A quantitative morphological study of the optic nerve of pre-weanling rats[1]

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Summary. A quantitative histological estimation of the optic nerve of pre-weanling rats has revealed that the total fiber number decreases rapidly between 7 and 25 days of age.

In a recently published study Lam et al.[3] reported that newborn albino rats had a total of about 242,000 non-myelinated fibers per optic nerve. By 6 days of postnatal age a few of the fibers had become myelinated and the total fiber number had dropped by about 60% to 99,000. This latter figure was almost identical to the 100,000 fibers they estimated to be present in adult rats. In previous experiments Bedi and Warren[4] found that in black and white hooded Lister rats the number of optic nerve fibers increases from a value of about 90,000 at 25 days of age to the adult value of about 115,000 by 100 days of age. There may be several possible ways to explain these apparently contradictory observations. For instance Bedi and Warren[4] have suggested that it is possible that during postnatal development of the rat optic nerve there may be a loss of some fibers and growth of new ones occurring more or less simultaneously. Changes in the magnitude of the overall loss or gain of fibers with age could first result in a drop in the total number of fibers (as observed by Lam et al.[3]) but that this could later be followed by a small increase. In order to test this possibility we decided that it would be worthwhile to extend our study to include rats aged between birth and 25 days of age. At birth, litters of black and white hooded Lister rats were standardized to contain 8 pups, of which between 4 to 6 were male. Six male pups, which were not siblings, were killed at each of 7, 15 and 25 days of age by perfusion under anesthesia with 2.5% phosphate buffered glutaraldehyde (pH 7.3) warmed to 37 °C. Only male rats were used so that the overall variance of our results would not be influenced by any possible sex differences in the features under study. The right optic nerve of each rat was dissected out and left in fresh fixative for a further 16 h. The tissue was then washed in buffer, post-fixed in 1% osmium tetroxide, dehydrated in a series of alcohols and embedded in Spurr's resin.

The procedures used for sampling and estimating the total fiber number in the optic nerve have been described in detail in a previous paper[4]. They are however outlined briefly here. A 0.5 µm thick transverse section was cut from each nerve and stained with toluidine blue. Light micrographs of the whole area of these sections were taken on a Vickers M17 light microscope. These were used to obtain the total cross-sectional area of each nerve. This was measured on a sonic digitizer interfaced to a Commodore Pet Personal computer. These measurements were later used to estimate the total number of nerve fibers in each optic nerve, from the estimates of fiber density (see below).

An ultrathin (approx. 70 nm thick) section was subsequently cut from each nerve, stained with uranyl acetate and lead citrate and examined in a Philips 201C electron microscope. A point on the periphery of each nerve was chosen at random and a series of micrographs was taken right across the nerve section. It was ensured that this track passed through the center of each nerve. The electron micrographs of the 7, 15 and 25 day old optic nerves were taken at nominal magnifications of × 15,000, × 10,000 and × 3000 and printed at final magnifications of about × 30,000, × 20,000 and × 6000 respectively. A micrograph of a cross-grating replica having 2160 lines/mm was included in each film taken as a magnification standard. Pilot experiments had revealed that these magnifications were suitable for counting and measuring the optic nerve fibers at the different ages.

The electron micrographs for any given nerve were assembled into photomontages. The approximate centre of a given nerve section was located and sectors were drawn from this point to the periphery. The areas of these sectors were determined and used later to obtain an estimate of the number of fibers per unit sectional area for each optic nerve. All nerve fibers whose centers fell within these sectors were counted and classified as either myelinated or non-myelinated. The minimum diameter (i.e. true diameter; Sima and Sourander[5]) of the axon plus myelin sheath was measured using the computer aided digitizer. These measurements and counts were used to estimate the total number of nerve fibers per optic nerve, their mean fiber diameter and the proportion of myelinated to non-myelinated fibers. The results were analyzed by a 1-way analysis of variance and a test for linear regression.

Our results indicated that myelination of the optic nerve fibers had not commenced by 7 days of postnatal age. Myelination after this age seemed to occur fairly rapidly so that by 15 days of age about 33% of the fibers were myelinated. By 25 days of age this figure had risen to about 93% (table 1). These results are similar to those published by Foster et al.[6] who reported that myelination in the rat optic nerve commenced between 6 and 8 postnatal days of age, and that about 25% and 85% of the fibers were myelinated by 16 and 28 days of age respectively.

We have also observed that the mean fiber diameter of the axon plus myelin sheath also increased rapidly between these ages. Thus at 7 days of age the mean fiber diameter was about 270 nm and had increased to about 760 nm by 25 days of age (table 1). This change almost certainly reflects the increasing degree of myelination of the fibers within the optic nerve.

It was estimated that on average there was a total of 226,622 fibers in the 7 day old rat optic nerve. This number declined rapidly with increasing age; by 15 days of age it had fallen to 167,020 and by 25 days of age to 84,280 (table 1). These changes with age were statistically significant; the linear correlation coefficient (r^2) was close to 1 (table).

Our results confirm the observations made by Lam et al.[3] that there is a rapid decline in the number of fibers per optic nerve during the early development of the rat. However, important differences remain. For instance, Lam et al.[3] found that the decline in fiber numbers occurred between 0 and 6 days of age, at the end of which stable adult values were reached. In the present study, although newborn rats were not examined, we found that the loss in the number of optic nerve fibers continued until about 25 days of age. A possible explanation for this discrepancy may be the strain of the rat used in the 2 experi-

	Myelinated fibers (%)	Mean fiber diameter (nm)	Total fiber number
7 days (n = 6)	0	270.4 ± 16.2	226,622 ± 8067
15 days (n = 6)	32.95 ± 1.91	462.6 ± 24.2	167,020 ± 11,406
25 days (n = 6)	93.30 ± 0.48	761.0 ± 25.1	84,280 ± 7256
F value	999***	113.7***	60.5***
r^2 (linear regression)	0.98**	0.95**	0.91**
b ± SE (slope)	5.21 ± 0.15	27.48 ± 1.51	−7.80 ± 0.61

Results are expressed as mean ± SE. ** $p < 0.01$; *** $p < 0.001$.

ments; Lam et al.[3] used albino rats whilst we have examined hooded rats. This possibility requires further investigation.

It is interesting to note that the value for the total fiber number obtained for the 25 day old rats (i.e. 84,280 ± 7562; see table 1) is not significantly different from the value of 89,778 ± 6625 that we obtained for a separate group of identically aged well-fed rats in a previous study[4]. This seems to indicate that the experimental procedures and methods that we have used for estimating the number of optic nerve fibers are capable of yielding consistent results. This in turn suggests that the observations we have made on the change in nerve fiber number with age, both in this and the previous study[4] are due to biological effects rather than to any vagaries of the experimental methods.

We agree with the suggestion made by Lam et al.[3] that the loss of axons from the optic nerve probably accompanies the withdrawal of axons from inappropriate terminal sites either in the opposite retina and/or in the visual centres of the brain. This procedure may be necessary for the 'adult pattern' of connections to be established. A similar pattern of events has been described for the development of the axons in the optic nerves of chickens[17].

In conclusion, it seems that our present results lend support to the suggestion that during the development of the rat optic nerve there is an initial overall loss in the total number of optic nerve fibers. This loss could however be followed by a net gain in the number of fibers after 25 days of age, at least in hooded Lister rats[1].

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Effects of fasting on villi along the small intestine: a stereological approach to the problem of quantifying villus 'shape'

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Summary. We present stereological methods for establishing the shapes of villi from simple measurements on histological sections. Villi at different intestinal locations are analyzed in control and fasted rats. Villus shape factors are sensitive indicators of the effects of fasting but estimates of villus height alone are not.

The mucosal surface area of the small intestine is increased by villi which display considerable variations in size and shape according to species, location, age, disease and experimental treatment[3–13]. Many studies of villus size have limited measurements to a single dimension, namely villus height[3,8,12,13]. This is relatively easy to estimate on histological sections and can be used to detect gradients of villus morphology along the intestine[3–5,12,13]. Unfortunately, villus height has certain disadvantages. Being only 1 dimension, it is comparatively insensitive to alterations in villus shape because this depends on several dimensions in space. Indeed, it is difficult to see how height alone could describe the forms of irregular villi such as those which appear after irradiation[11].

Though specimens of intestine can be viewed by microscopical techniques which reveal the shapes of intact villi[5,10,11], mere qualitative descriptions are, by definition, inexact. In fact, there have been no attempts to quantify changes in villus shape independently of changes in their size. The present investigation describes a stereological approach to this problem. Essentially, the method relies on a dimensionless coefficient determined by mean villus surface area and volume[14]. The coefficient is used to study the influence of fasting on villi at several sites along the small intestine of the rat. A practical advantage of the coefficient is that it requires no previous assumptions about villus morphology. Consequently, the use of simplistic geometric approximations[5,9] can be avoided.

Materials and methods. A group of 6 hooded Lister rats weighing 260–310 g was allowed access to water but deprived of food for 48–51 h. Together with a group of 6 control animals matched for strain, sex, age and body weight, they were killed under anesthesia by intracardiac perfusion with buffered glutaraldehyde at the same time and on the same day. Intestines were excised and their lengths were measured from pylorus to ileocaecal valve. Simple random samples[15] of tissue from each third of intestinal length (proximal, middle and distal segments) were postfixed in osmium tetroxide, dehydrated in graded ethanols and flat-embedded in Araldite moulds. These were subsequently affixed to dummy Araldite blocks and 1 tissue block per segment was selected by lottery from each animal. A single arbitrary semithin section (about 1 μm) was cut from each block to provide a complete transverse section through the intestine. All sections were stained with toluidine blue, photographed and printed to a final magnification of × 56 with the aid of a calibration scale. Light micrographs were analyzed by conventional point and intersection counting procedures[15] using a square test lattice of spacing d equivalent to 0.18 mm on the specimen. Estimates of total villus volume per segment V were obtained from $V = P.d^2.L$ where P is the number of test points falling on villi and L the segment length. Total villus surface area per segment S was estimated using $S = I.d.L$ where I is the number of intersections between villus borders and the horizontal and vertical test lines. The latter relationship holds for transverse sections if villi display collective isotropy (i.e. if the overall villus surface has no preferred direction of orientation in space). For control and fasted laboratory rats, there is empirical evidence that this assumption is valid[14,16].

Values of the mean volume $\bar{V}$ and mean surface area $\bar{S}$ of villi