbit (patient 1, Table II). The thickness of the rim of neural tissue as it enters the superior or inferior nerve head (arcuate) at the disc rim was

FIGURE 12

A: Over 11 months of elevated IOP, monkey M620U45 developed severe diffuse atrophy, shown both by progressive loss of striations and by abnormally clear view of first- and second-order blood vessel branches. B: Normal NFL appearance in left eye of same monkey for comparison with histology in C. C: These photomicrographs compare histologic appearance of inferior NFL in right, glaucoma eye (top) and superior NFL in left, normal eye (middle). In top photo, remaining NFL overlying four vessels is nowhere as thick as 25 μ (A = arteriole, V = venule). These same vessels are seen in clinical photo, Fig 12A, bottom, to have no apparent NFL blurring over them. By contrast, there is approximately 50 μ of NFL thickness over superior arteriole and venule in left eye, superiorly (middle photo and Fig 12B) and 75 μ overlying the arteriole (A) in inferior, left retina seen histologically in bottom photo (and clinically in Fig 12B) (paraphenylenediamine, × 175 and × 400, respectively).

approximately 400 μ in thickness. This is somewhat greater than the thickness in the macaque monkey at this position in a previous report.[43] The thickness of the NFL at the disc rim on the foveal side was somewhat less, 275 μ. At the standard position measured in the arcuate retina, approximately 1 mm up or down from the nerve head, the NFL was 150 to 200 μ thick in this normal.

Neurologic Disease (2 Eyes)

In two eyes, clinical photographs were available in patients with a meningioma of the sella (patient 2) and presumed optic neuritis (patient 3, right eye). Patient 2 had easily detected NFL striations above and below the disc (Fig 13) and NFL thickness of 125 to 150 μ. The actual optic nerve counts for this eye showed a loss of about ⅓ in the total number of axons expected, with a slight preponderance for loss in the temporal quadrant that was not statistically significant.

The other patient had a prior history compatible with optic neuritis in the right eye (3 R), and had typical open-angle glaucoma in the fellow eye (3 L). The right eye had temporal pallor of the disc, decreased visual acuity, and a central scotoma. The arcuate zones of the NFL were well seen (Fig 14) and measurements of NFL thickness in the corresponding arcuate retinal zones was normal at 100 to 150 μ. Note that the foveal side of the disc rim was dramatically thinner than the normal eye above (50 compared to 275 μ) and was even narrower than its fellow eye (3 L) that had much more significant overall optic nerve damage from glaucoma. The photograph of this right eye suggests a loss of the NFL pattern toward the fovea (Fig 14), but this area demands red-free photographic technique for adequate definition, and only standard color slides were available.

Ocular Hypertension (2 Eyes)

Patient 4 was followed for 3 years in a prospective study of NFL appearance with serial examinations, including detailed perimetry on the Goldmann instrument. No defect was defined by this method, though several single points were abnormal in the superior field by 1 log unit on the Peritest automated test in each eye. The NFL was rated normal by repeated clinical examination. The optic nerves are not yet studied for precise nerve count, but the area of remaining neural tissue suggests mild loss. The NFL thickness was in the same range as in the preceding normals in the arcuate area, 125 to 250 μ (Fig 15).

Asymmetric Glaucoma Damage (5 Eyes)

In this group, there was asymmetric loss of NFL pattern clinically and a

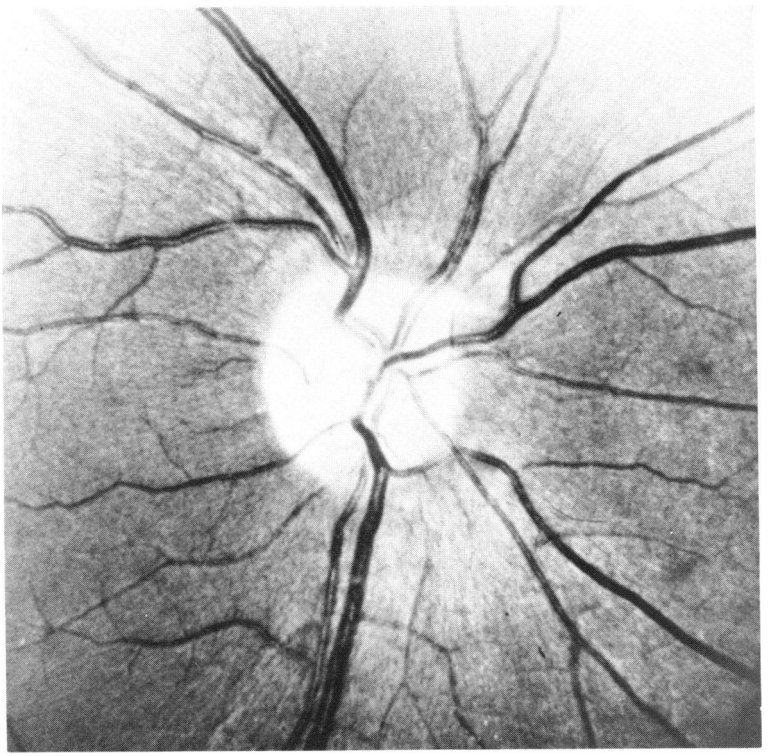


FIGURE 13

This young woman had a tumor of sella and had normal NFL clinical appearance in this eye with a retinal NFL thickness equal in most areas to our control retina. Optic nerve counts suggested a modest, diffuse loss of ganglion cells less than one-third below the normal number. This loss, if accurate, was undetected by NFL examination (patient 2).

corresponding histologic difference either in one eye compared to the other (patient 5) or in the superior compared to the inferior retina (patients 6 to 8). Patient 5 had an inferior nasal step in the right visual field, while the left eye had a generally contracted field, 20/70 cataract, but no localized defect. The cups were symmetrical. Careful inspection of the color photographs of this case show a lack of NFL pattern in the 11 o'clock zone above and a lack of normal blurring of vessel walls there by the NFL pattern (Fig 16). Histologically, the NFL at the disc in the right eye was thinner superiorly by 100 μ compared to inferiorly, and the latter itself is somewhat lower than the normal, control eye. Furthermore, the arcuate retina NFL thickness was 50 μ superiorly compared to 100 μ inferiorly (Table II).

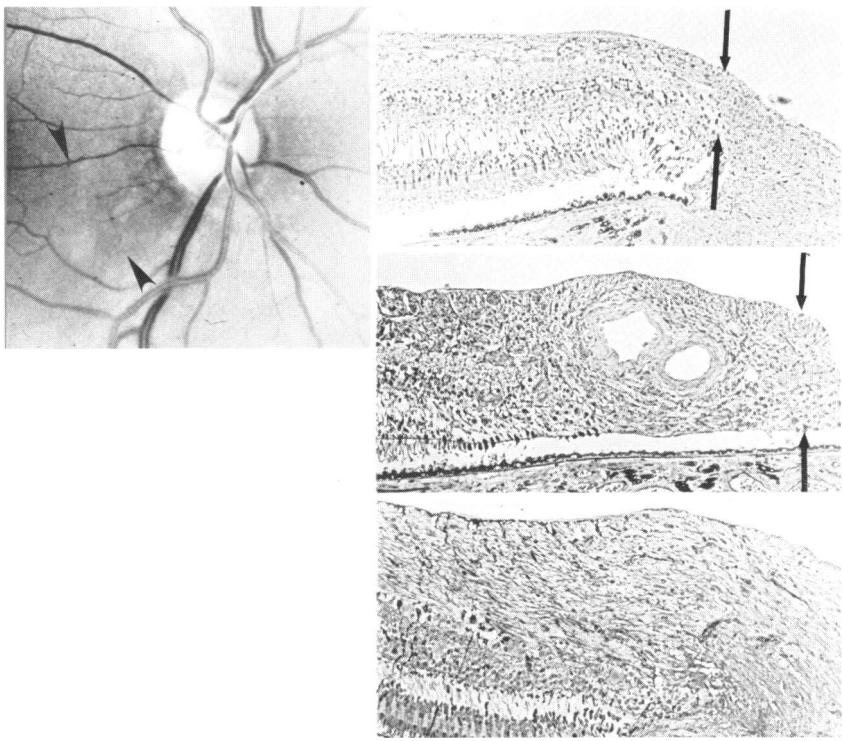
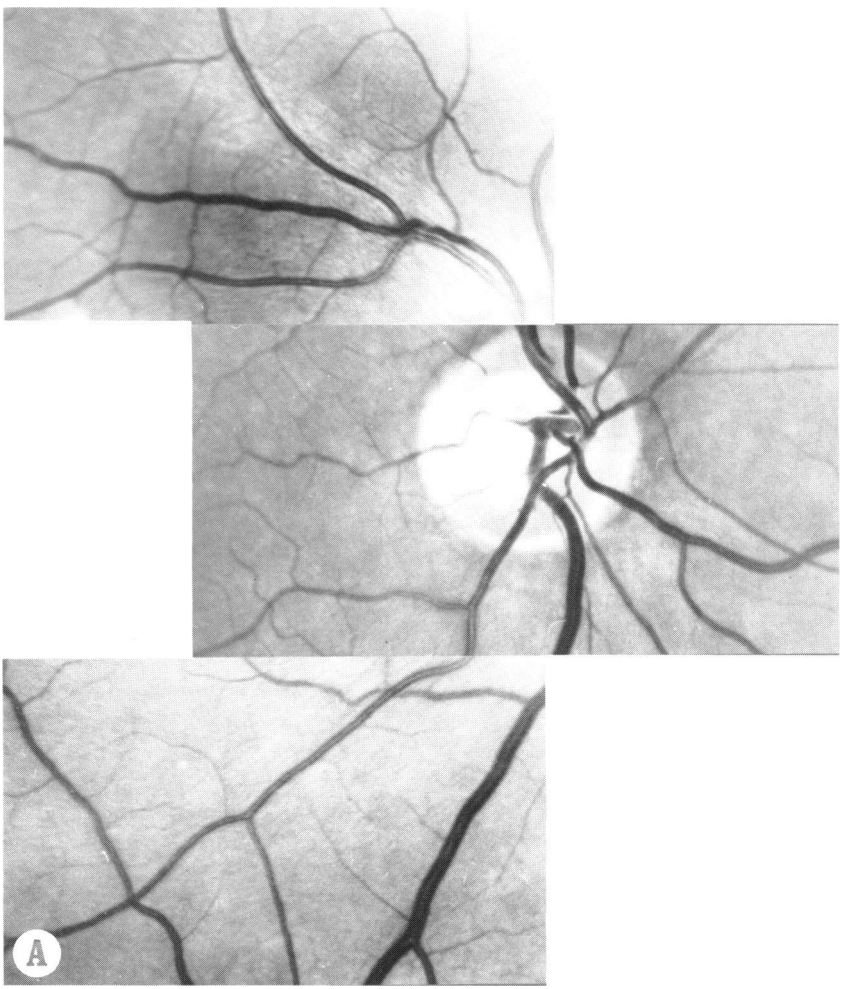


FIGURE 14

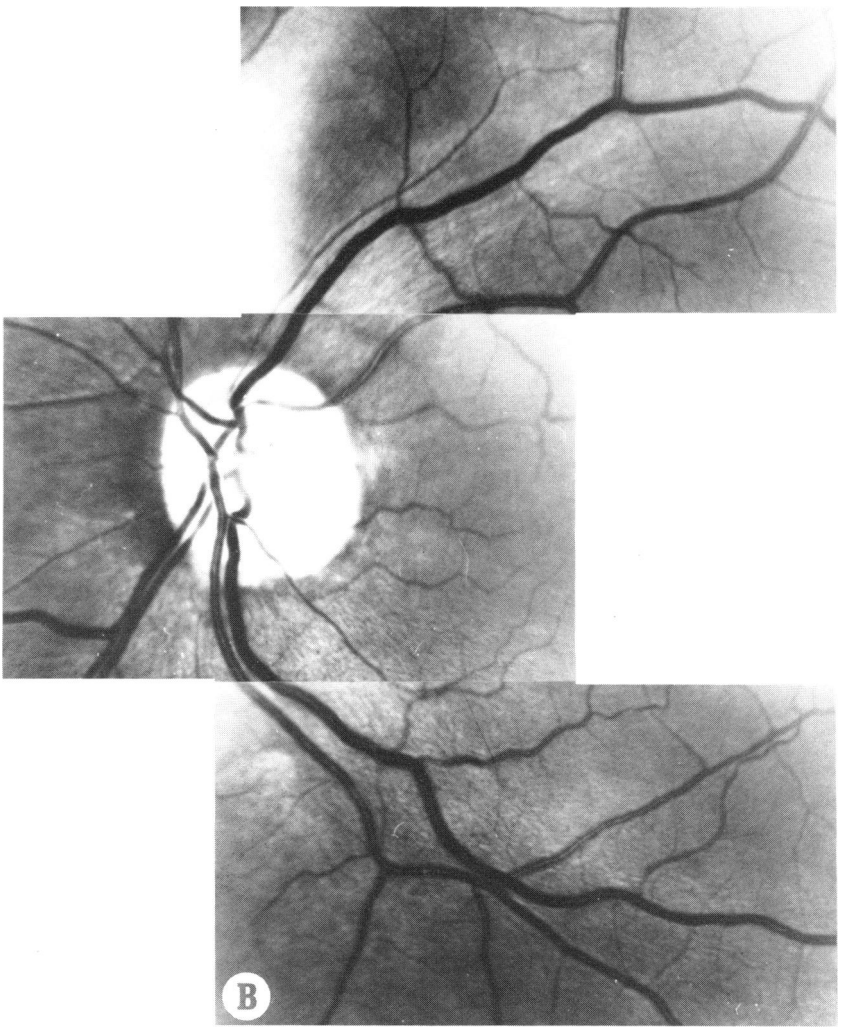
A: Clinical photograph of patient 3, right eye, with history compatible with optic neuritis, exhibiting visual acuity of 20/50, relative central scotoma, and loss of one-half of fibers in optic nerve cross-section (mostly in temporal quadrant of nerve). The arcuate NFL areas extending out from 12 and 6 o'clock have no detectable loss of pattern, and this is confirmed by the histological finding of up to 150 μ of NFL thickness. Toward the fovea, there is a subtle darkening of the NFL pattern beginning just clockwise from the 6 o'clock venule and extending up to nearly 9 o'clock (between *arrowheads*). The technique of this photograph (originally a color slide) is barely adequate to show this finding. B: Three photomicrographs of disc rim on the edge of nerve head facing the fovea (temporal). At top, rim of patient 3, right eye, showing narrowing to only 50 μ of NFL (between *arrows*). The fellow eye (middle photograph, 3L) that had severe glaucoma damage, and had major loss of fibers at the vertical disc poles, nonetheless retained 150 μ of NFL at the foveal rim (between *arrows*). Below, normal eye (1) shows 250 μ of NFL thickness at the foveal rim edge, filling the photomicrograph from top to bottom at the same magnification (paraphenylenediamine, × 200).

FIGURE 15

A & B: Clinical photographs comprising the right eye (A) and left eye (B) of patient 4, with no detectable abnormality. The pattern of the NFL is brightest at the vertical poles, and of similar brightness comparing superior with inferior, and superior in one eye with superior in the other eye. C: Retinal sections from right eye superior (above) and inferior (below) in

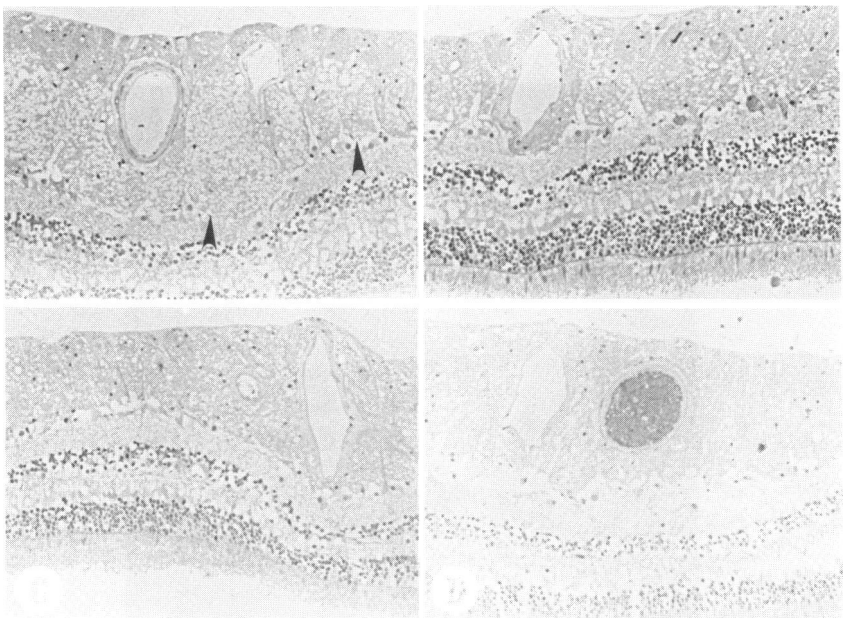


same patient. Range of NFL thickness is normal (150 to 250 μ) in these areas (*arrows* show NFL limits in upper photograph for orientation in this and other histologic sections). Large open areas are blood vessels. In top photo, larger arteriole seen is that found in A at 11 o'clock position. Note that NFL does not greatly blur vessel wall clinically, though there is 30 μ of NFL tissue over it. Similarly, bottom picture of C shows large venule at 6 o'clock in same right eye. Clinically, it had no overlying striations, as is true of many large vessels near disc. Histologically, there was almost no NFL tissue over it, as the vessel occupies nearly entire 200 μ of NFL thickness (paraphenylenediamine, × 160). D: Histology of left eye, patient 4 (compare B), showing normal thickness NFL. Upper photo contains 1 o'clock venule and lower has 6 o'clock arteriole and venule. With less than 50 μ of tissue over them, the vessels have no blurring of their walls (paraphenylenediamine, × 160).



Patient 6 had long-standing open-angle glaucoma with complete loss of inferior disc rim, dense upper field loss, and major loss of neural area of the disc, especially the inferior nerve. The remaining blurring of vessels by the NFL pattern was visible superiorly, while no NFL pattern was seen inferiorly. The disc rim showed this asymmetry, with 125 μ of NFL left superiorly, but only 65 μ inferiorly.

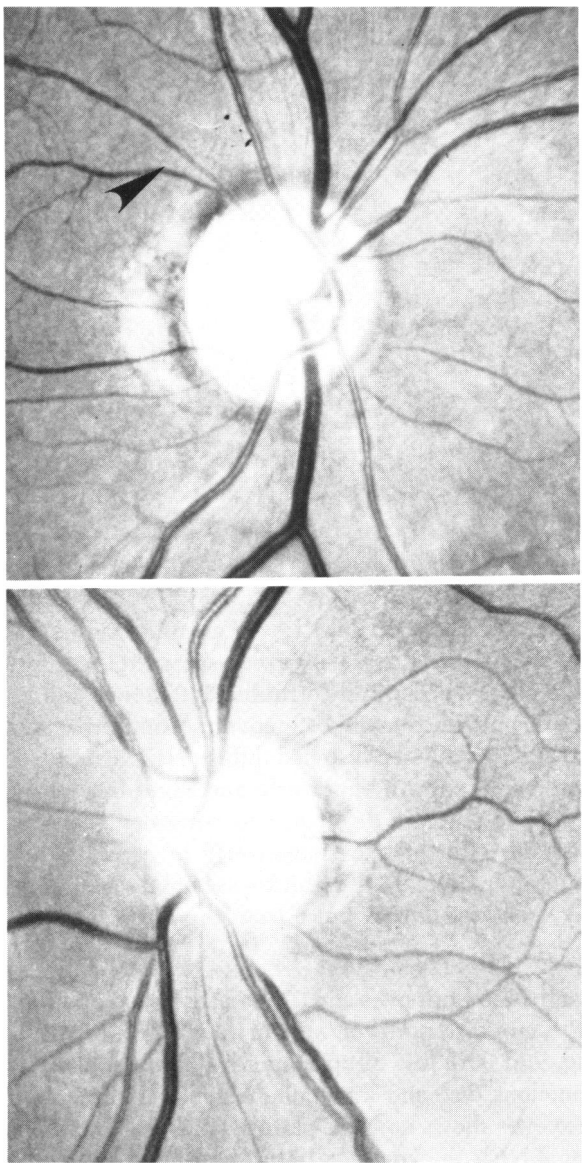
Other NFL photographs of patients 7 and 8 have been previously published.[13] Both had iris melanomas and secondary glaucoma. Both had



enlarged cups and superior field loss of a mild degree associated with major loss of optic nerve fibers with greatest loss in the inferior nerves. In patient 7, the NFL was clinically atrophic both above and below the disc, but some NFL pattern remained to cover second-order vessel branches superiorly (Fig 17). Patient 8 also had diffuse NFL atrophy clinically, with a barely detectable pattern superiorly and none left inferiorly (Fig 18). The NFL thickness at the disc rim was consonant with these findings, with 140 μ more rim thickness superiorly in patient 7 and 50 μ more above in patient 8. Retinal NFL thicknesses of 50 μ or less in these eyes were poorly seen at 1 mm or more from the disc.

Severe Glaucoma Damage (5 Eyes)

Patients 9 and 10 in both eyes and 3L had advanced open-angle glaucoma damage, with cup/disc ratios of 0.9, threatened or lost central fixation in field testing, and 90% loss of optic nerve fibers. In addition, patient 9R had an anomalous disc and a macular hole. The clinical photographs in each of these eyes shows no NFL pattern (Fig 19). Only in patient 9R was there a retinal NFL thickness > 100 μ, and in most areas it was < 75 μ thick. The disc rim thicknesses were also depressed to ⅓ or normal or less in all but one area of patient 10L.



DISCUSSION

THE TYPICAL COURSE OF NFL LOSS

These studies detected the course of NFL loss in two ways. In individual monkey eyes, the stages of NFL atrophy were photographed. In human eyes, the time course of atrophy is more often much longer, and examples of progressive atrophy are fewer. Therapy to reduce IOP probably also (hopefully) slows the rate of progression. Thus, the human data are dependent on cross-sectional study to determine the timing of NFL loss.

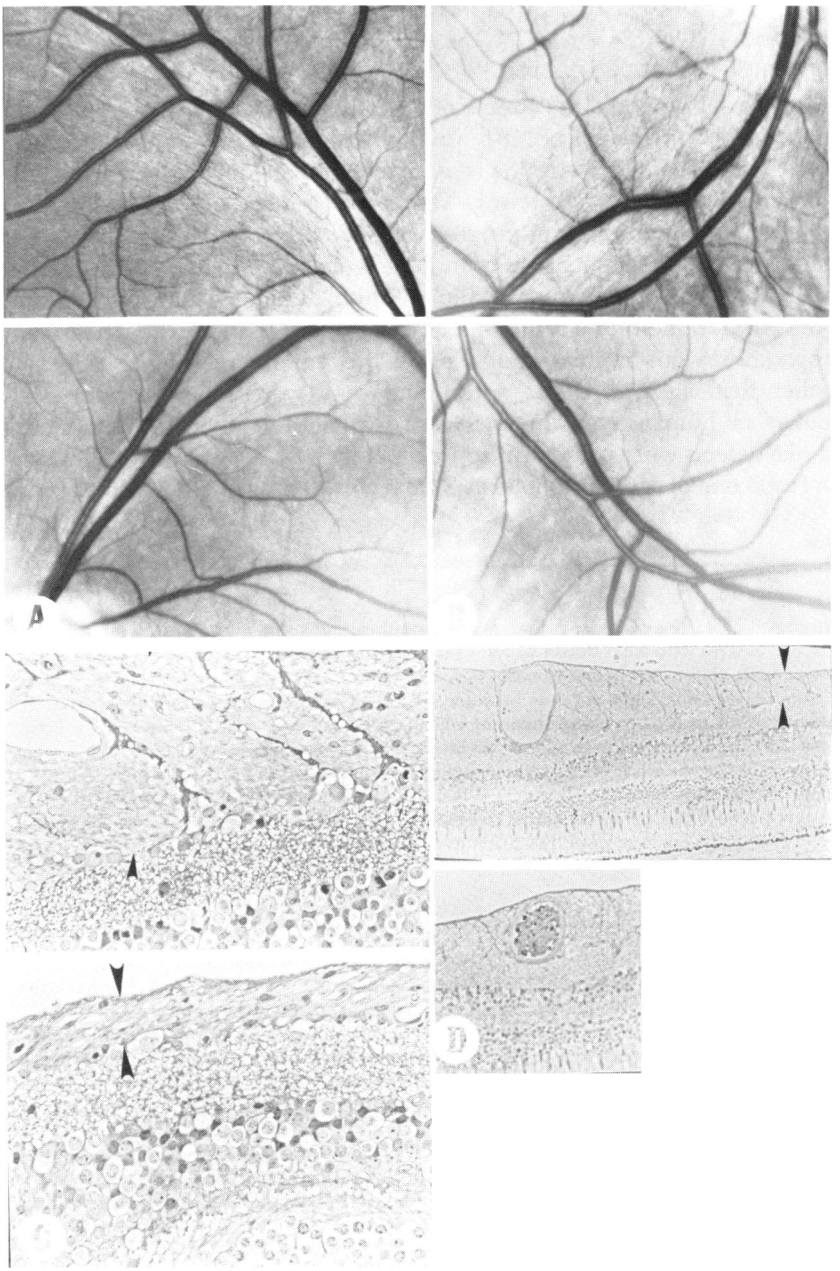
The first surprising result was the finding that narrow, slit-like defects in the NFL are neither a common nor reliable sign of the earliest phase of atrophy. Previous reports had stressed the importance of slit defects, smaller than an arteriole in width, but they were rare in either our monkey or human eyes. Furthermore, in another clinical study, such slit-like defects were present in approximately 10% of normal eyes.[40] One can reach one of several conclusions from this finding. First, normal eyes

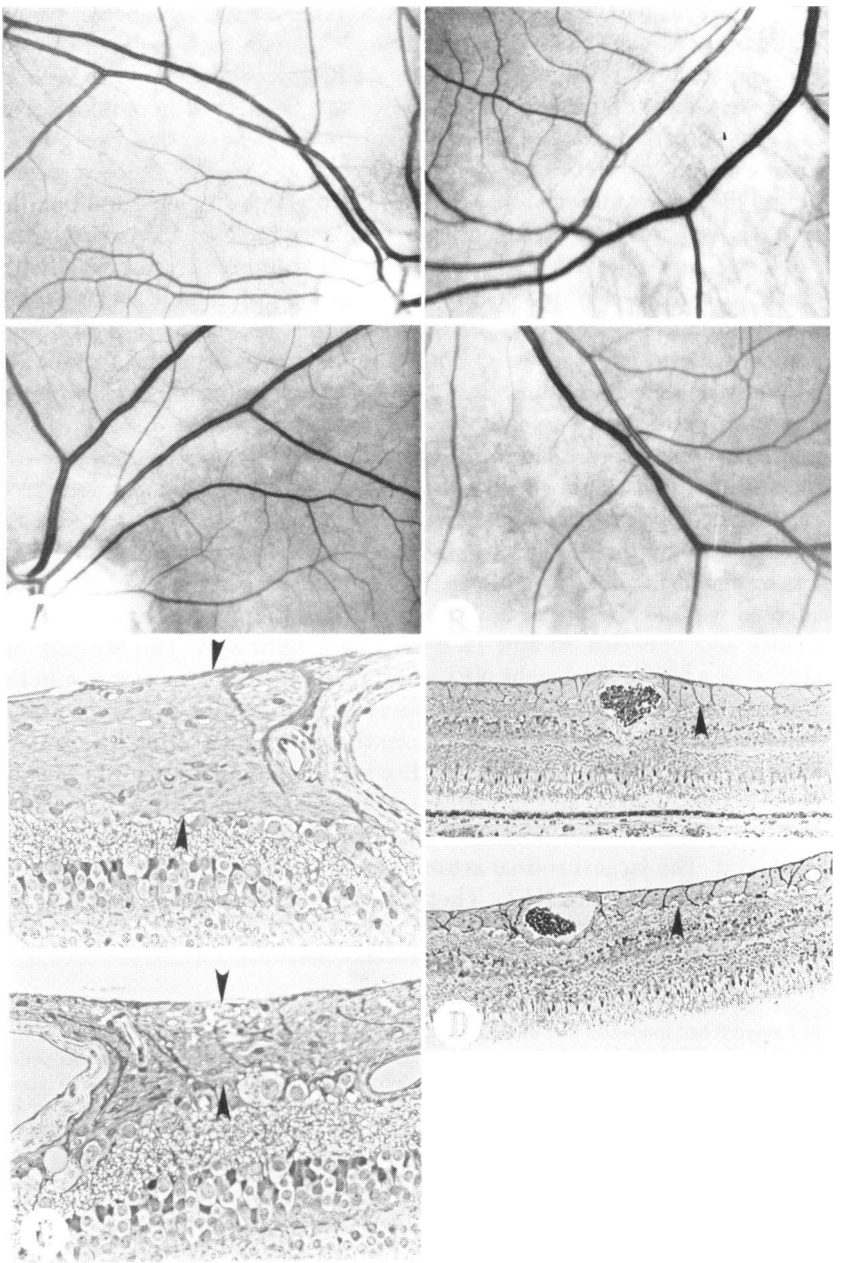
FIGURE 16

Patient 5, with early cataract and open-angle glaucoma, had a shallow nasal step inferiorly in right eye and no local field defect in left eye. Careful inspection shows an NFL pattern is present both above and below in each eye, but if one considers in which zone it is least prominent, superior, right eye area between 10 and 11 o'clock is less distinct than expected (*arrowhead*). This is particularly apparent when comparisons are made to other areas. First, upper NFL is usually just as bright or brighter than inferior (see left eye, below). This is not true in right eye. Second, NFL pattern should be brightest in right eye between 10 and 12 o'clock, but in zone between 10 and 11 it is no more apparent than in foveal zone from 8 to 10 o'clock. This finding is quite similar to monkey eye in Fig 2. Histologically, upper NFL in right eye was from one-third to one-half thinner than lower NFL.

FIGURE 17

A & B· Patient 7 had diffuse loss of NFL striations above and below disc. In A, normal right eye is compared to atrophic left eye in superior NFL. Note slight remaining cover of NFL for second order blood vessels in lower photo, with complete baring of first-order branches. In B, inferior NFL is seen in normal eye (top) and glaucoma eye (bottom). Atrophy is complete clinically. C: Asymmetric diffuse atrophy seen clinically is mirrored in retinal NFL thickness in left eye. Above, superior disc rim retained 190 μ of NFL (less than half of normal), while more atrophic appearing inferior rim had only 50 μ left (both shown between *arrowheads*) (paraphenylenediamine, × 400). D: Histology of superior NFL in same patient. In upper photo, superior venule seen in A has little overlying NFL, so appears clinically bare, but has over 100 μ of surrounding NFL, explaining somewhat retained striations seen on either side of it. However, temporal to this vessel, NFL striations disappeared clinically, corresponding to zone to right in upper photo where NFL thinned to < 50 μ (*arrowheads*). Below, photograph of arteriole from inferior arcuate retina, showing no NFL overlying this clinically bared vessel (paraphenylenediamine, × 160 and 400).


A
D



not infrequently have single tiny zones of thinner NFL as a normal variant that appear as dark slits. Even if this were also an early pattern of NFL loss in glaucoma, the specificity of the finding would be too low. Second, since we do not detect the finding with great frequency in monkey eyes, the degree of localization of early glaucoma damage is not likely to be great enough to produce knife-thin slit dark zones. For these to occur, neural damage would have to be limited entirely to single neural bundles in a highly localized area, with no damage at all to the surrounding bundles. The selectivity of glaucoma damage is apparently not usually this great. However, one must bear in mind that clinical detection of NFL loss is far from perfect. Possibly, the phase at which individual nerve bundles are lost without effect on neighboring bundles does occur, but cannot be clinically detected with any frequency. Studies are in progress to determine the pattern and selectivity of glaucomatous damage at its earliest phase in monkey eyes. These studies may clarify this point. Nonetheless, the specificity of single slit-like dark defects in the NFL for detection of early glaucoma is highly questionable.

The photographs shown here indicate that the earliest detectabe NFL loss occurred in zones as wide as 2 or 3 venules near the disc. The areas affected were at the upper and lower poles of the disc, between 6 and 8 o'clock and between 10 and 12 o'clock in a right eye. The atrophy appeared as a loss of the bright NFL striation pattern and an increase in the visibility of the walls of retinal blood vessels. Since the NFL consists of bundles that converge on the disc from the peripheral retina, the zone of atrophy always is narrowest at the disc and broadens toward the periphery. Both in monkey and human eyes, the sensitivity of detecting abnormality improved when both NFL pattern itself and blood vessels were examined. The largest retinal arterioles and venules near the disc are not normally buried in the NFL. Their diameters are great enough that the

FIGURE 18

A: Patient 8 had moderate loss of NFL pattern in left eye (below) compared to normal right eye (above). While NFL striations are not extremely striking in normal eye in this patient due to light retinal pigment epithelial pigmentation, blurring of vessel walls is almost completely lacking in affected eye. B: Inferior NFL in same patient, with even greater loss of NFL pattern in affected left eye (below) compared to normal eye inferior NFL (above) or same eye's superior NFL (A, below). C: NFL thickness in upper disc rim (above) in patient 8 was 100 μ, or 25% of normal, while thickness was even less (50 μ) in inferior disc rim of same eye (below). *Arrows* delimit NFL margins. Ths asymmetry corresponded to moderate diffuse NFL atrophy superiorly and severe atrophy seen inferiorly by clinical examination (paraphenylenediamine, × 400). D: NFL thickness averaged 50 μ or less in both superior (above) and inferior (below) arcuate retina at 1 mm from disc in patient 8. Outer limit of NFL marked by *arrowhead* (paraphenylenediamine, × 175).

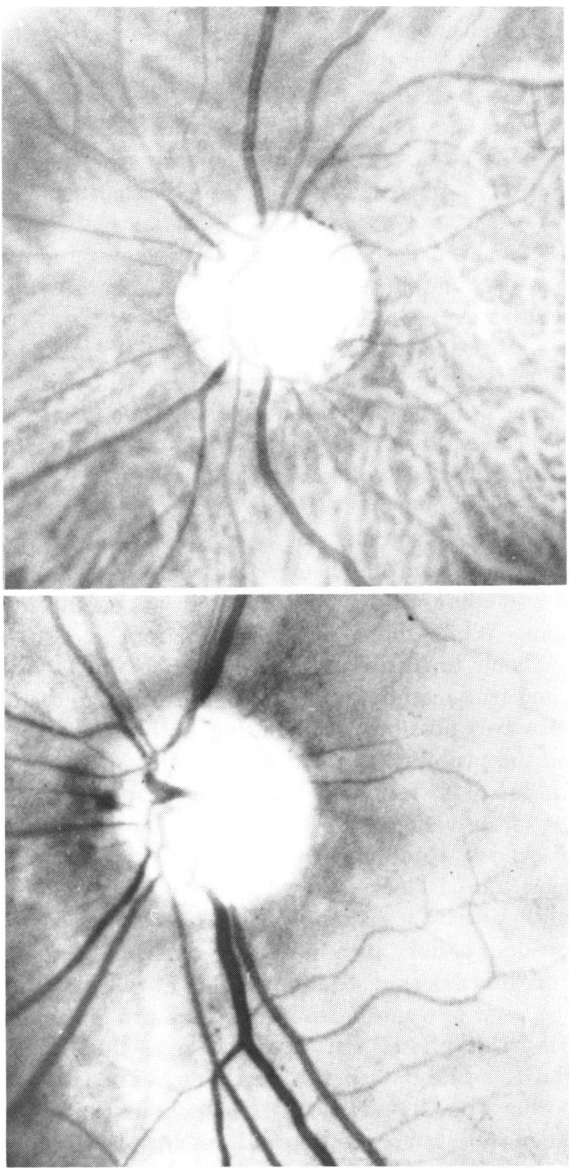


FIGURE 19

Clinical photographs of patients 9, left eye (above) and 10, left eye (below). While both were color photographs taken through moderate cataract, clarity of blood vessel walls is so sharp that little NFL would be expected to be present. Both eyes had a maximum of 100 μ of NFL thickness remaining in arcuate zones, with many areas in both eyes equalling only 50 μm.

portion of their walls nearest the inner retinal surface has little overyling neural tissue. As a result, the red column of blood in these vessels is seen clearly. However, by 1 disc diameter from the nerve head, vessels become narrower and have their first-order branch vessel. At this location, both the narrower main arteriole (and venule) and especially their first-order branches often have striations of the NFL pattern overlying them. In normal eyes this occurs when 50 μ or more of NFL thickness is between the vessel wall and the internal limiting membrane of the retina. Normally, one sees only the red column of blood in the vessels; the walls of the vessels are grey-white in color. Because the walls are the same color as the white reflexes from the NFL, they are obscured in normal retina by the surrounding and overlying NFL pattern.

When NFL atrophy occurs, the decreasing bulk of NFL on top of the vessel leads to a readily distinguishable sequence of changes in vessel appearance. First, the red column of blood is seen not to have the linear striations of the NFL over it. It is seen crisply. Second, as further loss of NFL occurs, the vessel actually stands up in relief due to recession of the NFL. Not only is it seen much more distinctly than normally, but the white vessel wall comes into view with the disappearance of surrounding NFL striations. While the loss of the striations is also evident, it is sometimes difficult to distinguish between poor NFL visibility due to poor media and true moderate NFL atrophy. One can distinguish better between these two possibilities by using both the brightness of NFL striations and the visibility of blood vessel walls.

After the phase of NFL pattern loss in a localized wedge, the next step is broadening of the wedge to include 2 to 3 hours of the clock centered near 12 or 6 o'clock, combined with a greater loss of the NFL pattern. Often, a single zone 1 clock hour wide develops complete loss of NFL striations, while nearby areas are less affected. Not uncommonly, multiple smaller wedges of dark atrophy appear superimposed on a less severe pattern of mild diffuse loss of striations at this stage. The visibility of blood vessels can be used as a semiquantitative measure of the degree of NFL atrophy during this phase. Large vessels near the disc are often not covered by NFL, while their first-order branches usually are so covered, and second-order branches always are. When a first-order branch is bare and its wall seen well, but second-order branches still are blurred by NFL pattern, the damage is likely to be moderate. When even second-order branch vessels have no overlying NFL pattern, the damage is severe.

At a later stage of damage, nearly the entire upper and lower NFL is gone. At this time, the bundles directed toward the disc from the fovea can sometimes be seen to remain. Their normal faint visibility is seem-

ingly enhanced by the contrast of no pattern at all visible in the arcuate zones. Blood vessels stand up in sharp relief and their walls can be seen as white lines out a considerable distance from the disc. Sometimes the reflex from the internal limiting membrane draped over the vessels is seen.

HOW MUCH NERVE FIBER LOSS CAN BE DETECTED?

Examination of the NFL would be worth little if it detected damage after other available methods. Its sensitivity can be measured in terms of actual neural loss or comparatively with other techniques.

A previous report[43] suggested that loss of 50% of th NFL thickness could be detected after orbital nerve injury. The new data presented here allow a more precise measure of the extent of NFL loss that can be detected under ideal circumstances in monkey and human eyes. It is now clear that we require between 25 and 50 μ of NFL thickness for any striated pattern to be discernable. In normal eyes, this is confirmed by noting that the arcuate NFL pattern becomes difficult to see from 2 to 3 disc diameters from the nerve head, where the normal NFL thickness declines to < 50 μ. The normal NFL thickness between the disc and fovea is at or below 50 μ within 1 disc diameter of the nerve head. This largely explains the difficulty in identifying the normal NFL pattern in this area except under ideal conditions.

A second confirmation that 25 to 50 μ of NFL thickness is a critical amount for detection is NFL observation over blood vessels. In the monkey and human eyes, vessel walls appeared to have an overlying NFL pattern that blurred the view of their walls when at least 50 μ of NFL thickness was measured over them. Those vessels that appeared to have no NFL cover had less than this much tissue. Furthermore, in those monkey eyes that had just detectable NFL loss, the absolute amount of thinning of NFL in the clinically atrophic zones was between 50 and 70 μ.

If it is possible to detect a loss of 50 μ of NFL loss in the anterior/posterior direction, and if one of our localized wedges is approximately 250 μ wide near the nerve head, how many nerve fibers would be involved? The average optic nerve fiber is approximately 1 μ^2,[44] placing 12,500 axons in such a zone. The volume of the tissue occupied by astrocytes and capillaries is less than 5% of the total, so 12,500 fibers represents about 1% of the axons in a monkey or human optic nerve. While NFL examination may theoretically be capable of detecting damage at this level, glaucoma damage may typically be more diffuse than this. It seems unlikely that 10,000 axons in one zone die with no loss anywhere else. In fact, the three monkeys in the mild damage group that had NFL damage detected

clinically had lost ¼, ⅓, and ½ of the optic nerve fibers overall. In the zones of NFL measured histologically, we could detect loss of 28% to 45% of the NFL thickness, but the overall neural loss exceeded that accounted for by the decrease in the zones that we measured. This indicates again a fact stressed by previous neural studies[12,13]—glaucoma damage selectively kills fibers in the upper and lower arcuate zones (the vertical disc poles), but damage is also going on to a lesser degree in the remainder of the nerve as well. Loss of fibers in the nonarcuate areas goes undetected by NFL examination longer because it occurs more slowly and because the NFL is more difficult to see there. As a result, the total loss of fibers that is present when damage is detected by NFL examination is greater than the loss calculated to have occurred in the specific zone of NFL with a local wedge defect. On the other hand, damage begins to be routinely apparent in the examples we studied when only a minority of the optic nerve has been lost.

It can also be concluded from the quantitative data that NFL examination reliably detects nearly every example of neural loss amounting to more than ½ of optic nerve fibers. The moderate and severely damaged monkey eyes all had optic nerve fiber loss estimated to be near or greater than 50%. The abnormality in the NFL in these eyes was no longer subtle. Likewise, in each human eye with damage greater than 50% of nerve fibers, the NFL was definitively atrophic.

Did the optic disc and its cup signal the damage equally well? In the monkeys, five mildly abnormal NFL eyes had cup/disc ratios from 0.3 to 0.7. The smallest of these was not detectably enlarged, even in comparison to its preglaucoma size. While the other cups were larger than their baseline pictures, the configuration of the final pictures alone was not striking enough to be more than suspicious for damage. By contrast, only one of five eyes with mildly abnormal NFL could not have been detected without the serial pictures for comparison. In all cases of monkey or human eyes with moderate diffuse atrophy, the cup/disc ratio was 0.8 or larger. One can conclude that the NFL examination appears well correlated with cup/disc ratio and that both begin to indicate neural loss during the loss of the first 50% of the optic nerve fibers. Sommer and co-workers[34,35] concluded that the sensitivity of the NFL examination was greater than disc examination in detecting eyes that subsequently developed field loss. More extensive, prospective data are needed to examine this question.

All human eyes with field defects on the Goldmann perimeter had abnormal NFL examinations. This agrees with larger clinical studies showing that the NFL examination is abnormal with great frequency prior

to the stage of (Goldmann) visual field loss.[33,40] It remains to be shown whether the apparent increase in sensitivity of automated perimetry will change this conclusion. Early studies have suggested that the sensitivity of NFL examination continues to compare favorably even with quantitative static perimetry.[38]

A SYSTEMATIC APPROACH TO THE NFL EXAMINATION

The findings of this study, as well as experience with NFL examination in several thousand photographs of human eyes, have suggested an approach for looking at the NFL that uses the histological results presented here as effectively as possible.

Begin with a dilated pupil and as bright a direct ophthalmoscope with green filter or contact lens/slit lamp with green filter. Obtain opposite eye fixation and position the eye at different angles to obtain best reflex from the NFL. With the slit lamp, the light source should be optimally placed to one side with a moderately thin beam. The NFL pattern is near or slightly posterior to the retinal blood vessels.

Examine the pattern of the NFL near the 12 o'clock position and determine if there are striations or not. If there are, see if they cover the largest blood vessels by blurring the vessel wall image near the disc. If there is a bright pattern, and large vessels are covered, the NFL is normal. If the pattern is difficult to see and first-order branches of vessels are bare (sharp wall images), mild or moderate NFL atrophy is present. If the pattern is absent and even second-order vessels are clearly not covered by the pattern, severe diffuse atrophy is present.

Remember at this stage that comparison of one area to another is very helpful. In a right eye, the pattern should be brightest between 6 and 8 o'clock and 10 and 12 o'clock, since the NFL is thickest there. Look in these areas and see if the normal progression from brighter to dimmer pattern toward the horizontal meridian is present. If the pattern is difficult to see at 11 o'clock, becomes brighter inferiorly at 10 o'clock, then dims again at 9 o'clock, an abnormality is present. Probably closer inspection will disclose a local wedge centered at 11 o'clock. Compare next the superior to the inferior NFL pattern. Typically, there is a lighter pigment epithelial background below, making the inferior NFL pattern slightly harder to see. However, this comparison of up to down is the best way to see whether a somewhat attenuated NFL pattern is due to media haze or mild atrophy. Cataract rarely makes it difficult to see only one half of the NFL.

Next, look at the fellow eye, going through the individual steps mentioned above. Then, compare each area in the second eye with that seen

in the first eye. Often, the superior NFL in one eye is a better yardstick for the same zone of the fellow eye than is its own inferior NFL. This comparison is easiest in photographs, relatively easy with the direct ophthalmoscope, and somewhat cumbersome with a contact lens/slit lamp (but worth the effort).

Finally, it is helpful to correlate the NFL finding with the appearance of the cup/disc ratio and with the visual field (an advantage that was not possible in this study until after the readings were recorded). In the clinical setting, a cup/disc ratio of 0.6 is not definitive, and a 7 decibel loss of sensitivity in two adjacent inferior points in a static, automated field are also of questionable significance. When a mild NFL abnormality in the corresponding superior zone is present in this eye, but not in the fellow eye with the same cup and no perimetric defect, the strength of each finding is greater.

CONCLUSION

This clinical/pathological comparison study extends the histological basis for our understanding of visible changes in the retinal NFL examination. This method can be performed simply, rapidly, and objectively, with equipment available in every ophthalmic office. The histological studies show that the level of optic nerve damage detected in monkey and human eyes is comparable to that of presently utilized perimetry and at least similar in sensitivity to examination of the cup/disc ratio. In some cases, the information from NFL examination is not simply a duplication of data obtainable from other methods, but is truly additive. The course of progressive NFL atrophy does not seem to begin with extremely thin, slit-like defects in the NFL pattern. Rather, local zones of decreased NFL pattern and increased visibility of blood vessel walls occur in zones that are 1 to 2 clock hours wide. While 3 degrees of atrophy can be distinguished by criteria outlined, further quantitative analysis must await improvements in instrumentation. The method described for evaluating the NFL requires patience and experience, but is no more difficult to learn than other ophthalmic examination techniques.

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