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

were calculated from estimates of villus number[17]. On the basis of these values, a coefficient $(\bar{S})^{1.5}/\bar{V}$ was obtained for the average villus in each segment[14]. This coefficient was employed to test for anisomorphic alterations of villus dimensions within and between the 2 groups of rats. Our rationale for adopting the coefficient may be illustrated as follows: Imagine a cube of edge length 2 mm. It is easy to calculate that this has a coefficient equivalent to $(24)^{1.5}/8$ or approximately 14.7 mm^3/mm^3. If this cube alters its size isomorphically to a new edge length of 1 mm, the coefficient becomes $(6)^{1.5}/1$ which is still 14.7 mm^3/mm^3. However, if the dimensions of the cube alter by different amounts, say only a single dimension decreases to 1 mm, the decrease in size is anisomorphic and the result is a rectangular slab with a coefficient of 16 mm^3/mm^3. In a sense, therefore, the coefficient is a shape factor but it is important to note that the exact shape is not specified because alternative geometric shapes can have the same coefficient. Thus, a cube has the same coefficient as a right circular cylinder with a diameter:length ratio of about 1:2.5. Fortunately, alterations of villus shape are more subtle than this.

To examine the nature of these alterations, we estimated the mean villus height $\bar{h}$ per segment. Together with our estimates of mean villus volume $\bar{V}$ per segment, we were then able to calculate villus profile areas per segment using the expression $\bar{a} = \bar{V}/\bar{h}$. Here, $\bar{a}$ represents the mean profile area of a villus of volume $\bar{V}$ sectioned perpendicular to its height axis by parallel slices. This derived area affords a convenient, albeit crude measure of villus length X breadth which, in combination with $\bar{h}$, allows shape changes to be characterized more completely.

Results and discussion. Over the 48–51-h period, fasted animals lost roughly 10% of body weight in comparison to controls. On average, the small intestine of fasted rats had a total villus volume of 1600 mm^3 (SEM 115 mm^3) and a total villus surface area of 400 cm^2 (26 cm^2). These values were significantly lower than corresponding control values of 2100 mm^3 (245 mm^3) and 500 cm^2 (43 cm^2). Segmental estimates of villus 'shape factors', heights and profile areas are provided in table 1. The values are based on a total of 135,000 villi per rat and numerical proportions of 1:1.1:1.9 in proximal:middle:distal segments. Absolute and relative numbers of villi were the same for control and fasted groups. Results of 2-way analysis of variance tests[18] are summarized in table 2.

There were significant differences between segments for all variables, with proximal segments tending to have taller villi of greater profile area. Gradients of villus shape showed a similar pattern of decline from pylorus to terminal ileum. These differences persisted after fasting. Moreover, fasting itself had a significant influence on villus shape factors, coefficients being consistently smaller than those in control animals regardless of intestinal location. The shape discrepancies cannot be explained by changes in villus height since mean heights per segment were not altered significantly in response to fasting. Rather, they were due to villi becoming thinner. In no instance did we detect significant interactions between segmental differences and treatment effects. We interpret this as indicating that fasting had no preferential effect on villi in particular regions. Villi in all regions responded to fasting in the same manner. Decreases in total villus surface area and volume can therefore be attributed to changes in the length X breadth of individual villi rather than a decline in villus number or height.

These findings are consistent with the known transition of villus morphology in the rat. Scanning electron microscopy has demonstrated that villi in the duodenum and jejunum tend to be tall and broad and those in the ileum short and narrow[10]. Morphometric studies on tissue sections have shown regional variation in villus height[3–5, 12, 13]. However, our results indicate that height is not a good discriminator of variation between control and fasted rats. Indeed, villus height is unsatisfactory from other points of view. Thus, it provides an inadequate index of villus surface area[4] and shows a poorer correlation with functional parameters[9]. For future studies, it seems preferable to combine height measurements with estimates of villus profile area when attempting to compare villi in different groups of animals. Alternatively, it may be even better to estimate shape factors since, in this investigation, the shape factor was a more powerful discriminator than villus height. Therefore, it offers a potentially useful way of quantifying those changes which occur during development[19] and irradiation[11]. We have shown that it can be estimated from simple counts performed on transverse sections through the intestine.

It is natural to enquire about possible mechanisms for the observed decreases in villus volume and surface area. Parallel studies in this laboratory have suggested that villus epithelial cell height varies according to the intestinal segment but does not alter significantly after fasting. However, there may be local changes, particularly at the tips of villi in proximal regions[16]. It appears that the thinner (and less voluminous) villi in fasted animals have a smaller lamina propria core and this suggestion is supported by our volumetric analyses of the histological composition of villi in the 2 groups of rats. These observations are consistent with the known physiological importance of intestinal blood flow but the possibility that the number of villus enterocytes also declines during fasting cannot be discounted at present. It has been established, for instance, that cell production rates diminish during reduced food intake and starvation[12, 20]. Such a hyperproliferative response could also explain the decreases in villus surface area. It remains to be seen whether volumes and surface areas, like cell cycle times[20], return towards normal values on subsequent nutritional rehabilitation.

Table 1. Morphometric data for villi in different intestinal segments of control and fasted rats (group means ± SEM)

Variable	Group	Proximal	Middle	Distal
Shape factor (mm^3/mm^3)	Control	18.2 ± 0.91	16.7 ± 1.41	14.2 ± 1.92
	Fasted	15.1 ± 0.87	15.0 ± 0.69	12.8 ± 0.76
Height (μm)	Control	585 ± 25.5	523 ± 28.6	278 ± 15.8
	Fasted	543 ± 45.7	485 ± 25.1	293 ± 21.4
Profile area ($mm^2 \cdot 10^2$)	Control	3.54 ± 0.31	4.21 ± 0.20	2.95 ± 0.56
	Fasted	3.37 ± 0.26	3.23 ± 0.16	2.29 ± 0.36

Table 2. Summary of 2-way analysis of variance results. Values are variance ratios

Variable	Segment effects (df = 2.30)	Treatment effects (df = 1.30)	Interaction effects (df = 2.30)
Shape factor	3.8*	4.4*	0.3 (ns)
Height	52.6***	0.9 (ns)	0.6 (ns)
Profile area	5.8**	4.8*	0.7 (ns)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; (ns) indicates not significant.

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Antagonism by haloperidol of the suppression of exploratory locomotor activity induced by the local application of (-)3-(3-hydroxyphenyl)-N-*n*-propylpiperidine into the nucleus accumbens of the rat[1]

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Summary. The injection of (-)3-PPP into the nucleus accumbens, 10 µg/side, produced a suppression of exploratory locomotor activity without affecting treadmill locomotion. Furthermore, the suppression of exploratory locomotor activity produced by (-)3-PPP was antagonized by the administration of haloperidol, 25–50 µg/kg i.p.

The 2 enantiomers of 3-(3-hydroxyphenyl)-N-*n*-propylpiperidine (3-PPP) are both biologically active as central dopamine (DA) receptor agonists. The (-) enantiomer selectively activates DA autoreceptors, whereas (+)3-PPP is a DA agonist at both autoreceptors and postsynaptic receptors. In addition to its actions at autoreceptors, (-)3-PPP appears also to block postsynaptic DA receptors[3]. Recently we demonstrated that the local injection of 3-PPP enantiomers into the nucleus accumbens of the rat produced a suppression of exploratory locomotor activity[4]. In agreement with biochemical and pharmacological evidence as mentioned above, (-)3-PPP was the most potent enantiomer. In the work described in the present report we investigated the specificity of the response to the local application of (-)3-PPP in 2 ways: by (1) an examination of the ability of animals thus treated to display coordinated forward locomotion on a treadmill and, (2) an attempt to antagonize the (-)3-PPP-induced suppression of exploratory locomotor activity by pretreatment with low doses of the DA receptor antagonist haloperidol (HPD)[5]. HPD may block DA autoreceptors preferentially when administered at low doses[6,7].

Materials and methods. Adult male Sprague-Dawley rats (Anticimex, Sollentuna, Sweden), 280–320 g, were used. The animals were housed under a constant dark-light cycle (dark 11.00–23.00 h), temperature and relative humidity, with food and water available ad libitum. The animals arrived in the laboratory at least 1 week before they were used in the experiments. Cranial cannulation for injections into the nucleus accumbens was carried out under deep anesthesia as previously described[4]. Intracerebral injections of (-)3-PPP·HCl (synthesized at Reseach and Development Laboratories, Astra Läkemedel AB) or physiological saline were made 24–30 h after surgery. Injection volume was 1 µl/side, injected over 45 sec and the cannula was left in place for an additional 30 sec before being retracted. HPD (generously donated by Janssen Leo Farma AB, Helsingborg, Sweden) was dissolved in a few drops of glacial acetic acid with 5.5% glucose added to final volume. HPD was administered i.p. in a volume of 2 ml/kg. The animals tested for motor coordination were trained to walk on a rotating drum (∅ = 166 mm) (treadmill) in 2 consecutive days (3–6 min training/day). On the following day, the animals were given a pretest and an animal which was not able to walk continuously on the treadmill for 3 min was excluded. The open field observations were made in a square arena (0.49 m^2) as previously described[8]. The injection site was checked by standard histological procedures after completion of the experiments. Each animal was used once only.

Results and conclusions. With the exception of the highest dose of (-)3-PPP, 160 µg/side, there were no statistically significant effects of local injection of (-)3-PPP into the nucleus accumbens on treadmill performance (fig. 1). This observation indicates that the decrease in exploratory locomotor activity previously observed after local injection of (-)3-PPP into the nu-

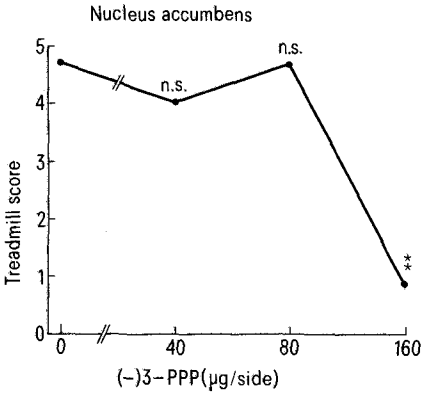

Figure 1. Effects of local injection of (-)3-PPP into the nucleus accumbens on treadmill performance in the rat. Animals trained to the criterion (> 3 min continuous walk) were tested on the treadmill 6 min after completion of bilateral application of (-)3-PPP or saline into the nucleus accumbens. The animals were scored 0–5 according to time spent on the treadmill using a square root transformation (> 2.25 min = maximal score). There were 5 animals/group and the figure shows median values. The data were subject to the nonparametric Kruskal-Wallis 1-way ANOVA followed by the Mann-Whitney U-test when comparing drug effects with saline controls[9]. $H(3) = 9.40$, $p < 0.05$; n.s., $p > 0.05$; ** $p < 0.02$.