A COMPARATIVE MORPHOLOGICAL AND MORPHOMETRIC STUDY OF THE CHIROPTERAN SMALL INTESTINE.

by

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A thesis submitted in partial fulfillment for the requirements of a Master of Science degree of the University of Nairobi.

DEDICATION

To

Grace & Mark

For their Love and Care

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

.....

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This work has been submitted with my approval as a University supervisor.

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Prof. J. N. Maina.

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SUMMARY

The structural characteristics of the intestinal tract of three species of bats namely the fruit bat *Epomophorus wahlbergi* and the entomophagous bats *Miniopterus inflatus* and *Rhinolophus hildebrandti* have been examined macroscopically, with the light microscope and with the scanning and transmission electron microscopes. Stereological techniques and formulae have been modified and utilized to estimate the surface characteristics of the intestinal mucosa on two species of bats. In all the bats examined, neither a caecum nor an appendix was observed and the intestine was a narrow tube of almost uniform diameter save for the rectum whose diameter was greater than that of the rest of the intestine. A colon was only observed in the fruit bat but could only be distinguished from the rest of the intestine from its characteristic mucosal surface after opening the intestine. The villi in the cranial 20 % of the intestine of the fruit bat branched and interconnected while those occurring in the rest of the intestine were generally finger-like discrete projections. In the insectivorous bats, the villi were ridge-like and were transversely oriented, the only deviation from this pattern being observed in a small posterior part of the intestine of the horseshoe bat. In the latter, the villi anastomosed profusely forming shallow hexagonal compartments semblant of the reticular cells of the ruminant stomach. In the two insectivorous species of bats, a short segment of the

mucosa immediately posterior to the pylorus had numerous hexagonal and cylindrical pits which were thought to be either involved in enzyme secretion, absorption of nutrients or both.

The ultrastructural picture of the intestinal mucosa in the frugivorous bat showed remarkable cellular and pericellular modifications that were absent in the entomophagous bats. The enteric epithelium was made of tall columnar cells between which were large intercellular spaces. These columnar cells had large intracellular vacuoles and sent long and tortuous cytoplasmic projections (pseudopodia) into the intercellular spaces. Adjacent cells were joined by means of desmosomes formed between two apposing cytoplasmic processes. The role of the cytoplasmic processes besides structural reinforcement, was thought to be pinocytosis. In the insectivorous bats, intercellular spaces were narrow and epithelial cells were devoid of vacuoles and no specializations were observed on the lateral membranes of the absorptive cells. In both groups of bats, the epithelial cells had numerous mitochondria distributed over the apical and basal sides of the centrally located nucleus.

Macroscopic morphometric comparisons showed that the intestine of the frugivorous bat is longer than that of the insectivorous bat but when intestinal length is normalized with body mass, the insectivore had a longer intestine. The microvillous dimensions (mean length,

diameter and surface) in the fruit-eating bat and insectivorous bat showed no significant trends but such trends were significant for segmental microvillous numbers, microvillous amplification factors and segmental surface areas with these values being highest in the proximal intestinal segments and lowest in the posterior segments of the intestine. In the fruit bat, the average values for microvillous height, diameter and surface were 2.87 μm, 0.097 μm and 0.8739 μm^2 respectively with an uncorrected total microvillous surface area of 2.50×10^{12} μm^2. The mean microvillous height, diameter, and surface area for the insectivorous bat were 1.09 μm, 0.088 μm and 0.3069 μm^2 respectively with an uncorrected absolute intestinal surface area of 1.32×10^{11} μm^2. The microvillous packing density and the absolute number of microvilli in the fruit bat were 58 μm^{-2} and 3.24×10^{12} respectively. In the insectivorous bat the microvillous packing density was 88 μm^{-2} with an absolute number of 3.90×10^{11}.

This study indicates that the chiropteran intestine is structurally and hence functionally better adapted for absorption than that of the land-based mammals for which similar studies have been conducted. The qualitative and quantitative results of this study indicate that the frugivorous bat has a superior intestine than the entomophagous bat. This may in part be explained in terms of the differences in their energetic demands of flight and the differences in the types of diet on which these bats thrive. Although an attempt has been made to explain

the adaptive characteristics of the chiropteran intestine and the observed species differences, conclusive explanations of these peculiarities must not only await broader morphometric studies on a wider range of species, but also detailed observations of their ecological, physiological and flight biomechanical characteristics.

ABBREVIATIONS

Parameters

W	average width of the intestine. This is the average circumference of the segment.
L	length of intestinal segment
S, s	surface area
I	intersections
N	Number
h	height
d	diameter
t	total

Reference Spaces

pm	primary mucosa
V	villus
mv	microvillus
em	enterocyte membrane

Stereological Expressions

S(pm)	primary mucosal surface, cross-sectional internal surface area of the intestine.
I(v)	number of intersections between the villous profiles and the test lines.

I(pm)	number of intersections between the test lines and an imaginary line running along the crypt-villus axis.
S(v)/S(pm)	villous amplification factor.
S(v)	secondary surface area, villous surface area.
I(mv)	intersection counts between microvillous profiles and the test lines
I(em)	intersection counts between the test lines and an imaginary line running along the boundary between microvilli and the apical cell membrane.
S(mv)/S(v)	microvillous amplification factor.
S(mv)	tertiary surface area, absolute surface area, surface area of microvilli (of an intestinal segment).
S(mv)t	total (microvillous) surface area of the entire small intestine.
h(mv)	height, length of microvilli.

d(mv)	diameter of microvillus.
s(mv)	surface area of the average microvillous, mean microvillous surface area.
N(mv)/S(v)	microvillous packing density.
N(mv)	number of microvilli per intestinal segment.
N(mv)t	total number of microvilli in the entire small intestine of the bat.

Non-Stereological Abbreviations

SD	standard deviation
SE	standard error
H & E	Hematoxylin and Eosin
TB	Toluidine Blue
LM	light microscopy
TEM	transmission electron microscopy
SEM	scanning electron microscopy

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1.0. INTRODUCTION AND LITERATURE REVIEW

1.1. General Introduction

Bats belong to the order Chiroptera which comprises closely related species of flying mammals. They are the only true flying mammals and are superior to birds in their great capability of maneuverability in space and dexterity in flight with some species being able to negotiate tangled thickets which most birds would be forced to hop over (Kingdon, 1974). All bats are nocturnal and this may be attributed to competition for the same ecological niches with birds and also to physiological limitations related to heat and water balance (Kingdon, 1974). Bats, however, have developed methods of overcoming the various physiological and environmental problems by, for example, developing the capacity to fly, migrate and for the greater majority the capacity for echolocation.

The order chiroptera is subdivided into two suborders; the Megachiroptera comprising mainly large fruit eating bats and Microchiroptera comprising numerous genera of mainly small sized entomophagous bats and groups exhibiting diverse feeding habits including frugivory, piscivory, carnivory and hematophagy.

The suborder Megachiroptera consists of large bats which feed principally on fruits, though some have shown carnivorous behavior (Van Deusen, 1968) and a large number also feed on nectar. They are all restricted to the Old World tropics and subtropics where an yearlong supply of

fruit is available. Some such as *Eidolon* and *Hypsignathus* are known to migrate (Yalden & Morris, 1975). The megachiroptera have got an increased eye sensitivity and acuity so that they can see the faintest of lights and their sense of smell which is normally used to detect fruits is acute (Kingdon, 1974). Most species have to drink water though fruit and nectar provide adequate supply of water for many bats. Most fruit bats feed on the pulp and juice of a variety of fruits and these require a capacious stomach. Digestion and excretion are, however, accomplished in a few hours (Rodhain & Bequert, 1916; Rosevar, 1965; Kingdon, 1974).

Wahlberg's fruit bat (*Epomophorus wahlbergi*, Halowell, 1846) which is one of the species studied here, belongs to the family Pteropodidae. In Africa, it is mainly found in southeastern regions in savanna woodland and forest margins at altitudes 2000 metres or below. The bats roost in forests, farm and garden trees and feed on ripe, sweet fruits of many kinds such as mangoes, bananas and wild figs. These bats roost close to the feeding site and fly low, seldom rising above the forest canopy (Kingdon, 1974; Wickler & Seibt, 1976) though some individuals may travel for long distances in search of fruit in one night (Fenton *et al*, 1985).

The suborder Microchiroptera comprises numerous species of bats that are generally and relatively smaller in size compared to the Megachiroptera. They have small, often inconspicuous eyes but their ears are well

developed (Norberg & Rayner, 1987). Generally, they feed on insects but a good number have developed divergent and specific feeding habits and adaptations. The phyllostomatids which belong to this suborder, for example, thrive on fruits and have evolved flat crowned teeth and a ridged palate just like the frugivorous Megachiroptera (Yalden & Morris, 1975).

The other feeding habits observed in this suborder are piscivory, carnivory, nectarivory and sanguivory. The greater majority of the Microchiroptera are, however, insectivores that capture their prey mainly by echolocation (Yalden & Morris, 1975), most of them being opportunistic feeders that capture the available insects rather than selected species (Yalden & Morris, 1975; Belwood & Fenton, 1976; Fenton & Morris, 1976). The ears are quite elaborate and noseleaves, earlobes and earfolds are all structural adaptations to serve the echolocation system.

The rhinolophid horseshoe bat [*Rhinolophus hildebrandti* (Peters, 1878)] and the verspertilionid longfingered bat [*Miniopterus inflatus* (Sanborn, 1936)] which are reported in this study fall under the group Microchiroptera, are both insectivores and are not known to feed on any other type of diet (Kingdon, 1974).

The rhinolophids derive their name from their noseleaves which are horseshoe in shape. Flight in this group of bats is slow and manoeuverable with some hovering often in a clutter. They are mainly specialized

for flycatching and in some cases for gleaning (Norberg & Rayner, 1987). The rhinolophids have evolved a very highly effective sonar which has entailed development of elaborate noseleaves and emission of sound through the nostrils (Kingdon, 1974). *Rhinolophus hildebrandti* is a large highly evolved species apparently restricted to the eastern part of Africa. It often roosts singly but is sometimes found in small groups occasionally associated with other species (Kingdon, 1974). It is a slow flyer alternating between continuous and short flights from perches foraging just above the ground in woodland or riverine forests (Fenton & Rautenbauch, 1986). It is capable of flying for up to 53 minutes without perching while foraging and capturing moths and beetles, the only known food on which this bat thrives (Fenton & Rautenbauch, 1986).

The longfingered bats (Verspertilionidae) on the other hand, inhabit most of the tropical world but they are notably absent from the deserts. They are primarily cave bats, preferring to roost in very dark holes and crevices well away from light. In their roost they congregate into large groups or clusters, occasionally hanging free and often clinging to each other (Kingdon, 1974). The longfingered bats mostly feed on high flying insects including small beetles. They are early flyers and resemble martins or swallows in their very rapid flight with abrupt swoops and changes of altitude and direction. These bats can swerve round corners and

artificial obstacles with unabated speed and precision (Kingdon, 1974).

Miniopterus inflatus, the subject of this study is often found together with its other closely associated species such as *Miniopterus schreibesi*, with which it is normally confused, the only distinguishing characteristics being the length of the skull which is relatively longer for *M. inflatus* than for *M. schreibesi*.

1.2. Morphology of the gastrointestinal tract.

The morphology of the chiropteran gastrointestinal tract has received considerable attention since the mid-nineteenth century when Huxley (1865) described the tubular stomach of *Desmodus rotundus*. Owen (1866-1868), Dobson (1878), Robin (1881) and Mitchell (1905, 1916) described the stomachs and intestines of varied species of bats. These studies, however, described mainly the gross morphology and were deficient of details. The stomach in particular has since received extensive interest as seen in the studies of Fischer (1909), Hamperl (1923), Mathis (1928 a,b), Eisentraut (1950), Park & Hall (1951), Kolb (1954), Ito & Winchester (1963), Schultz (1965), Forman (1971; 1972; 1973), Phillips *et al* (1984). The intestine was, however, only mildly studied with Owen (1868), Dobson (1878), Mitchell (1905), Madkour (1976; 1977a, b) describing the gross morphology only. McMillan & Churchill (1947) made one of the earliest attempts at describing the histology of the chiropteran intestine and compared it with that of the mouse. They

contended that a caecum was not a feature in the chiropteran gut and that the transition from the small to the large intestine was marked by a constriction or brief narrowing of the intestine. The absence of the caecum and an appendix in the chiropteran intestine had, however, been pointed out earlier by Allen (1939). Subsequent studies (Park & Hall, 1951; Klite, 1965; Madkour, 1976) highlighted the absence of a caecum and an appendix in the chiropteran intestine. However, there is controversy on the existence of the various parts of the large intestine in the chiropteran gut with some workers mentioning the presence of a colon without any histological or ultrastructural verification (see for example, Forman, 1972; 1974a,b; Halstead & Segun, 1975; Danguy *et al*, 1987). Studies employing histology alone (Okon, 1977), both histology and transmission electron microscopy (Tedman & Hall, 1985) or histology, transmission and scanning electron microscopy (Ishikawa *et al*, 1985) show that the morphology of the chiropteran intestine is remarkably diverse with the colon being absent in some species (Okon, 1977) and an ileo-colon being encountered in others (Ishikawa *et al*, 1985). Madkour (1976) on the other hand observed that the disposition of viscera in bats had both inter- and intra-species variations and that a caecum was present in only one species of bats, *Rhinopoma hardwickei*. This indicates that the gut morphology in bats probably bears strong generic or even specific inclinations.

Stutz & Ziswiler (1983-1984) found topography of the digestive tract of bats to be species specific without sexual dimorphism. They categorized microchiropteran intestinal mucosal relief as having transverse zigzag folds and villi or as having net-like connected zig-zag folds, or isolated villi. This study aims among other things, to examine the variation in the intestinal topography of the chiropteran gut mucosa in an attempt to identify the various parts of the large intestine present.

1.3 Flight and Flight Energetics.

As a form of locomotion, active flight is energetically very expensive (see for example, Tucker, 1972; Carpenter, 1975; Thomas, 1975). Many investigators cite bats as being unique among mammals in their capacity for flight. This feature is well pointed out by among others Greenhall and Paradiso (1968), Wimsatt (1970), Thomas and Suthers (1972), Dawson (1975), Yalden and Morris (1975), Thomas (1975, 1980) and Jurgens *et al.*(1981). Birds evidently evolved flight long before the bats and hence colonized the diurnal niche (Jepsen, 1970), thus relegating the bats to the nocturnal niche. Consequently, all bats are nocturnal, roosting during the daytime and foraging at night.

Aerial locomotion has enabled bats to occupy different ecological niches, exploit diverse and widely dispersed food sources and avoid quite a number of would be predators (Thomas, 1980). The metabolic cost of flight

in bats is comparable to that of birds (Thomas & Suthers, 1972) and the highest metabolic rates of flying bats are essentially the same as those predicted for flying birds of comparable body mass but are 2.5-3.0 times greater than the highest metabolic rates of which similar sized exercising terrestrial mammals are capable (Thomas, 1975). Flight, however, is reported to be a form of locomotion superior to either walking or running since in flight a greater distance is covered per unit of energy consumed. Thomas (1980) observed that the metabolic capabilities of bats are greater than those of any group of mammals. Bats have both respiratory (see Maina *et al.*, 1982; Maina *et al*, 1991) and cardiovascular (Jurgens *et al*, 1981; Ayettey *et al.*,1991) adaptations to which such high metabolic rates are attributable . The blood oxygen carrying capacity of bats, for example, particularly with respect to hemoglobin concentration and hematocrit is remarkably high (Jurgens *et al.*, 1981) and blood supply to flight muscles is also notably high (Ayettey *et al.*, 1981; Mathieu-Costello *et al.*, 1992). Studies on the respiratory system of bats (Maina & King, 1984; Maina *et al.*, 1991) indicate that the morphometric characteristics of the chiropteran lung are comparable to those of flying birds and that for small sized bats, the lung is even superior to that of birds. For metabolic rates as high as observed in bats, it is expected that there is an adequate supply of the necessary nutrients and hence efficient digestive and absorptive processes to provide

the required raw materials for energy production.

1.4 Food and Feeding Habits.

Although bats are all fundamentally similar, there has been great evolutionary pressures for them to develop different feeding habits than in most other mammals (Yalden & Morris, 1975). Consequently, bats have established themselves in most mammalian feeding niches and developed one, hematophagy, which is unique among terrestrial vertebrates (Yalden & Morris, 1975). The trend, however, in evolution from the ancestral insectivory has been to adopt only the highly nutritious types of foods while avoiding the bulky, poorly digested types (Yalden & Morris, 1975). Various feeding habits such as frugivory, nectarivory, piscivory, carnivory and sanguivory have been developed in bats. A lot of them exhibit more than one feeding habits with species such as *Phyllostomus hastatus* (Yalden & Morris, 1975) and *Hypsignathus monstrous* (Van Deusen, 1968) which are primarily frugivores showing carnivorous behavior and a remarkable number of frugivores also feed on nectar (Kingdon, 1974). In general, all bats are restricted to nutritionally concentrated and easily absorbed foods with high energy returns. Most fruit bats feed on the pulp and juice of a variety of fruits (Kingdon, 1974) while a majority of insectivores are opportunistic feeders (Yalden and Morris, 1975), preying on the available insects rather than a few selected species (Belwood &

Fenton, 1976; Fenton & Morris, 1976). Larger insects are more efficiently digested and bats may tend to show preference for large insects (Barclay *et al.*,1991). Various factors such as the size of the bat and echolocation specializations (Barclay, 1986; Shiel *et al.*, 1991) will, nevertheless, determine the types of insects eaten and variation in size is usually correlated with variation in food-particle or prey size (e.g. Ashmole, 1968). The diversity in feeding habits of bats varies from frugivory to faunivory to the very unique hematophagy. Though bats have restricted themselves to highly nutritious and easily digestible foods, the very fast food transit times reported (see for example, Klite, 1965; Morrison, 1980; Tedman & Hall, 1985; Laska, 1990) indicate that the chiropteran gut must be very efficient in food absorption if appreciable amounts of energy are to be obtained from the ingested food.

1.5. Foraging Strategies.

Fruit bats and insectivorous bats exhibit diverse foraging strategies. Though most fruit bats roost close to the source of food, some such as *Eidolon helvum* and *Hypsignathus monstrous* are known to migrate in search of fruits (Kingdon, 1974). Some fruit bats have extensive foraging territories and cover large distances in one night. *Epomophorus wahlbergi*, for example, is known to cover over 4 km per move and makes over thirty such moves in one night while foraging (Fenton *et al.*, 1985).

The mobility of bats permits exploitation of

patchily distributed food resources (McNab, 1971). Flying insects are taken by hawking or flycatching while non flying prey is taken by hovering or gleaning (Norberg & Rayner, 1987). Smaller bats are more manoeuverable and agile and can fly more slowly than the larger ones and their energetic demands of flight are low compared with other components of metabolism (Norberg & Rayner, 1987). Hovering is also employed by a few flower-visitors but they are small in size for aerodynamic and mechanical reasons since taking fruit or nectar while hovering may be more costly in terms of energy than flight hunting. Large nectarivores perch while feeding.

Large fruit bats handle larger fruits which they carry to their roosts and since frugivores may find difficulties in obtaining enough protein from fruits some such as phyllostomatids feed on insects (Norberg & Rayner, 1987) while pteropids take larger quantities of fruits than their energy requirements (Thomas, 1984), and perhaps dissipate the excess energy during flight.

In the insectivorous group, most bats feed mainly on insects but use different foraging techniques such as fast, long-range hawking; slow short-range hawking; trawling; hovering and/or gleaning and fly catching (Norberg & Rayner, 1987). Foraging behavior may, however, be dependent on energy demand (eg. Barclay, 1991). The prey detection system of aerial insectivorous bats renders small prey unavailable to larger bats and aerial insectivorous bats are all relatively small (Barclay &

Brigham, 1991).

1.6. Digestive Physiology.

Various reports (Rodhain & Bequert, 1916; Rosevar, 1965; Kingdon, 1974; Okon, 1977) indicate that digestion in bats occurs very fast. The passage of food through the gastrointestinal tract of bats, in particular, has been shown to be remarkably rapid (Klite, 1965; Keegan, 1975; Okon, 1977; Morrison, 1980; Wolton, *et al.*, 1982; Tedman & Hall, 1982; 1985). It was further shown that the intestine of the bat absorbs sugars faster than that of the rat (Keegan, 1975; 1980). Keegan and Mödinger (1979) attributed the fast absorption rate to the very elaborate microvilli they demonstrated in the intestine of the bat. Griffin (1958) suggested that the very rapid intestinal transit observed in the bat indicated a highly efficient digestive system. Studies on the enzyme activity of the chiropteran alimentary tract (Ogunbiyi and Okon, 1976; Okon and Ogunbiyi, 1979) indicated that the digestive enzymes were present all along the alimentary canal, including the oesophagus and the colon. Since no active transport mechanism was demonstrable in the intestines of the bat (see Keegan, 1980; Keegan *et al*, 1980), it is possible that the high absorption rates are due to increased effective intestinal surface area and probably extensive distribution and increased amounts of digestive enzymes.

1.7. Intestinal Morphometry.

Methods have been developed for quantitative characterization of intestinal structure and hence for relating such features to function (see Buschmann & Manke, 1981a; Mayhew, 1984) and these have been employed to investigate a few species (for example, Buschmann & Manke, 1981a, b; Stenling & Helander 1981; Mayhew, 1987; Mayhew & Middleton, 1985; Mayhew & Carson, 1989). Surface area of intestinal mucosa is one parameter that indicates high performance in terms of absorptive capacity (Mayhew & Carson, 1989). Intestinal surface area varies both with age (Bastie, *et al*, 1982) and with fasting (Mayhew, 1987) and there are intrinsic factors that determine villous size in specific regions of the gut (Gabriel & Leplond, 1970) with decrease from the duodenum to the ileum.

Villi and microvilli in the intestine are structures that are said to amplify surface area. These surface modification structures are, however, both anisotropic and earlier studies on intestinal morphometry were short of the recent techniques in stereology that solve the problem of anisotropy (see Baddeley *et al*, 1986). By using vertical sections and specially designed test systems (Baddeley *et al*., 1986), unbiased estimates of surface areas of anisotropic structures such as intestinal villi and microvilli can be obtained. These techniques have been used effectively to determine the surface areas of the avian coprodeum (Mayhew, *et al*., 1990) and reversed segments of the rat intestine (Sondenaa *et al*., *1991)*.

Morphometric information on the chiropteran intestine is scarce and the only reported data was based on microvilli alone (Keegan & Mödinger, 1979) or on gross specimens only (e.g. Madkour, 1976) in contrast to those of hens and rats (see for instance, Mayhew, 1990; Elbrond *et al.*, 1991; Mayhew *et al.*, 1992; Williams & Mayhew, 1992).

In this study, a tissue sampling protocol has been adopted and modified and, together with various morphologic techniques such as SEM and TEM, employed to study the morphology of the intestine of a megachiropteran and a microchiropteran bat. The morphology of another closely related microchiropteran bat is also presented. The results have been related to and compared with the dietary and flight characteristics of each species in an attempt to correlate structure and function in this unique group of mammals.

1.8. Aims and Objectives

1. To investigate the morphology of the chiropteran intestine in an attempt to resolve the controversy on the presence or absence of the various parts of the intestine.
2. To investigate the structural modifications of the bat intestine to which the high digestive performance may be attributed.
3. To compare and contrast the morphologic and morphometric characteristics of the intestine of frugivorous and the insectivorous bats and correlate the possible differences with function.
4. To explain major deviations of the chiropteran gut morphology from the general design of the mammalian digestive system.

2.0.0 MATERIALS AND METHODS

2.1.0 Experimental Animals

Three species of bats belonging to the two suborders namely Megachiroptera and Microchiroptera were employed in the study. Details of the number of specimens used for each study technique are given in table 1 (page 38).

2.1.1. Megachiroptera

Nine epauleted fruit bats [*Epomophorus wahlbergi* (Halowell, 1846)] five adult males and four adult females were caught in forests in and around Nairobi by spreading mist nets next to small streams. Bats got entangled in the nets as they descended upon the streams to drink water at dusk. Two of the specimens were used for scanning electron microscopy (SEM), one for histology, one for transmission electron microscopy (TEM) and five for morphometric studies.

2.1.2. Microchiroptera

Two species belonging to the suborder Microchiroptera, *Miniopterus inflatus*, Sanborn, 1936 and *Rhinolophus hildebrandti*, Peters, 1878 were studied. Bats were obtained from caves in Naivasha (Kenya) during the day by spreading a mist net at the cave entrance and stirring the bats from their roosts. A detailed account of the methods is available in Kunz & Kurta (1988). Nine longfingered bats (*Miniopterus inflatus*), six adult males and three adult females were employed in the study. While trapping the longfingered bats (*Miniopterus inflatus*) the targeted species for this study, five horseshoe bats

(Rhinolophus hildebrandti) were found to be co-roosting with the longfingered bats. These were used for comparative qualitative and quantitative studies.

2.2.0. Preparation of the Tissues.

Bats were killed by intraperitoneal injection of sodium pentobarbital at 50 mg/kg body weight and the abdominal cavity opened up through a ventro-median incision to reveal the digestive organs which were studied *in situ*. The oesophagus was severed cranial to the diaphragm and the pelvic bones carefully cut to reveal the rectum and then the entire gastrointestinal tract was dissected out by tearing off the mesenteries and immediately transferred to a bath of 0.85% sodium chloride. The intestinal tract was opened by a longitudinal incision along the mesenteric border, ingesta/digesta washed off with fresh saline and then dealt with as outlined below.

2.2.1. Scanning Electron Microscopy (SEM).

From each of the three species of bats, two individuals, one male and one female, were used. The saline-washed intestinal tracts were cut into 1 cm long serial segments and immediately immersed in 2.3% or 2.5% phosphate buffered glutaraldehyde (pH 7.4 and 450 mOsm) for at least four hours. The segments were then dehydrated in ascending concentrations of ethanol starting from 50% through 70%, 80%, 90%, and finally two changes of 100%. The segments were left in absolute ethanol for 24 hours and then critical-point dried in liquid carbon dioxide,

mounted on brass stubs, sputter-coated with gold-palladium complex and viewed on a Jeol (JSM-T100) scanning electron microscope.

2.2.2. Light Microscopy (LM).

From each of the three species, one individuals male was used for histological studies. Intestinal serial segments measuring 1 cm in length were prepared from each individual. The segments were dehydrated in ascending concentrations of ethanol (see 2.2.1. above) and subsequently embedded in paraffin wax and sections measuring 5μm thick were obtained using a Rotary (Leitz Wetzlar) microtome and stained in hematoxylin and eosin (H&E).

2.2.3. Transmission Electron Microscopy (TEM).

Three individual bats each representing one of the three species indicated above were employed for the ultrastructural study. Intestinal tracts were obtained as previously described (above). Intestinal serial segments were prepared and fixed in 2.3% or 2.5% phosphate buffered glutaraldehyde at 450 mOsm and pH 7.4, as outlined above under SEM (ranges of 2-3% glutaraldehyde are recommended for fixing gut, eg Hayat, 1981). The segments were washed three times in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide and then dehydrated through ascending concentrations of acetone starting from 20% through 40%, 60%, 80%, 90%, two changes of 100% and finally infiltrated with and embedded in Transmit resin (Taab, UK). These tissues were used for both light and TEM studies.

Semithin sections were obtained at 1μm and ultrathin ones at 60 nm by use of a Reichert-Jung (Austria) ultramicrotome. Ultrathin sections were stained with uranyl acetate (in the dark) and counter stained with lead citrate in presence of sodium hydroxide pellets and viewed on a Phillips EM 300 or Phillips EM 410 Transmission Electron Microscope.

2.3.0. Morphometry.

Two species, *Epomophorus wahlbergi* (Megachiroptera) and *Miniopterus inflatus* (Microchiroptera) were used for comparative morphometry. *Rhinolophus hildebrandti* was only considered in as far as gross measurements were concerned.

2.3.1. Tissue Sampling Protocol.

Five adult epauleted fruit bats (3 females and 2 males) and five adult longfingered bats (4 males and 1 female) were used for the stereological study. Entire gastrointestinal tracts were obtained as outlined above and after washing with saline the length of the intestine and parts thereof were measured. The junction between the foregut and the hindgut was identified and the foregut separated by severing the intestine at this junction and at the pyloro-intestinal junction. The foregut was subsequently divided into five equal segments, the average width (w) of each determined and hence the cross sectional area (WL) obtained by multiplying the length (L) of each segment by the average width (W). Each of these five segments was divided into five approximately equal subsegments. The latter were

immersed in 2.5% phosphate buffered glutaraldehyde and allowed to fix for at least four hours. One subsegment from each set of five was picked at random and processed for TEM as outlined above but before embedding, the subsegment was put at the centre of a petri dish placed on a square lattice grid on which one side was previously chosen to represent the microtome knife edge. The petri dish was then spun about its centre and on coming to rest, the subsegment was picked and embedded with the now predetermined edge on the face to be sectioned. This ensured that IUR (isotropic uniform random) vertical sections of the intestine were obtained. A detailed account of the method and its justification is available in Baddeley *et al.*(1986). These tissues were used for both light and TEM studies. Semithin sections were obtained at 1μm and ultrathin ones at 60 nm by use of a Reichert-Jung (Austria) ultramicrotome. Semithin sections were viewed on a Nikon or Carl Zeiss photographic light microscope while the ultrathin ones were stained with lead citrate and uranyl acetate and viewed on a Phillips EM 300 or a Phillips EM 410 electron microscope at an accelerating voltage of 80 kv. These steps are summarized in figs. 1, 2, 3a & 3b.

The intestines of the rhinolophid bat were cleaned and fixed as outlined above. The lengths of the various parts of the intestine and the average width were determined. The intestines were then used for qualitative studies as outlined above.

2.3.2. Intersection Counting

From the blocks obtained above, two toluidine blue-stained sections were prepared from every segment and subsequently two micrographs printed at a final magnification of x 100 for the insectivorous bats and x 80 for the frugivorous bats. At TEM, five micrographs were prepared at final magnifications of x 50000 for the insectivorous bats and x 18500 for the frugivorous bats. These magnifications were the lowest at which the villi and microvilli could be adequately resolved. At both LM and TEM levels, micrographs were taken randomly but in such a way that the vertical direction was maintained. Fields that were devoid of the structures of interest (villi or microvilli) were ignored. Intersection counts between the test lines and profiles of the villi, I(v), on one hand and those between the primary mucosal surface and the test lines, I(pm), on the other hand were made. The primary mucosal surface was taken to be the imaginary line (parallel to the long axis of the intestine) running between villi and crypts. This was used as the horizontal reference at LM level. Similarly, intersection counts between the microvilli profiles and the test lines, I(mv), and those between the apical enterocyte membrane, I(em), were counted. The apical membrane was taken to be an imaginary line running at the level of the bases of microvilli and the basement membrane in this case served as a convenient horizontal reference. The values I(v) and I(pm) were totalled for each segment and the totals

provided the intersection ratio, I(v)/I(pm). Total intersection counts on TEM fields of view, I(mv) and I(em), were obtained for each segment and the microvillous amplification factor, S(mv)/S(v), obtained directly from the intersection ratio (eg. Mayhew, 1984). This relies on consistency in definition of the villous and apical enterocyte surfaces when moving between LM and TEM levels respectively. Absolute surface area of microvilli per segment, S(mv) was taken to be the product:

$$W \times L \times S(v)/S(pm) \times S(mv)/S(v).$$

2.3.3. Calculation of the Stereological Parameters

A multi-level sampling scheme was employed (Cruz-Orive & Weibel, 1981). The surface area of the intestine was calculated at three levels.

Level I. Macroscopic level, primary mucosal surface area, S(pm)

This was the primary surface area obtained by multiplying the average internal circumference of each intestinal segment (W) by the length (L) of the same:

$$S(pm) = W \times L.$$

Level II. Light microscopic level, villous surface area, S(v)

This was the surface area of the intestine after considering the amplification of the primary mucosal surface by villi. The villous amplification factor,

S(v)/S(pm), was taken to be numerically equal to the intersection ratio, I(v)/I(pm). Therefore, the villous surface area was estimated as

$$S(v) = S(pm) \times I(v)/I(p).$$

Level III. Electron microscopic level

A. Microvillous surface area, S(mv)

The intersection ratio for the test lines hitting microvillous and apical membranes was taken to be the microvillous amplification factor, S(mv)/S(v), and subsequently gave an estimate of the absolute microvillous surface area per segment, S(mv).

Thus, $S(mv) = S(v) \times I(mv)/I(em)$.

B. Surface areas per intestine

The absolute surface areas per whole intestine were calculated simply by summing the values for all the five segments in a given animal.

For example, total microvillous surface was estimated as

$$S(mv)t = s(mv)1 + s(mv)2 + s(mv)3 + s(mv)4 + s(mv)5.$$

C. Microvillous diameter, d(mv)

This was estimated by measuring the diameter of at least ten favorably sectioned microvilli and computing the average across all micrographs representing a given segment. If cut obliquely, the short axis of a profile was measured. By favorably sectioned microvilli, it is

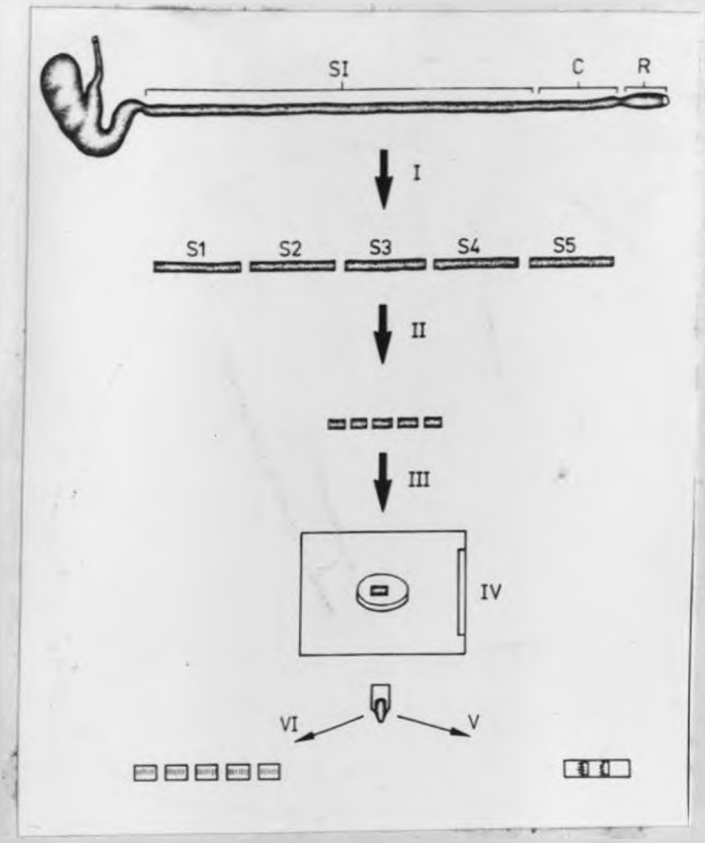

Fig. 1: A schematic drawing showing the intestine of the fruit bat and the steps employed in sampling. The intestine comprises a small bowel (SI) a colon (C) and a rectum (R). The small intestine is sampled in steps after separating it from the rest of the gut and opening it into a flat sheet. The intestine is divided into five equal segments and the average width of each determined (I). Each of the five segments is divided into five smaller approximately equal subsegments (II). The subsegments are processed for TEM up to and including the infiltration stage. One of the subsegments is picked at random (III), placed at the middle of a petri dish and given a spin (IV). The subsegment is then embedded and sectioned for TEM and LM. Two LM micrographs (V) and five TEM micrographs (VI) are prepared for intersection counting.

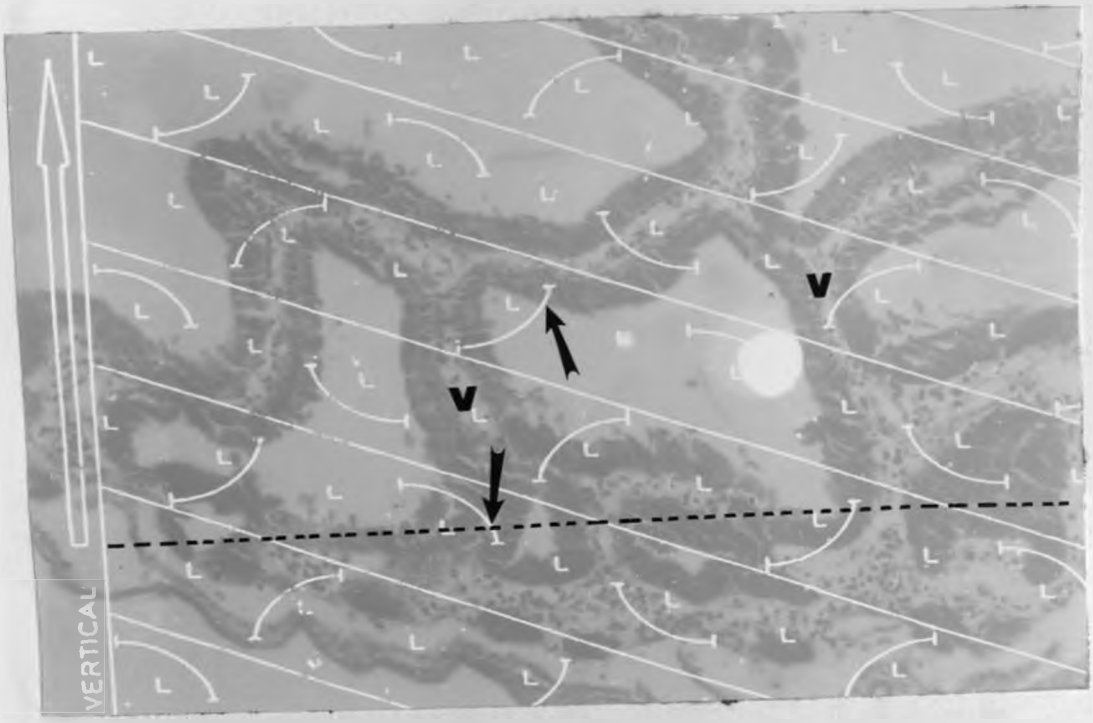

Fig. 2. A photomicrograph showing a section of the cranial part of the intestine of the epauleted fruit bat, *Epomophorus wahlbergi*, with a superimposed cycloid lattice grid to show the interaction between the test lines and the mucosal surface. Note the branching and joining of villi (V). The hatched line represents the imaginery reference line on which intersection counts are made.The white arrow indicates the vertical direction while the dark ones indicate examples of intersections counted.(Mag. X 125, H & E).

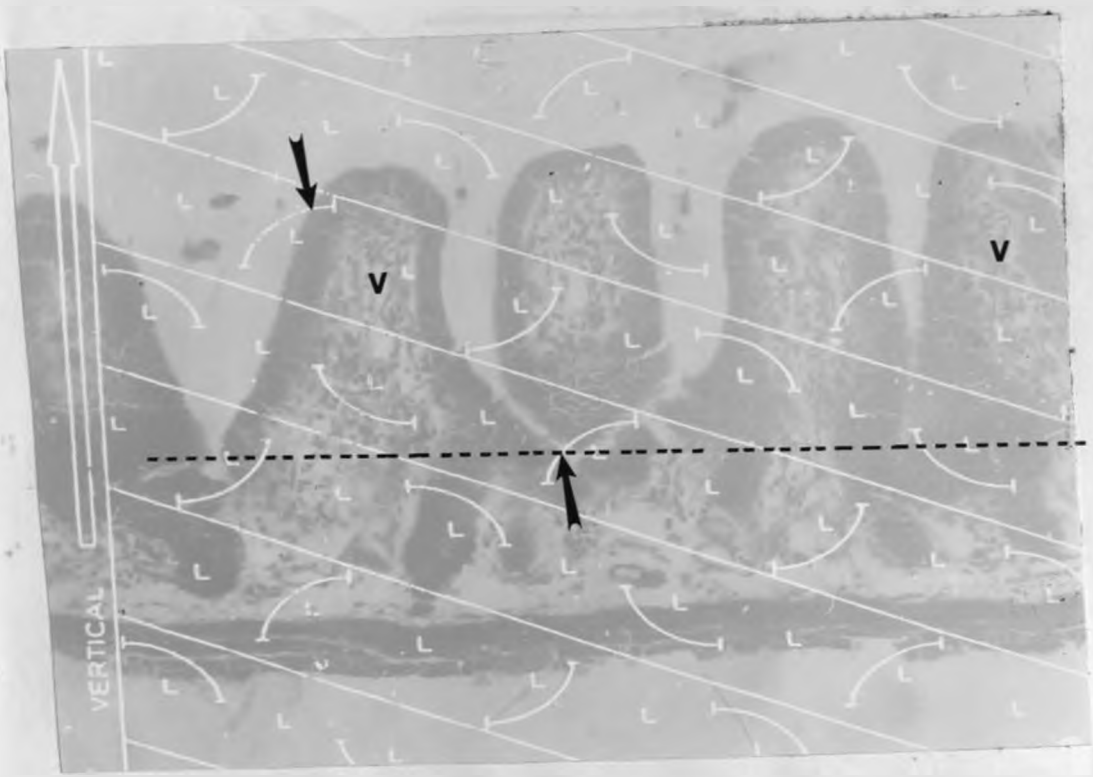

Fig. 3a. A transverse section of the intestine of the fruit bat, *Epomophorus wahlbergi*, showing the interaction between the cycloid arc test lines and the villous surface in the mid-intestinal level. At this level, there is no branching or anastomosis of the villi (V). The hatched line represents the imaginery reference line on which intersection counts are made. The white arrow indicates the vertical direction while the dark ones indicate examples of intersections counted. (Mag. X 125, H & E)

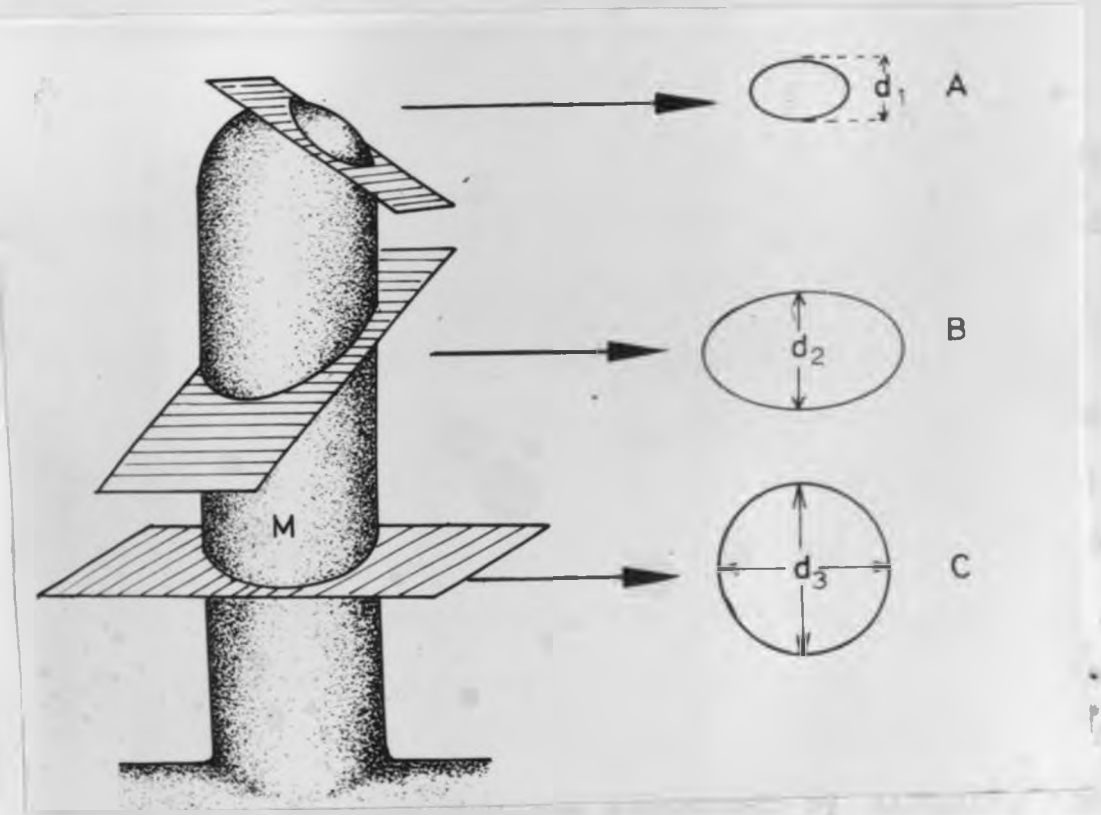

Fig. 3b. Possible profiles of the microvillus generated by the microtome Knife when the microvillus (M) is transected across the long axis. Small profiles nipped off from the tip of the microvillus (A) have an exceptionally small diameter (d_1) and are ignored in measurement of the microvillous diameter. A tangential section results with an ellipsoid profile (B) and in this case the short axis (d_2) is taken to be the diameter. Transverse sections result in circular profiles (C) with a uniform diameter (d_3).

meant those that reveal the entire diameter. Profiles of sections nipped from microvillous tips have an exceptionally small diameter, are rare and are ignored when encountered (fig. 3b).

D. Microvillous height, h(mv)

Estimated by measuring the profiles of at least 30 individual microvilli sectioned along their long axis and computing the average for a given segment.

E. Surface of the average microvillus, s(mv)

This was computed on a per segment basis and was estimated using the formula, $s(mv) = \pi \times d(mv) \times h(mv)$ where d(mv) and h(mv) were the average microvillous height and diameter per intestinal segment respectively.

F. Packing density of microvilli, N(mv)/S(v)

This is the number of microvilli per unit surface area of apical cell membrane and was estimated by dividing the microvillous amplification factor by the surface area of the average microvillus. It is obtained by the formula,

$$N(mv)/S(v) = \{S(mv)/S(v)\}/s(mv).$$

G. Total number of microvilli, N(mv)

Estimated by dividing the total surface of microvilli by the surface of the average microvillus in the same segment. Segmental values were summed in order to calculate values per intestine. The formulae for this variable is

$$N(mv) = S(mv)t/s(mv)$$

The total number of microvilli per intestine, N(mv)t is the summation of segmental values.

Thus; $N(mv)t = N(mv)1 + N(mv)2 + \ldots\ldots + N(mv)5$

H. Check-up Calculation of Microvillous Surface Area.

This was obtained by multiplying the surface area of the average microvillous, s(mv) by the total number of microvilli , N(mv) per segment. This was employed as a checkup calculation to see how much the directly estimated values differ from the stereological estimates.

I. Statistical Analysis.

The variation in microvillous dimensions along the small gut was evaluated by Page's L trend test for related samples (eg. Miller, 1975). This is a non parametric test assuming an ordinal level of measurement and is used to evaluate a predicted trend across k related samples.

Differencs between average morphometric values were estimated by the use of Student-t-test. This test evaluates differences between mean values when the population variances are unknown.

3.0.0. RESULTS.

3.1.0. Macroscopic Observations.

In the three species of bats examined, the abdominal viscera were tucked at the posterior end of the coelomic cavity, the thoracic viscera taking the greater anterior part of the cavity. The intestine was a coiled thin tube bound by a thin mesentery with scanty fat deposits. The stomach was situated at the proximal left hand corner of the abdominal cavity largely covered by the liver and the cranial segment of the intestine coursed towards the right. The disposition of the greater mass of the intestine was variable within and among the three species but the position of the rectum at the middle of the pelvic cavity was constant. The varied disposition of the intestinal loops for the longfingered bat is shown in figures 4 and 5. In all the species the part of the large intestine discernible externally was a rectum, distinguished only by its obviously greater diameter.

3.1.1. The Frugivorous Bat.

The stomach in the fruit bat was roughly U-shaped and elongate with a large cardiac caecum, a large fundus and a long pylorus (figs. 6 & 7). The pyloro-intestinal junction was marked by a prominent constriction externally (fig. 8). Grossly, the gastric mucosa was thrown into longitudinal rugae which extended down to the pylorus and ceased abruptly at the very prominent transverse fold that marked the pyrolic sphincter (fig. 8).

The intestine in the fruit bat was an almost uniform tube with the proximal part showing a slightly greater diameter and the small intestine inconspicuously gave way to a short rectum, their difference grossly being an increase in diameter in the latter, which was gradual and non uniform. An appendix, a caecum, or a colon were not observable externally. The cranial part of the intestine was characterized by large, grossly visible papillae like villi (fig. 8) which diminished in size caudally and towards the posterior fifth of the intestine gave way to longitudinal folds of the colon (fig. 9). The latter extended distally to the rectum becoming less conspicuous towards the anus (figs. 9 & 10). Grossly, the intestine of the fruit bat was divisible into a foregut and a hindgut, the latter comprising a colon and a rectum only (fig. 6).

3.1.2. The Insectivorous Bats.

The gastrointestinal tracts of the two insect-feeder bats were grossly similar and indistinguishable, save for their differing lengths. The stomach was of the simple type with a short pylorus whose aperture was very close to the cardiac opening of the oesophagus (figs. 11 & 12). The intestine was a coiled thin tube with an almost uniform diameter, except for the slightly wider proximal part and the rectum which had an obviously greater and non uniform diameter. No mucosal structures were grossly discernible and even the pyloro-intestinal junction was not very conspicuous.

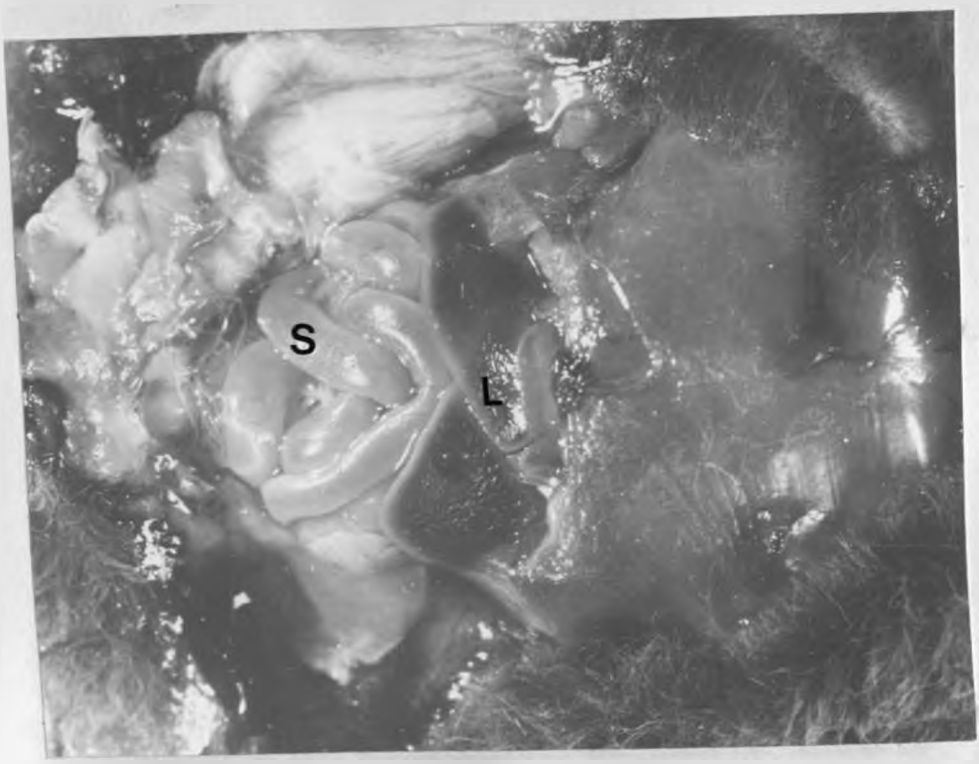

Fig. 4. Photograph of the abdominal cavity of *Miniopterus inflatus* showing the disposition of viscera *in situ*. The intestine (S) is a small mass of loops tightly packed posterior to the liver (L). (Mag. x 4)

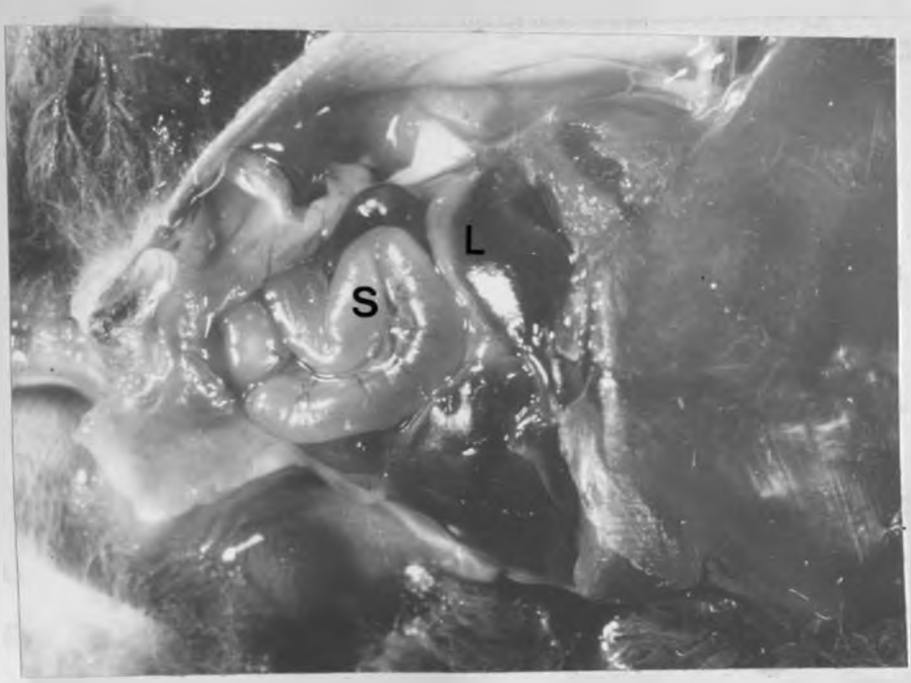

Fig. 5.*In situ* disposition of the abdominal viscera in the longfingered bat, *Miniopterus inflatus*, is variable. Note that the disposition of the viscera in this micrograph differs remarkably from that shown in Fig.4 above as shown by the intestinal loops (S) but the position of the liver (L) is unchanged. (Mag. x.4).

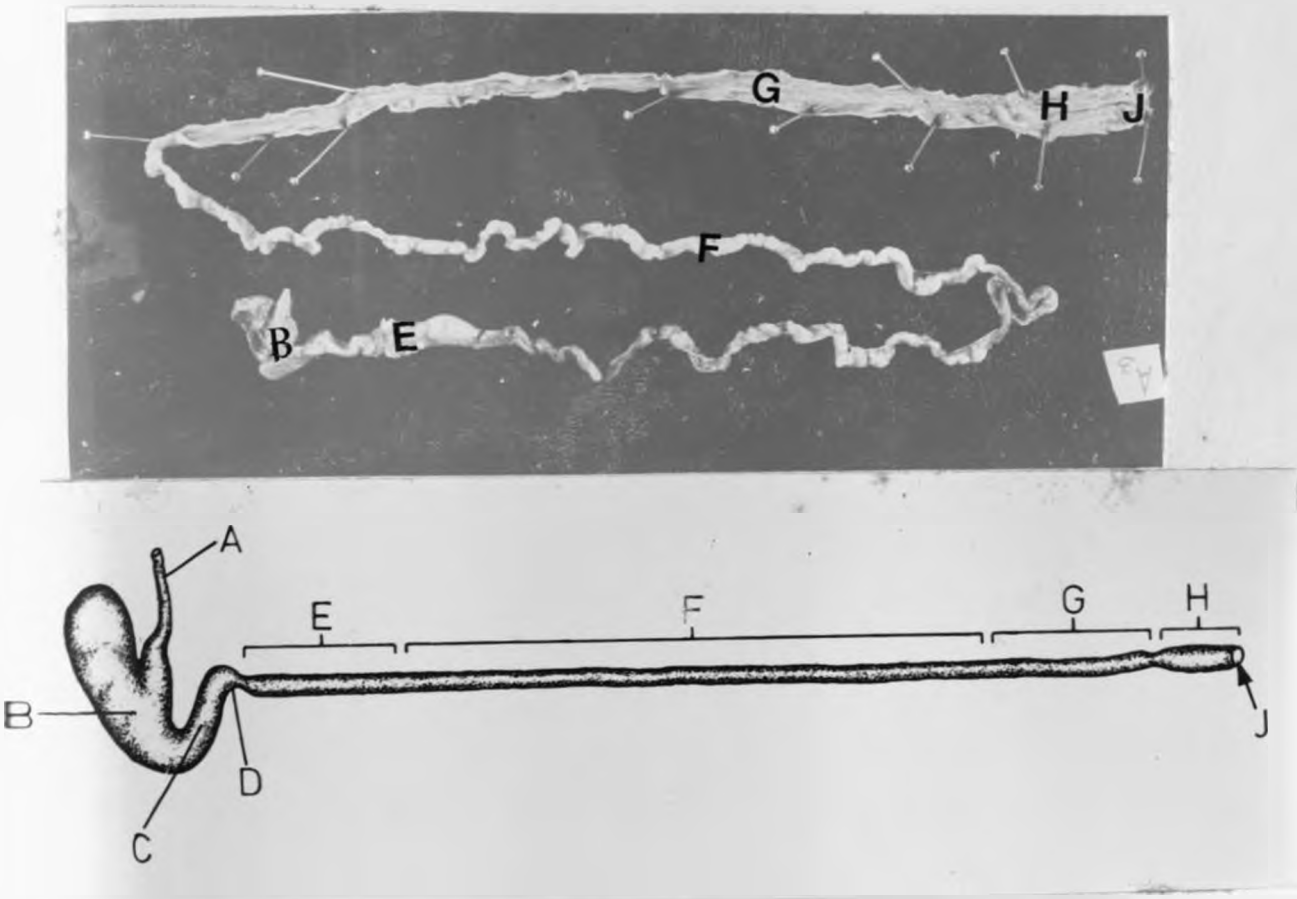

Fig. 6. A photomicrograph (I) and a schematic drawing of the gastrointestinal tract of the epauleted fruit bat *Epomophorus wahlbergi*, showing the various parts of the gastrointestinal tract. A- Oesophagus; B- Stomach; C- Pylorus; D- Pyloro-intestinal junction. The parts E, F, and G represent regions with special mucosal architecture. E- represents the region with long branching and anastomosing villi; F- the region with discrete finger-like villi and G the region with longitudinal folds. H and J represent the rectum and anus respectively. (Mag. I = X 0.5; II = x 0.25).

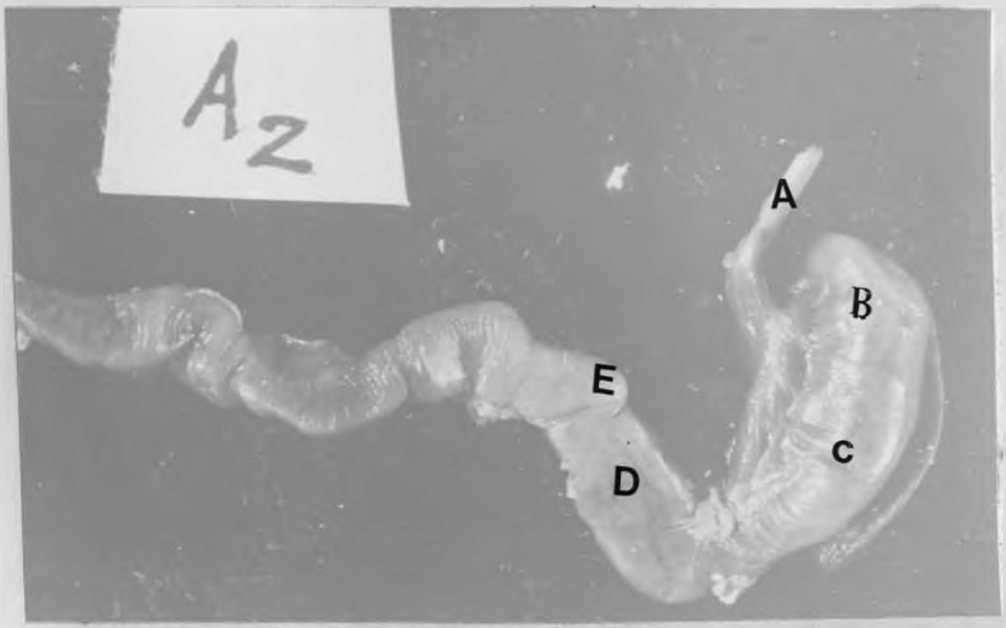

Fig. 7. Photograph showing the stomach and the cranial part of the intestine of the epauleted fruit bat (*Epomophorus wahlbergi*). A-oesophagus, B- cardiac caecum, C- Fundus, D- Pylorus, E-Pyloro-intestinal junction. The mesenteries have been separated out. (Mag. x 2).

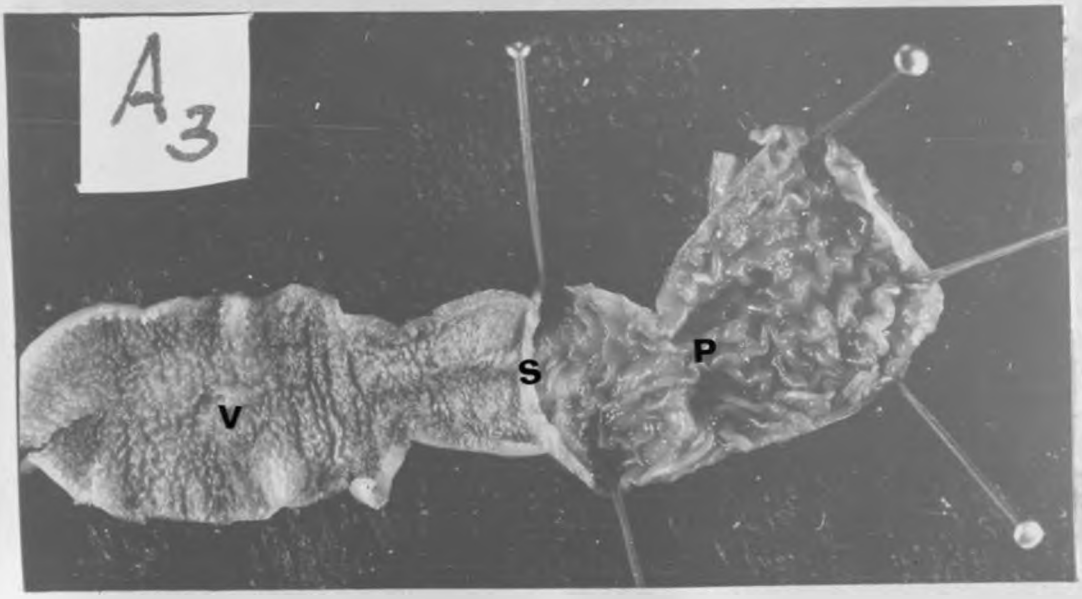

Fig. 8. A photograph of the pylorus and the cranial part of the intestine of the epauleted fruit bat (*Epomophorus wahlbergi*). The intestine and pylorus have been opened along the mesenteric border to show the pyloric rugae (P), the pyloric sphincter (S) and the papillae-like villi (V). (Mag. x 2).

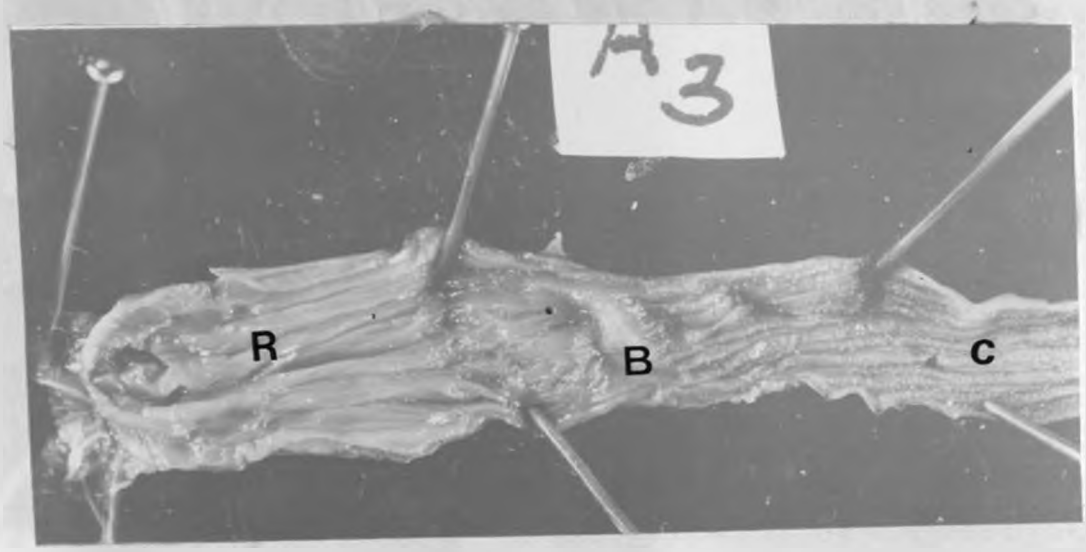

Fig. 9. Photograph of the posterior part of the intestine of the fruit bat, *Epomophorus wahlbergi* showing the colon (C) and the rectum (R). Note the boundary between the rectum and the colon (B) is marked by an increase in circumference of the former.(Mag. x 2)

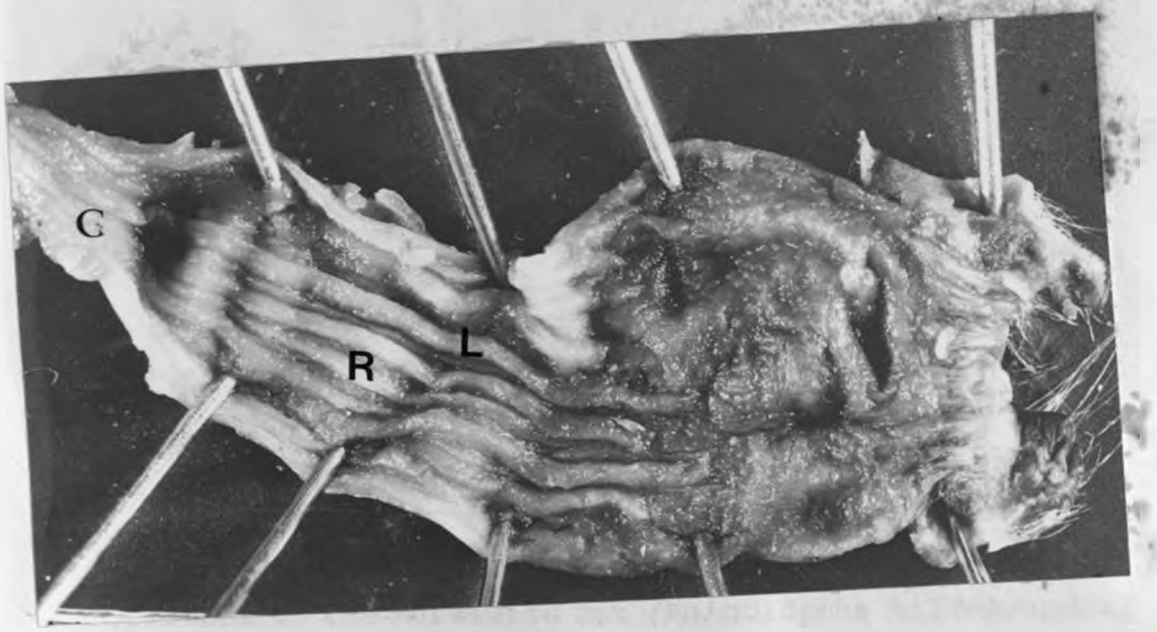

Fig. 10. A higher magnification of the large intestine of the fruit bat, *Epomophorus wahlbergi*, showing the very conspicuous longitudinal folds (L) found in the colon and rectum. The change in diameter from the colon (C) to the rectum (R) here is dramatic.(Mag. x 10).

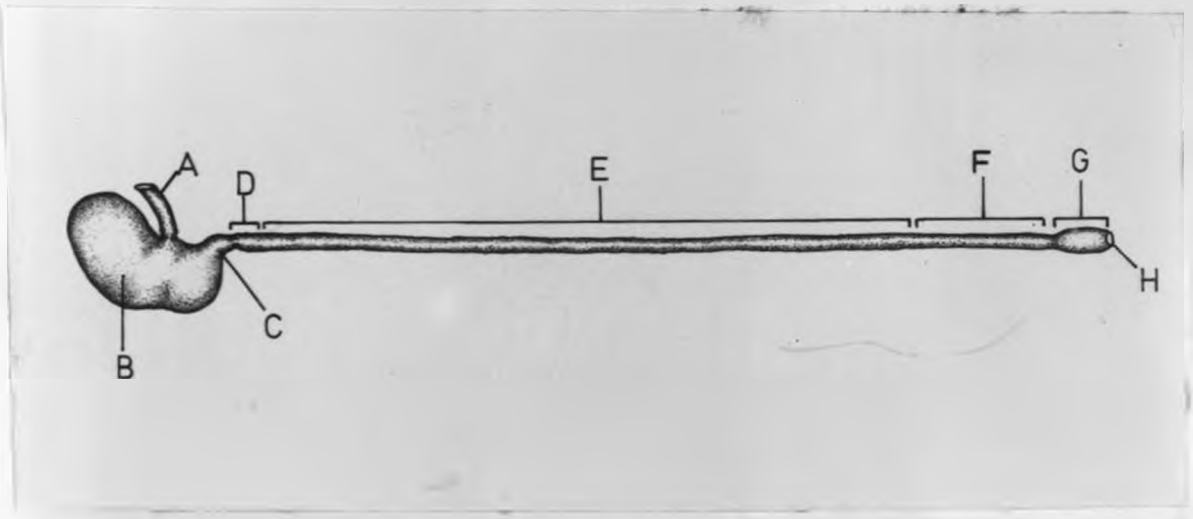

Fig. 11. A schematic diagram showing the stomach and the intestine of the horseshoe bat *(Rhinolophus hildebrandti)*. The oesophagus (A), the pylorus (C) and the stomach (B) are also shown. The various parts of the intestine are represented whereby (D) represents the honey comb segment, (E) the greater part with transverse ridge-like villi and (F) the posterior segment exhibiting hexagonal compartments. The letters G and H represent the rectum and the anus respectively. (Mag. x 0.5).

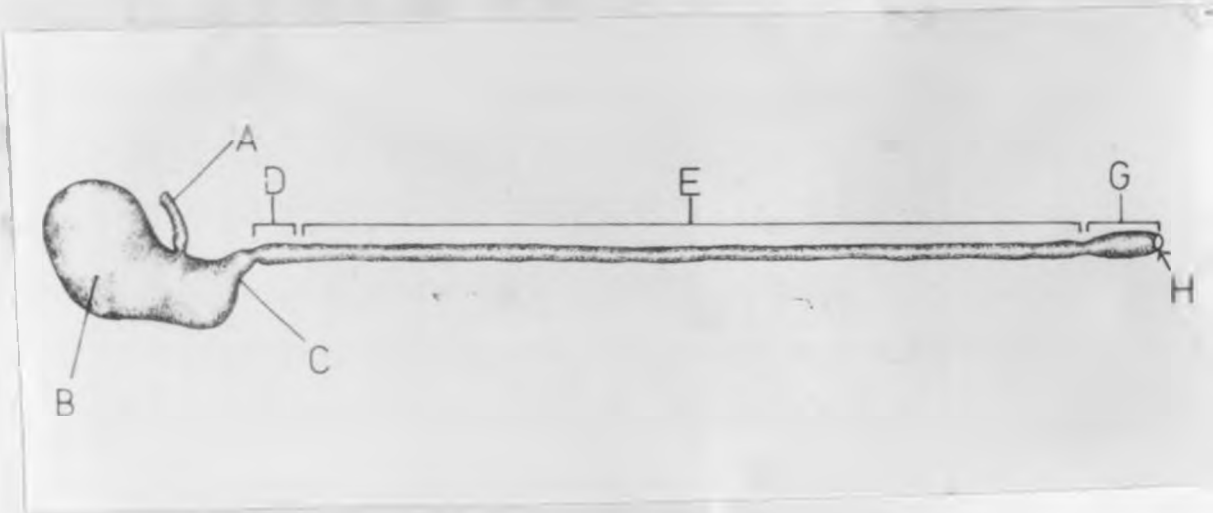

Fig. 12. A schematic drawing of the intestine of the insect-feeder bat, *Miniopterus inflatus*. The parts of the alimentary tract shown are A- Oesophagus, B- Stomach, C- Pylorus, D- the Cranial honey comb segment and E- the greater part of the intestine characterized by transverse ridge-like villi. The rectum (G) and the anus (H) are also shown.(Mag. x 0.5).

3.2.0. Light Microscopy.

The intestinal structure of the three species of bats was fundamentally similar to that of the terrestrial mammals with the wall comprising the four main layers, namely, the serosa, tunica muscularis, submucosa and the mucosa. Brunner's glands were notably absent from the submucosa of the entire intestine in all the three species and lymphoid tissues was restricted to the posterior half of the intestine. Structures characteristic of specific parts of the large intestine in mammals such as taenia coli, taenia caeci, appendices epiploicae or haustrae were notably and universally absent.

In the frugivorous bat, the villi in the most cranial part of the intestine (approximately cranial fifth) were long branching and adjoined one to the next (fig. 13). The lacteals of the villi were continuous through the adjoining branches. The villi were covered with an elaborate columnar epithelium which was occasionally interrupted by scattered goblet cells. The cells of the columnar epithelium were well developed and had a very prominent brush border. Caudally, the villi lost the branching and adjoining characteristic and assumed a more finger-like structure (fig. 3). The height of the villi on the other hand decreased cranio-caudally diminishing completely at the level of the colon. The intestinal glands were prominent and characterized the entire intestinal tract but were most prominent in the colon and rectum (fig. 14). The number of goblet cells in the mucosa

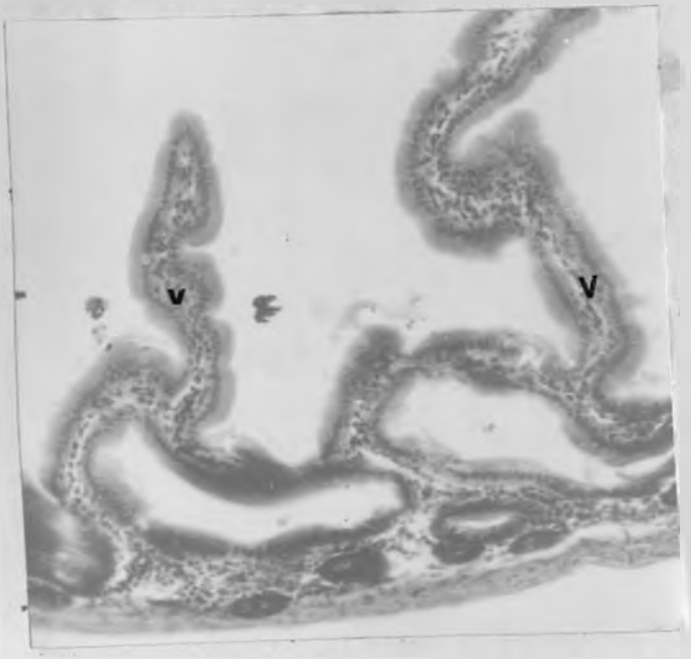

Fig. 13. A photomicrograph showing a longitudinal section from the cranial one fifth of the intestine of wahlberg's fruit bat (*Epomophorus wahlbergi*) showing the anastomosing and branching villi (V). (Mag. x 125, H & E)

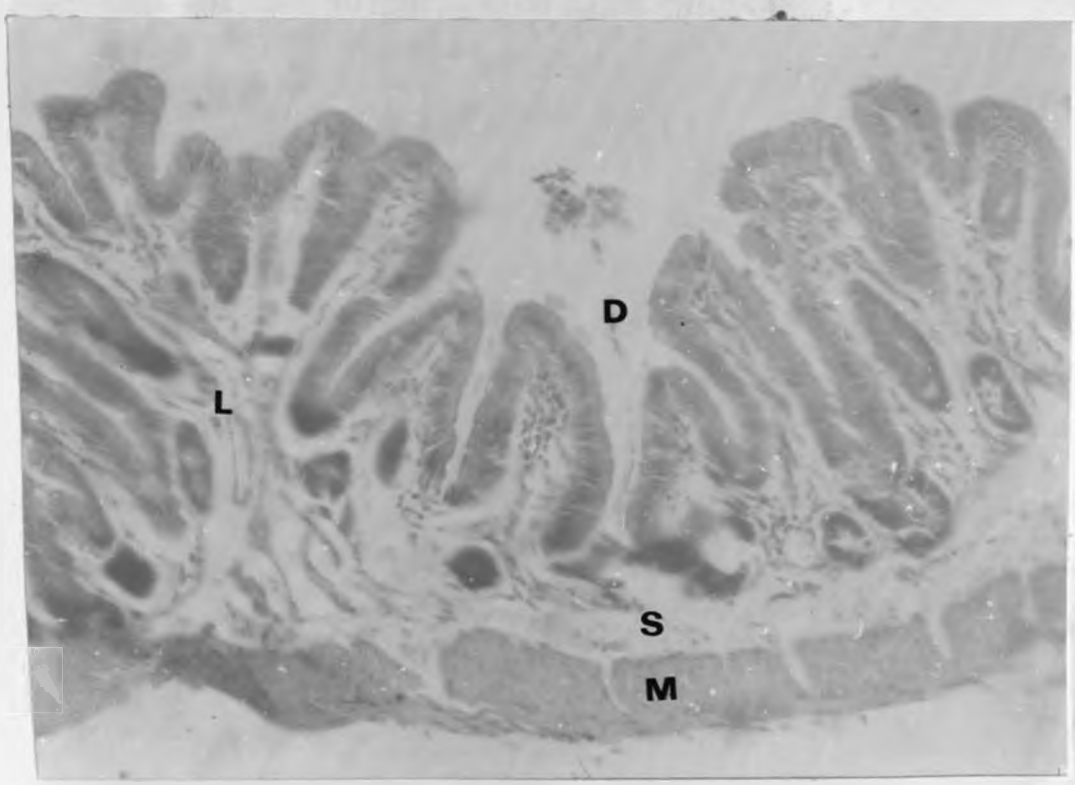

Fig. 14. A transverse section across the colon of the fruit bat (*Epomophorus wahlbergi)* showing longitudinal folds (L) and the intervening depressions (D). The submucosa (S) and the muscularis mucosa (M) are also shown. (Mag. X 420; H & E).

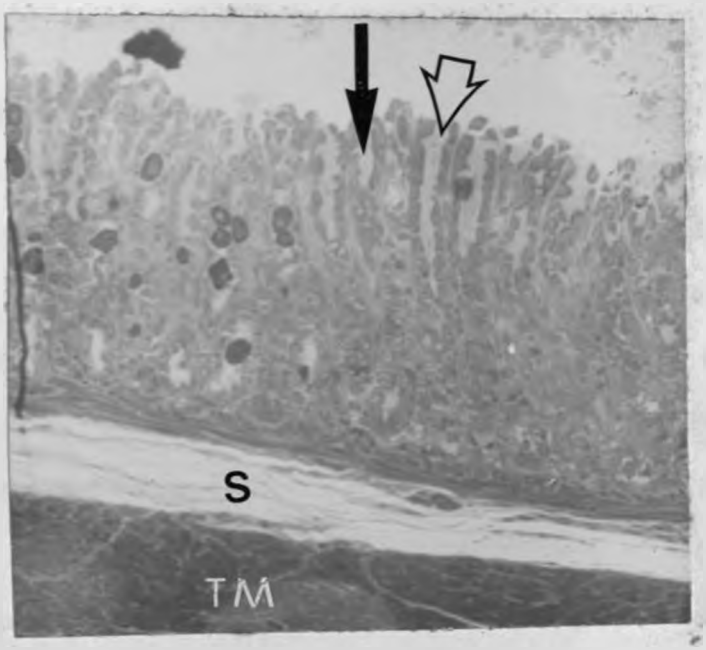

Fig. 15. A longitudinal section showing the pitted segment in cranial part of the intestine of the longfingered bat (*Miniopterus inflatus*). These pits (arrows) closely resemble those described in the mammalian gastric mucosa but are devoid of mucus secreting cells. The submucosa (S) and tunica muscularis (TM) are also shown. (Mag. X 100, H & E)

increased cranio-caudally, reaching a maximum in the rectum.

The histology of the intestine of the two microchiropteran bats was almost similar. In both cases there was a short proximal segment characterized by pits similar to those of the gastric mucosa (fig. 15). Villi were short and demonstrated a well developed columnar epithelium. The villi were tightly packed in the anterior parts of the intestine (fig. 16) and in between them were numerous, short intestinal glands. Posteriorly, the villi were shorter and less tightly packed (fig. 17). The villous core had well developed lacteals, blood capillaries and the cells normally encountered in this region such as fibroblasts and smooth muscle cells were abundant (fig. 18). The villi had a prominent columnar epithelium with a conspicuous brush border (fig. 18). Goblet cells were few in the cranial part of the foregut but increased gradually, reaching a peak in the rectum. The villi decreased in height in a cranio-caudal direction ceasing abruptly at the junction between the foregut and the rectum (fig. 19), the latter being the only apparent part of the hindgut present. The rectum gave way to the anus at the mucocutaneous junction. Intestinal lymphoid tissue in both cases was only observed in the posterior half of the intestine.

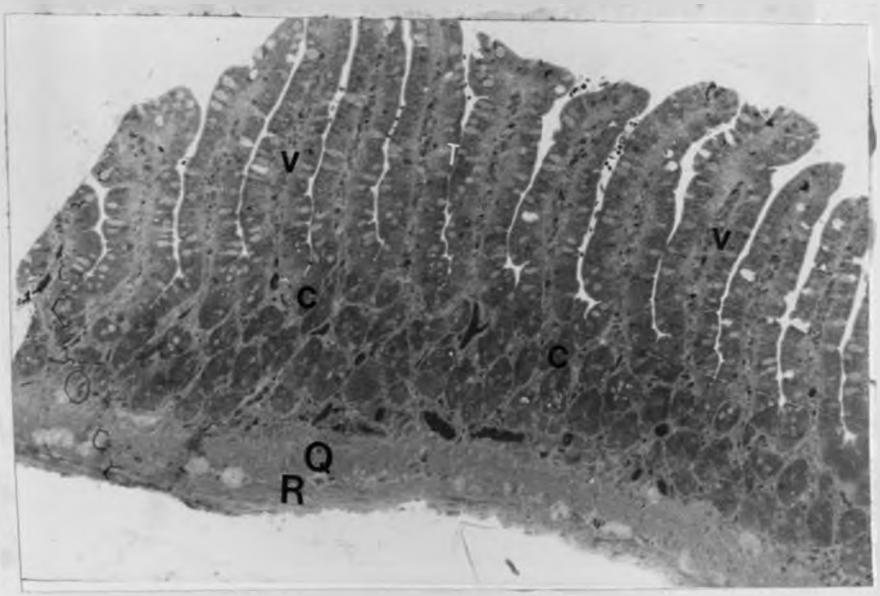

Fig. 16. Longitudinal section of the intestine of the horseshoe bat *(Rhinolophus hildebrandti)* showing tightly packed villi (V) crypts of Lieberkuhn (C), inner circular muscle layer (Q) and outer longitudinal muscle layer (R). The arrangement of the villi under the light microscope is the same for both insectivorous bats. (Mag. X 420, TB).

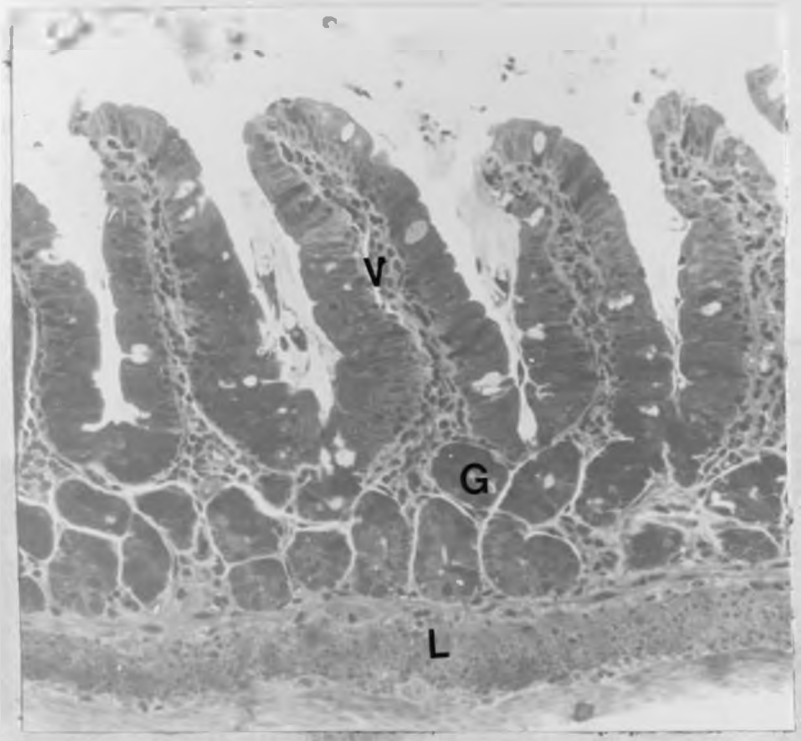

Fig. 17. A light micrograph of a longitudinal section of the posterior part of the intestine of the longfingered bat (*Miniopterus inflatus*) showing the villi (V), intestinal glands (G) and the muscle layer (L). At this level, the villi are stumpy and less tightly packed. (Mag. X 200, TB).

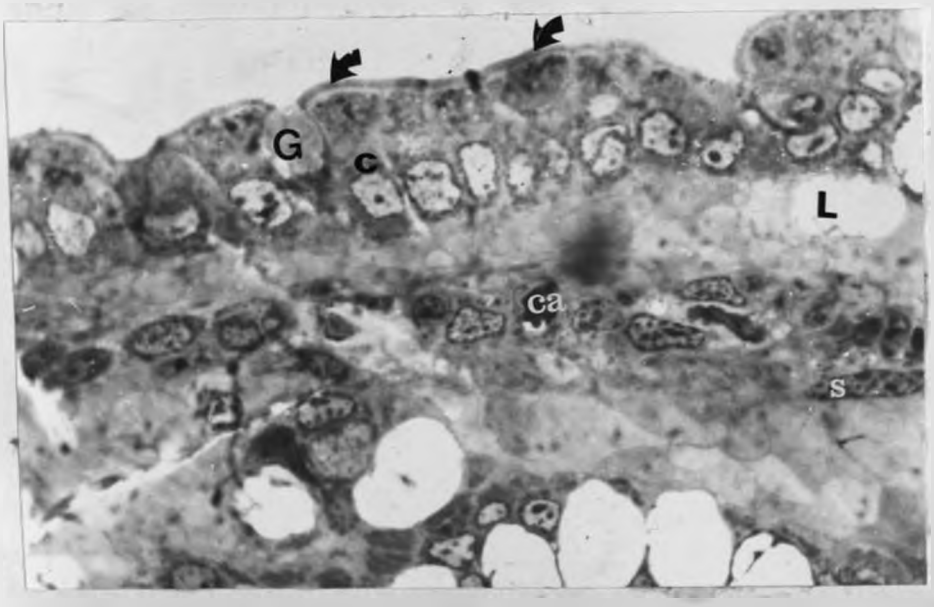

Fig. 18. Mid segment of the villus taken from the middle of the intestine of *Rhinolophus hildebrandti* showing a prominent brush border (arrows) and goblet cells (G) entrenched between columnar cells (C). The lacteal (L), blood capillaries, (Ca) and smooth muscle cells (S) feature quite prominently in the villous core.(Mag. X 1000, TB).

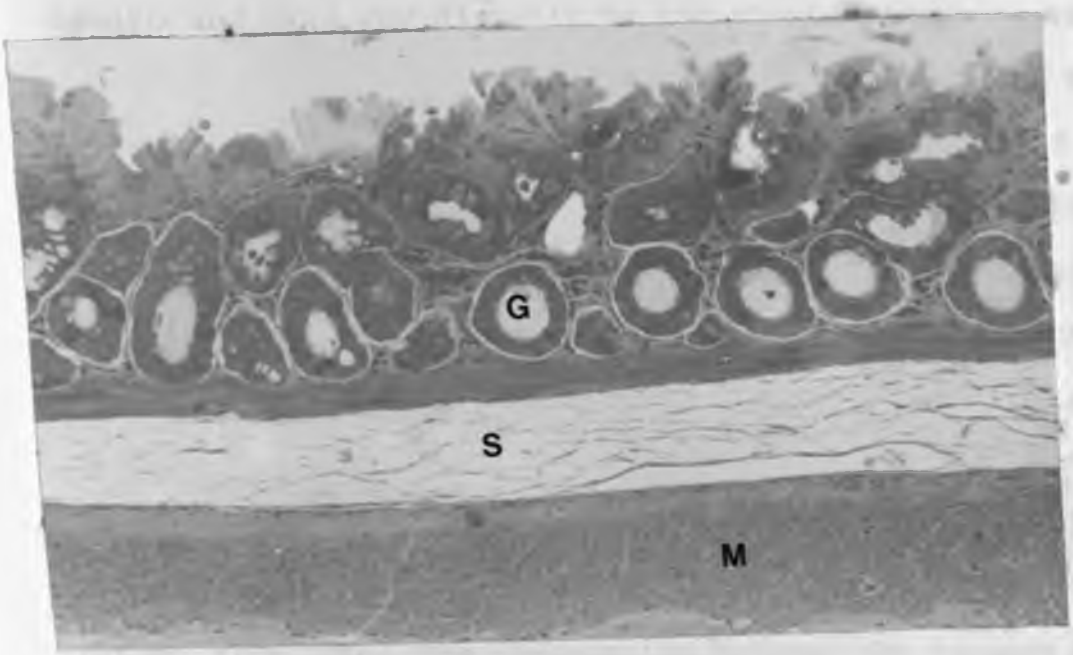

Fig. 19. Transverse section of the rectum of the longfingered bat (*Miniopterus inflatus*) showing very prominent intestinal glands (G). The submucosa (S) and tunica muscularis (M) are also very well developed. (Mag. X 200, TB).

3.3.0. Mucosal Topography.

3.3.1. The Frugivorous Bat.

The mucosa of the cranial 20% of the intestine of the fruit bat was characterized by long branching and interconnecting villi (fig. 13). The height of the villi and degree of branching and joining decreased gradually in a cranio-caudal direction giving way to tall and further caudally to short, finger-like villi (fig. 20). The latter segment occupied about 80% of the entire intestinal length and gave way distally to the short colon with wavy longitudinal folds. The transition between the colon and the small intestine was gradual and was characterized by two categories of villi; those occurring in the regions corresponding with longitudinal folds were bigger, broader and further distally tended to merge into each other forming wavy longitudinal folds seen in the colon (figs. 21 & 22). The second category of villi comprised shorter finger-like and tongue like discrete structures that typified the depressions between the longitudinal folds (figs. 21 & 23). The demarcation between the foregut and the hindgut was the point where continuous longitudinal folds began and at this point two categories of villi were evident (already described). The longitudinal folds were continuous from the colon to the rectum and in the latter, they were larger and straight (fig. 24). The mucosa of the rectum was smooth with numerous openings to goblet cells (fig. 24) and was occasionally interrupted by pits (fig. 25). These pits (rectal pits) were

Fig. 20. A scanning electron micrograph showing finger-like villi (V) in the depressions between longitudinal folds in the transitional zone when moving from the small to the large intestine in the epauleted fruit bat *(Epomophorus wahlbergi)*. (Mag. X 310).

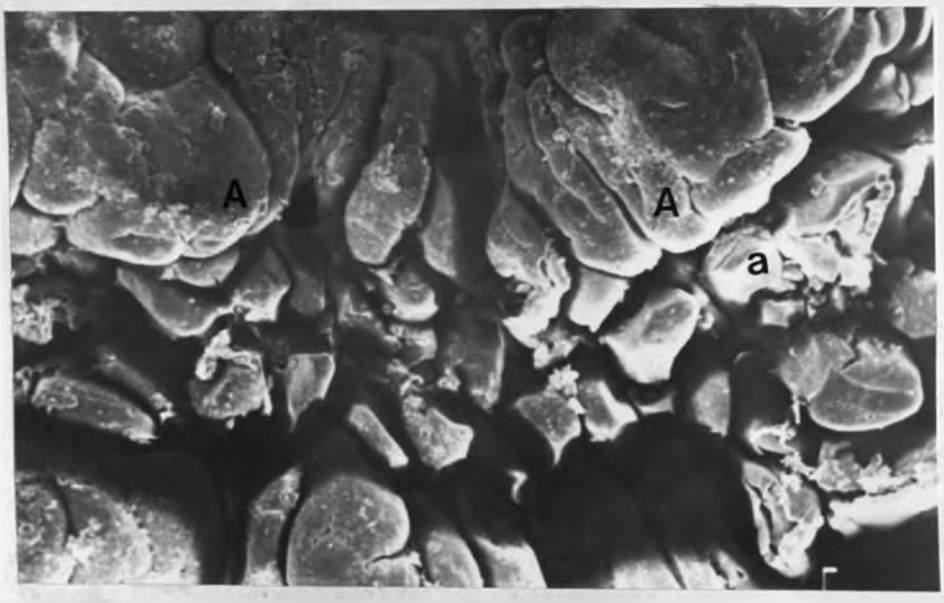

Fig. 21. Two types of villi characterizing the intestine of the fruit bat *(Epomophorus wahlbergi)*. Note that the villi on the longitudinal folds (A) are bigger and tend to merge one into the next while those in the depressions (a) are smaller and discrete. This forms the transition zone between the small intestine and the large intestine. (Mag. X 75).

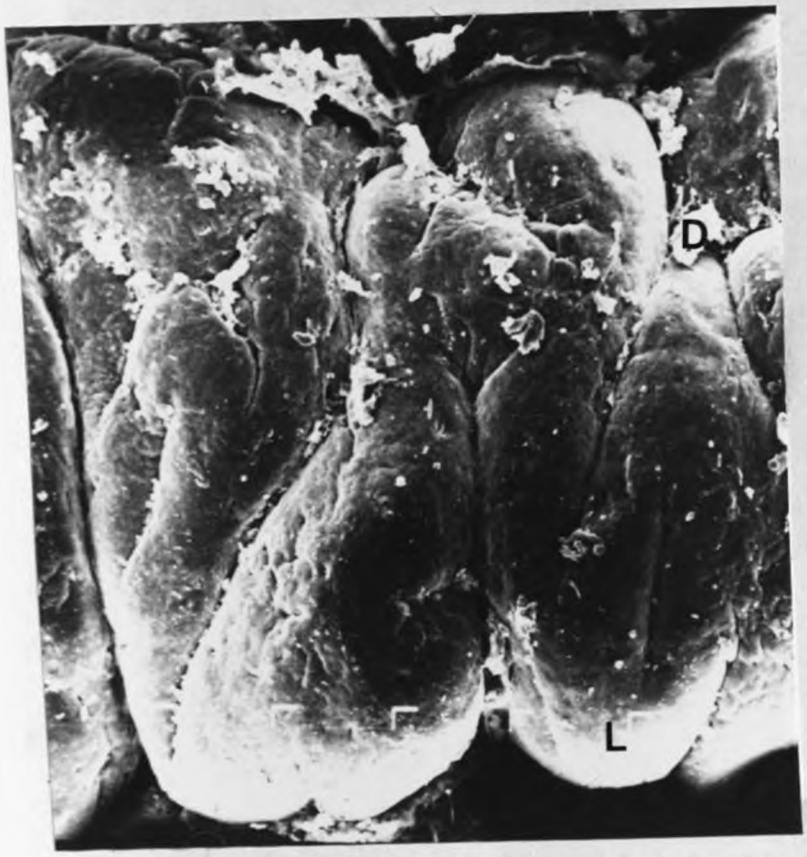

Fig. 22. The colonic longitudinal folds (L) of the fruit bat (*Epomophorus wahlbergi*) are broad and tend to have a wavy appearance. At this level there are numerous goblet cells and mucus debris (D) is abundant. (Mag. X 130).

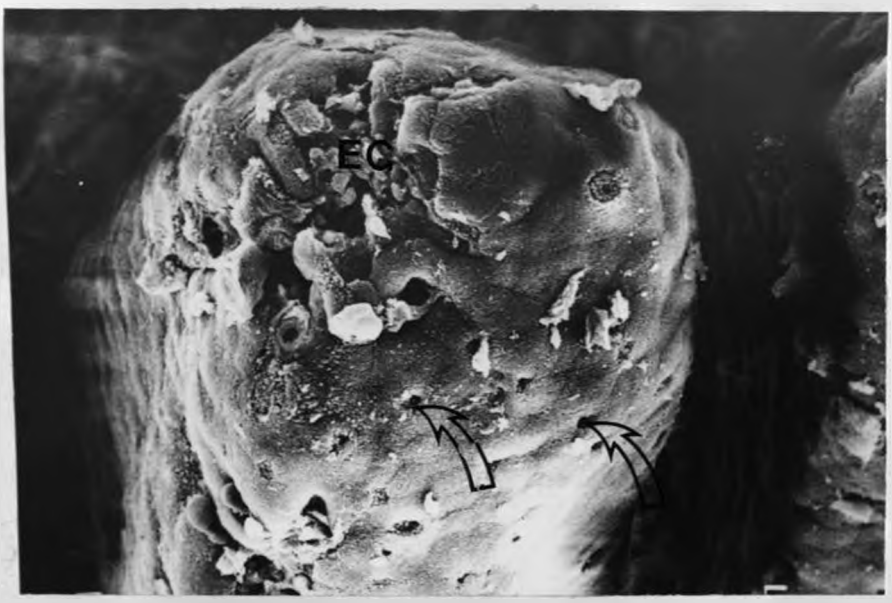

Fig. 23. Individual villus in the longitudinal depression showing exfoliating cells (Ec) and openings to goblet cells (arrows), in the mid-intestine of the fruit bat *(Epomophorus wahlbergi)*. (Mag. X 920).

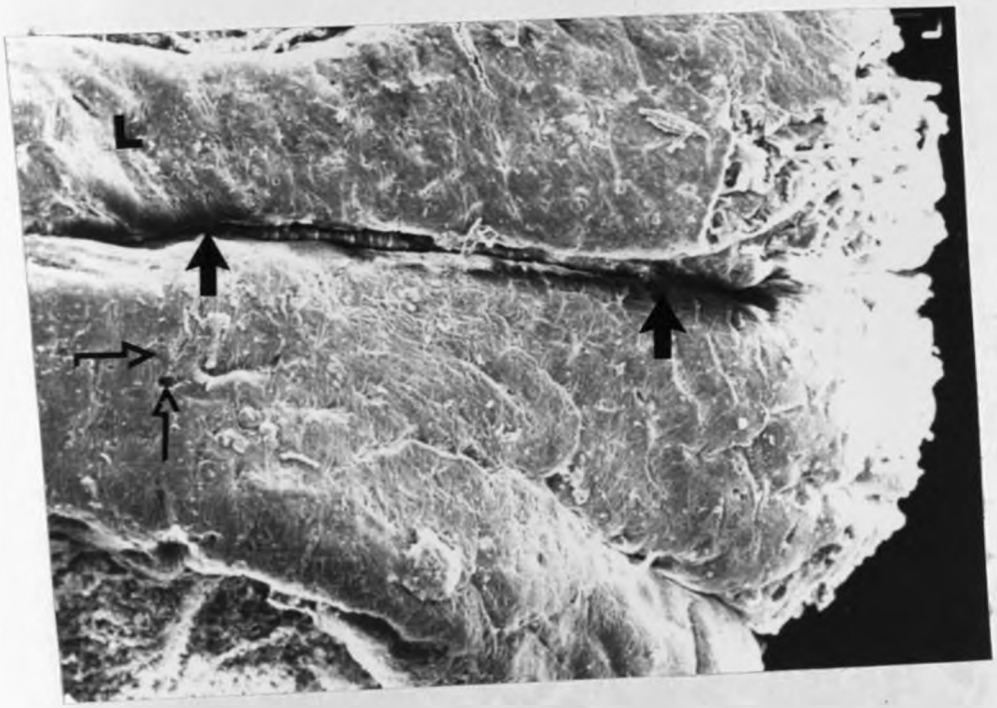

Fig. 24. The rectum in the fruit bat *(Epomophorus wahlbergi)* showing the longitudinal folds (L) which tend to merge towards the anus. The intervening depressions (big arrows) and rectal pits (small arrows) are also shown. (Mag. X 47).

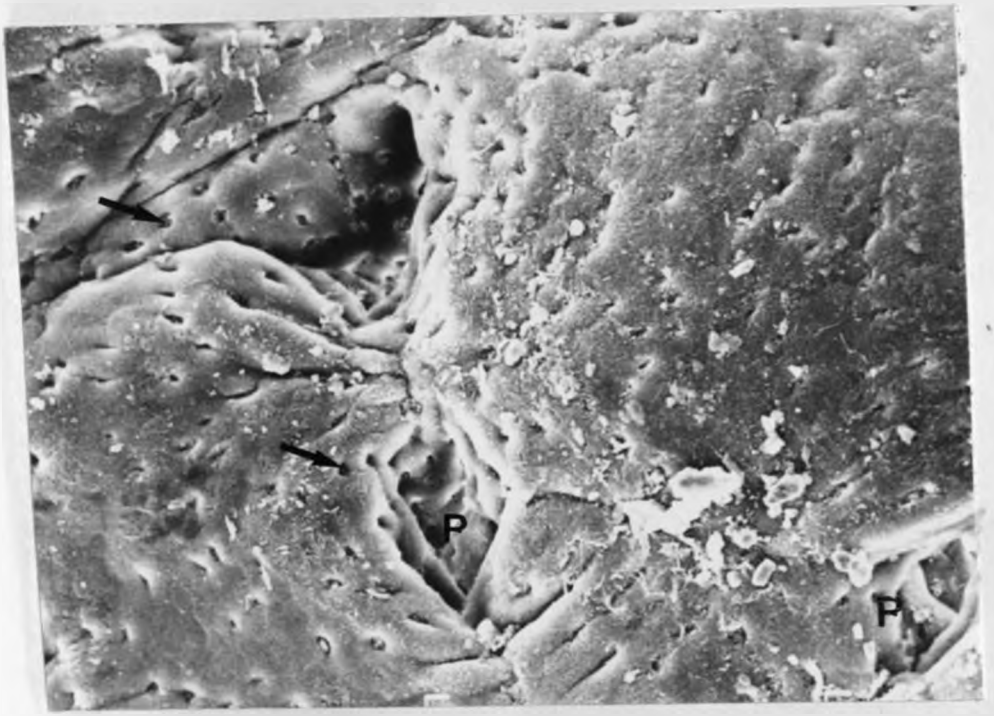

Fig. 25. A higher magnification of the rectal mucosa of the fruit bat (*Epomophorus wahlbergi*) shows numerous openings to goblet cells (arrows) and larger pits (P), which have numerous openings to Goblet cells. (Mag. X 470).

Fig. 26. A close up of an individual "rectal pit" shown in fig. 25 above showing an enormous number of openings to goblet cells (arrows). (Mag. X 2100).

characterized by numerous openings to goblet cells (figs. 25 & 26). The surrounding cells were covered with numerous microvilli. There were occasional aggregations of crypts of Lieberkuhn in the rectum (figs. 27 & 28) that were separated only by thin ridges of mucosa (fig. 28). The rectum gave way to the anus at the mucocutaneous recto-anal junction. The absorptive enterocytes had polygonal dome-shaped profiles with a rich microvillous cover (figs. 29 & 30). Generally, the intestine of the fruit bat was divisible into a small intestine and a large intestine comprising a colon and a rectum only.

3.3.2. The Insectivorous bats.

The mucosa of the intestine of the two entomophagous bats was characterized by transverse ridge-like villi, a small deviation from this pattern being observed in a very short segment bordering the pylorus in both bats. This segment comprised numerous hexagonal and cylindrical pits (figs. 31, 32 & 33). This segment was about 10 mm long in the longfingered bat and was also characterized by low longitudinal ridges (fig. 31). The "pitted" segment gave way caudally to a region of transverse villi (figs. 34, 35 & 36). This design was maintained along the mucosa of the small intestine of the longfingered bat (fig. 36), the only change being a gradual decrease in height of the villi. In the horseshoe bat, however, the pattern was changed just cranial to the rectum where the villi joined in a network pattern to form shallow hexagonal chambers (figs. 37 & 38).

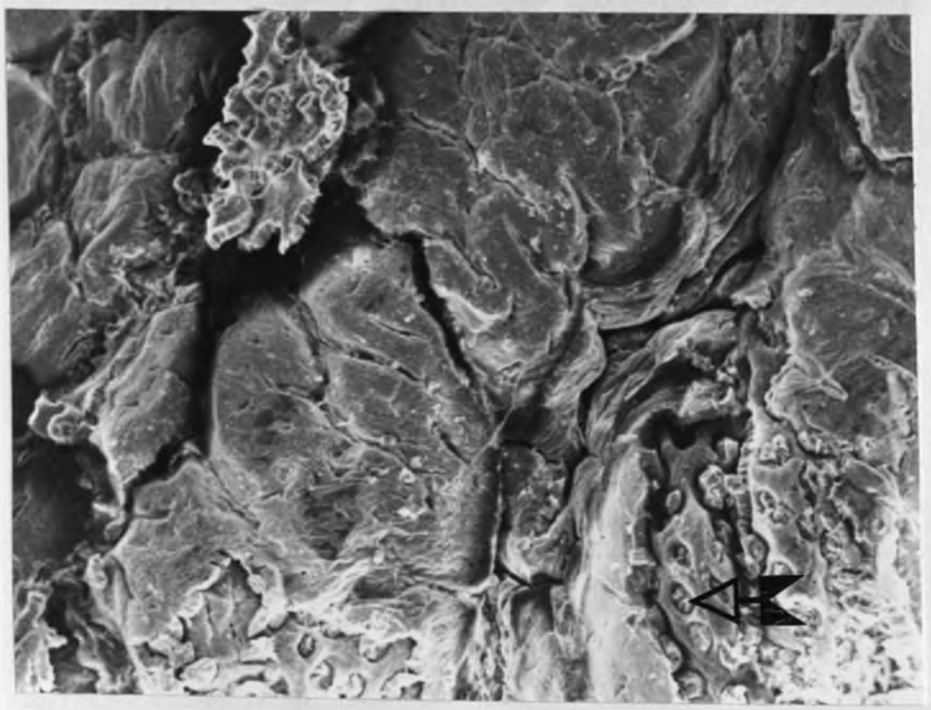

Fig. 27. A scanning electron micrograph showing aggregations of intestinal glands in the rectum of the fruit bat, *Epomophorus wahlbergi*, (arrows). (Mag. X 45).

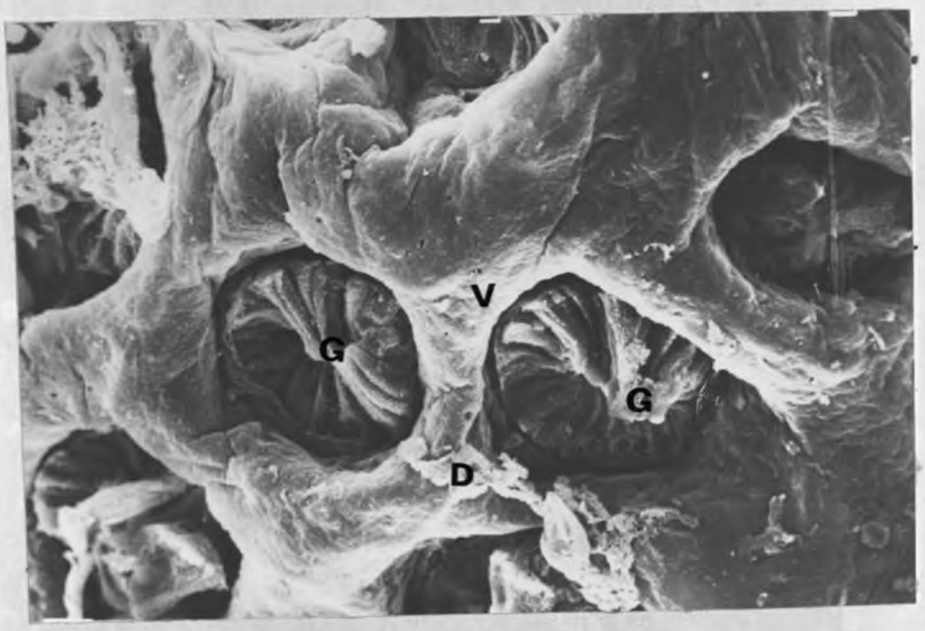

Fig. 28. A Close up of a group of intestinal glands (shown in fig. 27) in the fruit bat (*Epomophorus wahlbergi*) rectum shows the epithelial cell septae (V) that separate adjacent glands (G). Mucus debris (D) is sometimes seen covering these septae. (Mag. X 450). (Photograph was taken at 2 cm from anus).

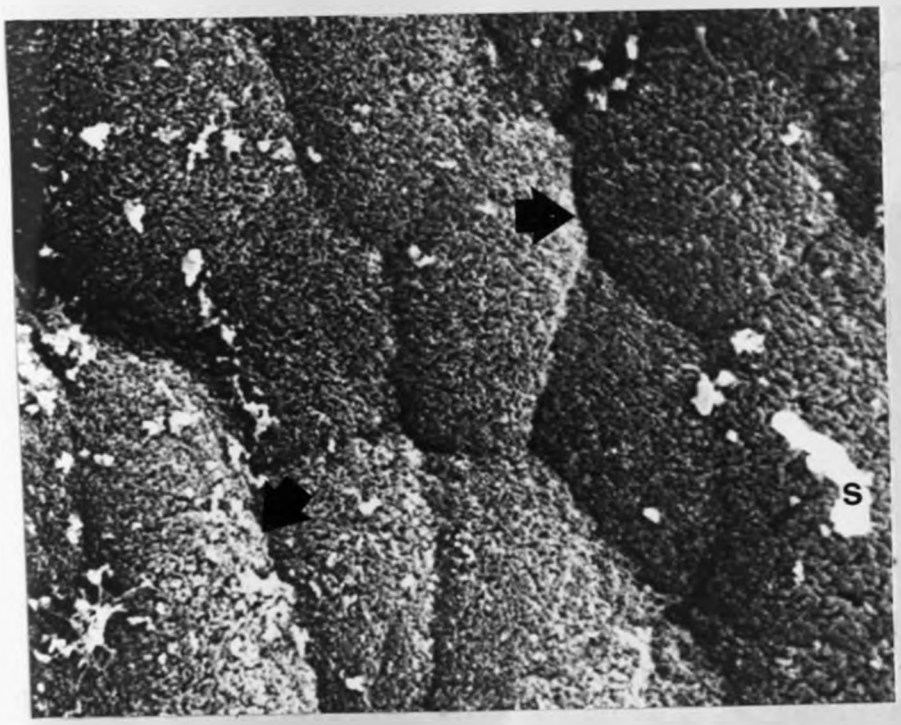

Fig. 29. Individual epithelial cells in the fruit bat (*Epomophorus wahlbergi*) intestine form polygonal profiles separated by shallow depressions (arrow heads). Mucus strands (S) are commonly found on the luminal surfaces of the cells. This photograph was taken at a level 4 cm from the pyloro-intestinal junction. (Mag. x 4600).

Fig. 30. A higher magnification of the apical aspects of the epithelial cells showing the "forest"of microvilli forming the luminal dome shaped surface (D) of the epithelial cell in the intestine of the fruit bat (*Epomophorus wahlbergi*). (Mag. X 9500).

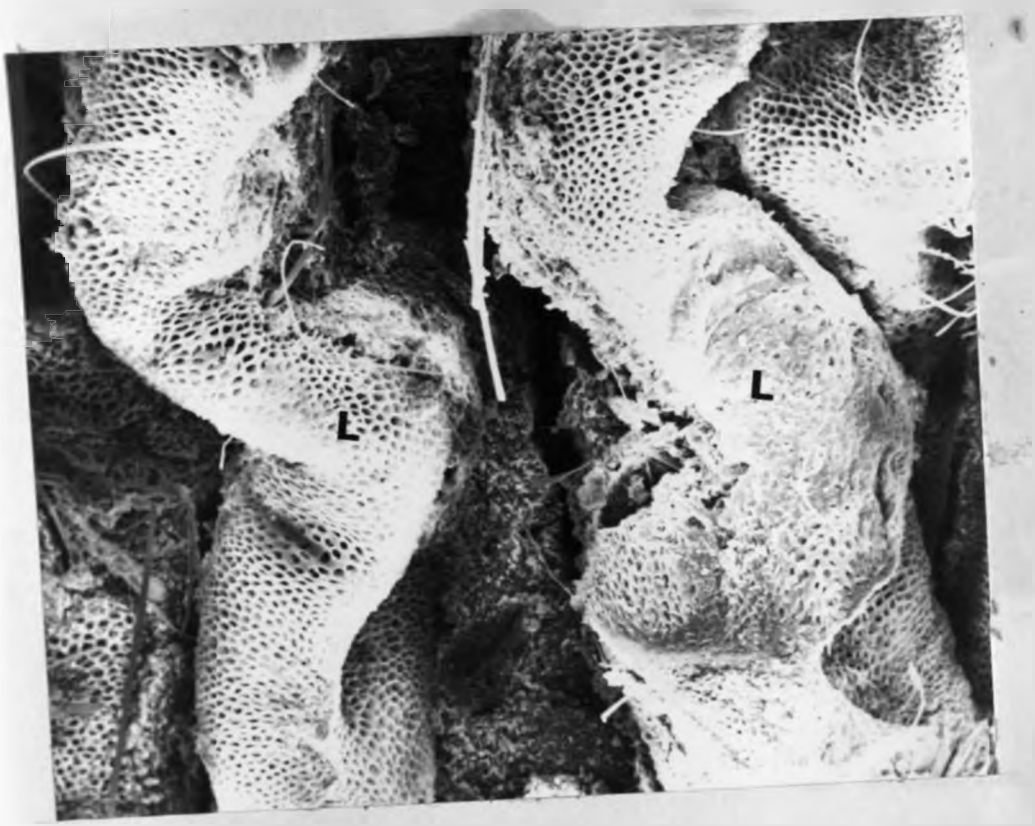

Fig. 31 The cranial pitted segment in the intestine of the longfingered bat (*Miniopterus inflatus)* is characterized by both longitudinal folds (L) and hexagonal pits (evident on the longitudinal folds); the latter give the mucosa a honey comb appearance. (Mag. X 36).

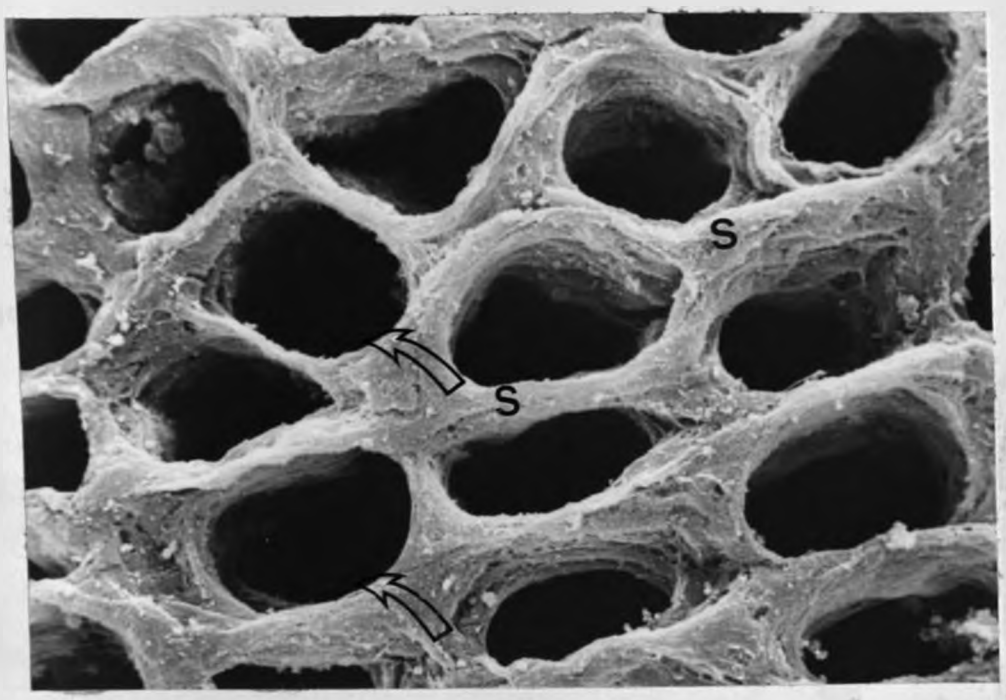

Fig. 32. A close up of the honeycomb intestinal segment in *Miniopterus inflatus* shows deep cylindrical and hexagonal pits (arrows) separated by septae (S) measuring about 5-19 μm thick on average. (Mag. X 770).

Fig. 32. A close up of the honeycomb intestinal segm in *Miniopterus inflatus* shows deep cylindrical hexagonal pits (arrows) separated by septae (S) measu about 5-19 μm thick on average. (Mag. X 770).

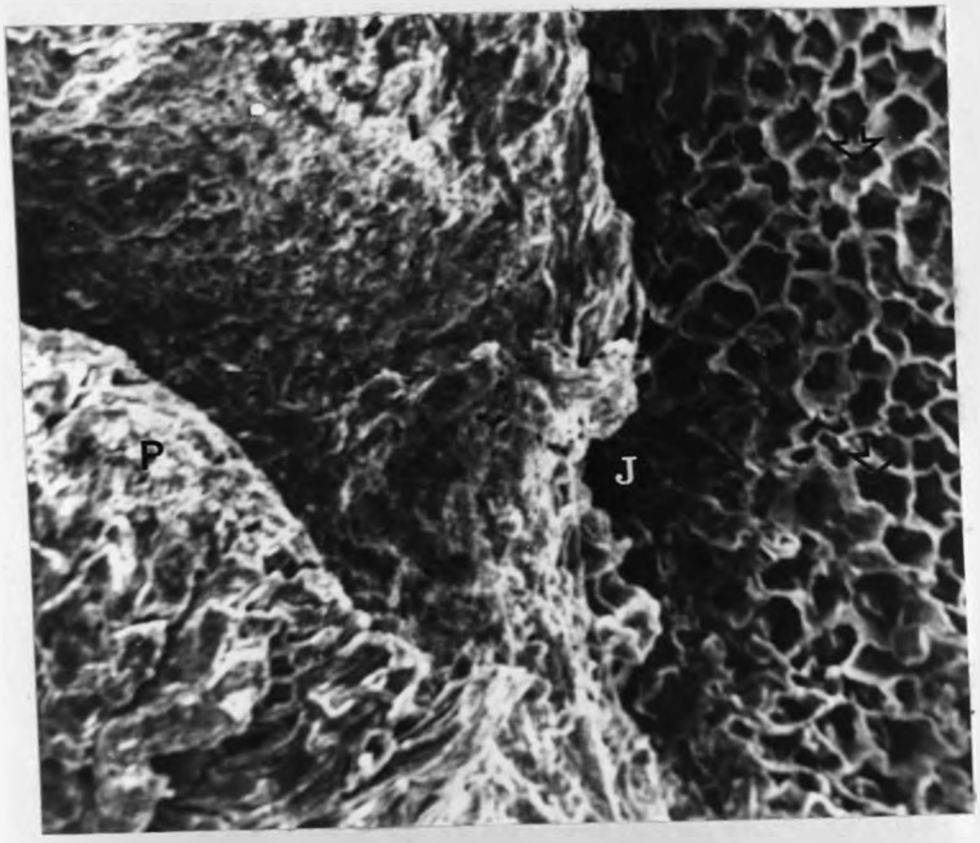

Fig. 33. A scanning electron micrograph showing part of the honeycomb segment in the horseshoe bat (*Rhinolophus hildebrandti*) with the hexagonal cylindrical pits (arrow heads), the pyloro-intestinal junction (J) and the pyloric folds (P). The pits have an approximate diameter of 19-36 µm and the septae are 4-9 µm thick, on average. (Mag. X 225)

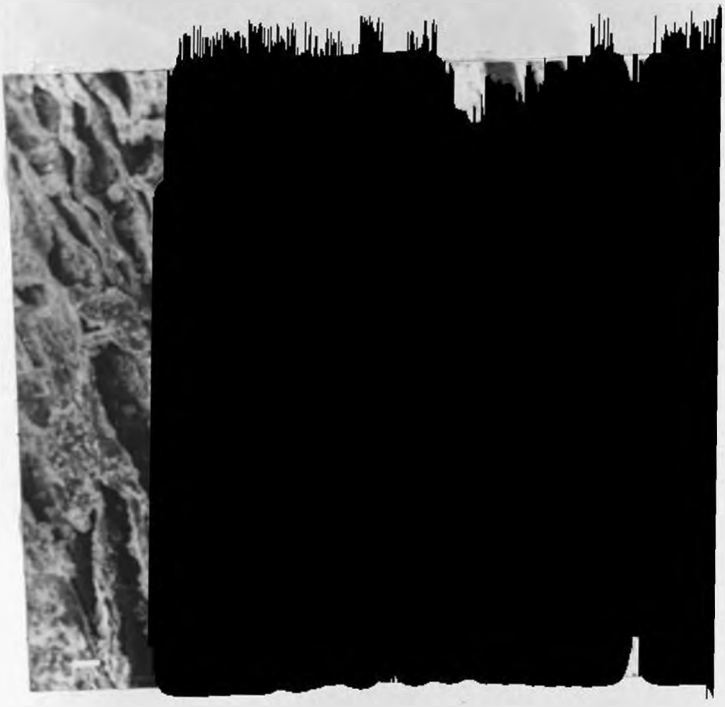

Fig. 34. Transverse ridge-like villi
rhinolophid (*Rhinolophus hildebrandti*
characterizes about 76% of the small
this species. (Mag. X 65).

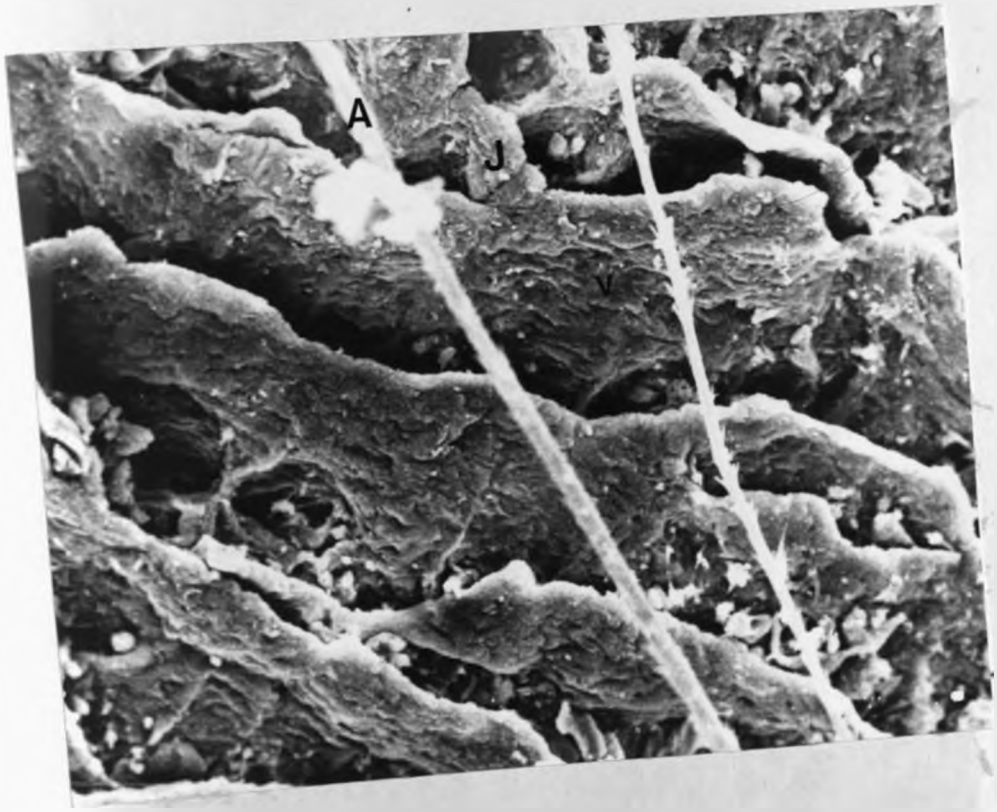

Fig. 35. The ridge-like villi (V) in the horseshoe bat (*Rhinolophus hildebrandti*) are occasionally joined by plicae (J); possible antenna of a previous ingested insect (A). (Mag. X 420).

Fig. 36. The transverse ridge-like villi (V) in the intestine of *Miniopterus inflatus* closely resemble those of *Rhinolophus hildebrandti* (see fig. 35 above). (Mag. X 150).

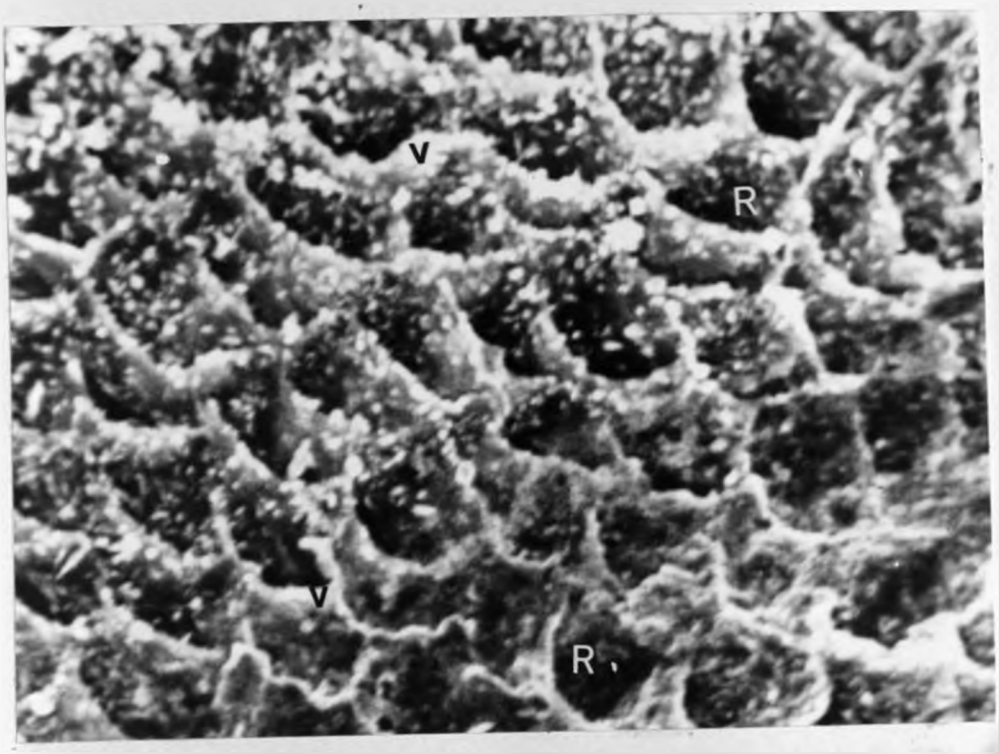

Fig. 37. The posterior part of the foregut of *Rhinolophus hildebrandti* is characterized by anastomoses of the ridge-like villi (V) forming shallow hexagonal and cylindrical compartments (R) that resemble reticular cells in the ruminant stomach. (Mag. X 220)

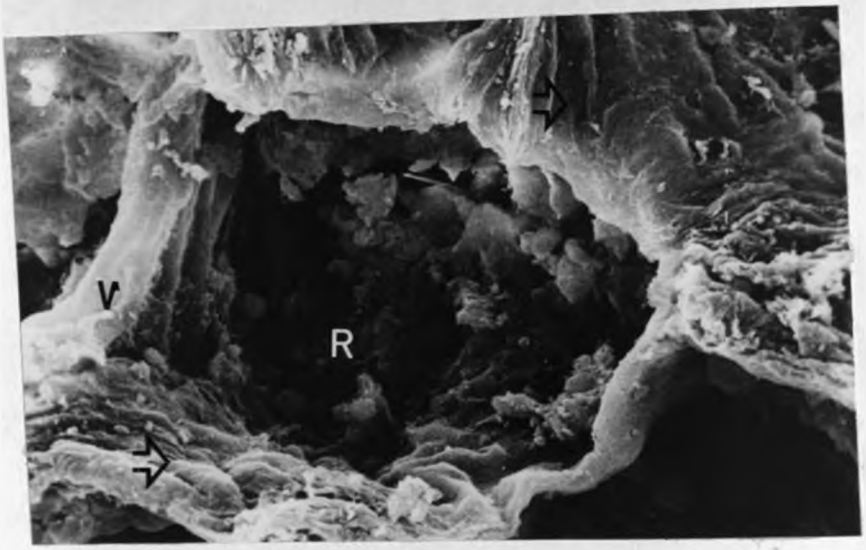

Fig. 38. A close up of the hexagonal compartment (R) found in the posterior part of the small intestine of *Rhinolophus hildebrandti* (fig. 37 above) shows the rough topography of the floor and the convolutions (arrows) forming the surface of the villi (V). The greatest diameter of the chamber is 47.1 μm. (Mag. X 1700).

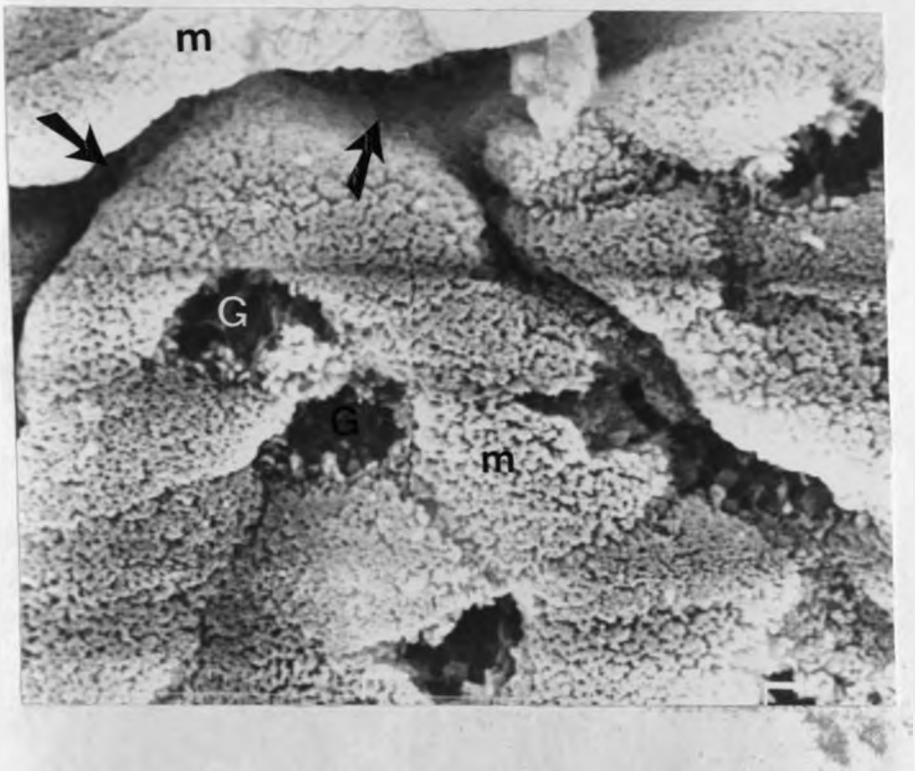

Fig 39. A scanning electron micrograph showing the mucosal surface of the rectum of the horseshoe bat *(Rhinolophus hildebrandti)* with openings to intestinal glands (arrows) and openings to Goblet cells (G). The epithelial cell profiles form the mucosal surface convolutions (M) with a rich microvillous cover. (Mag. X 4500).

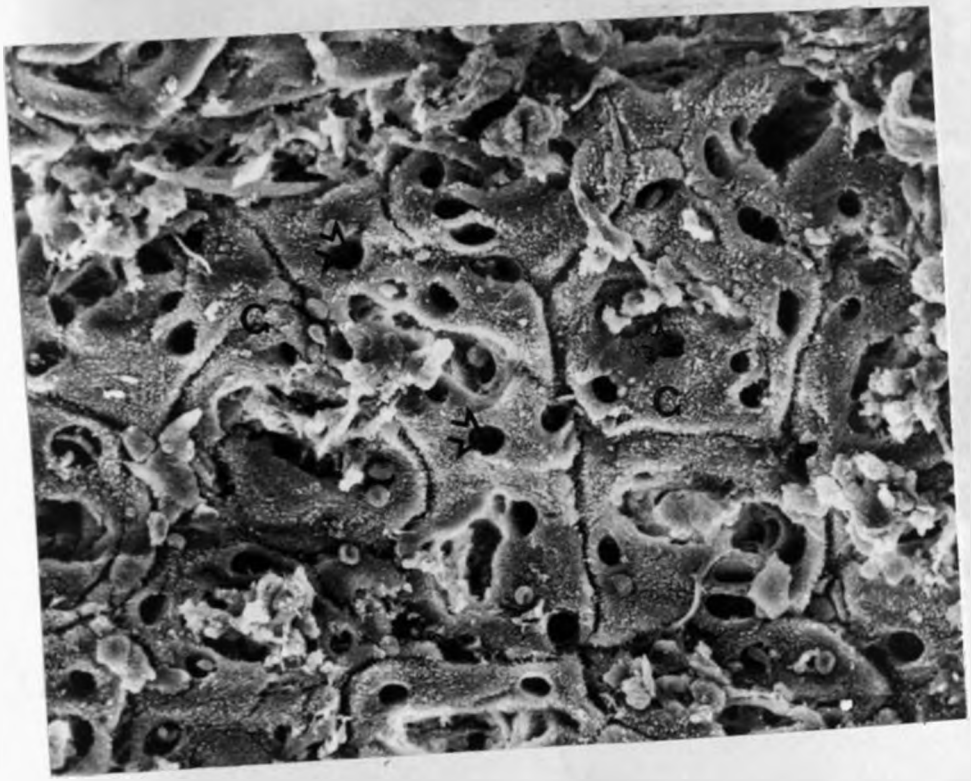

Fig. 40. The pattern of the mucosal surface convolutions (C) in the miniopterine bat *(Miniopterus inflatus)* differs from that of the rhinolophid bat with the convolutions being more regular in shape and pattern in the former (see fig. 39 above). Each convolution shows several openings leading to goblet cells (arrows). (Mag. X 3600).

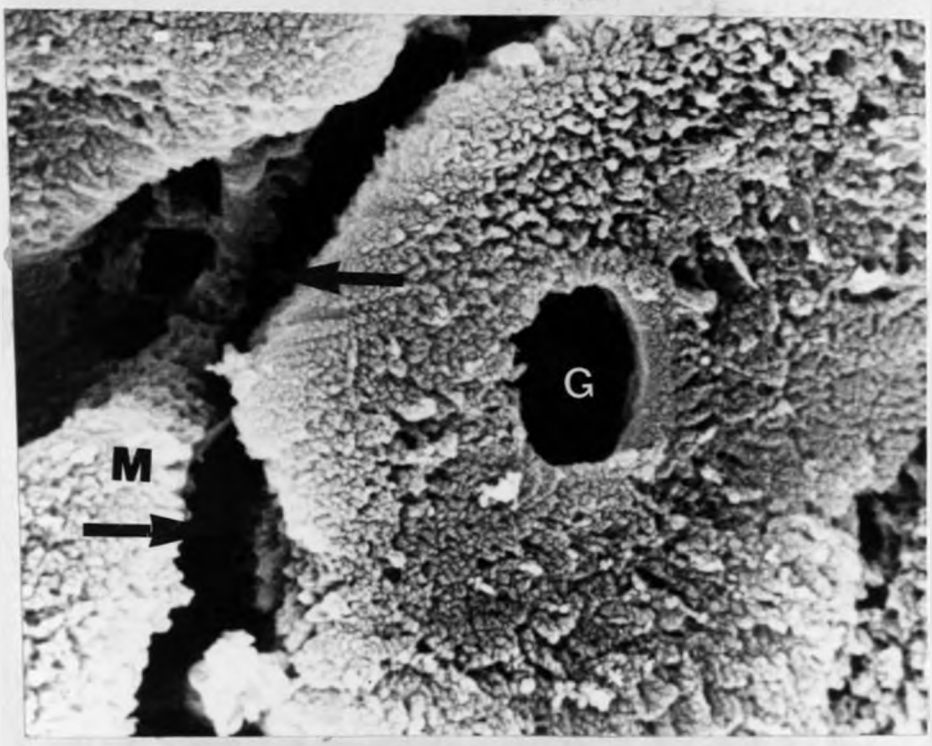

Fig. 41. A higher magnification of the mucosal surface convolutions in the rectum of the longfingered bat (*Miniopterus inflatus*) showing an individual opening to the goblet cell (G) and openings to intestinal glands (arrows). Note the smooth margin of the goblet cell opening, the numerous microvilli (m) on the convolutions. (Mag. X 3800).

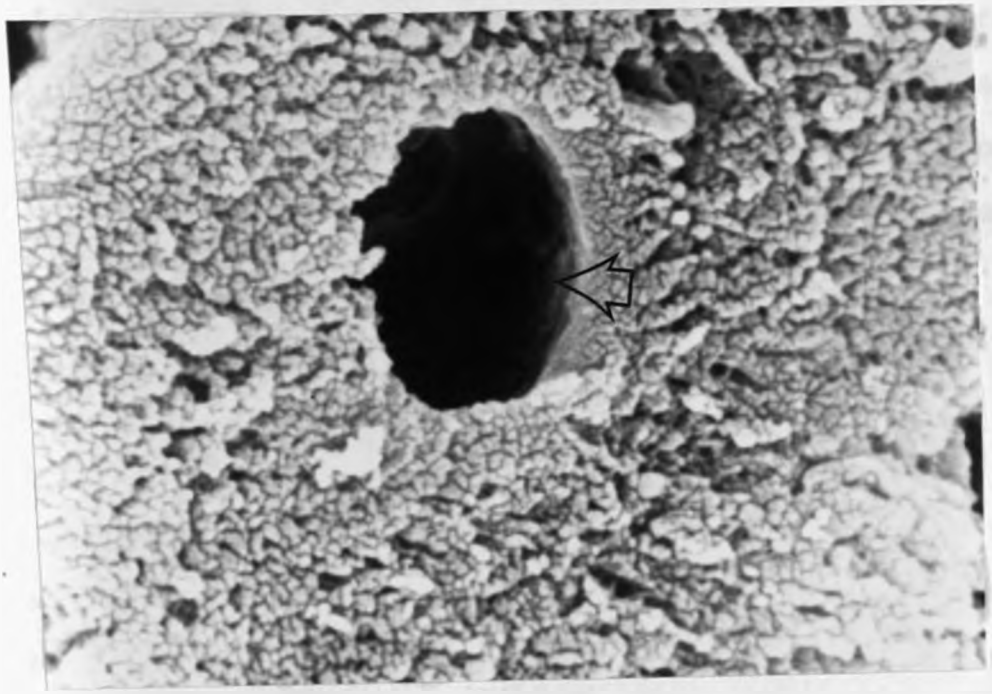

Fig. 42. Micrograph showing a high magnification (fig. 41 above) of the mucosa of the rectum (cranial part) of the longfingered bat (*Miniopterus inflatus*). Note the numerous microvilli surrounding the opening to goblet cell (arrow). (Mag. X 7500).

The change in the mucosal architecture from the small intestine to the large intestine was both dramatic and specific for each of the two species of bats. In both cases, the ridge-like villi diminished in height caudally and gave way to the rectum, a region of prominent mucosal convolutions, intestinal glands and numerous goblet cells (figs. 39, 40, 41 & 42). The shapes of the convolutions differed remarkably with those of the *Miniopterus inflatus* forming rounded mounds each with numerous openings to goblet cells (fig. 40). Openings to goblet cells in *Miniopterus* had a smooth margin while those in the *Rhinolophus* were rough (figs. 39 & 41). In both cases, the rectal mucosa gave way to the anus at the mucocutaneous junction.

3.4.0. Ultrastructure.

The ultrastructural design of the intestinal epithelium in all the three species of bats was essentially similar to that of terrestrial mammalian species which have been studied. The epithelium was mainly simple columnar and the cells rested on prominent basal lamina. The villous core had a rich supply of blood capillaries embedded in collagenous connective tissue. At ultrastructural level, there were cellular and pericellular specialisations (described below) that were more developed in the frugivorous bat than in the insectivorous bats with the situation in the latter tending to resemble that seen in terrestrial mammals.

3.4.1. The Frugivorous Bat.

The intestine revealed a well developed columnar epithelium with relatively tall enterocytes. Tight cell junctions were limited to the apical lateral walls of the cells (fig. 43) below which there were numerous, capacious intercellular spaces (figs. 43, 44 & 45). Migrating lymphocytes were occasionally encountered in the intercellular spaces (fig. 44). The columnar cells showed a very prominent brush border and nuclei were centrally located and mitochondria distributed diffusely in the cytoplasm (fig. 44). Adjacent cells sent long and tortuous cytoplasmic projections (lateral pseudopodia) into the large intercellular spaces (figs. 43, 46 & 47). The luminal compartment was well delineated from the intercellular compartment by well developed junctional complexes (fig. 45). The epithelium rested on a basal lamina, the connective tissue of which separated the epithelial cells and intercellular spaces from the numerous blood capillaries in the villous core. The intercellular spaces were larger at the subnuclear level and occasionally, cells were held together by desmosomes formed between cytoplasmic processes of adjacent cells (figs. 46 & 47). The epithelial cells had large vacuoles (figs. 43 & 47) located mainly at the supranuclear level and apparently formed by fusion of numerous pinocytotic vesicles (fig. 47). The apposition of two cytoplasmic processes appeared to facilitate formation of such pinocytotic vesicles (fig. 47). Mitochondria were occasionally encountered in the cytoplasmic projections

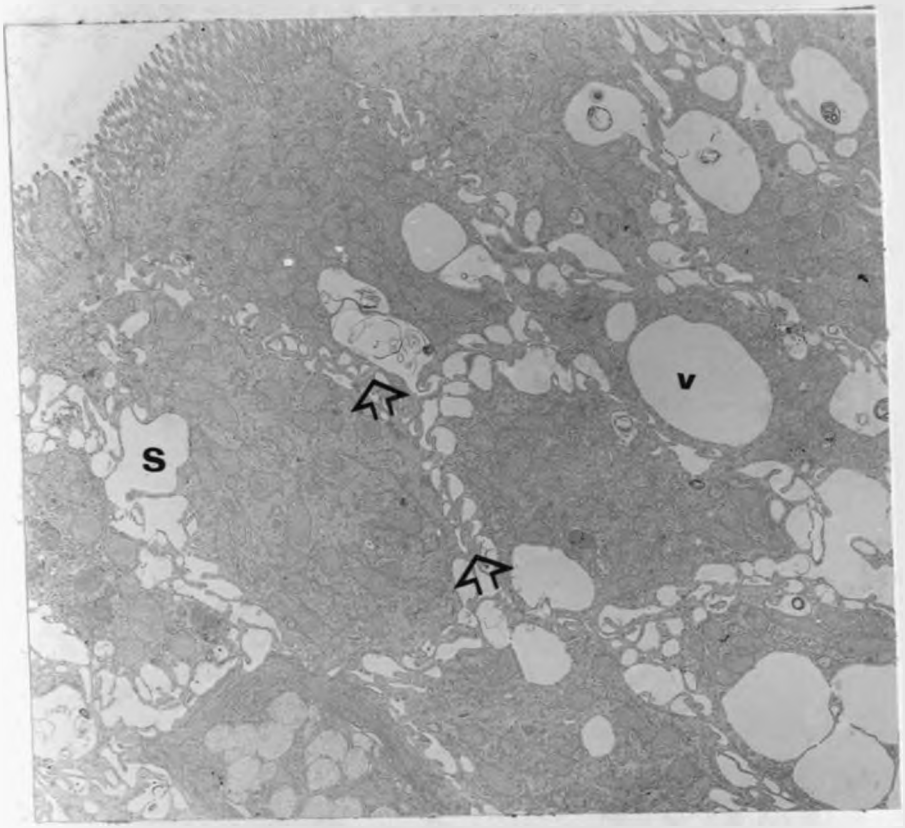

Fig. 43. An electron micrograph of the columnar epithelium in intestine of the fruit bat, *Epomophorus wahlbergi*, showing very prominent intercellular spaces (S), intracellular vacuoles (V) and pseudopodia-like cytoplasmic projections extending from the lateral membranes of the cells into the intercellular spaces (arrows). (Mag. X 2100).

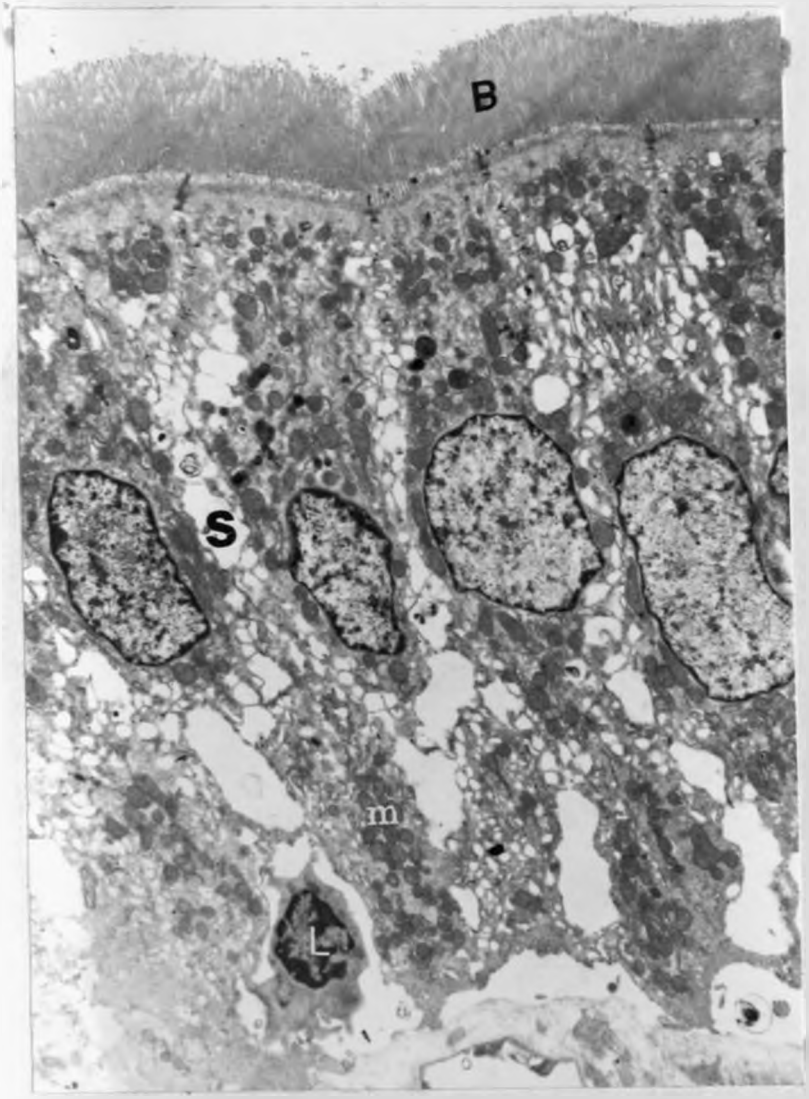

Fig. 44. A higher magnification of the columnar epithelium in the fruit bat (*Epomophorus wahlbergi*) showing the prominent brush border (B), the intercellular spaces (S), and the numerous mitochondria (M) distributed diffusely in the cytoplasm. A migrating lymphocyte (L) in the intercellular spaces is also shown.
(Mag. X 3540).

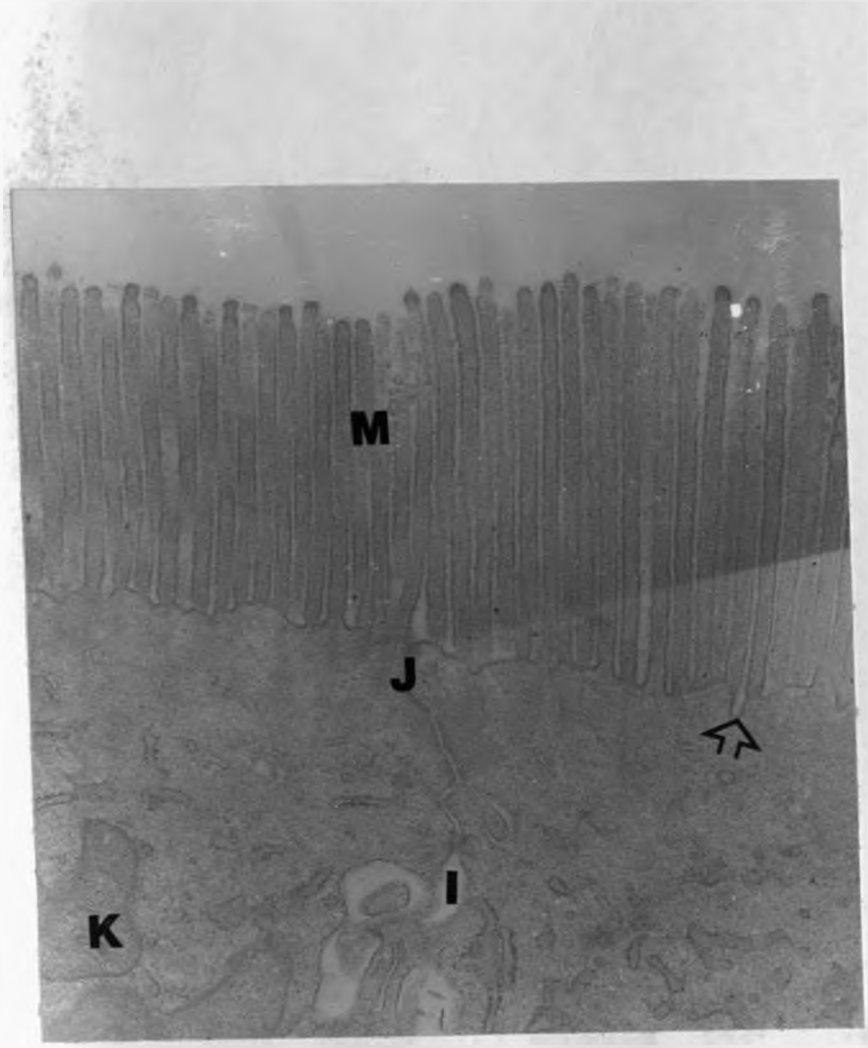

Fig. 45. A higher magnification of the enterocyte apical surface in the fruit bat (*Epomophorus wahlbergi*) showing very prominent microvilli (M), junctional complexes (J) and intercellular spaces (I). Caveolae (arrows) and mitochondria (K) are also shown. (Mag. X 18500).

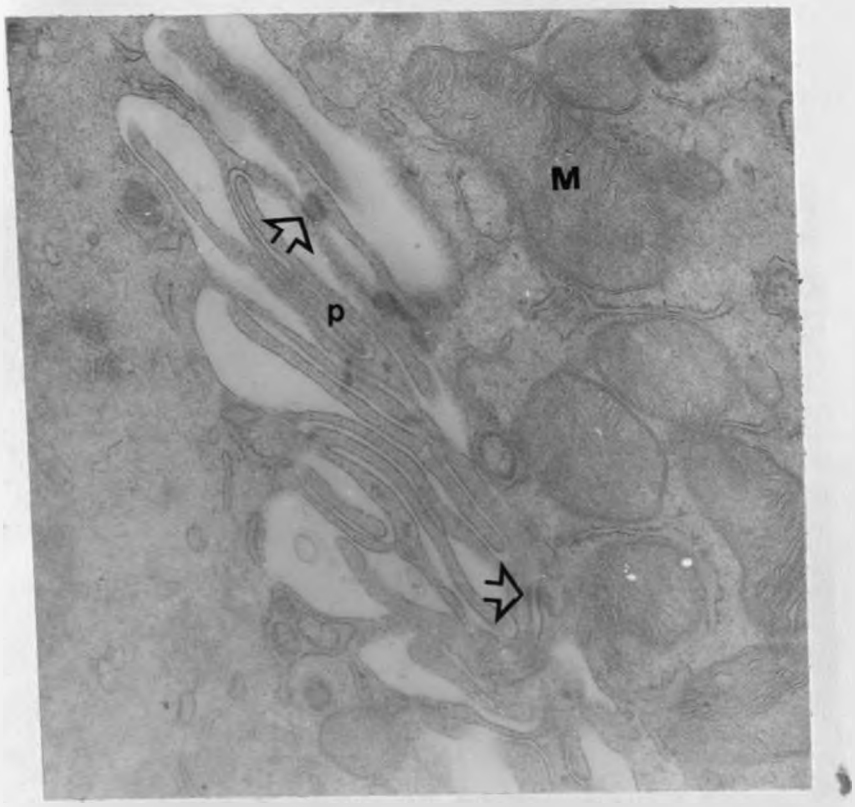

Fig. 46. A higher magnification of the intercellular space in the intestinal epithelium of Wahlberg's fruit bat, *Epomophorus wahlbergi* showing the very prominent cytoplasmic processes projecting into the intercellular spaces (P). Note the numerous desmosomes between the processes of adjacent cells (arrows) and the numerous mitochondria (M) lying close to the cell membrane. (Mag. X 37,000)

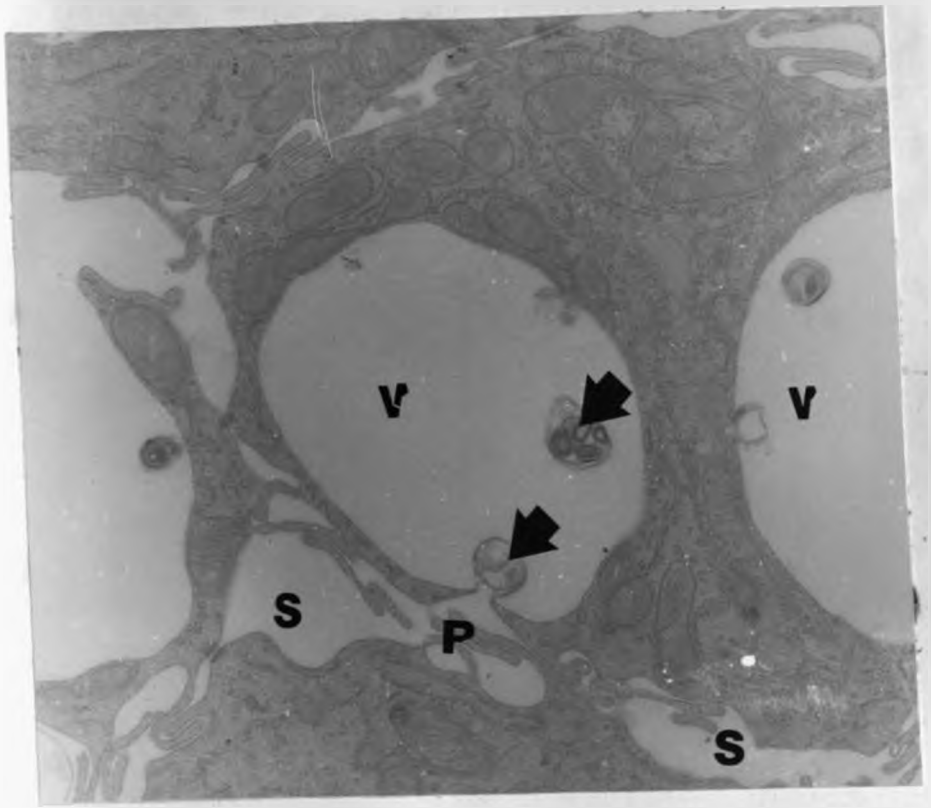

Fig. 47. An electron micrograph showing intracellular vacuoles (V) pinocytotic vesicles (arrows) intercellular spaces (S) and cytoplasmic processes (P) in the enteric epithelium of the fruit bat (*Epomophorus wahlbergi*). The cells have been cut across their vertical axis. (Mag. X 11,800).

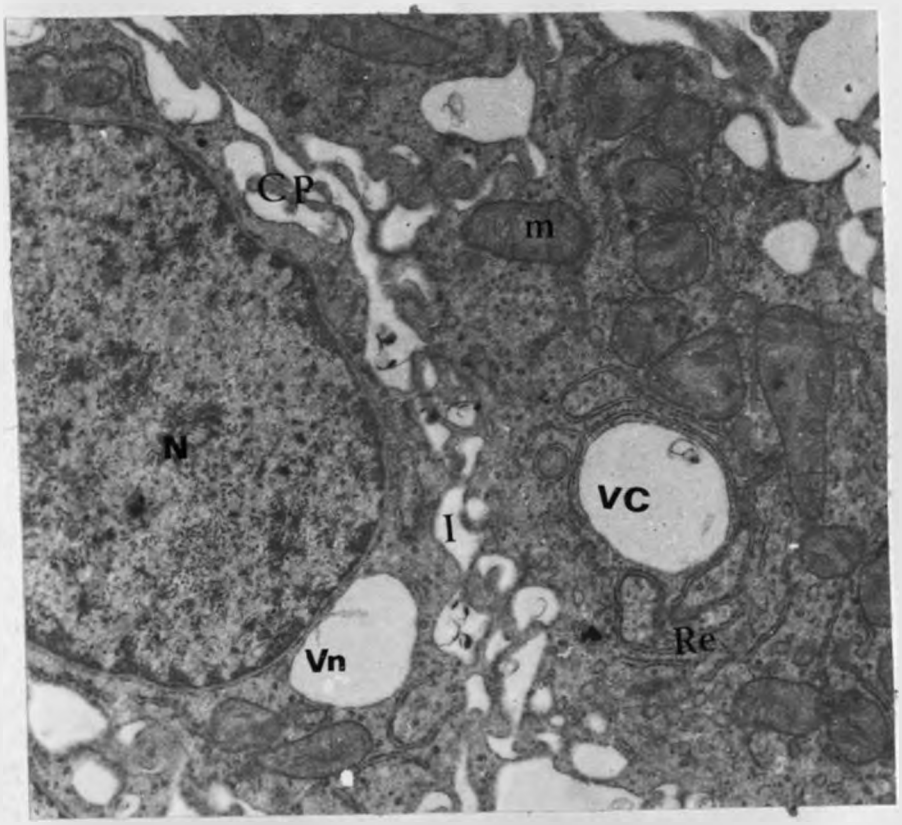

Fig. 48. An electron micrograph of the intestinal epithelium of the epauleted fruit bat *(Epomophorus wahlbergi)* showing a prominent nucleus, (N) , a perinuclear vacuole (Vn), rough endoplasmic reticulum (Re), mitochondria (M), intercellular spaces (I), cytoplasmic processes (Cp) and a cytoplasmic vacuole (Vc). (Mag. X 13,800)

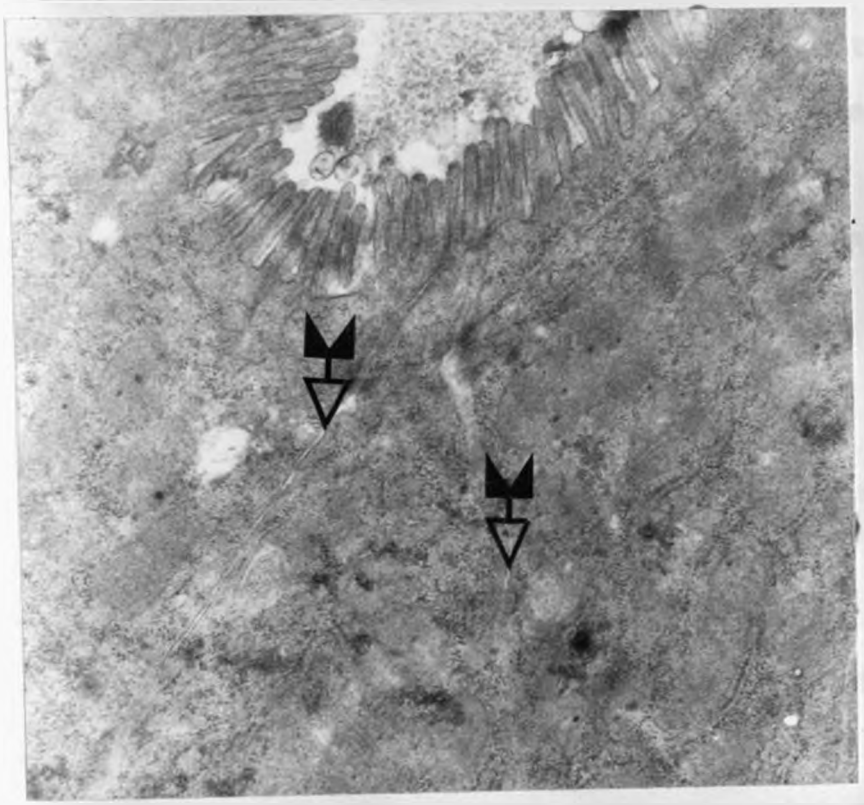

Fig. 49. Micrograph showing the enteric epithelium in the longfingered bat (*Miniopterus inflatus*) with very narrow intercellular spaces (arrows). (Mag. X 3300).

(fig. 47). Leucocytes were common in the villous core and were sometimes found in the intercellular spaces just above the basal lamina (fig. 44). Microvilli were densely packed (fig. 45) and between their bases were numerous caveolae and subsequent supranuclear micropinocytotic vesicles were frequent (fig. 45). Perinuclear vacuoles were occasionally encountered (fig. 48).

3.4.2. The Insectivorous Bats.

In both the microchiropteran bats, the enteric ultrastructure was closely related to and resembled that described for the rat (eg. Rhodin, 1974) to a large extent. Epithelial cells were closely apposed and intercellular spaces were narrow.

3.4.2.1 The Longfingered Bat.

The enteric epithelium was well developed with tall columnar cells and a prominent brush border, (fig. 49). The epithelium rested on a prominent basal lamina and intercellular spaces were small (figs. 49 & 50). Lateral cytoplasmic projections were not observed. Nuclei were basally located and there were numerous mitochondria below the fuzzy coat and were distributed diffusely in the cytoplasm. Junctional complexes separating the luminal compartment from the intercellular spaces were very prominent and restricted to the apical lateral membranes (fig. 50).

3.4.2.2. The Horseshoe Bat.

The enteric epithelium in the horseshoe bat resembled

that of *M. inflatus* closely. The epithelium was made up of tall closely adhered columnar cells interrupted by occasional goblet cells (fig. 52). Tight junctions were restricted to the apical lateral membranes and intercellular spaces were extremely narrow (figs. 52 & 53). The brush border was well developed and microvilli were densely packed on the enterocyte surfaces (fig. 53).

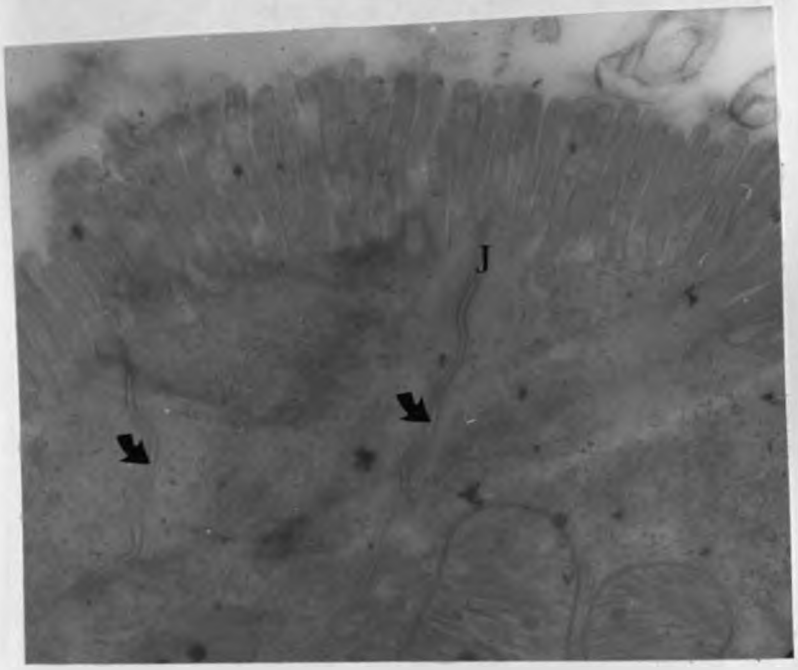

Fig 50. An electron micrograph showing the apical part of the intestinal epithelium in the longfingered bat *(Miniopterus inflatus)*. Note the narrow intercellular spaces (arrows) and the apical junctional complexes (J) and the relatively short microvilli. (Mag. X 30,000).

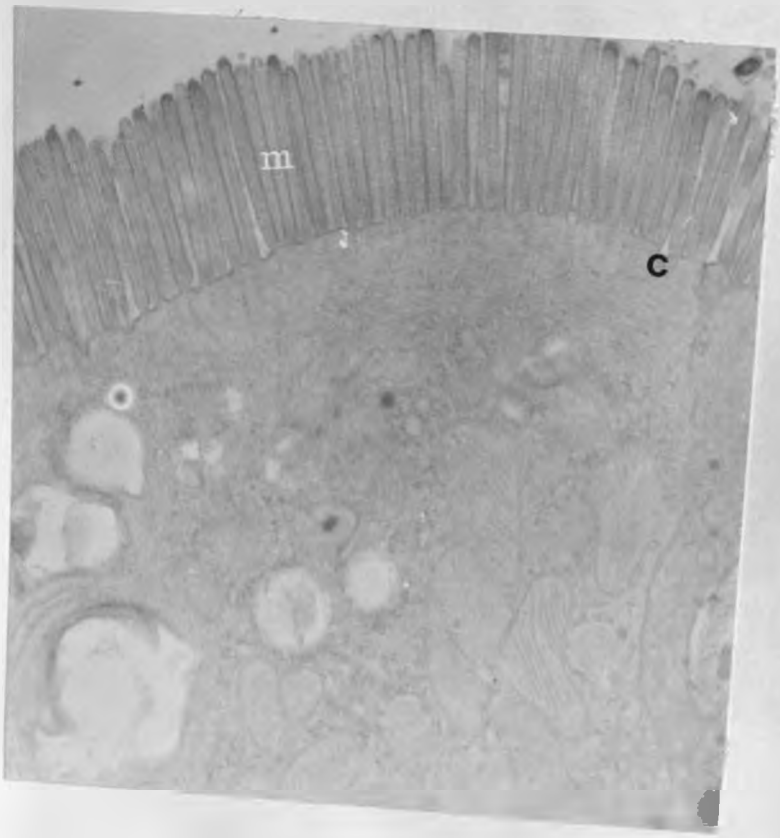

Fig. 51. The enteric brush border in the longfingered bat (*Miniopterus inflatus*). The microvilli (M) and caveolae (C) are quite prominent. (Mag. X 30,000)

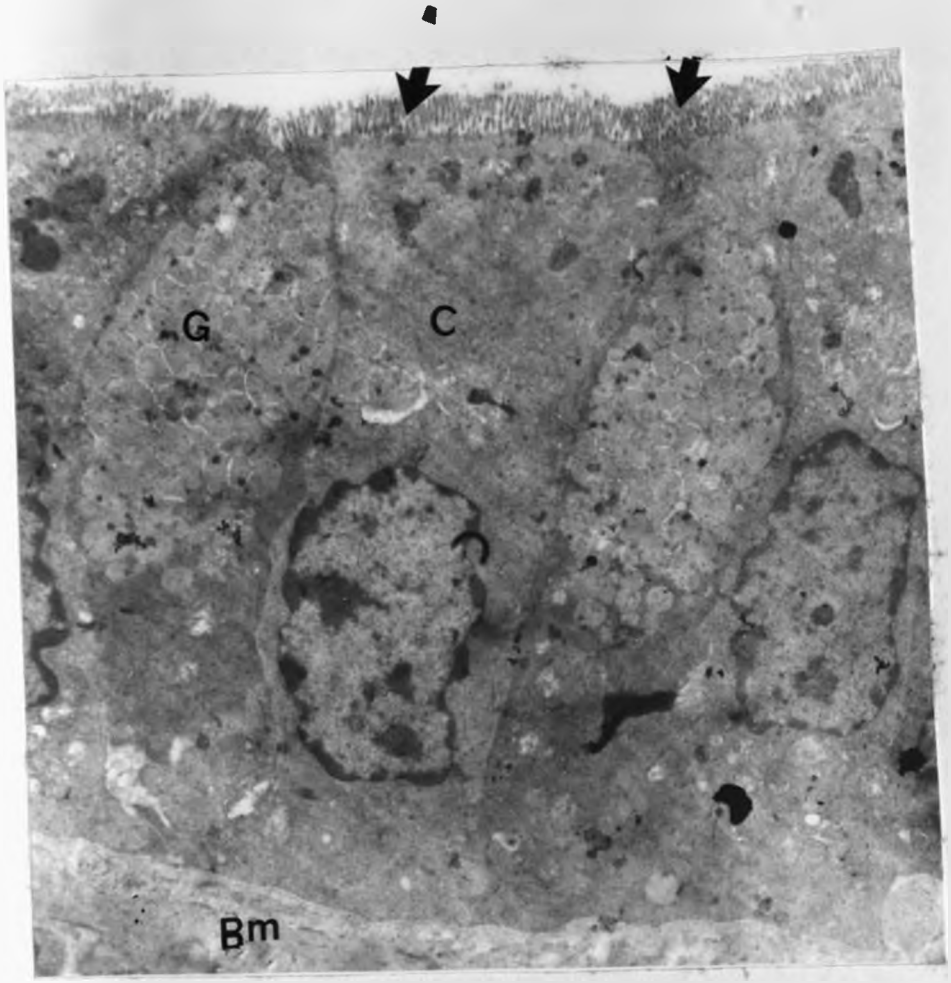

Fig. 52. The columnar epithelium in the posterior part of the intestine of the horseshoe bat (*Rhinolophus hildebrandti*) showing very tightly packed columnar cells (C) interspersed with occasional goblet cells (G). A brush border (arrows) and basal lamina (BM) feature quite prominently. (Mag. X 4200)

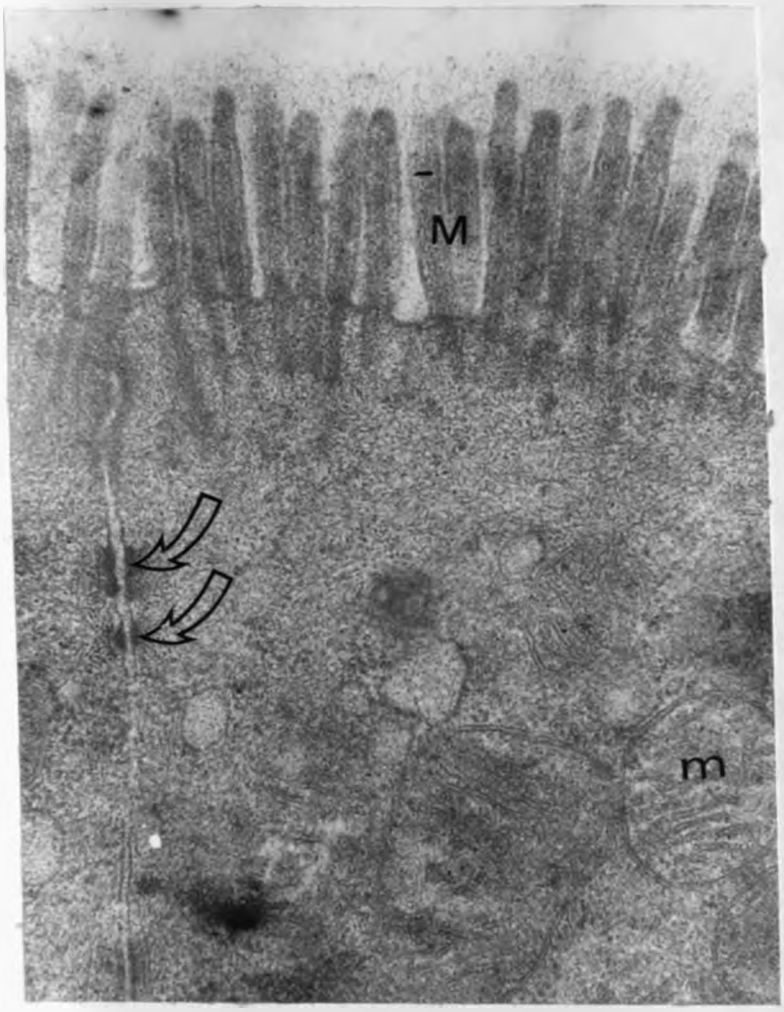

Fig. 53. A close up of the apical part of the rhinolophid *(Rhinolophus hildebrandti)* enterocyte showing junctional complexes (arrows), mitochondria (M) and microvilli (m). (Mag. X 18,000).

3.5.0. Morphometry.

Morphometric results are given in tables 2-21. All average values in tables and text are given as mean ± SD, unless otherwise stated.

3.5.1. The frugivorous bat

The fruit bat had an average body weight of 76.04 ± 13.45 g with an accompanying body length of 147 ± 14.80 mm. The intestinal length in the fruit bat averaged 788 ± 129.50 mm and comprised a small intestine measuring 733 ± 116.13 mm, a colon of 32.20 ± 10.94 mm and a rectum measuring 22.40 ± 2.41 mm in length. The intestinal length to body mass and to body length ratios were 10.40 ± 1.01 mm/g and 5.33 ± 0.38 mm/mm respectively. This information is presented in tables 2 and 5. The average small intestinal width ranged from 12.80 ± 1.30 mm in the cranial parts to 8.20 ± 1.92 mm in the posterior parts with a resultant primary mucosal surface area of the foregut being 7687.80 ± 2018.62 mm^2 (see tables 11 & 12). The average villous amplification factors ranged from 10.48 in the cranial segments to 3.78 in the posterior part of the small intestine resulting with a secondary surface area of 548 ± 161 cm^2 (mean ± SD, see tables 11 & 12). The microvilli further amplified the surface area by a factor of 50.35 ± 16.15 in the anterior parts of the intestine and 38.02 ± 11.55 in the posterior part resulting with an absolute surface area of 24951 ± 5628 cm^2 or 2.50 x 10^{12} μm^2 (tables 11 & 12). Body mass weighted absolute microvillous surface area was 3.29 x 10^{10}

μm^2 per gram body weight or 3.29×10^{-2} m^2 per gram body weight (table 21). The microvillous height averaged 2.87 $\pm$ 0.35 μm and had an average diameter of 0.097 $\pm$ 0.008 μm. Subsequently the surface area of the average microvillus was 0.8739 $\pm$ 0.119 μm^2 with a microvillous packing density of 58 $\pm$ 7.87 microvilli per square micron. These values are presented in table 13. The resultant absolute number of microvilli per animal on average was $3.24 \times 10^{12} \pm 6.70 \times 10^{10}$. These morphometric data are provided in tables 7 to 10 and summary values in tables 11, 12 and 13. Body mass normalized parameters and comparisons between the frugivorous bat and the insectivorous bat are available in table 21.

Page's L trend test (with k = 5 segments and N = 5 animals) shows that there were significant trends in microvillous dimensions (mean height and surface area) and also in the microvillous packing densities (see figs. 54 - 56). These together with the villous and microvillous amplification factors decreased cranio-caudally, a trend also observed in segmental microvillous numbers. For microvillous amplification factors (L = 250) and microvillous numbers (L = 272) there was a statistically significant trend at 5% and 1% levels respectively (for N = 5 and k = 5). Segmental microvillous numbers were highest in the cranial segments and decreased posteriorly (see fig. 57). However, there were no significant trends for microvillous diameters.

Table 1. Number of specimens used in the three species investigated in the study.

Species* No.	Gross**	LM	TEM	SEM	Morpho -metry	Total
1	2	1	1	2	5	9
2	2	1	1	2	5	9
3	2	2	1	2	-	5

** Specimens used for macroscopic studies were the also used for SEM studies.

*The Species: 1. *E. wahlbergi*
2. *M. inflatus*
3. *R. hildebrandti*

Table 2 shows the sex, body weights, body lengths and the intestinal lengths in the epauleted bat, *Epomophorus wahlbergi*. The ratios of the intestinal lengths to body mass and to body lengths are also presented.

Abbreviations to tables 2, 4, 5 and 6

FGL: foregut length	BL: body length
IL: intestinal length	RL: rectal length
HGL: hind gut length	BM: body mass
CL: colonic length	Spec.: specimen

Table 2: *Epomophorus wahlbergi*

Spec. No.	sex	BM (g)	BL (mm)	IL (mm)	IL:BL ratio (mm/mm)	IL:BM ratio (mm/g)
1	male	99.93	170	965	5.676	9.66
2	female	68.83	140	689	4.921	10.01
3	male	68.22	130	642	4.938	9.41
4	female	72.23	150	849	5.66	11.75
5	male	71.00	145	793	5.469	11.16
mean		76.04	147	787.6	5.333	10.40
SD		13.45	14.80	128.64	0.377	1.01

Table 3 shows the lengths of the various parts of the intestine of the fruit bat, *Epomophorus wahlbergi*.

Specimen No.	Foregut length (mm)	Colonic length (mm)	Rectal length (mm)
1	890	50	25
2	645	25	19
3	598	23	21
4	790	35	24
5	742	28	23
mean	733	32.2	22.4
SD	116.13	10.94	2.41

Table 4 shows the sex, body weights, body lengths and the intestinal lengths of the various components of the intestine in *Miniopterus inflatus*. The ratios of the intestinal lengths to body mass and to body lengths are also presented.*

Spec .No.	sex	BM (g)	BL (mm)	IL (mm)	FGL (mm)	HGL (mm)	IL :BL ratio (mm/mm)	IL:BM ratio (mm/g)
1	male	8.98	100	190	180	9.8	1.90	21.35
2	male	9.35	120	196	190	6.0	1.63	20.97
3	female	8.69	110	211	200	11.0	1.92	24.28
4	male	9.26	110	222	210	12.0	2.02	23.97
5	male	8.33	100	208	200	8.0	2.087	24.97
mean		8.92	108	205	196	9.36	1.71	23.11
SD		0.42	8.37	12.6	11.4	2.40	0.49	1.82

* See table 2 for definition of symbols.

Table 5 shows the sex, body weights, body lengths and the intestinal lengths of the various components of the intestine in *Rhinolophus hildebrandti*. The ratios of the intestinal lengths to body mass and to body lengths are also presented[♣].

spec. No.	Sex*	BM (g)	BL (mm)	IL (mm)	FGL (mm)	HGL (mm)	IL:BL ratio (mm/mm)	IL :BM ratio (mm/g)
1	f	12.25	62	138.7	130	8.7	2.24	11.32
2	f	17.45	65	189.5	180	9.5	2.92	10.86
3	f	16.23	64	147.9	140	7.9	2.31	9.11
4	m	11.28	61	149.6	140	9.6	2.45	13.26
5	m	8.05	58	127.4	120	7.4	2.20	15.83
6	m	15.3	65	179.7	170	9.7	2.76	11.75
mean		13.43	62.5	155.5	147	8.8	2.49	12.02
SD		3.5	2.74	24.11	23.4	0.97	0.30	2.3

* The letters "m" and "f" represent male and female respectively; ♣ See table 2 for definition of symbols.

Table 6: A summary table for comparing the gross morphometric data for the three species of bats examined. Values are given as mean ± SD. SD is given in parentheses[♀].

species	FGL (mm)	CL (mm)	RL (mm)	IL:BL ratio (mm/mm)	IL:BM ratio(mm/g)
E. wahlbergi	733 (6.13)	32.2 (10.94)	22.4 (2.41)	5.33 (0.38)	10.40 (1.01)
M. inflatus	196 (11.40)	—	9.36 (2.40)	1.71 (0.49)	23.11 (1.82)
R. hildebrandti	147.0 (23.4)	-	8.8 (0.97)	2.49 (0.30)	12.02 (2.30)

[♀] See table 2 for definition of symbols

Table 7: Segmental intestinal widths, W, primary mucosal surface areas, S(pm), and villous amplification factors, S(v)/S(pm), for the five fruit bats, *Epomophorus wahlbergi*.

		Gut Segment				
Variable	Bat No.	1	2	3	4	5
W, mm	1	14	12	11	13	8
	2	14	11	8	11	9
	3	12	9	10	8	5
	4	13	12	11	10	10
	5	11	10	9	9	8
S(pm), mm^2	1	2492	2136	1958	2314	1602
	2	1806	1419	1032	1419	1162
	3	1440	1080	1200	960	600
	4	2054	1896	1738	1580	1580
	5	1632	1480	1336	1336	1187
S(v)/S(pm),	1	9.8	9.3	8.0	5.8	3.7
mm^2/mm^2	2	9.3	7.4	5.8	4.0	3.3
	3	11.8	8.4	7.5	4.3	3.0
	4	9.8	10.3	6.8	4.0	3.3
	5	11.8	6.5	6.6	5.6	2.4

Table 8. Villous surface areas, S(v), microvillous surface areas, S(mv) and microvillous amplification factors, S(mv)/S(v), in the intestine of the fruit bat, *Epomophorus wahlbergi*.

		Gut Segment				
Variable	Bat No.	1	2	3	4	5
S(v), mm^2	1	24297	19929	15664	13306	5927
	2	16596	10501	5986	5677	3831
	3	16992	9072	9000	4128	1800
	4	20129	19586	11731	6320	5214
	5	19176	9620	8818	7482	2849
S(mv)/S(v)						
mm^2/mm^2	1	33.54	56.00	42.39	36.32	28.22
	2	46.7	39.80	35.10	39.30	33.10
	3	76.99	54.46	50.74	45.55	28.49
	4	50.37	53.55	57.15	42.62	46.43
	5	44.17	61.90	71.55	62.12	53.88
S(mv), cm^2	1	8149	11160	6640	4833	1673
	2	12931	5719	3037	2586	1092
	3	7934	3609	3160	1624	595
	4	1014	10488	6703	2694	2421
	5	8470	5955	6309	4648	1319

Table 9. Diameters of microvilli, d(mv), microvillous heights, h(mv) and mean microvillous surface areas, s(mv), in the foregut of the frugivorous bat, *Epomophorus wahlbergi*.

Variable	Bat No.	Gut Segment 1	2	3	4	5
d(mv), nm	1	81	108	108	135	135
	2	81	81	81	81	81
	3	108	97	81	81	87
	4	81	108	95	108	108
	5	86	108	90	108	108
h(mv), μm	1	2.06	2.67	3.24	2.52	2.16
	2	2.97	3.24	3.24	2.48	1.80
	3	2.70	2.47	2.05	2.05	2.70
	4	3.13	3.19	3.24	2.70	1.98
	5	2.10	4.21	4.42	4.59	3.46
s(mv), μm^2	1	0.52	0.89	1.10	1.05	0.92
	2	0.76	0.82	0.82	0.63	0.46
	3	0.91	0.75	0.52	0.52	0.74
	4	0.80	1.08	0.97	0.92	0.67
	5	0.57	1.43	1.25	1.56	1.17

Table 10. Microvillous packing densities, N(mv)/S(v) and numbers of microvilli, N(mv), in the foregut of the frugivorous bat, *Epomophorus wahlbergi*.

Variable	Bat No.	Gut Segment 1	2	3	4	5
N(mv)/S(v),	1	64	63	39	34	31
μm^{-2}	2	101	66	62	72	72
	3	51	53	67	75	45
	4	63	50	59	47	69
	5	78	43	57	40	46
N(mv),	1	15.50	12.60	6.04	4.52	1.83
x 10^{11}	2	17.10	6.94	3.68	4.10	2.38
	3	8.66	4.79	6.06	3.11	0.81
	4	12.70	9.69	6.93	2.94	3.60
	5	14.90	4.17	5.05	2.98	1.12

Table 11: Summary of the primary mucosal surface areas, S(pm), villous surface areas, S(v), microvillous surface areas, S(mv) and numbers of microvilli, N(mv) in the intestine of the fruit bat, *Epomophorus wahlbergi*. S(pm) is given in mm^2 while S(v) and S(mv) are given in cm^2

	Variable			
Bat No.	S(pm)	S(v)	S(mv)	N(mv) x 10^{12}
1	10502	791	32435	4.05
2	6838	428	25364	3.42
3	5280	410	16920	2.34
4	8848	630	23321	3.59
5	6971	480	26698	2.82
Mean	7687.8	548	24951	3.24
SD	2018.62	161	5628	0.67

Tables 12 and 13 (below) provide the summary of the morphometric data (intestinal widths, primary, secondary and tertiary surface areas, microvillous dimensions, and villous and microvillous amplification factors) for the fruit bat averaged across the intestinal segments of the various individuals.

Abbreviations appertaining to the tables:

S(v,p) = villous amplification factor, S(v)/S(pm)

S(m,v) = microvillous amplification factor, S(mv)/ S(v)

N(m,v) = microvillous packing density, N(mv)/S(v)

All the other abbreviations are as described in the earlier tables.

Table 12. Summary of width, surface areas and amplification factors (mean ± SD) averaged across the intestinal segments of five epauleted fruit bats, *Epomophorus wahlbergi*. The standard deviation (SD) is given in parentheses. Abbreviations are described above.

Segment	W mm	S(pm) mm^2	S(v,p)	S(v) mm^2	S(m,v)	S(mv) cm^2
1	12.8 (1.30)	1976 (320.30)	10.48 (1.20)	19478 (3046.00)	50.35 (16.15)	5492 (4551)
2	10.8 (1.30)	1671 (327.40)	8.77 (1.44)	13741.43 (5516.62)	53.14 (8.14)	7386 (3277)
3	9.8 (1.30)	1529 (358.70)	6.93 (0.85)	10239.74 (3650.52)	51.39 (14.02)	5170 (1897)
4	10.2 (1.92)	1583 (425.50)	4.73 (0.87)	7382.22 (3525.78)	45.19 (10.08)	3277 (1401)
5	8.2 (1.92)	1264 (337.40)	3.78 (1.05)	3411.50 (2352.03)	38.02 (11.55)	1420 (683)
mean	10.4	1605		10850.58		4549
SD	(1.68)	(257.15)		(6133.53)		(5091.53)

Table 13. Summary of the mean microvillous diameter, d(mv), height, h(mv), packing densities, N(mv)/S(v), microvillous numbers, N(mv) and mean microvillous surface area, S(mv), averaged across the intestinal segments (mean ± SD) of the small intestines of five epauleted fruit bats, *Epomophorus wahlbergi*. The standard deviation (SD) is given in parentheses.

Segment No.	d(mv) μm	h(mv) μm	s(mv) μm^2	N(m,v)	N(mv)
1	0.087 (0.01)	2.59 (0.49)	0.7120 (0.16)	71 (19.11)	1.38×10^{12} (3.26×10^{11})
2	0.10 (0.01)	3.16 (0.68)	0.9947 (0.27)	55 (9.46)	7.63×210^{11} (3.52×10^{11})
3	0.091 (0.01)	3.24 (0.84)	0.9324 (0.28)	57 (10.64)	5.55×10^{11} (1.24×10^{11})
4	0.103 (0.02)	2.87 (0.99)	0.9391 (0.41	54 (18.77)	3.53×10^{11} (7.30×10^{10})
5	0.104 (0.02)	2.42 (0.67)	0.7915 (0.27)	51 (15.04)	1.95×10^{11} (1.11×10^{11})
mean	0.097	2.87	0.8739	58	6.32×10^{11}
SD	(0.008)	(0.35)	(0.119)	(7.87)	(4.82×10^{11})

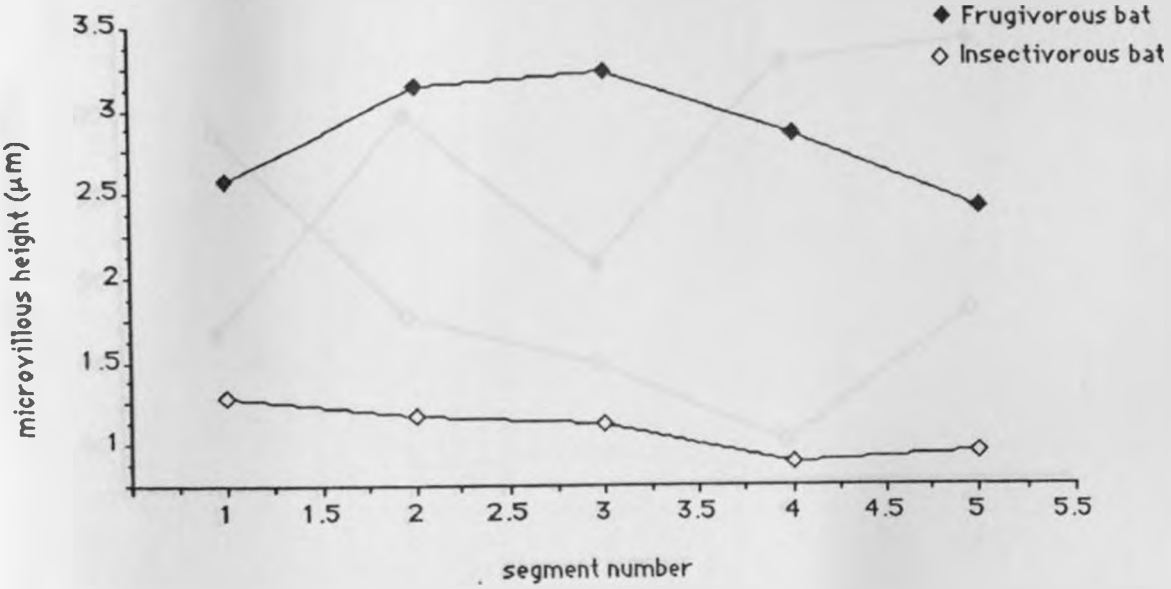

Fig. 54 : Variation in mean microvillous height, h(mv), along the intestine of the fruit bat *Epomophorus wahlbergi* and the insectivorous bat *Miniopterus inflatus*.

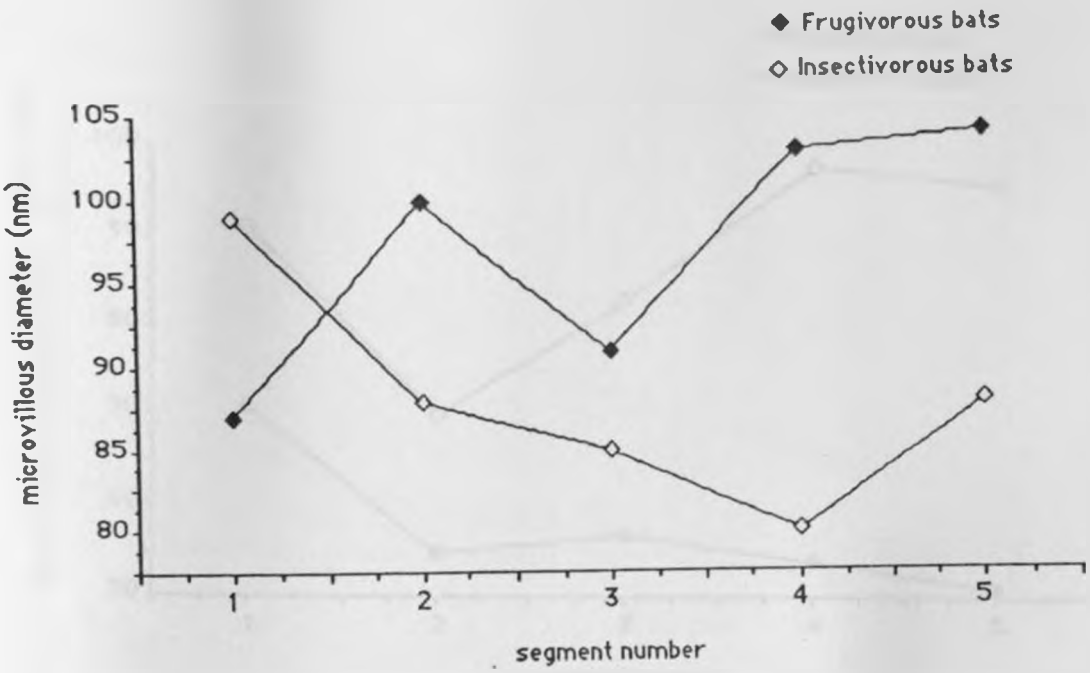

Fig. 55:. Changes in microvillous diameter, d(mv), along the small gut of the fruit bat, *Epomophorus. wahlbergi* and that of the insectivorous bat, *Miniopterus inflatus.*

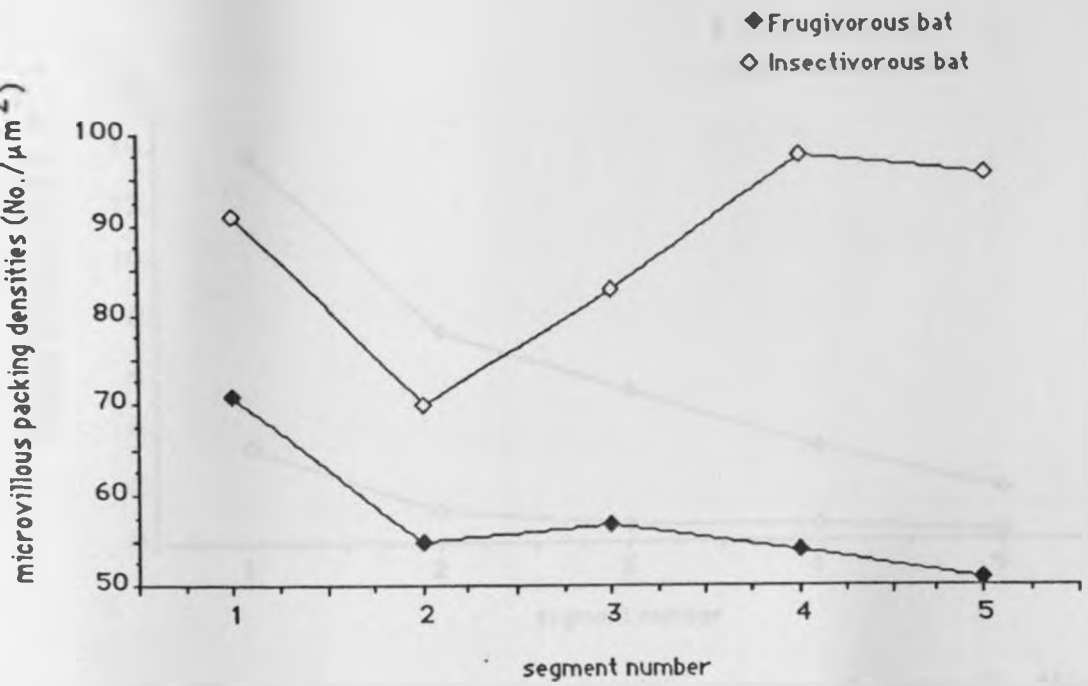

Fig. 56 : Changes in microvillous packing densities, (N(mv)/S(v), along the small gut of *Epomophorus wahlbergi* and that of *Miniopterus inflatus*..

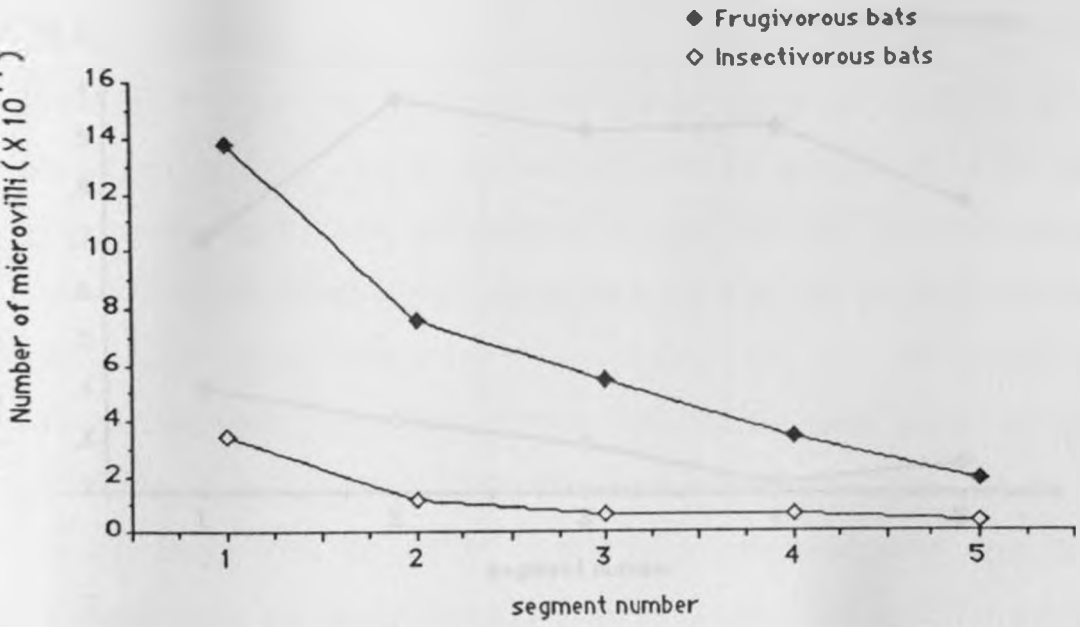

Fig. 57. A comparison of the total number of microvilli, N(mv), per intestinal segment in the small gut of *Epomophorus wahlbergi* and that of *Miniopterus inflatus*.

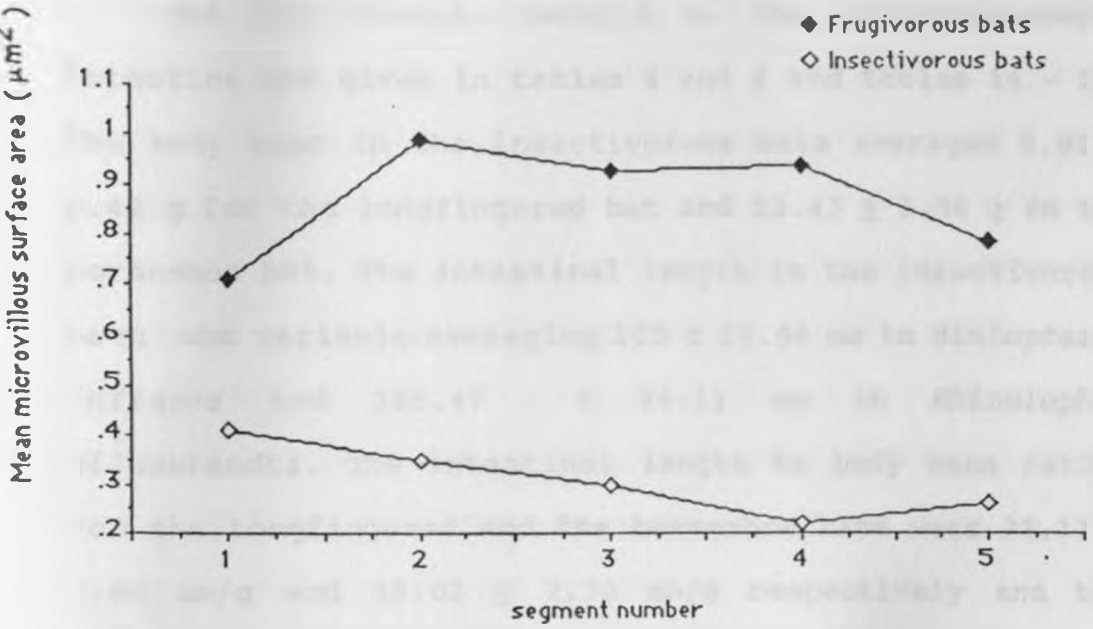

Fig. 58: Changes in surface areas of the average microvillous, s(mv), along the intestine of *Epomophorus wahlbergi* and that of *Miniopterus inflatus*.

3.5.2. The Insectivorous Bats.

The morphometric details on the microchiropteran intestine are given in tables 4 and 6 and tables 14 - 21. The body mass in the insectivorous bats averaged 8.92 ± 0.42 g for the longfingered bat and 13.43 ± 3.50 g in the horseshoe bat. The intestinal length in the insectivorous bats was variable averaging 205 ± 12.64 mm in *Miniopterus inflatus* and 155.47 ± 24.11 mm in *Rhinolophus hildebrandti*. The intestinal length to body mass ratios for the longfingered and the horseshoe bats were 23.11 ± 1.82 mm/g and 12.02 ± 2.30 mm/g respectively and the intestinal to body length ratios were 1.71 ± 0.49 mm/mm and 2.49 ± 0.30 mm/mm respectively. The intestine comprised a foregut averaging 196 ± 11.40 mm in length in *M. inflatus* and 147 ± 23.40 mm in *R. hildebrandti* with a rectum measuring 9.40 ± 2.40 mm and 8.80 ± 0.97 mm respectively. The above details are presented in tables 4, 5 and 6. The primary small intestinal surface area of the longfingered bat was 920.80 ± 76.59 mm^2 and the villous amplification factors in *M. inflatus* ranged from 7.78 ± 1.33 in the anterior parts of the intestine to 2.22 ± 0.29 in the caudal parts (table 19) with a resultant secondary (villous) surface area of 48 ± 4.08 cm^2 (table 18). The microvilli amplified the intestinal surface area by a factor of 37.53 ± 14.57 in the cranial parts to 24.56 ± 4.66 in the posterior parts (table 19) with a resultant absolute (microvillous) surface area of 1324.40 ± 238.50 cm^2 or 1.32 x 10^{11} μm^2 (table 18). Body mass normalized

microvillous surface was 1.48×10^{10} μm^2 g^{-1} (square microns per gram body weight) or 1.48×10^{-2} m^2 g^{-1} (table 21). The microvilli measured 1.09 ± 0.161 μm in height on average and had a mean diameter of 0.088 ± 0.0007 μm (table 20). The surface area of the average microvillus was 0.3069 ± 0.007 μm^2 and the resultant microvillous packing density was 88 ± 11.40 microvilli per square micron (table 20). The absolute number of microvilli per animal on average was $3.90 \times 10^{11} \pm 1.02 \times 10^{11}$. These morphometric details for individual bats are presented in tables 14 - 17 and summary values in tables 18, 19, 20 and 21.

Page's L trend test (see Miller, 1975) shows that there were significant trends (with k = 5 segments and N = 5 animals) in microvillous dimensions (mean height, and surface area) and also in the microvillous packing densities. Villous and microvillous amplification factors decreased cranio-caudally, a trend also observed in segmental microvillous numbers(figs 54 & 56-58). For microvillous amplification factors (L = 252) and microvillous numbers (L = 270) there was a statistically significant trend at 1% level. Segmental microvillous numbers were highest in the cranial segments and decreased posteriorly (see fig. 57). There were no significant trends in the diameter (see fig. 55).

Table 14. Segmental intestinal widths, W, primary mucosal surface areas, S(pm), and villous amplification factors, S(v)/S(pm) in the foregut of the insectivorous bat, *Miniopterus inflatus*.

Variable	Bat No.	Gut Segment 1	2	3	4	5
w, mm	1	5.5	5.0	4.5	4.0	4.0
	2	5.0	5.0	4.5	4.0	4.0
	3	4.0	5.5	6.0	5.0	4.5
	4	4.5	4.5	3.5	4.0	4.0
	5	6.0	5.5	5.0	4.5	4.5
S(pm), mm^2	1	198	180	162	144	144
	2	210	210	189	168	168
	3	152	209	228	190	171
	4	189	189	147	168	168
	5	240	220	200	180	180
S(v)/S(pm),	1	7.0	7.0	6.0	3.0	2.7
mm^2/mm^2	2	6.7	6.2	4.0	3.8	2.2
	3	10.0	6.8	4.2	4.8	2.0
	4	7.2	7.8	6.1	3.4	2.2

Table 15. Villous surface areas, S(v), microvillous surface areas, S(mv) and microvillous amplification factors, S(mv)/S(v), in the intestines of the entomophagous bat, *Miniopterus inflatus*.

Variable	Bat No.	Gut segment 1	2	3	4	5
S(v), mm^2	1	1386	1260	972	432	389
	2	1407	1302	756	638	397
	3	1520	1421	958	912	342
	4	1361	1474	897	571	370
	5	1920	1452	920	720	360
S(mv)/S(v), mm^2/mm^2	1	30.05	26.15	17.89	19.71	22.56
	2	36.05	25.31	17.15	22.89	26.08
	3	32.99	26.73	21.32	22.59	30.11
	4	62.72	30.78	19.09	22.55	26.33
	5	25.86	22.44	20.05	20.58	17.72
S(mv), cm^2	1	416	329	174	85	88
	2	507	330	130	146	103
	3	501	380	204	206	103
	4	854	454	171	129	97
	5	497	326	184	148	64

Table 16. Diameters of microvilli, d(mv), microvillous heights, h(mv) and mean microvillous surface areas, s(mv), in the foregut of the insectivorous bat, *Miniopterus inflatus*.

Variable	Bat No.	Gut Segment 1	2	3	4	5
d(mv), nm	1	100	80	80	80	93
	2	90	97	84	81	91
	3	99	79	84	79	90
	4	117	104	104	80	80
	5	88	79	72	80	86
h(mv), μm	1	1.10	0.81	0.75	0.79	0.81
	2	1.37	0.98	1.14	0.89	1.03
	3	1.11	1.10	0.89	0.82	0.78
	4	1.70	1.72	1.38	0.90	1.13
	5	1.12	1.26	1.40	1.06	0.98
s(mv), μm^2	1	0.35	0.20	0.19	0.20	0.24
	2	0.38	0.30	0.30	0.23	0.29
	3	0.35	0.35	0.24	0.20	0.22
	4	0.62	0.45	0.56	0.23	0.28
	5	0.33	0.31	0.32	0.27	0.26

Table 17. Microvillous packing densities, N(mv)/S(v) and numbers of microvilli, N(mv), in the foregut of the insectivorous bat, *Miniopterus inflatus*.

Variable	Bat No.	Gut Segment 1	2	3	4	5
N(mv)/S(v), μm^{-2}	1	64	63	39	34	31
	2	101	66	62	72	72
	3	51	53	67	75	45
	4	63	50	59	47	69
	5	78	43	57	40	46
N(mv), $\times 10^{11}$	1	12.00	16.10	9.26	4.30	3.64
	2	13.40	11.00	4.31	6.45	3.51
	3	14.50	10.80	8.68	10.10	4.67
	4	13.70	10.10	3.05	5.69	3.43
	5	15.00	10.40	5.82	5.56	2.41

Table 18: Summary of the primary mucosal surface areas, S(pm), villous surface areas, S(v), microvillous surface areas, S(mv) and numbers of microvilli, N(mv), in the intestine of the insectivorous bat, *Miniopterus inflatus*. S(pm) is given in mm^2 while S(v) and S(mv) are given in cm^2

	Variable			
Bat No.	S(pm)	S(v)	S(mv)	N(mv) x 10^{11}
1	828	44	1093	4.53
2	945	45	1216	3.90
3	950	52	1395	4.90
4	861	47	1705	2.23
5	1020	54	1213	3.90
Mean	920.8	48	1324.4	3.90
SD	76.59	4.08	238.5	1.02

Table 19. Summary of width, surface areas, and surface amplification factors averaged across the intestinal segments of *Miniopterus inflatus*. Abbreviations are as described under *E. wahlbergi* above (tables 12 & 13). Values are given as mean ± SD. SD is given in parentheses.

segment No.	W mm	S(pm) mm^2	S(v,p)	S(v) mm^2	S(m,v)	Smv mm^2
1	5 (0.79)	198 (32.03)	7.78 (1.33)	1519 (232.38)	37.53 (14.57)	55506 (17097.55)
2	4.8 (0.76)	202 (16.50)	6.88 (0.59)	1382.2 (95.10)	26.28 (3.01)	36369.81 (5507.60)
3	4.7 (0.96)	181 (30.78)	4.98 (1.00)	901 (86.16)	19.10 (1.67)	17268 (2731.7)
4	4.3 (0.45)	170 (17.20)	3.80 (0.68)	655 (178.43)	21.66 (1.43)	14285 (4346.68)
5	4.2 (0.27)	166 (13.35)	2.22 (0.29)	372.48 (22.14)	24.56 (4.66)	9107 (1651.08)
mean	4.6	183	-	955.64	_	26507
SD		(16.19)	-	(482.10)	_	(19207.40)

Table 20: Summary of the mean microvillous diameter, d(mv), height, h(mv), packing densities, N(mv)/S(v), microvillous numbers, N(mv) and mean microvillous surface areas, S(mv), averaged across the intestinal segments (mean ± SD) of the small intestines of five longfingered bats, *Miniopterus inflatus*. Values are given as mean ± SD. SD is given in parentheses.

1	0.99 (0.01)	1.28 (0.26)	0.4055 (0.124)	91 (8.63)	3.50×10^{11} (4.6×10^{10})
2	0.088 (0.01)	1.18 (0.36)	0.3455 (0.13)	70 (22.73)	1.17×10^{11} (2.5×10^{9})
3	0.085 (0.001)	1.13 (0.27)	0.2983 (0.10)	83 (27.31)	6.22×10^{10} (2.7×10^{10})
4	0.080 (0.001)	0.89 (0.10)	0.2242 (0.03)	98 (12.51)	6.42×10^{10} (2.2×10^{10})
5	0.088 (0.005)	0.95 (0.16)	0.2609 (0.03)	96 (25.42)	3.53×10^{10} (8.03×10^{9})
mean	0.088	1.09	0.3609	88	1.26 x 1011
SD	(0.0007	(0.161	(0.007)	(11.40)	(1.29×10^{11})

Table 21. Comparison of weight specific morphometric values between the intestine of the frugivorous bat, *Epomophorus wahlbergi* and that of the insectivorous bat, *Miniopterus inflatus*.

	Bat Species	
Variable	*E. wahlbergi*	*M. inflatus*
Intestinal length, L, mm g^{-1}	10.29	23.11
Primary mucosal surface, S(pm) mm^2 g^{-1}	101.10	103.23
Secondary mucosal surface, S(v), cm^2 g^{-1}	7.20	5.38
Tertiary Mucosal surface, S(mv), $m^2 g^{-1}$	3.29×10^{-2}	1.48×10^{-2}
Total number of microvilli, N(mv), microvilli/g.	4.27×10^{10}	4.37×10^{10}

4.0.0. DISCUSSION

4.1.0. Comparative Morphology.

4.1.1. General characteristics.

Reports on the chiropteran gut morphology (Mathis, 1928a; Park & Hall, 1951; Okon, 1977; Madkour *et al.*, 1982; Ishikawa *et al.*, 1985) indicate that the small intestine and the large intestine are indistinguishable from external aspects only. The results of this study show that in the three species examined, the rectum, which is part of the large intestine, is easily identified due to its obviously greater diameter. Its precise demarcation from the rest of the gut may, however, be elusive especially in the insectivorous bats since the change in diameter is very gradual. This is in contrast to the earlier findings cited above. The caecum and the appendix were universally absent in the three species of bats and this confirms numerous earlier observations (eg. Mathis, 1928a,b; Allen, 1939; McMillan & Churchill, 1947; Okon, 1977; Ishikawa *et al.*, 1985).

Many researchers have mentioned the presence of a colon in the chiropteran gut without any microscopic verification (eg. Forman, 1972, 1974a, b; Halstead & Segun, 1975; Danguy *et al.*, 1987). Okon (1977) observed that a colon was present in the fruit bat, *Eidolon helvum*, but absent from the intestine of the entomophagous bat, *Tadarida nigeriae*. This study confirms the presence of the colon in the frugivorous bat and the absence of the same in the insectivorous bats, the latter in complete contrast

to the observations of Ishikawa *et al.* (1985) who described a colon and an ileocolon in one insectivorous bat, *Myotis frater kaguae*. It is worthwhile to note here that mucosal topography alone is not sufficient for the purposes of identifying a colon in the chiropteran gut since even villi are sometimes disposed as transverse ridges. The colon was easily discernible grossly from the mucosal surface of the opened intestine in the fruit bat and was characterized by large longitudinal folds. The transition zone, however, shows very gradual changes and at some point, villi and longitudinal folds occur together as revealed by SEM. Ishikawa *et al.* (1985) observed a similar segment in the insectivorous bat which they named ileocolon. The segment that forms the transition between foregut and hindgut in the fruit bat, however, is too short to qualify for an independent name and although two categories of villi are recognized, the histological picture is fundamentally that of the small intestine.

Investigations on gut show that there is a correlation between complexity of gut morphology and type of diet (Chivers & Hladik, 1980; Wrong *et al.*, 1981) and a number of workers also suggest that there are taxonomic inclinations (Schultz, 1965; Chivers & Hladik, 1980). In general, the chiropteran intestine is reported to be shorter than that of similar sized terrestrial mammals (Klite, 1965) and that the insect-feeder bat has an even shorter gut than the frugivorous bat (Park & Hall, 1951; Madkour, 1977a; Okon, 1977; Madkour *et al.*, 1982). The

comparative methods employed to draw these conclusions were, nevertheless, inadequate since most comparisons involved linear measurements or body length normalized parameters only. Body mass normalized parameters employed in this study are considered to be superior since they are not affected by body designs. The values obtained for this study, however, show that the fruit bat (*E. wahlbergi*) had a shorter intestine (10.40 mm g^{-1}) than either of the two insectivorous bats, *Rhinolophus* (12.02) mm g^{-1}) and *Miniopterus* (23.11 mm g^{-1}). This is in complete contrast to the previous observations already cited.

The gross and/or light microscopic morphology has been reported for various species of bats (e.g. McMillan & Churchill, 1947; Rouk & Glass, 1970; Forman, 1972; 1973; Halstead & Segun, 1975; Kamiya & Pirlot, 1975; Madkour, 1976; 1977a,b; Okon 1977; Madkour, *et al.*, 1982; Tedman & Hall, 1982; 1985; Stutz & Ziswiler, 1983-84). Controversy, however, still exists on whether parts of the large intestine such as the caecum, the colon and the rectum are present in the chiropteran gut. The external morphological characteristics of the bat's small and large intestine are noted to be similar and hence indistinguishable from external aspects only (see Mathis, 1928a, b; Okon, 1977; Madkour *et al.*, 1982) and although many researchers mention the presence of the colon, few (Okon, 1977; Tedman & Hall, 1985; Ishikawa *et al*, 1985) have tangible morphological proof of the presence or

absence of the parts of the large intestine they name.

4.1.2. The Small Intestine.

The small intestine is a highly differentiated organ that accomplishes both digestive and absorptive functions with great efficiency (Komuro & Hashimoto, 1990). This calls for an adequate supply of digestive enzymes and expansive surface area. In bats, the gut is both short and small but shows characteristics suggestive of modifications to enhance efficiency.

In the fruit bats, the villi in the cranial fifth were long, branched and interconnected thus increased the surface area tremendously. The epithelial cells occurred in groups and each epithelial cell presented a polygonal profile projecting into the luminal surface. A similar topographical picture of enteric absorptive cells was described by Demling *et al.* (1969), Anderson & Taylor (1970) and Balcerzak *et al.* (1970). Demling *et al.* (1969) suggested that the projection of the epithelial cells into the intestinal lumen increases the surface area by about 25%. The microvilli on the other hand were relatively very long (averaging 2.87 μm) compared to those of the rat estimated at 1.14 μm (Keegan & Mödinger, 1979) or 1.076 μm (Penzes & Regius, 1985) thus further amplifying the absorptive surface area tremendously. The epithelium of the intestine of the frugivorous bat showed cellular and pericellular specialisations to which the high efficiency could be ascribed. The large intercellular spaces into which numerous cytoplasmic processes project may be

reservoirs for absorbed nutrients. Contrary to observations of Johnson (1975) that the lateral border of the absorptive cell is relatively unspecialized, the absorptive cells of the fruit bat intestine showed numerous long and tortuous microvillus-like cytoplasmic processes, thus named due to the absence of the microfilaments found in microvilli and their apparent role in pinocytosis. Johnson (1975) further observed that virtually all cells of the mammalian organism are capable of pinocytosis. In the fruit bat, evidence for pinocytosis is represented by the cytoplasmic vesicles and the large vacuoles seen in the epithelial cells which probably result from fusion of several pinocytotic vesicles. These results confirm earlier observations by Manley and Williams (1979) that the fruit bat intestinal epithelium has large absorption vacuoles.

Besides participating in binding adjacent cells together through "interpseudopodial" desmosomes (fig. 46), the cytoplasmic processes apparently also facilitate pinocytosis as may be deduced from the vesicles seen next to apposing cytoplasmic processes. Since the luminal compartment is well delineated from the intercellular compartment by tight junctions, transfer of materials from the intestinal lumen takes place through the enterocyte apical membrane. It is imperative that the lateral cytoplasmic processes play a role in the subsequent transfer of materials from the absorptive cell to the intercellular spaces and possibly any subsequent exchanges

between these two compartments. Though there is morphological evidence for pinocytosis, the materials transferred and the precise sequence of events remain unknown. A hypothesis is put forward to try and explain the possible events that take place: that if transport of sugars from the intestinal lumen is solely by diffusion (eg. Keegan, 1980) and given that the absorption rates are exceptionally high (Keegan, 1975; 1977a, b) then a concentration gradient has to be established in the absorptive cell to facilitate such high absorption rates. Therefore, absorbed sugars need to be removed from the absorptive cell very fast to maintain the concentrations within the cell low (thus sustaining a high concentration gradient) hence the reported fast absorptive rates. It is predicted that the role of the cytoplasmic projections is to secrete sugars into the intercellular spaces besides, of course, binding adjacent cells together through the evident desmosomes. The other possible role of these projections is most probably re-admittance of the intercellular contents by pinocytosis as may be deduced from the various intracellular vacuoles and the apparent role of the processes in formation of these vacuoles (fig. 47). Comparable intercellular spaces described in the rat (Rhodin, 1974) are thought to be important in the absorptive process.

In the insectivorous bats, a small segment of the intestine bordering the pylorus and measuring about 0.2 mm in the horseshoe bat and about 10 mm in the longfingered

bat comprises numerous hexagonal and cylindrical pits. These pits resemble those found in gastric mucosa but appear to be larger and devoid of mucus secreting cells (figs. 15, 31, 32 & 33). They probably trap the fluid part of the digesta exposing it to extensive surface area for digestion and absorption or may be involved in enzyme secretion. Their precise role, however, remains to be investigated.

Anatomically constant folds are structures that are said to reduce digesta transit time (Langer, 1989). In both the horseshoe bat and the longfingered bat, the villi took a transverse direction and spanned the entire internal circumference of the intestine. While these may be too low to withhold the bulk of the semi solid digesta, the liquid nutrients may be retained for appreciable durations to allow enzymatic digestion and subsequent absorption. In the horseshoe bat, the hexagonal chambers in the posterior part of the foregut closely resemble the reticular cells in the ruminant stomach and are probably used for retaining digesta and thus allowing absorption of water and possibly electrolytes. The gut appears to be shorter in the horseshoe bat than in the longfingered bat. The specimens examined for the former species were, however, of different ages as may be deduced from the body weight values and the average values obtained may not have been representative of the situation in adults (coefficient of variation is 26%: estimated from results in table 4). In general, the arrangement of the digestive

tract in the abdominal cavity and the mucosal surface architecture observed in the microchiropteran bats are consistent with previous observations by Stutz and Ziswiler (1983-1984) with the stomach resting in left cranial aspect of the abdominal cavity and the cranial part of the intestine coursing towards the right side. The variation seen in the disposition of the mid-segment of the intestine was partly due to differing lengths and the constraints to pack the gut in a very small cavity. The variation in the intestine can further be attributed to peristaltic movements which shift loops of the mid segment of the intestine. The general anatomical picture of the small intestine in the two insectivorous bats was closely related, and the ultrastructural characteristics were much simpler than those of the fruit bat and closely approached those of the rat intestine (eg. Pfeiffer *et al.*, 1974). The rate of passage of food in the intestine of bat is the same as that in mice but transit time in the former is far much shorter (Klite, 1965). This has been attributed to the short gut in bats. Relatively slow transit of food is advantageous for efficient digestion (Clemens & Stevens, 1980; Knight & Knight-Eloff, 1987). It thus appears that the assimilatory process in bats is very efficient to facilitate their high metabolic rates (Griffin, 1958) and to compensate for the remarkably short gastrointestinal transit times and the results of this study strongly support this hypothesis.

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4.1.3. The Large Intestine.

The parts of the mammalian large intestine have a definite histological structure that enables them to be distinguished from each other and from the rest of the intestine (Okon, 1977). Taenia caeci and coli, for example, are specific to the caecum and colon respectively while haustrae and appendices epiploicae are characteristic of the large intestine in general (Bloom, 1986). In the bats examined in this study, none of these structures was observed but the longitudinal folds found in the colon and rectum of the fruit bat and the accompanying long and straight intestinal glands evident at light microscopic level were characteristic. In the insectivorous bats, the large intestine was represented by the rectum only. It has, however, been observed that a distinct rectum may be missing in some frugivorous bats (Tedman & Hall, 1985).

The colon is responsible for absorption of water, electrolytes and short chain fatty acids besides allowing microbial fermentation (Kerlins & Phillips, 1983). In carnivores and insectivores, the intestine is unimportant as a fermentation chamber except in breakdown of chitin in cetaceous animals and the caecum is very simple or absent (Wrong *et al.*, 1981). As reported by many workers, most bats lack a colon and the hindgut extensions such as the caecum and appendix (McMillan & Churchill, 1947; Park & Hall, 1951; Klite, 1965; Forman, 1973; Madkour, 1976) and it is only on a few occasions that a colon has been

convincingly described (e.g. Okon, 1977, Ishikawa *et al.*, 1985; Tedman & Hall, 1985). The presence of a caecum was described in only one species of entomophagous bats, *Rhinopoma hardwickei,* by Madkour (1976).

The fruits on which most fruit bats thrive contain copious amounts of water estimated to be 70-90% (Altman & Dittmer, 1968) and the epauleted fruit bat also drinks water. While the colon in the fruit bat may not be of appreciable fermentative value, its role in water and probably electrolyte absorption is of significant value. Furthermore, the presence of digestive enzymes in the fruit bat, *Eidolon helvum,* colon has been reported by Ogunbiyi and Okon (1976) and Okon and Ogunbiyi (1979) indicating a possible participatory role of the colon in digestion. In contrast, the insects on which most bats thrive do not offer much in terms of structural carbohydrates and contain low water levels (Okon, 1977) estimated at 60% (Carpenter, 1969; Vogel, 1969; Geluso, 1975; Anthony & Kunz, 1977). Fermentation chambers such as an extended colon or a caecum are thus unnecessary. The portion of the large intestine seen in entomophagous bats in particular is small and may not be involved in breakdown of chitin and chitosans since parts of the exoskeleton of previously ingested insects appear in faeces and have actually been used in the assessment of the type of insects eaten (see for example Shiel *et al.*, 1991). Furthermore, bats are capable of discarding undigestible portions of large prey such as legs, wings

and in some cases heads (Shiel *et al.*, 1991).

One of the principal functions of the large intestine is lubrication and as such the rectum in the bats studied had numerous goblet cells, both in the mucosal convolutions and the walls of the very numerous intestinal glands. While the intestinal glands occurred in aggregations in specific regions in the rectum of the fruit bat, they were distributed evenly in the recta of the entomophagous bats. The arrangement in the latter is the one normally encountered in mammals. On the other hand, the rectum of the fruit bat was characterized by numerous pits ("rectal pits") that had abundant goblet cells. The arrangement in the latter may be attributed to the enormous quantities of water found in fruits that the bat has to cope with. Since absorptive function is performed by columnar cells, it is necessary to have an adequate surface of such cells in contact with the water to be absorbed. Nevertheless, the need to have lubricating mucous is paramount and thus the numerous goblet cells, and in addition the rectal pits. The role of the rectal pits was presumably to increase the numbers of goblet cells and hence the amount of mucus. In the insectivorous bats, the rectal mucosa was thrown into convolutions of varied shapes with a high number of goblet cells. The pattern of the convolutions, however, appeared to be species specific but in each case, they were separated by openings into intestinal glands. The packing of goblet cells in the insectivorous bats appeared more compact than

that of the fruit bat. This is possibly because their diets are low in water quantity and as such would require more lubricants for smooth passage of egesta. The exoskeletons of insects on the other hand are made up of indigestible chitin and are as such passed in faeces.

4.2.0. Quantitative comparisons

Identification of the boundary between the small and the large intestine in the bat can be difficult since neither a caecum nor an appendix is present. Furthermore, the external characteristics of the chiropteran small and large intestine are reported to be the same (Mathis, 1928a; Okon, 1977; Madkour *et al.*, 1982). In the fruit bat this boundary was established as the point of origin of the macroscopically visible longitudinal folds that characterize the colon. However, these were only discernible on the mucosal surface of the opened intestine. The boundary between the foregut and the hind gut in the entomophagous bats was taken to be a point a few millimetres cranial to the anus where the intestinal diameter starts to increase.

For comparative purposes, body mass normalized parameters are an ideal indicator of variations in animals in biomedical experiments. The bats examined in this study that is, *Epomophorus, Miniopterus* and *Rhinolophus* had contrasting mean body weight values of 75.24, 8.92 and 13.43 g respectively. On normalizing the parameters with body mass, it was observed that the fruit bat had a shorter foregut than the insectivorous bats with values of

10.29 mm/g compared to 12.02 mm/g and 23.11 mm/g (see tables 2 - 6) for the rhinolophid and the miniopterine bats respectively. Student t-test ($t = 13.65$, $df = 8$, $p < 0.001$) shows a significant difference in intestinal length between the longfingered bat and the epauleted fruit bat. This is in complete contrast with the previous observations that the frugivorous bat has a longer intestine than the insectivorous bat (see Park & Hall, 1951; Madkour, 1976; Madkour *et al.*, 1982). Most of these previous observations were based on linear measurements, their percentages or body length normalized measurements. The latter varies considerably with body designs and based on these values, the horseshoe bat which has a shorter tail would appear to be having a longer intestine, the discrepancy being in its relatively smaller body length. More investigations based on a wide range of species would, however, give a more reliable picture.

The primary intestinal surface area of the fruit bat of 7688 mm^2 was greater than that recorded for the insectivorous bat of 921 mm^2 but the body mass normalized primary mucosal surface areas were not much different (table 21). The villous and microvillous amplification factors were, however, superior to those of the entomophagous bat resulting in a similarly superior secondary (villous) and absolute (microvillous) surface areas. The absolute surface area of 24951 cm^2 on average or 332 cm^2/ g for the fruit bat is far much greater compared to 1324 cm^2 or 14.8 cm^2/g for the entomophagous

bat. Student t-test ($t = 85.76$, $df = 8$ and $p < 0.001$) shows that the average surface area of the fruit bat intestine is greater than that of the insectivorous bat. The body mass normalized absolute intestinal surface area value of 3.29×10^{-2} m^2 g^{-1} for the fruit bat is about three times that of the insectivorous bat of 1.48×10^{-2} m^2 g^{-1} and about ten times that of the rat of 3.61×10^{-3} m^2 g^{-1} (value for the rat computed from Mayhew & Middleton, 1985). The dimensions of the microvilli (mean length and surface) in both bats showed significant trends in Page's L-trend test (see Miller, 1975) comparable to that described for the rat by Mayhew (1990). These parameters decreased cranio-caudally. Similar trends were recognized in the microvillous packing densities and segmental microvillous numbers. Microvillous packing densities (fig. 56) and microvillous numbers decreased sharply in a cranio-caudal direction (fig. 57). Trends were statitically insignificant in mean microvillous diameters. These trends are represented in figures 54-58.

The qualitative and quantitative results of this study indicate that the fruit bat has a superior intestine than the insectivorous bat. This may in part be explained in terms of the differences in energetic demands of flight and the differences in the types of diet on which these bats thrive. *Epomophorus wahlbergi*, for example, is known to cover long distances of up to 4 km per move and makes many such moves in one night while foraging (Fenton *et al*, 1985) while the maximum distance recorded for *R.*

hildebrandti, is only 2 km (Fenton & Rautenbauch, 1986).

The present study was prompted by a desire to compare the intestinal morphology of bats with different lifestyles. Differences between bats are achieved by intestinal adaptations at several levels of organization. These include increase in intestinal length and circumference and also increase in villous and microvillous amplification factors. Observed differences are most conspicuous at the villous and microvillous levels with the fruit bat having branching and interconnecting villi with relatively higher villous amplification factors than the entomophagous bat. At TEM level, the frugivorous bat has achieved a high surface area by increasing microvillous dimensions (mainly the microvillous length) while the entomophagous bat has attained a comparable surface area by increasing the microvillous packing density rather than the dimensions (see N(mv)/S(v) values of 58 μm^{-2} for the frugivore and 88 μm^{-2} for the insectivorous bat). In birds and mammals, microvillous elongation is part of the process of enterocyte maturation as cells migrate along the crypt-villus axis (Brown, 1962; Van Dongen *et al.*, 1976; Stenling & Helander, 1981; Smith & Brown, 1989) and a feature of variation in cell morphology along the intestine (Mayhew, 1990). In the avian coprodeum, the length and packing density of the microvilli vary with dietary salt load (Mayhew *et al.*, 1992). In rats and hamsters, length may also vary in response to reduced food

(Misch *et al*, 1980; Buschmann & Manke, 1981a, b; Mayhew, 1987). The data obtained in this study show that the chiropteran intestine is much more superior to that of the land-based mammals for which similar data are available (eg. Mayhew & Middleton, 1985; Mayhew, 1987; 1988; 1990) and the fruit bat in particular has values that are remarkably higher than those of the insectivorous bat. The morphometric values for the microvillous dimensions for *Epomophorus* are very close to those reported earlier for another fruit bat, *Rousettus aegypticus,* by Keegan and Mödinger (1979). Microvillous heights of 3.6 μm (Keegan & Mödinger, 1979) and 5.7μm (Tedman & Hall, 1985) reported earlier are comparable to the values for the fruit bat obtained in this study. Although the quantitative methods employed in the current study are stereologically superior to those of Keegan and Mödinger (1979), the microvillous dimensions they obtained (length, diameter and surface area respectively) of 3.6 μm, 0.099 μm and 1.0 μm^2 are not very different from the values obtained in this study (see table 13). Morphometric studies on the chiropteran lung (Maina *et al.*, 1982; Maina & King, 1984; Maina *et al.*,1991) indicate that *E. wahlbergi* did not only have pulmonary diffusing capacity values superior to those of birds and terrestrial mammals, but that it was outstanding among the studied chiropteran species. Data on enteric morphometry in bats is, however, scarce and it is not possible to rank *E. wahlbergi* among other members in this taxon.

The differences in morphometric and structural characteristics between the intestine of the frugivorous bat and the insectivorous bat are notably diverse. Such differences have only been partly explained. Though the nutrient requirements of fruit bats are unknown, they are thought to be comparable to those normally required by other mammals though the precise proportions may be species specific (Wilson, 1988). The diet on which most fruit bats thrive is rich in carbohydrates (Watt, 1968) but low in protein and fat (Morrison, 1980) and protein requirements of most fruit bats probably cannot be met by unsupplemented diets (Wilson, 1988). Various observations have been made in support of the latter contention with reports that some fruit bats may ingest leaves or buds (Cunningham van Someran, 1972; Wickler & Seibt, 1976) while others take in insects in their diets (Wilson, 1973; Gardner, 1977). Thomas (1984) suggested that frugivorous bats consume high levels of fruit to meet critical values of nutrients that are deficient in their diets and probably dissipate the excess energy during flight. This appears to be a more likely method by which fruit bats would make for the deficient nutrients in their natural diets since leaves would most likely result in digestive problems. Capturing insects at night on the other hand may prove difficult for fruit bats since majority of them are incapable of echolocation (Kingdon, 1974). The entomophagous bat has little problems obtaining the required nutrients since insects are rich in proteins

(Bodenheimer, 1951; Morton, 1973) and, though low in carbohydrates, may provide essential levels of the other nutrients. The levels of carbohydrates in insects vary from as low as 0% in crickets to 1.4% in caterpillars (Morton, 1973). Consequently, to meet their energy demands, insectivorous bats must consume greater quantities of prey than was previously estimated and their foraging strategies may be geared towards maximizing energy intake (Barclay *et al.*, 1991).

Although an attempt has been made to explain the superior characteristics of the chiropteran intestine and the observed species differences, conclusive explanations of these peculiarities must not only await broader morphometric studies on a wider range of species, but also additional detailed ventures into the ecological, physiological and flight biomechanical characteristics of the individual species.

This study provides methods for estimating intestinal surface areas in such a way that the results are subject to minimal sampling and estimation biases. The estimates are obtained on per segment basis rather than per micrograph basis. By opening the intestine into a flat sheet and then cutting it into small rectangular segments, an opportunity is created for spinning the tissues without compromising the vertical direction. Spinning allows completely random sections to be obtained. Taking vertical sections and applying cycloid arc test lines provides practically unbiased estimates of surface areas (Baddeley

et al., 1986). Nevertheless the results are not devoid of the various types of technical bias associated with morphometric studies performed on material processed routinely for TEM (Mayhew, 1983) such as Holmes effect (Weibel, 1979). Holmes effect has little impact on the surface area of villi since the latter are relatively large but the microvillous amplification factors are appreciably influenced due to the relatively small size of the microvilli (Mayhew *et al.*, 1990). The microvilli vary in both length and number in different regions and as such it is difficult to estimate this bias. In the rat intestine, this bias has been estimated to be as high as +54% (see Mayhew & Middleton, 1985; Mayhew, 1987).

The other possible source of bias would be tissue shrinkage due to glutaraldehyde fixation and resin treatment. These two have, however, been shown to produce minimal distortion (Hayat, 1981; Burton & Palmer, 1988). The effect of shrinkage on amplification factors is taken to be unimportant since these are ratios that would remain unchanged provided the tissue shrinkage is uniform. Subsequently, estimates of absolute values of surface area would be unaffected since the primary mucosal surface area was estimated on fresh (unfixed) tissues.

The final source of bias was the use of local vertical windows. This is difficult to avoid when dealing with the small gut since the villous surface can be tortuous. In this case, the basal lamina is taken as the best reference for vertical direction.

5.0.0. CONCLUSION.

This study indicates that the chiropteran intestine is structurally and hence functionally better adapted for absorption than that of the land-based mammals for which similar studies have been conducted. The qualitative and quantitative results of this study show that the fruit bat has a "superior" intestine to that of the insectivorous bat. The fruit bat has achieved high intestinal surface areas by increasing villous and microvillous dimensions while the insectivorous bat has achieved slightly lower but comparable surface areas by increasing microvillous packing densities. Though these observed species differences may partly be attributed to differing lifestyles, the role of ecological and phylogenetic constraints remain unknown.

6.0.0. CRITIQUE OF THE METHODS.

This study employed only three species of bats, one representing the suborder Megachiroptera and the other two the suborder Microchiroptera for qualitative and coarse morphometric comparisons. The number of species is thus too few for any general conclusions to be made on either of the two suborders. On the other hand, intensive morphometric analysis was only done in one species in either of the suborders. It is possible that although each of the species would show characteristics representative of the suborder, most of the results obtained here represent mainly the specific rather than generic or taxonomic characteristics.

The specimens studied for the horseshoe bat were of varying body weights, and possibly also varied similarly in age. The morphometric results obtained as such may not be precisely representative of the species. The qualitative observations are, however, not appreciably affected by the age. It is suggested that a study employing more species in either of the suborders, especially those occupying diverse ecological niches need to be carried out. A stereological survey of the volumes and volume densities of the various components of the intestinal tissue would further shed light on the functional capabilities of the intestine of this very unique group of animals.

Problems associated with the sampling procedure and the microscopic techniques are fully addressed under

discussion. A quantification of the enterocyte and organelle volumes and volume densities would, however, have thrown more light on to the functional capabilities of the chiropteran intestine.

7.0.0. REFERENCES

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8.0.0. APPENDIX.

A Worked Example.

One of the bats had a small intestinal length, *L*, of 180 mm. The length of each of the five segments obtained in macroscopic sampling thus was 36 mm. The circumference (average width, W) of the first segment (S1) was 5.5 mm.

Level I: The primary mucosal surface area, S(pm), for S1 therefore was 5.5 x 36 = 198 mm^2. This step is repeated for all the segments i.e. S1, S2 .....S5, bearing in mind that the only variable is W.

Level II: At light microscopic level, two micrographs were prepared from each segment.

The total number of intersections between the villous surface profiles and the cycloid lines for S1 was 42 while those between the latter and the primary mucosal surface was 6. The villous amplification factor, S(v)/S(pm), was 42/6 = 7. The surface of villi, S(v), thus was

198 x 7 =1386 mm^2.

Level III: At EM level, five micrographs obtained from each segment were used. The lattice grid with cycloid arcs was placed on each of the micrographs and intersection counts between the microvilli and the arcs, Imv, and those between the latter and apical enterocyte surface, Iem, estimated and summed over all the five micrographs. Imv was weighted twice by conveniently placing the lattice grid two times and making the counts twice but being careful to maintain the vertical direction.

In a similar manner, I(em) was weighted five times. The weighting redduces estimation errors when making the intersection counts. The estimates for S1 were as follows:

I(mv) = 589 intersection counts. The working total of the intersection counts is thus the product: 5 x 589 = 2945.

The intersection counts on the enterocyte apical membrane/microvilli interface, I(em) = 49. This was weighted twice with a resultant working value of 2 x 49 = 98.

The microvillous amplification factor was the ratio I(mv)/I(em).

Thus: S(mv)/S(v) = 2945/98

= 30.05

Subsequently, the surface area of microvilli in the segment was

S(mv) = S(v) x [S(mv)/S(v)]

= 1386 x 30.05

= 41649.30 mm^2 or 41649 x 10^6 μm^2

A calculation as outlined above was carried out for each of the other segments (i.e. S2 - S5). The surface area of microvilli for each of the segments is given below. All values are given in millimetres squared.

S2 = 52949.00, S3 = 17389.08, S4 = 8514.72

S5 = 8771.33

The total intestinal surface area for bat 1, therefore, is the summation of all these segmental values, thus S(mv)t

= 129273.43 mm^2 or 0.13 m^2. The average microvillous diameter, d(mv), and the average microvillous height, h(mv), were 0.0998 and 1.103 microns respectively.

The resultant average microvillous surface area was therefore given by the formula:

$$\pi \times d(mv) \times h(mv)$$

$$= 3.14 \times 0.0998 \times 1.103 = 0.3458\ \mu m^2$$

The microvillous packing density, i.e. number per unit area was the ratio of the microvillous amplification factor to the surface area of the individual microvillus in the same segment, thus:

30.05/0.3458 = 87 microvilli per square micron

The total number of microvilli was the ratio of the total microvillous surface area of the segment to the surface area of the average microvillous in the same segment, thus:

$41650 \times 10^6/0.3458 = 1.2 \times 10^{11}$ microvilli.

Check up calculation: The total surface area was checked directly by multiplying the surface area of the average microvillous by the total number of microvilli in a particular segment. For *Miniopterus inflatus* bat No. 1 , the microvillous surface area for the first segment (S1) was estimated to be $41649 \times 10^6\ \mu m^2$.

The surface area of the average microvillous in the same segment was 0.3458 μm^2 and the total number of microvilli was 1.2×10^{11}.

The directly estimated microvillous surface area therefore was

$$= s(mv) \times N(mv)$$

$$= 0.3458 \times 1.2 \times 10^{11} = 41496 \times 10^{6}\ \mu m^2$$

This shows an error of only about 0.37%.