The Journal of Experimental Biology 200, 2415–2423 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
JEB0995

A STEREOLOGICAL COMPARISON OF VILLOUS AND MICROVILLOUS SURFACES IN SMALL INTESTINES OF FRUGIVOROUS AND ENTOMOPHAGOUS BATS: SPECIES, INTER-INDIVIDUAL AND CRANIOCAUDAL DIFFERENCES

A. N. MAKANYA[1,2,*], J. N. MAINA[2], T. M. MAYHEW[3], S. A. TSCHANZ[1] AND P. H. BURRI[1]

[1]*Institute of Anatomy, University of Berne, Bühlstrasse 26, CH-3000, Berne 9, Switzerland,* [2]*Department of Veterinary Anatomy, University of Nairobi, Box 30197, Nairobi, Kenya* and [3]*Department of Human Anatomy and Cell Biology, University of Nottingham, Nottingham NG7 2UH, UK*

Accepted 30 June 1997

Summary

The extents of functional surfaces (villi, microvilli) have been estimated at different longitudinal sites, and in the entire small intestine, for three species of bats belonging to two feeding groups: insect- and fruit-eaters. In all species, surface areas and other structural quantities tended to be greatest at more cranial sites and to decline caudally. The entomophagous bat (*Miniopterus inflatus*) had a mean body mass (coefficient of variation) of 8.9 g (5 %) and a mean intestinal length of 20 cm (6 %). The surface area of the basic intestinal tube (primary mucosa) was 9.1 cm^2 (10 %) but this was amplified to 48 cm^2 (13 %) by villi and to 0.13 m^2 (20 %) by microvilli. The total number of microvilli per intestine was 4×10^{11} (20 %). The average microvillus had a diameter of 89 nm (10 %), a length of 1.1 μm (22 %) and a membrane surface area of 0.32 μm^2 (31 %). In two species of fruit bats (*Epomophorus wahlbergi* and *Lisonycteris angolensis*), body masses were greater and intestines longer, the values being 76.0 g (18 %) and 76.9 g (4 %), and 73 cm (16 %) and 72 cm (7 %), respectively. Surface areas were also greater, amounting to 76 cm^2 (26 %) and 45 cm^2 (8 %) for the primary mucosa, 547 cm^2 (29 %) and 314 cm^2 (16 %) for villi and 2.7 m^2 (23 %) and 1.5 m^2 (18 %) for microvilli. An increase in the number of microvilli, 33×10^{11} (19 %) and 15×10^{11} (24 %) per intestine, contributed to the more extensive surface area but there were concomitant changes in the dimensions of microvilli. Mean diameters were 94 nm (8 %) and 111 nm (4 %), and mean lengths were 2.8 μm (12 %) and 2.9 μm (10 %), respectively. Thus, an increase in the surface area of the average microvillus to 0.83 μm^2 (12 %) and 1.02 μm^2 (11 %) also contributed to the greater total surface area of microvilli. The lifestyle-related differences in total microvillous surface areas persisted when structural quantities were normalised for the differences in body masses. The values for total microvillous surface area were 148 $cm^2\,g^{-1}$ (20 %) in the entomophagous bat, 355 $cm^2\,g^{-1}$ (20 %) in *E. wahlbergi* and 192 $cm^2\,g^{-1}$ (17 %) in *L. angolensis*. This was true despite the fact that the insect-eater possessed a greater length of intestine per unit of body mass: 22 $mm\,g^{-1}$ (8 %) *versus* 9–10 $mm\,g^{-1}$ (9–10 %) for the fruit-eaters.

Key words: bats, entomophagous, frugivorous, intestine, villi, microvilli, craniocaudal variations, *Miniopterus inflatus*, *Epomophorus wahlbergi*, *Lisonycteris angolensis*.

Introduction

Stereology is the approach of first choice when three-dimensional information about structural quantities (volume, surface area, length and number) must be extrapolated from tissue sections (Cruz-Orive and Weibel, 1990; Mayhew, 1991, 1992). Although sampling protocols have been devised for obtaining stereological data on the functional surfaces of avian and mammalian intestines (Mayhew, 1987, 1988, 1990, 1996; Elbrønd *et al.* 1991; Mayhew *et al.* 1992*a*,*b*; Warren, 1991; Makanya *et al.* 1995), little quantitative three-dimensional information is available for the chiropteran small intestine (Makanya *et al.* 1995; Mayhew, 1996). Yet, amongst mammals, bats are unique in their capacity for flapping flight (Greenhall and Paradiso, 1968; Wimsatt, 1970; Thomas and Suthers, 1972; Dawson, 1975; Thomas, 1975; Jurgens *et al.* 1981). This mode of flight is extremely energy-expensive (Tucker, 1972; Carpenter, 1975; Thomas, 1975, 1980), and it might be expected that strategies and adaptations might have evolved for meeting the high calorific demands. These could include higher feeding rates and higher absorption rates mediated by, for example, increases in absorptive surface areas or the densities or activities of transport sites at those surfaces (Karasov and Diamond, 1983). Some of these strategies and

*Author for correspondence at address 1 (e-mail: makanya@ana.unibe.ch).

adaptations are known to occur in bats (see, for example, Keegan, 1977).

Amongst bats and other vertebrates, carnivores and herbivores are adapted by having different intestinal lengths and nutrient transport rates. Carnivores tend to have shorter intestines and lower glucose transport rates than herbivores (see review by Karasov and Diamond, 1983). Bats have evolved ecological and feeding habits geared towards meeting high nutrient requirements. They have restricted their feeding scope in favour of nutritionally concentrated foods and away from bulky, poorly digestible and poorly absorbable types of food (Yalden and Morris, 1975). For example, whilst some bats ingest leaves and whole flowers or parts thereof (Ratcliffe, 1932; Jaegar, 1954; van der Pijl, 1956, 1957; Nelson, 1965; Rosevar, 1965; Cunningham van Someran, 1972; Funmilayo, 1976; Wickler and Seibt, 1976; Cheke and Dahl, 1981), only the juices are swallowed and fibrous ingredients are rejected (Marshall, 1983).

The intestines of bats also display extremely short transit times (Klite, 1965; Morrison, 1980; Tedman and Hall, 1985; Laska, 1990) and appear to be well adapted for accelerated digestive and absorptive activities. Although certain active transport systems may be lacking (Keegan, 1980; Keegan *et al.* 1980), absorption rates are very high (Keegan, 1977) and digestive enzyme distributions are extensive (Ogunbiyi and Okon, 1976). As a morphological basis for this relatively high activity, it has been noted (Keegan and Mödinger, 1979) that the sizes, numbers and packing densities of enterocyte microvilli are greater in bats than in rats of comparable body mass. However, these studies were constrained by limitations in morphometric techniques and lack of hard information about effective surface areas. More recently, we have attempted to redress some of these inadequacies by designing a sampling protocol for stereological quantification of villous and microvillous surface areas in bat intestine (Makanya *et al.* 1995).

In the present investigation, we exploit these developments to quantify the villous and microvillous surface areas of the intestines of three species of bats: *Epomophorus wahlbergi* and *Lisonycteris angolensis* (frugivorous) and *Miniopterus inflatus* (entomophagous). Comparisons are drawn between different bat types and lifestyles as well as between bats and terrestrial mammals.

Materials and methods

Bat provenance

Two frugivorous species (Pteropodidae) were taken to represent the suborder Megachiroptera and one species (Verspertillionidae) to represent the suborder Microchiroptera. In the case of the Megachiroptera, ten specimens were captured in Kenya: five epauletted fruit bats (*Epomophorus wahlbergi* Halowell) caught in forests near Nairobi and five Angola fruit bats (*Lisonycteris angolensis* Eisentraut) obtained from Kakamega by spreading mist nets next to small streams. Specimens became entangled in the nets at dusk as they descended to the streams to drink. To represent Microchiroptera, five long-fingered insectivores (*Miniopterus inflatus* Sanborn) were caught in Naivasha (also in Kenya) during the day by spreading mist nets at cave entrances and stirring the bats from their roosts. Trappings were conducted with the assistance of experts from the National Museums of Kenya who had the requisite permits. Capture methods are described in Kunz and Kurta (1988).

Tissue preparation

Animals were transported live to research laboratories and, after weighing, gastrointestinal tracts were obtained under sodium pentobarbitone anaesthesia (intraperitoneal dosage 50 mg kg^{-1} body mass) *via* a ventromedian incision in the abdominal wall. The oesophagus was severed cranial to the diaphragm, and the pelvic bones were cut carefully to reveal the rectum. Gastrointestinal tracts were dissected free of mesenteries and immediately transferred to a bath of 0.85 % sodium chloride. Each was opened by a longitudinal incision along the mesenteric border and ingesta/digesta were washed away with fresh saline.

In bats, the boundary between the small and large intestine can be difficult to identify since there is neither a caecum nor an appendix. In addition, the external appearances are very similar (Mathis, 1928; Okon, 1977; Madkour *et al.* 1982). In frugivores, the boundary can be taken as the beginning of macroscopically visible longitudinal rugae in the colon. Most entomophagous bats lack a colon (Okon, 1977; Makanya and Maina, 1994; Makanya, 1997), although one has been identified in one species (Ishikawa *et al.* 1985). In contrast, the rectum in insect-eating bats is conspicuous because of its greater width (Makanya and Maina, 1994). The foregut–hindgut boundary was taken to be where the width started to increase slightly cranial to the anus.

Tissue sampling

After washing, the small intestine was isolated by cutting at the ileocolic and pyloro-intestinal junctions, and its length was measured. It was then divided into five segments of roughly equal length, numbered craniocaudally from 1 to 5. The mean width and length of each segment were determined. Next, each segment was divided into five approximately equal subsegments, one of which from each set was picked at random to represent the segment as a whole and processed for light microscopy (LM) and transmission electron microscopy (TEM) as outlined below.

Subsegments were immersion-fixed in 2.5 % phosphate-buffered glutaraldehyde (350 mosmol l^{-1}, pH 7.3) for at least 4 h. They were washed repeatedly in 0.1 mol l^{-1} phosphate buffer, postfixed in 1 % osmium tetroxide and dehydrated through ascending concentrations of acetone before being embedded in resin (Transmit, Taab, UK). Prior to embedding, each piece was placed at the centre of a transparent dish lying on a square test grid, and the dish was rotated so as to randomise the orientation between the subsegment and the lines of the test grid. Once a random direction had been

selected, blocks of tissue were cut at the microtome in this direction. This ensured that vertical sections of small intestine were cut so as to be isotropic on the reference plane (the workbench surface) and satisfied sampling requirements for the unbiased estimation of surface areas (Baddeley *et al.* 1986).

Vertical sections were used for both LM (semithin sections cut at a nominal 1 μm thickness) and TEM (ultrathin sections cut at approximately 60 nm thickness). Semithin sections were stained with Toluidine Blue for light microscopy. Two sections per segment were sampled randomly and fields of view were printed at final linear magnifications of ×80 and ×100. TEM sections were viewed using Philips EM 300 or EM 410 microscopes operated at an accelerating voltage of 80 kV. Five micrographs per segment were sampled randomly and prepared at final magnifications of ×18 500 (fruit-eaters) and ×50 000 (insect-eaters).

Stereological methods

Organs were sampled at three levels of magnification as detailed elsewhere (Makanya *et al.* 1995). At level 1, macroscopic estimates of the surface area of the primary mucosa (the basic tube unmodified by villi and microvilli) were derived for each intestinal segment as the product of circumference and segment length. At level 2, we obtained LM estimates of the extent to which villi amplify the surface area of the primary mucosa. To this end, grids of cycloid test lines were superimposed on fields of view (Baddeley *et al.* 1986), and chance intersections were counted between the test lines and profiles of the villi and the primary mucosa. The latter was taken to be the interface between the bases of villi and the openings of crypts. Intersections were summed for each segment, and the villous amplification factor was estimated as the intersection ratio. When multiplied by the surface area of primary mucosa, this ratio provided the total villous surface area in a given segment.

At the TEM level (level 3), we estimated the extent to which microvilli amplify the villous surface area. Again, intersections were counted between cycloid test lines and the traces of microvilli and the apical membrane of enterocytes (taken to be the surface on which the bases of microvilli are situated). Total intersections on TEM fields of view were counted for each segment, and the microvillous amplification factor was computed as the intersection ratio. When multiplied by the total surface area of villi in a given segment, this ratio provided an estimate of the total microvillous surface area in the same segment.

Also at this level, estimates were made of the sizes and numbers of microvilli. The mean diameter of microvilli was estimated by measuring at least ten favourably sectioned microvilli on the micrographs representing a given segment. If a microvillus was cut obliquely, the minor axis of its profile was measured. The mean height of microvilli in a given segment was estimated by measuring at least 30 individual microvilli sectioned through their long axis. The presence of clear membrane traces was taken to indicate longitudinal sectioning. The surface area of the average microvillus in a segment was calculated from mean diameter and mean height (Mayhew, 1990). Finally, the number of microvilli per unit surface area of apical cell membrane was estimated by dividing the microvillous amplification factor by the mean area of a microvillus (Mayhew, 1990). In a similar fashion, we estimated the total number of microvilli in a segment by dividing the total surface area of microvilli by the surface area of the average microvillus in the same segment. Where appropriate, segmental values were summed in order to calculate values per intestine.

To estimate the biases introduced by the Holmes or overprojection effect, we used the formula described by Gundersen (1979) in which the overestimation was approximated using lengths and diameters averaged over all microvilli in the entire small intestine and taking into account a section thickness of 60 nm.

Statistics

Group means for each bat type were estimated together with their coefficients of variation (coefficient of variation, CV=standard deviation divided by the corresponding group mean and expressed as a percentage). CVs were selected because these provide a sensible way of comparing the observed subject-to-subject variation within a given species and of comparing different species. To compare body masses and intestinal lengths between species (degrees of freedom, d.f.=2), one-way analyses of variance (ANOVAs) were employed (Sokal and Rohlf, 1981). Comparisons between species (d.f.=2) and between intestinal segments (d.f.=4) were undertaken using balanced-design two-way ANOVAs (Sokal and Rohlf, 1981). This test generates an interaction term (species × segment, d.f.=4), which indicates the extent to which the effects of one factor (e.g. segment location) depend on the effects of the other (e.g. species of bat). Given that five segments were sampled within each subject, the segments are related. To exploit the greater statistical efficiency of tests for related samples, Page's *L*-trend test (Miller, 1975) was applied. This test assesses whether apparent trends between segments (k=5) within a species (N=5 subjects) are significant. Data were handled and analysed using Unistat 4.72 software. In all cases, the null hypothesis was rejected at a probability level of $P<0.05$.

Results

Detailed morphometric results on the various gut parameters are presented as group means together with coefficients of variation (CV %) in Tables 1–5.

Body masses and intestinal lengths

From the results in Table 1, the mean mass of the insectivorous bat (*M. inflatus*) was 8.92 g (CV 5 %). Although the two fruit bats (*E. wahlbergi* and *L. angolensis*) were both heavier than the insect-eaters, they were similar in mean mass to each other at 76.04 g (18 %) and 76.93 g (4 %) respectively. Similar differences between species were noted for intestinal

Table 1. *Body masses and intestinal lengths in the three species of bats*

Species name	Body mass (g)	Small intestinal length (mm)
Miniopterus inflatus	8.92 (4.7 %)	196 (5.8 %)
Epomophorus wahlbergi	76.04 (17.7 %)	733 (15.8 %)
Lisonycteris angolensis	76.93 (3.5 %)	722 (7.1 %)

Values are group means and coefficients of variation (CV %).

lengths. The three bat types had mean small intestine lengths of 196 mm (6 %), 733 mm (16 %) and 722 mm (7 %) respectively.

Intestinal morphometry

Effects of craniocaudal location

Segmental values of intestinal circumferences, villous and microvillous amplification factors and surface areas are summarised in Table 2. The dimensions and packing densities of microvilli are given in Table 3.

Except for the packing density of microvilli at the apical surface of enterocytes in *E. wahlbergi*, significant regional differences were detected for all variables in all species. Values tended to be greatest in cranial segments and smallest in caudal segments. Two-way ANOVAs indicated that there were significant interaction (segment × species) effects involving intestinal circumference, villous and microvillous surface areas, the mean dimensions of microvilli and the total number of microvilli.

Differences between species

Two-way ANOVAs confirmed that the apparent differences between species were statistically significant. Table 4 presents data for the entire intestine of each bat type. The results indicate that, on average, the entomophagous bat (*M. inflatus*) had a villous surface of 4791 mm^2 (13 %). Although the two species of fruit bats (*E. wahlbergi* and *L. angolensis*) were similar in mean body mass, they showed remarkably different villous surface areas at 54 670 mm^2 (29 %) and 31 370 mm^2 (16 %) respectively. Comparable differences among these species were noted for total microvillous surface areas and numbers, with the insectivorous bat showing respective values of 1316 cm^2 (20 %) and 4.2×10^{11} (20 %), while for *E. wahlbergi* the respective values were 26 850 cm^2 (23 %) and 32.5×10^{11} (19 %). The corresponding values for *L. angolensis* were 14 800 cm^2 (18 %) and 14.6×10^{11} (24 %). As well as possessing the shortest intestines, the insect-eater (*M. inflatus*) also tended to a have smaller intestinal circumference, a smaller amplification factor and, hence, less extensive villous and microvillous surface areas than the fruit-eaters (*E. wahlbergi* and *L. angolensis*). The microvilli were less numerous, shorter and marginally thinner, but more densely packed, in the insect-eater.

Differences between the two species of fruit-eaters were also detected. Whilst *E. wahlbergi* and *L. angolensis* had similar body masses and intestinal lengths, intestinal circumference

Table 2. *Segmental intestinal surface areas and amplification factors at various sampling levels in the three species of bat*

Variable	Species	Gut segment 1	2	3	4	5
Circumference (mm)	*Mi*	5.0 (15.8 %)	5.1 (8.2 %)	4.7 (19.3 %)	4.3 (10.4 %)	4.2 (6.5 %)
	Ew	12.8 (10.2 %)	10.8 (12.1 %)	9.8 (13.3 %)	10.2 (18.9 %)	8.0 (23.4 %)
	La	8.2 (5.5 %)	6.8 (6.6 %)	6.0 (11.8 %)	5.2 (8.6 %)	5.0 (0 %)
Primary mucosal surface area (mm^2)	*Mi*	195 (14.7 %)	200 (9.4 %)	184 (20.0 %)	169 (13.2 %)	165 (9.9 %)
	Ew	1884 (21.7 %)	1602 (26.0 %)	1452 (26.5 %)	1521 (32.8 %)	1190 (31.4 %)
	La	1183 (7.6 %)	980 (6.2 %)	869 (15.7 %)	751 (10.8 %)	722 (7.1 %)
Villous amplification factors	*Mi*	7.78 (17.1 %)	6.88 (8.6 %)	4.98 (20.1 %)	3.80 (17.8 %)	2.22 (12.9 %)
	Ew	10.50 (11.5 %)	8.38 (17.9 %)	6.94 (12.2 %)	4.74 (18.7 %)	3.14 (15.4 %)
	La	9.60 (9.6 %)	7.98 (18.8 %)	5.72 (6.7 %)	5.82 (27.0 %)	3.86 (22.4 %)
Villous surface area (mm^2)	*Mi*	1508 (17.2 %)	1372 (40.0 %)	896 (14.1 %)	652 (30.7 %)	363 (5.4 %)
	Ew	19510 (15.9 %)	13720 (10.5 %)	10250 (35.7 %)	7402 (48.3 %)	3791 (39.8 %)
	La	11410 (16.1 %)	7812 (18.6 %)	4956 (15.0 %)	4389 (30.4 %)	2803 (24.7 %)
Microvillous amplification factor	*Mi*	37.5 (38.8 %)	26.3 (11.4 %)	19.1 (8.7 %)	21.7 (6.6 %)	24.6 (19.0 %)
	Ew	50.4 (32.1 %)	53.1 (15.3 %)	51.4 (27.3 %)	45.2 (22.3 %)	38.0 (30.4 %)
	La	56.1 (9.2 %)	46.1 (28.9 %)	45.9 (18.5 %)	37.1 (14.4 %)	33.5 (15.0 %)
Microvillous surface area (cm^2)	*Mi*	551 (31.7 %)	361 (17.7 %)	172 (20.5 %)	142 (33.6 %)	89 (18.5 %)
	Ew	9543 (22.4 %)	7331 (44.1 %)	5271 (37.6 %)	3264 (42.9 %)	1444 (47.3 %)
	La	6456 (22.6 %)	3564 (29.6 %)	2258 (21.4 %)	1576 (18.4 %)	944 (31.9 %)

Values are group means and coefficients of variation (CV %).
The data are presented on a per segment basis (five segments per animal). The segments are identified craniocaudally from 1 to 5.
Mi, *Miniopterus inflatus*; *Ew*, *Epomophorus wahlbergi*; *La*, *Lisonycteris angolensis*.

Table 3. *Microvillous packing density, segmental number, microvillous diameter, microvillous length and the surface area of the average microvillus in various segments of the bat small intestine*

Variable	Species	Gut segment 1	2	3	4	5
Microvillous packing density (μm^{-2})	*Mi*	92 (7.3 %)	88 (31.9 %)	70 (32.3 %)	98 (12.7 %)	96 (26.3 %)
	Ew	70 (14.4 %)	55 (21.5 %)	59 (39.3 %)	54 (39.6 %)	51 (36.0 %)
	La	40 (15.6 %)	40 (36.3 %)	47 (21.5 %)	64 (27.7 %)	65 (26.2 %)
Microvillous number $\times 10^{-10}$	*Mi*	1.38 (13.6 %)	1.19 (28.7 %)	0.64 (42.1 %)	0.64 (38.3 %)	0.35 (27.0 %)
	Ew	13.59 (15.4 %)	7.55 (44.6 %)	5.87 (39.2 %)	3.52 (18.6 %)	2.00 (58.6 %)
	La	4.60 (25.5 %)	3.08 (37.6 %)	2.31 (26.2 %)	2.75 (32.7 %)	1.84 (39.1 %)
Microvillous diameter (nm)	*Mi*	99 (11.6 %)	88 (13.5 %)	85 (13.9 %)	80 (0.9 %)	88 (5.8 %)
	Ew	87 (13.4 %)	100 (11.8 %)	91 (12.4 %)	103 (22.0 %)	104 (20.5 %)
	La	123 (6.7 %)	111 (7.5 %)	113 (8.2 %)	98 (10.9 %)	108 (10.4 %)
Microvillous length (μm)	*Mi*	1.28 (20.4 %)	1.17 (29.5 %)	1.11 (26.1 %)	0.89 (11.7 %)	0.95 (15.7 %)
	Ew	2.59 (19.0 %)	3.16 (21.4 %)	3.24 (25.9 %)	2.87 (34.6 %)	2.42 (27.8 %)
	La	3.70 (12.0 %)	3.39 (8.8 %)	2.79 (8.4 %)	1.9 (12.4 %)	1.56 (18.2 %)
Microvillous surface area (μm^2)	*Mi*	0.40 (31.6 %)	0.33 (41.2 %)	0.30 (33.4 %)	0.22 (12.0 %)	0.26 (12.0 %)
	Ew	0.71 (23.0 %)	1.00 (27.0 %)	0.93 (29.9 %)	0.94 (43.5 %)	0.79 (34.0 %)
	La	1.42 (13.0 %)	1.19 (12.5 %)	0.99 (7.6 %)	0.60 (20.3 %)	0.53 (16.2 %)

Values are group means and coefficients of variation (CV %).
The data are presented as in Table 2.
Mi, *Miniopterus inflatus*; *Ew*, *Epomophorus wahlbergi*; *La*, *Lisonycteris angolensis*.

was smaller in *L. angolensis* and this was sufficient to account for the smaller surface area of primary mucosa. It also contributed to the reduced total surface areas of villi and microvilli because the amplification factors for these two structures were similar in both types of fruit-eater. The smaller total surface area of microvilli in *L. angolensis* could not be attributed to any significant differences in organelle diameters, lengths or packing densities.

Standardising for body mass

The insect-eating bat harboured relatively longer intestines (Table 5). After normalising for the differences in body mass, mean intestinal lengths were 22 mm g^{-1} (8 %) for *M. inflatus*, 10 mm g^{-1} (10 %) for *E. wahlbergi* and 9 mm g^{-1} (9 %) for *L. angolensis*. In the case of relative total surface areas, the corresponding values were 540 mm^2 g^{-1} (17 %), 711 mm^2 g^{-1} (16 %) and 409 mm^2 g^{-1} (18 %) for villi and 148 cm^2 g^{-1} (20 %), 355 cm^2 g^{-1} (20 %) and 192 cm^2 g^{-1} (17 %) for microvilli. The insect-eater had the same area of primary mucosa per unit of body mass as *E. wahlbergi* but a greater relative surface than *L. angolensis*. The total villous surface area per unit of mass was less than that found in *E. wahlbergi*

Table 4. *Mean values of the various intestinal parameters per species (total values per whole intestine) for the three types of bat*

	Bat species *M. inflatus*	*E. wahlbergi*	*L. angolensis*
Intestinal length (mm)	196 (5.8 %)	733 (15.8 %)	722 (7.1 %)
Intestinal circumference (mm)	4.7 (8.7 %)	10.3 (11.5 %)	6.2 (4.2 %)
Primary mucosal surface area (mm^2)	913 (9.8 %)	7649 (25.7 %)	4505 (7.9 %)
Villous amplification factor	5.2 (5.6 %)	7.1 (8.4 %)	6.9 (10.7 %)
Total villous surface area (mm^2)	4791 (12.5 %)	54670 (29.2 %)	31370 (16.4 %)
Microvillous amplification factor	27.6 (19.2 %)	50.2 (6.13 %)	47.2 (12.6 %)
Total microvillous surface area (cm^2)	1316 (20.2 %)	26850 (22.8 %)	14800 (18.2 %)
Microvillous diameter (nm)	89 (9.7 %)	94 (8.3 %)	111 (3.7 %)
Microvillous length (μm)	1.12 (21.5 %)	2.79 (11.6 %)	2.89 (10.0 %)
Mean microvillous surface area (μm^2)	0.32 (31.4 %)	0.83 (11.7 %)	1.02 (11.2 %)
Microvillous packing density (μm^{-2})	87.6 (15.5 %)	61.4 (20.0 %)	46.6 (20.4 %)
Microvillous number	4.2×10^{11} (20.1 %)	32.5×10^{11} (19.1 %)	14.6×10^{11} (24.3 %)

Values are group means and coefficients of variation (CV %).

Table 5. *Body-mass-standardised intestinal lengths and surface areas at the various sampling levels*

	Bat species		
	M. inflatus	*E. wahlbergi*	*L. angolensis*
Intestinal length ($mm\,g^{-1}$)	22.0 (8.0 %)	9.7 (9.9 %)	9.4 (9.1 %)
Primary mucosal surface area ($mm^2\,g^{-1}$)	103 (14.4 %)	100 (16.2 %)	58.6 (8.4 %)
Total villous surface area ($mm^2\,g^{-1}$)	540 (16.8 %)	711 (16.2 %)	409 (18.2 %)
Total microvillous surface area ($cm^2\,g^{-1}$)	148 (19.7 %)	355 (20.1 %)	192 (17.3 %)

Values are group means and coefficients of variation (CV %).

but greater than that in *L. angolensis*. Finally, *E. wahlbergi* possessed relatively more total microvillous surface area than either of the other two species, whose values were not significantly different.

Discussion

This study has provided reasonably efficient and minimally biased estimates of the functional surfaces of bat small intestines using a sampling and estimation scheme designed for the purpose (Makanya *et al.* 1995). The results are not entirely free of some of the technical biases associated with quantifying images seen on sampled tissue sections, and these are discussed in Makanya *et al.* (1995).

Correcting microvillous surface area for section thickness effects

The magnitude of biases introduced by the overprojection effect (Gundersen, 1979; Weibel, 1979; Mayhew and Middleton, 1985) is governed by a combination of feature size and section thickness. Overprojection has little impact on villous surface area (since villi are large in comparison with section thickness), but microvillous amplification factors may be overestimated (owing to the relatively small diameters and lengths of microvilli). The overall extents of the biases may be approximated (Gundersen, 1979) using microvillous dimensions (lengths and diameters) averaged over the entire small intestine. Taking the section thickness of 60 nm used here, the relative biases in the present study would be approximately 48 % (*M. inflatus*), 43 % (*E. wahlbergi*) and 36 % (*L. angolensis*). However, these correction factors are only approximations because microvilli vary in length, diameter and number in different intestinal segments and species. For the average microvillous dimensions found in each segment, the segmental biases are likely to fall in the ranges 43–50 % (*M. inflatus*), 39–46 % (*E. wahlbergi*) and 33–42 % (*L. angolensis*). Similar degrees of bias have been encountered in studies on microvilli in rat small intestine (Mayhew and Middleton, 1985; Mayhew, 1987).

Biological interpretations

Craniocaudal differences

The gradients of craniocaudal morphology witnessed here are broadly similar to those reported in the small intestines of other mammals (see Mayhew and Middleton, 1985; Mayhew, 1996). Structural gradients involve intestinal circumference, villous amplification, absolute villous surface area, villous height, microvillous amplification and absolute microvillous surface area. The gradients are associated with changes in nutrient transport rates, and those involving microvillous surface area provide a basis for interpreting transport gradients associated with transport molecules and digestive enzymes located in or near the apical membrane domains of enterocytes (Mayhew, 1996; Makanya, 1997).

Lifestyle differences

A target of the present study was to compare intestinal morphology in bats with different lifestyles. The results demonstrate that intestinal adaptations occur at several levels of structural organisation. These include changes in intestinal length and circumference, villous amplification and microvillous amplification. The latter seems to be effected mainly by disproportionate alterations in the lengths of microvilli, although their diameters may also alter, with possible consequent changes in packing densities. As highlighted recently, villi in *M. inflatus* are disposed as transverse folds spanning the entire circumference of the intestine, which also has unique pits proximally (Makanya, 1997). The putative function of such pits has been discussed elsewhere (Makanya and Maina, 1994; Makanya, 1997) and is presumed to be either secretion of enzymes or absorption of nutrients or both, while the transverse disposition of the villi was thought to bear significance in withholding fluid-phase ingesta of minimal bulk. Precise elucidation of the functional significance of these regions must await further investigation by physiologists and others.

The entomophagous bat seems to invest more surface area in a relatively longer intestine per unit of body mass and smaller but more densely packed microvilli. In fruit bats, villi are taller and larger and may show anastomoses and branching (Manley and Williams, 1979; A. N. Makanya, personal observations) and have slightly less densely packed but longer microvilli at the apices of their enterocytes. Fruit bats have greater intestinal surface areas than insect-eaters. This may reflect the poorer diet on which frugivores thrive. They must eat large quantities of fruit each day in order to meet the critical levels of the nutrients that are deficient in their diets (Thomas, 1984). The nutrient requirements of frugivores are uncertain

but are presumed to be comparable to those of other mammals (Wilson, 1988). Their diet is rich in carbohydrates (Watt, 1968) but low in protein and fat (Morrison, 1980). Probably, protein requirements cannot be met by unsupplemented fruit diets (Wilson, 1988). Various observations support this since these bats have been seen to ingest leaves and insects (van der Pijl, 1957; Cunningham van Someran, 1972; Wilson, 1973; Wickler and Seibt, 1976; Gardner, 1977). Insect remains have been detected in the guts of fruit bats (Lim, 1973; Start and Marshall, 1976), but their ingestion may be accidental (Marshall, 1983). Insectivorous bats have few problems obtaining proteins since insects are protein-rich (Bodenheimer, 1951; Morton, 1973). Although low in carbohydrates, they also provide essential levels of other nutrients.

The fruit bat *E. wahlbergi* has total microvillous surface areas that are approximately 20 times those of the insect-eater *M. inflatus* and almost twice those of the fruit-eater *L. angolensis*, which is comparable in size. *M. inflatus* feeds on high-flying insects. Studies on the ecology of *E. wahlbergi* indicate that it may travel long distances in search of fruit in one night (Wickler and Seibt, 1976; Fenton *et al.* 1985). This bat stands out because of its high morphometric values, which are in agreement with previous investigations related to energy acquisition systems (Maina *et al.* 1991). A complete explanation of these findings is difficult since phylogeny is poorly understood in Chiroptera because of the paucity of fossil records (Jepsen, 1970) and ecological studies on tropical fruit bats are few. Consequently, little is known of the ecology of the bats studied here. Therefore, whilst the morphological differences observed here may be attributed to differences in lifestyle, the roles of foraging strategies, ecological and phylogenetic factors remain to be elucidated.

Adaptations of the microvillous surface

Changes in the overall surface area of microvilli depend on intestinal length and circumference, the available surface area of villi, the density of packing of enterocytes on those villi and the morphophenotypic maturation status of enterocytes as expressed in the sizes and numbers of their microvilli. In birds and rodents, elongation of microvilli is part of the process of enterocyte maturation which proceeds as cells migrate along the crypt–villus axis and is a feature of craniocaudal variation along the small intestine (Brown, 1962; van Dongen *et al.* 1976; Stenling and Helander, 1981; Smith and Brown, 1989; Mayhew, 1990, 1996). In the avian coprodaeum, the lengths and packing densities of microvilli vary with dietary salt load (Mayhew *et al.* 1992*a*,*b*; Mayhew, 1996). In rats and hamsters, length may also vary during adaptation to reduced food intake, but it does not alter in response to chemically induced diabetes mellitus (Misch *et al.* 1980; Buschmann and Manke, 1981*a*,*b*; Mayhew, 1987, 1990, 1996; Williams and Mayhew, 1992).

It must be emphasised that changes in absorptive surface areas in the avian coprodaeum are markedly effected, and in rodent small intestine during experimental diabetes they are effected exclusively, by cell recruitment onto villi (Mayhew *et al.* 1992*a*,*b*; Zoubi *et al.* 1995; Mayhew, 1996). Anatomical and functional adaptations are maximised when there has been sufficient time to replace enterocytes on villi. The extent to which species differences in cell complements explain differences in intestinal surface areas in Chiroptera has not been examined, but it seems reasonable to predict that larger villous and microvillous surface areas are due in part, if not in the main, to the presence of greater numbers of enterocytes. The ultrastructural complexity of the enterocytes (see, for example, Manley and Williams, 1979) and their relative volumes may also influence the functional capacity of the bat intestine. Unfortunately, there appear to be no reports on these parameters.

The packing densities and linear dimensions of microvilli vary not only between bat and rats but also between bat species. In the insect-eater examined here, the mean length of microvillus per intestine was 1.1 μm, the mean diameter was 89 nm and the packing density was 88 μm^{-2} of villous surface area. Corresponding values in fruit-eaters were 2.8–2.9 μm, 94–111 nm and 47–61 μm^{-2}. Microvillous lengths in rats (1.2–1.4 μm) are comparable to those found in the entomophagous bat but shorter than those in frugivores. Rat microvilli also tend to be slightly thicker (106–127 nm) and less densely packed (34–43 μm^{-2}, see Mayhew, 1990) than in bats. Despite these differences, absolute microvillous surface area in these bats is not too dissimilar from that found in rat small intestine once estimates are corrected for overprojection errors (0.1–1.9 m^2 *versus* 0.9–1.2 m^2; see Mayhew and Middleton, 1985; Mayhew, 1990). However, when normalised for body mass, the relative surface areas of microvilli are 2–18 times greater in the bat (100–250 $cm^2\,g^{-1}$ in bats *versus* 14–42 $cm^2\,g^{-1}$ in rats after correcting for overprojection bias; Mayhew, 1990). In general, the bats studied here display a greater intestinal surface than terrestrial mammals of similar body mass, and this may be related in part to their energy-expensive lifestyles and deficiencies in their natural diets. However, precise interpretation of these findings must await new ventures into chiropteran ecology, physiology and perhaps phylogeny. Further investigations on enterocyte volumes, ultrastructural complexity and turnover rates would throw more light on the remarkable enteric functional capacity of the Chiroptera.

These studies were supported jointly by The World Bank and the Deans' Committee of the University of Nairobi. We thank especially the late Joseph Kwambai (formerly of the Department of Mammalogy, National Museums of Kenya) for providing bat specimens and Alan Pyper, Tim Self and Barry Shaw for technical assistance.

References

BADDELEY, A. J., GUNDERSEN, H. J. G. AND CRUZ-ORIVE, L. M. (1986). Estimation of surface area from vertical sections. *J. Microsc.* **142**, 259–276.

BODENHEIMER, F. M. (1951). *Insects as Human Food*. Junk: The Hague. 352pp.

BROWN, A. L. (1962). Microvilli of the human jejunal epithelial cell. *J. Cell Biol.* **12**, 623–627.

BUSCHMANN, R. J. AND MANKE, D. J. (1981*a*). Morphometric analysis of the membranes and organelles of the small intestinal enterocytes. I. Fasted hamster. *J. Ultrastruct. Res.* **76**, 1–14.

BUSCHMANN, R. J. AND MANKE, D. J. (1981*b*). Morphometric analysis of the membranes and organelles of the small intestinal enterocytes. II. Lipid-fed hamster. *J. Ultrastruct. Res.* **76**, 15–26.

CARPENTER, R. E. (1975). Flight metabolism of flying foxes. In *Swimming and Flying in Nature*, vol. 2 (ed. C. J. Brokaw and C. Brennen), pp. 883–890. New York: Plenum Publishing Company.

CHEKE, A. S. AND DAHL, J. F. (1981). The status of bats on Western Indian Ocean islands with special reference to *Pteropus. Mammalia* **45**, 205–238.

CRUZ-ORIVE, L. M. AND WEIBEL, E. R. (1990). Recent stereological methods for cell biology: a brief survey. *Am. J. Physiol.* **258**, L148–L156.

CUNNINGHAM VAN SOMERAN, G. R. (1972). Some fruit bats eat leaves. *Bull. E. Afr. nat. Hist. Soc.* **1972**, 24–25.

DAWSON, R. W. (1975). Avian physiology. *A. Rev. Physiol.* **37**, 441–465.

ELBRØND, V. S., DANTZER, V., MAYHEW, T. M. AND SKADHAUGE, E. (1991). Avian lower intestine adapts to dietary salt (NaCl) depletion by increasing transepithelial sodium transport and microvillous membrane surface area. *Exp. Physiol.* **76**, 733–744.

FENTON, M. B., BRIGHAM, R. M., MILLS, A. M. AND RAUTENBACH, I. L. (1985). The roosting and foraging areas of *Epomophorus wahlbergi* (Pteropodidae) and *Scotophilus viridis* (Verspertilionidae) in Kruger National Park. *J. Mammal*. **66**, 461–468.

FUNMILAYO, O. (1976). Diet and roosting damage and environmental pollution by the straw coloured fruit bat in south western Nigeria. *Niger. Fld* **41**, 136–142.

GARDNER, A. L. (1977). Feeding habits. In *Biology of Bats of the New World Family Phyllostomatidae*, vol. 2 (ed. R. J. Baker, J. K. Jones Jr and D. C. Carter), pp. 293–352. Special Publication of the Museum, Texas Technical University: Lubbock.

GREENHALL, A. M. AND PARADISO, J. D. (1968). *Bats and Bat Banding*. Washington DC: Bureau of Sport, Fisheries and Wildlife Resource Publication **72**, 47pp.

GUNDERSEN, H. J. G. (1979). Estimation of tubule or cylinder L_V, S_V and V_V on thick sections. *J. Microsc.* **117**, 333–345.

ISHIKAWA, O. K., MATOBA, M., TANAKA, H. AND ONO, K. (1985). Anatomical study of the intestine of the insect feeder bat, *Myotis frater kaguae*. *J. Anat*. **142**, 141–150.

JAEGAR, P. (1954). Les aspects du problème de chiropterogamie. *Bull. Inst. Fr. Afr. Noir.* A **16**, 796–821.

JEPSEN, L. G. (1970). Bat origins and evolution. In *Biology of Bats*, vol. 1 (ed. W. A. Wimsatt), pp. 1–64. New York: Academic Press.

JURGENS, D. K., BARTELS, H. AND BARTELS, R. (1981). Blood oxygen transport and organ weights of small bats and non-flying mammals. *Respir. Physiol*. **45**, 243–260.

KARASOV, W. H. AND DIAMOND, J. M. (1983). Adaptive regulation of sugar and amino acid transport by vertebrate intestine. *Am. J. Physiol.* **245**, G443–G462.

KEEGAN, D. J. (1977). Aspects of assimilation of sugars by *Rousettus aegypticus*. *Comp. Biochem. Physiol.* **58**A, 349–352.

KEEGAN, D. J. (1980). Lack of active glucose transport system of the bat, *Rousettus aegypticus* intestine. *S. Afr. J. Sci.* **76**, 570–571.

KEEGAN, D. J., LEVINE, S. AND BALGOBIND, N. D. (1980). Absorption of calcium in the small intestine of the bat, *Rousettus aegypticus*. *S. Afr. J. Sci*. **76**, 328.

KEEGAN, D. J. AND MÖDINGER, R. (1979). Microvilli of the intestinal mucosal cells of *Rousettus aegypticus*. *S. Afr. J. Zool*. **14**, 220–223.

KLITE, P. D. (1965). Intestinal bacterial flora and transit time in three neotropical bat species. *J. Bacteriol.* **90**, 375–379.

KUNZ, T. H. AND KURTA, A. (1988). Capture methods and holding devices. In *Ecological and Behavioural Methods for the Study of Bats* (ed. T. H. Kunz), pp. 1–28. Washington DC, London: Smithsonian Institute Press.

LASKA, M. (1990). Food transit time and carbohydrate use in three phyllostomid bat species. *Z. Saeugetierk.* **55**, 49–54.

LIM, B. L. (1973). Breeding patterns, food habits and parasitic infections of bats in Gunong Brinchang. *Malay Nat. J.* **26**, 6–13.

MADKOUR, G. A., HAMMOUDA, E. M. AND IBRAHIM, J. G. (1982). Histology of the alimentary tract of two common Egyptian bats. *Annls Zool. (Agra)* **19**, 53–73.

MAINA, J. N., THOMAS, S. P. AND HYDE, D. M. (1991). A morphometric study of the lungs of different sized bats: correlations between structure and function of the chiropteran lung. *Phil. Trans. R. Soc. Lond. B* **333**, 31–50.

MAKANYA, A. N. (1997). Morphology of the intestine of the entomophagous longfingered bat, *Miniopterus inflatus*: mucosal topography and possible landmarks. *Acta biol. hung*. **48**, 15–27.

MAKANYA, A. N. AND MAINA, J. N. (1994). Morphology of the alimentary tract of the insectivorous Horseshoe bat, *Rhinolophus hildebradti*, Peters: a scanning and light microscopic study. *Afr. J. Ecol.* **32**, 158–168.

MAKANYA, A. N., MAYHEW, T. M. AND MAINA, J. N. (1995). Stereological methods for estimating the functional surfaces of the chiropteran small intestine. *J. Anat.* **187**, 361–368.

MANLEY, D. B. AND WILLIAMS, L. M. (1979). Structure of the gastrointestinal tract of the flying fox, *Pteropus poliocephalus*. *J. Anat.* **128**, 649–650.

MARSHALL, A. G. (1983). Bats, flowers and fruit: evolutionary relationships in the Old World. *Biol. J. Linn. Soc.* **20**, 115–135.

MATHIS, J. (1928). Beitrag zur Kenntnis des Fledermausdarmes. *Z. mikrosk. Anat. Forsch.* **8**, 595–647.

MAYHEW, T. M. (1987). Quantitative ultrastructural study on the response of microvilli along the small bowel to fasting. *J. Anat.* **154**, 237–243.

MAYHEW, T. M. (1988). Geometric model for estimating villous surface area in the rat small bowel is justified by unbiased estimates obtained using vertical sections. *J. Anat.* **161**, 187–193.

MAYHEW, T. M. (1990). Striated brush border of intestinal absorptive epithelial cells: stereological studies on microvillous morphology in different adaptive states. *J. Electron Microsc. Techn.* **16**, 45–55.

MAYHEW, T. M. (1991). The new stereological methods for interpreting functional morphology from slices of cells and organs. *Exp. Physiol.* **76**, 639–665.

MAYHEW, T. M. (1992). A review of recent advances in stereology for quantifying neural structure. *J. Neurocytol.* **21**, 313–328.

MAYHEW, T. M. (1996). Adaptive remodelling of intestinal epithelium assessed using stereology: correlation of single cell and whole organ data with nutrient transport. *Histol. Histopathol.* **11**, 729–741.

MAYHEW, T. M., ELBRØND, V. S., DANTZER, V. AND SKADHAUGE, E. (1992*a*). Quantitative analysis of factors contributing to expansion of microvillous surface area in the coprodaeum of hens transferred to a low-NaCl diet. *J. Anat.* **181**, 73–77.

MAYHEW, T. M., ELBRØND, V. S., DANTZER, V., SKADHAUGE, E. AND

MØLLER, O. (1992*b*). Structural and enzymatic studies on the plasma membrane domains and sodium pump enzymes of absorptive epithelial cells in the avian lower intestine. *Cell Tissue Res.* **270**, 577–585.

MAYHEW, T. M. AND MIDDLETON, C. (1985). Crypts, villi and microvilli in the small intestine of the rat. A stereological study of their variation within and between animals. *J. Anat.* **141**, 1–17.

MILLER, S. (1975). *Experimental Design and Statistics*. London: Methuen and Co. Ltd, 142pp.

MISCH, D. W., GIEBEL, P. E. AND FAUST, R. G. (1980). Intestinal microvilli: responses to feeding and fasting. *Eur. J. Cell Biol.* **21**, 269–279.

MORRISON, D. W. (1980). Efficiency of food utilisation by fruit bats. *Oecologia* **45**, 270–273.

MORTON, E. S. (1973). On the evolutionary advantages and disadvantages of fruit eating in tropical birds. *Am. Nat.* **107**, 8–22.

NELSON, J. E. (1965). Movement of Australian flying foxes (Pteropodidae: Megachiroptera). *Aust. J. Zool*. **13**, 53–73.

OGUNBIYI, O. A. AND OKON, E. E. (1976). Studies of the digestive enzymes of the African fruit bat *Eidolon helvum* (Kerr). *Comp. Biochem. Physiol*. **55**A, 359–361.

OKON, E. E. (1977). Functional anatomy of the alimentary canal in the fruit bat *Eidolon helvum* and the insect bat *Tadarida nigeriae. Acta zool. (Stockholm)* **58**, 83–93.

RATCLIFFE, F. (1932). Notes on fruit bats (*Pteropus spp*) of Australia. *J. Anim. Ecol*. **1**, 32–57.

ROSEVAR, D. R. (1965). *The Bats of West Africa*. London: British Museum (Natural History). 418pp.

SMITH, M. W. AND BROWN, D. (1989). Dual control over microvillus elongation during enterocyte development. *Comp. Biochem. Physiol.* **93**A, 623–628.

SOKAL, R. R. AND ROHLF, F. J. (1981). *Biometry*. San Francisco: W. H. Freeman and Co. 859pp.

START, A. N. AND MARSHALL, A. G. (1976). Nectarivorous bats as pollinators of trees in West Malaysia. In *Variation, Breeding and Conservation of Tropical Forest Trees* (ed. J. Burley and B. T. Styles), pp. 141–150. London: Academic Press.

STENLING, R. AND HELANDER, H. F. (1981). Stereologic studies on the small intestinal epithelium of the rat. I. The absorptive cell of the normal duodenum and jejunum. *Cell Tissue Res.* **217**, 11–21.

TEDMAN, R. A. AND HALL, L. S. (1985). The morphology of the gastrointestinal tract and food transit times in fruit bats, *Pteropus alecto* and *Pteropus poliocephalus* (Megachiroptera). *Aust. J. Zool.* **33**, 625–640.

THOMAS, D. W. (1984). Fruit intake and energy budgets of frugivorous bats. *Physiol. Zool.* **57**, 457–467.

THOMAS, S. P. (1975). Metabolism during flight in two species of bats, *Phyllostomus hastatus* and *Pteropus gouldii. J. exp. Biol.* **63**, 273–293.

THOMAS, S. P. (1980). The physiology and energetics of bat flight. In *Proceedings of the Fifth International Bat Research Conference* (ed. D. W. Wilson and A. Galdner), pp. 393–402. Texas: Texas Technical Press.

THOMAS, S. P. AND SUTHERS, R. A. (1972). The physiology and energetics of bat flight. *J. exp. Biol.* **57**, 317–335.

TUCKER, V. A. (1972). Respiration during flight in birds. *Respir. Physiol.* **14**, 75–82.

VAN DER PIJL, L. (1956). Remarks on pollination by bats in the genera *Freycinetia, Duabanga* and *Haplophragma* and on chirepterophily in general. *Acta bot. neerl.* **5**, 135–144.

VAN DER PIJL, L. (1957). The dispersal of plants by bats (chirepterochory). *Acta bot. neerl.* **6**, 291–315.

VAN DONGEN, J. M., VISSER, W. J., DAEMS, W. T. H. AND GALJAARD, H. (1976). The relation between cell proliferation, differentiation and ultrastructural development in rat intestinal epithelium. *Cell Tissue Res.* **174**, 183–199.

WARREN, M. A. (1991). Adaptations of the rat small intestine to a single and a double period of undernutrition. *J. Anat.* **176**, 89–97.

WATT, B. K. (1968). Composition of food, raw and processed: plant origin. In *Metabolism* (ed. P. L. Altman and D. S. Dittmer), pp. 26–47. Bethseda, Maryland: Federal Society of Experimental Biology.

WEIBEL, E. R. (1979). *Stereological Methods*, vol. 1, *Practical Methods for Biological Morphometry*. New York: Academic Press. 415pp.

WICKLER, W. AND SEIBT, U. (1976). Field studies of the African fruit bat *Epomophorus wahlbergi* (Sundeval), with special reference to male calling. *Z. Tierpsychol*. **26**, 726–736.

WILLIAMS, M. AND MAYHEW, T. M. (1992). Responses of enterocyte microvilli in experimental diabetes to insulin and an aldose reductase inhibitor (ponalrestat). *Virchows Archs B Cell Pathol*. **62**, 385–389.

WILSON, D. E. (1973). Bat faunas. A trophic comparison. *Syst. Zool.* **22**, 14–24.

WILSON, D. E. (1988). Maintaining captive bats. In *Ecological and Behavioural Methods for the Study of Bats* (ed. T. H. Kunz), pp. 247–261. Washington DC, London: Smithsonian Institution Press.

WIMSATT, W. A. (1970). *Biology of Bats*, vol. 1. New York, London: Academic Press. 406pp.

YALDEN, D. W. AND MORRIS, P. A. (1975). *The Lives of Bats.* Vancouver: New York Times Book Co. 247pp.

ZOUBI, S. A., MAYHEW, T. M. AND SPARROW, R. A. (1995). The small intestine in experimental diabetes: cellular adaptation in crypts and villi at different longitudinal sites. *Virchows Arch.* **426**, 501–507.