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Anatomical study of the intestine of the insect-feeder bat, *Myotis frater kaguae*

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INTRODUCTION

The bat is a very interesting mammal that is peculiar in its life style and flight. It is phylogenetically important to obtain knowledge of structural features of the bat intestine in relation to its adaptation to this life style. It is well known that the caecum is absent in the bat (Owen, 1868; Mitchell, 1905; Jacobshagen, 1967; Kent, 1978); moreover, the external aspects of the small and large intestine are similar and hence they are not distinguishable using these criteria (Mathis, 1928*a*; Okon, 1977; Madkour, Hammouda & Ibrahim, 1982). Using the light microscope, villi (Okon, 1977; Madkour *et al.* 1982) and/or folds (Mathis, 1928*a*) are observed on the mucosa of the small intestine. In the insect-feeder bat, it is reported that the colon is absent and that the large intestine is composed only of the rectum, the epithelium of which consists almost entirely of goblet cells (Okon, 1977). However, the demarcation between the small and large intestine is not easily seen and the mucosal structure is not properly elucidated.

Thus, in the present study, the whole intestine of the insect-feeder bat, *Myotis frater kaguae*, was investigated systematically using the naked eye, light microscopy, scanning and transmission electron microscopy.

MATERIALS AND METHODS

Nine long legged whiskered bats, *Myotis frater kaguae* (eight adult females and one young adult male), obtained in July in Kamikawa shrine, Asahikawa City, Hokkaido, Japan, were used in the present study. They weighed about 8 g each and had a forearm length of about 40 mm. Under ether anaesthesia, the abdominal cavity of each animal was opened to allow penetration of the fixative. They were killed immediately by cutting the common carotid artery and fixed *in toto* by immersion in 4% phosphate-buffered paraformaldehyde, pH 7·4, for at least 48 hours. The mesenteries, arteries and external aspects of the intestine were observed under an operation microscope and schematic drawings were made.

For scanning electron microscopical observations, the intestine was opened by longitudinal incision, and appropriate portions of it were removed and placed in modified Karnovsky's fixative (4% paraformaldehyde and 1·25% glutaraldehyde in 0·067 mol/l phosphate buffer, pH 7·4) for 2 hours. After washing in the same buffer, specimens were treated using the osmium-thiocarbohydrazide-osmium staining method (Takashio & Yamauchi, 1975; Ono & Takashio, 1978). They were then dehydrated in graded ethanol and transferred to isoamyl acetate for critical point

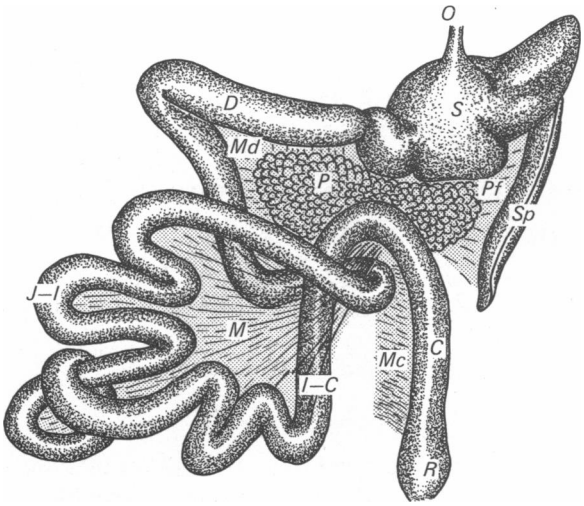


Fig. 1. Schematic drawing of the external aspects of the intestine and mesenteries. Ventral view. *O*, oesophagus; *S*, stomach; *P*, pancreas; *Sp*, spleen; *D*, duodenum; *J–I*, jejuno-ileum; *I–C*, ileo-colon; *C*, colon; *R*, rectum; *Md*, mesoduodenum; *Pf*, peritoneal folds extended to the stomach and spleen; *Mc*, mesocolon; *M*, mesentery. The greater omentum is removed.

drying with liquid carbon dioxide. The dried specimens were viewed in a scanning electron microscope without further metal coating.

For light and transmission electron microscopy, portions of intestine corresponding to the specimens taken for scanning electron microscopy were removed. Some blocks were washed overnight in buffer, dehydrated immediately in ethanol and embedded in a resin, Acrytron E (Mitsubishi Rayon Co). They were then cut into 3 μm sections. These sections were stained with haematoxylin and eosin and observed by light microscopy. Other specimens were fixed in modified Karnovsky's solution for 2 hours. After washing overnight in buffer, they were postfixed with 1% osmium tetroxide in phosphate buffer. They were then dehydrated in ethanol and embedded in Epon 812. Ultrathin sections were cut and stained with uranyl acetate and lead nitrate. Ultrathin sections were viewed in a transmission electron microscope.

RESULTS

Macroscopic observations

Figure 1 shows the topography, external features and mesenteries of the intestine in *Myotis frater kaguae*. The intestine was a short, convoluted tubule without a caecum and was about 12 cm in length. It was almost uniform in diameter throughout its length except for the distal end which was slightly dilated and did not possess an appendix, taeniae coli, haustra coli or appendices epiploicae. Therefore, the small and large intestines were almost similar in their external appearances. However, the intestine was differentiated into five portions according to their topographical situation and microscopic features. (1) *Duodenum*: From the distal end of the pylorus, the duodenum extended transversely toward the right abdominal wall along the visceral surface of the liver. It then turned caudally and ran along the right, dorsal, wall of the abdomen. Then, it curved again toward the midsagittal plane and continued to the jejuno-ileum beneath the transitional portion of the ileo-colon and

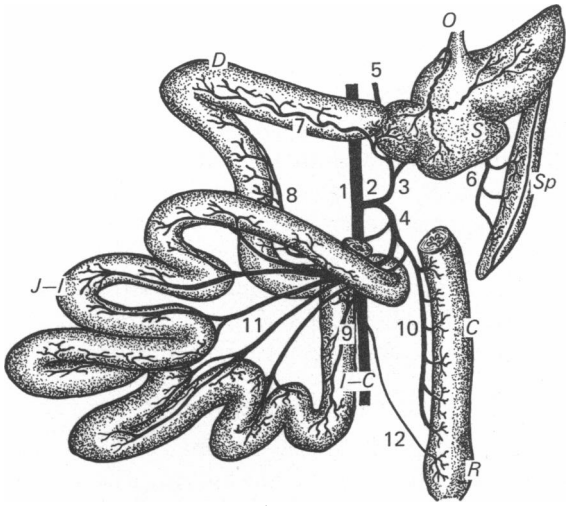


Fig. 2. Schematic presentation of the arterial supply to the intestine. 1, abdominal aorta; 2, common trunk of abdominal visceral artery; 3, coeliac trunk; 4, cranial mesenteric artery; 5, common hepatic artery; 6, splenic arteries; 7, cranial, pancreaticoduodenal artery; 8, caudal pancreaticoduodenal artery; 9, a branch to the ileo-colon; 10, a branch to the colon; 11, jejuno-ileal arteries; 12, caudal mesenteric artery; *O*, oesophagus; *S*, stomach; *Sp*, spleen; *D*, duodenum; *J–I*, jejuno-ileum; *I–C*, ileo-colon; *C*, colon; *R*, rectum. Pancreas, mesenteries and the transitional portion from ileo-colon to colon of the intestine are removed.

colon described below. The length of the duodenum was about 2·5 cm. (2) *Jejuno-ileum*: The jejunum and ileum were coiled tubes, the jejunum passing imperceptibly into the ileum, so that these portions were treated together as the jejuno-ileum in the present study. The total length of the jejuno-ileum was about 7 cm. Peyer's patches were not visible macroscopically in the jejuno-ileum. (3) *Ileo-colon*: The part of the intestine succeeding the jejuno-ileum ascended cranially for about 1 cm from the caudal abdominal cavity near the urinary bladder. Although this portion of the intestine was topographically equivalent to the ascending and transverse colon in other more common mammals such as rats, mice, etc., numerous villi were present on the mucosal surface. Therefore, it was convenient to call this portion the 'ileo-colon' in this study. (4) *Colon*: Near the common trunk of the visceral artery (Fig. 2), the intestine turned caudally and ran in an almost straight line toward the anus, as the colon. (5) *Rectum*: Immediately before reaching the anus, the intestine was somewhat dilated; this portion appeared to be the rectum.

The root of the mesentery was attached to the dorsal abdominal wall between the kidneys. From the attachment, thin folds spread cranially. The folds covered the right half of the pancreas and were attached to the duodenum forming a short mesoduodenum. The folds covered the left half of the pancreas and spread between the greater curvature of the stomach and the spleen. Another part of the peritoneal folds, the mesentery, extended caudally in the space between the duodeno-jejunal junction and the transition between ileo-colon and colon. It spread out with the jejuno-ileum attached to it and also suspended the ileo-colon. The colon was fixed by a short mesocolon to the midline of the dorsal abdominal wall along the right side of the abdominal aorta. The very short rectum was devoid of a mesentery.

Figure 2 shows the arterial supply to the intestine. Most of the arterial supply to the abdominal viscera arose from the abdominal aorta by a single common trunk. It

immediately bifurcated into the coeliac trunk and the cranial mesenteric artery. The coeliac trunk ran cranially and bifurcated again. One branch of the coeliac trunk approached the liver and gall bladder as the common hepatic artery and the other ran to the stomach and spleen as the splenic artery. On the dorsal aspect of the pylorus, the common hepatic artery gave off a branch to the pylorus and the proximal duodenum: this was the cranial pancreaticoduodenal artery. The cranial mesenteric artery bent caudally and gave off the caudal pancreaticoduodenal artery and then the cranial mesenteric artery bifurcated, one of its branches supplying the ileo-colon and the other the colon. The cranial mesenteric artery then divided into several branches which approached the jejuno-ileum as the jejuno-ileal arteries. A fine caudal mesenteric artery branched off from the abdominal aorta at the caudal level of the left renal artery. It descended toward the rectum and penetrated its wall.

Microscopic observations

Numerous villi were observed on the luminal surface of the duodenum and jejuno-ileum with the scanning electron microscope. These villi were tongue-like in shape (Fig. 3) and irregular shallow grooves and small droplets of mucus were seen on the surface. The light microscope revealed long villi on the luminal surface of the duodenum and jejuno-ileum, which were covered by a simple columnar epithelium with goblet cells interspersed among the columnar cells (Fig. 4). More distally in the jejuno-ileum, the goblet cells gradually increased in number. Brunner's glands were restricted to the initial portion of the duodenum just distal to the pylorus. In most of the duodenum and in the whole length of the jejuno-ileum, tubular intestinal glands (crypts of Lieberkühn) were present.

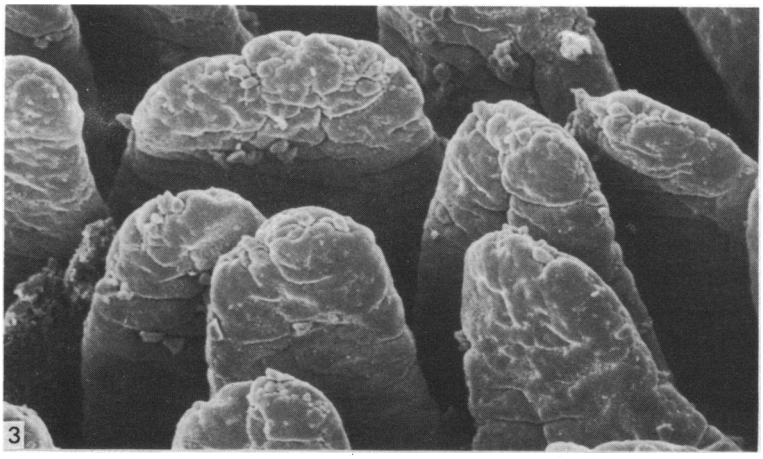
The muscularis mucosae and submucosa were not well developed and the muscular layers were thin in the duodenum and jejuno-ileum. Few lymph nodules were seen in these portions.

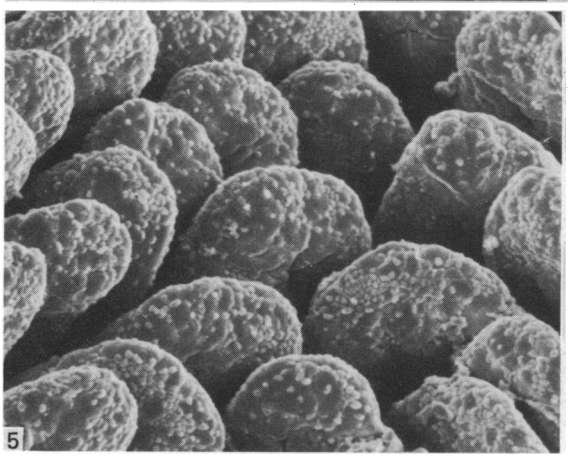
On the mucosal surface of the ileo-colon, tongue shaped villi were also observed (Fig. 5). On the surface of the villi, especially the apical parts, numerous mucous droplets were seen. Using the light microscope, the structure of the villi, crypts and other parts of the ileo-colon were very similar to those of the duodenum and jejuno-ileum. However, large lymph nodules were occasionally seen in the ileo-colon. Throughout the duodenum, jejuno-ileum and ileo-colon, no circular folds (plicae circulares) were observed.

With the transmission electron microscope, the tall columnar cells and goblet cells of the mucosal epithelium of the duodenum, jejuno-ileum and ileo-colon were found to have the typical structure seen in mammals. The apical surface of the tall columnar cells were covered with closely packed microvilli (Fig. 6) which were about 1·3 μm long and 0·1 μm wide.

Figure 7 shows the mucosal surface of the transitional portion from ileo-colon to colon. Villi decreased in height at the end of the ileo-colon and finally disappeared at the boundary between ileo-colon and colon where transverse folds were formed. Broad longitudinal folds were formed by deep grooves in the colon. The light microscope showed that the mucosa at the end of the ileo-colon was thrown into four to six broad folds which appeared to consist of narrow, short villi (Fig. 8). Numerous goblet cells were seen in the villi and crypts.

The mucosa of the colon had no villi and was thrown into broad longitudinal folds. Slit-like narrow openings of crypts and many mucous droplets were observed on the surface of the folds (Fig. 9). The openings of the crypts were surrounded by





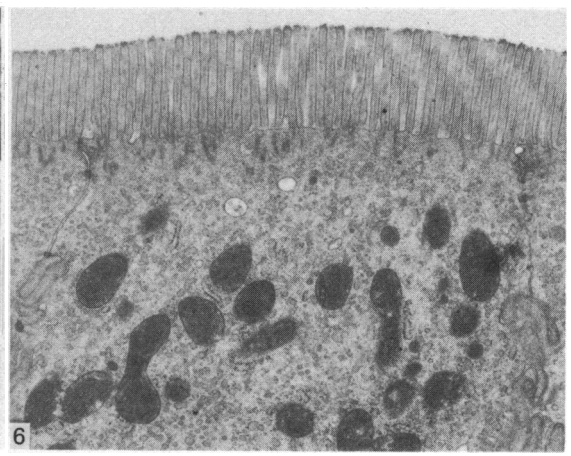


Fig. 3. Scanning electron micrograph of the luminal surface in the duodenum. Tongue shaped villi are observed. × 200.

Fig. 4. Light micrograph of the villi in the proximal portion of the jejuno-ileum. Villi are covered with simple columnar cells, and goblet cells are interspersed among the columnar cells. Haematoxylin and eosin staining. Goblet cells are seen darkly because a green filter was used to increase the contrast of the micrograph. × 200.

Fig. 5. Scanning electron micrograph of the luminal surface of the ileo-colon. Numerous droplets are seen on the apical surface of the tongue shaped villi. × 200.

Fig. 6. Transmission electron micrograph of the apical part of the columnar cells in the proximal portion of the jejuno-ileum. Closely packed parallel microvilli are seen. × 10000.

very low ridges, which resembled the stroma and guard cells of plants. As seen with the light microscope, the folds varied in height and width, and the submucosal layer invaginated into the larger folds (Fig. 10). The crypts of the colon formed straight tubes and were longer than those of the jejuno-ileum and ileo-colon. The muscular layers were also thicker than those in the ileo-colon. The mucosa was lined with simple columnar epithelium and goblet cells. Transmission electron microscopy demonstrated that the columnar cells possessed well developed microvilli similar to those of the jejuno-ileum and ileo-colon (Fig. 11).

In the rectum, the longitudinal mucosal folds were very low and the mucosal surface was relatively flat. Low ellipsoidal ridges surrounded the fissure-like openings

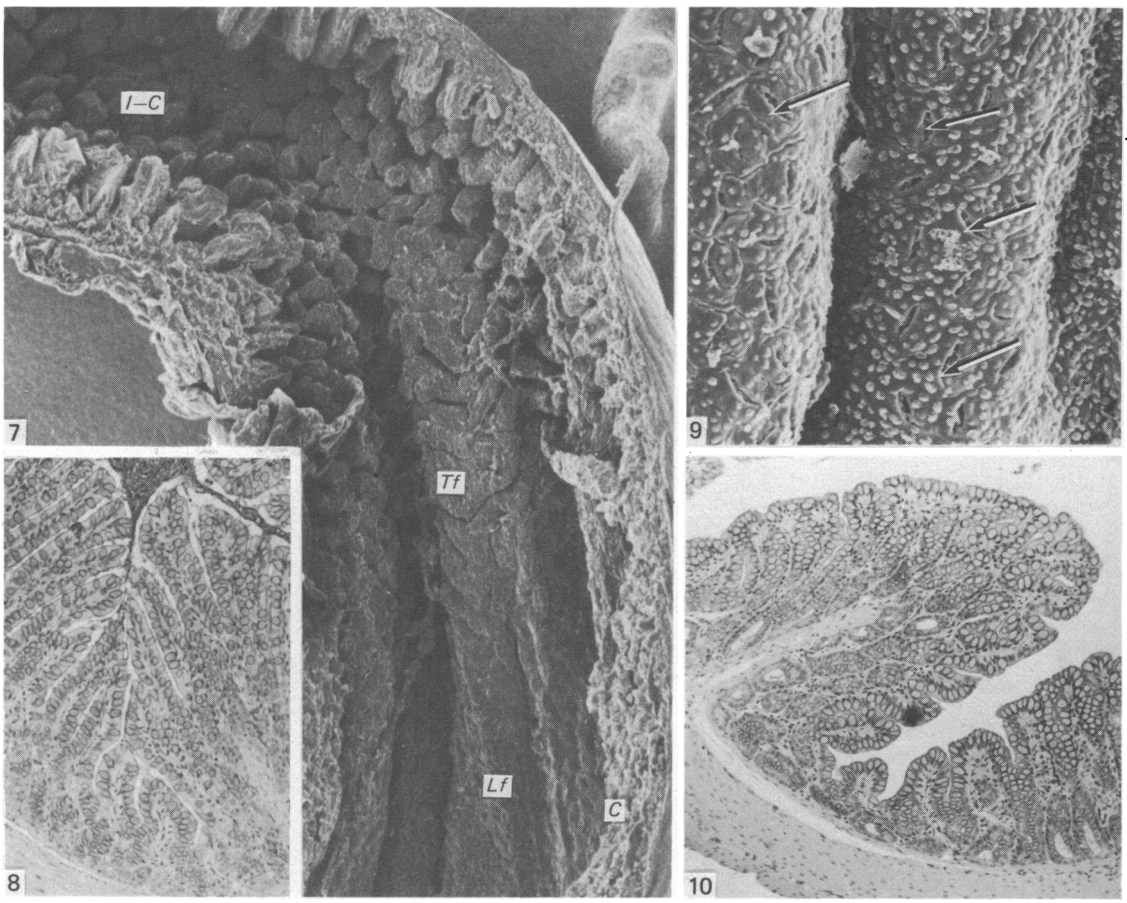


Fig. 7. Scanning electron micrograph of the luminal surface of the transitional portion from the ileo-colon (*I–C*) to the colon (*C*). The height of the villi gradually decreases in the end of the ileo-colon. The villi disappear and low transverse folds (*Tf*) are observed on the border between ileo-colon and colon. The longitudinal mucosal folds (*Lf*) are formed by deep grooves toward the colon. × 40.

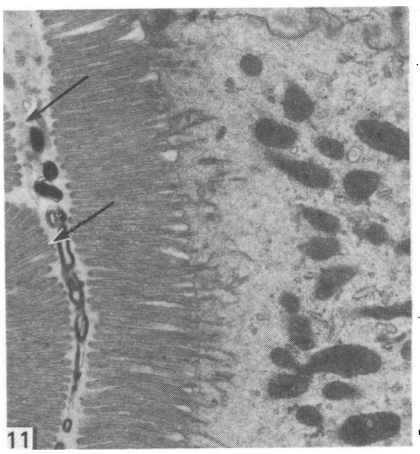
Fig. 8. Light micrograph of a cross section at the level of disappearance of the villi. Mucosa are thrown into broad folds which seem to consist of short, narrow villi. Goblet cells are abundant. Haematoxylin and eosin staining. × 100.

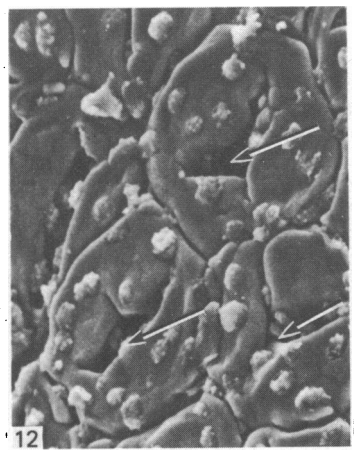
Fig. 9. Scanning electron micrograph of the luminal surface of the colon. Slit-like openings of the crypts (arrows) and a large number of mucous droplets are observed on the surface of the longitudinal mucosal folds. × 150.

Fig. 10. Light micrograph of a cross section of the colon. One fold is invaginated by submucosal layer. The folds vary in height and width. Enormous goblet cells are present. Haematoxylin and eosin staining. × 100.

of the crypts (Fig. 12). Mucous droplets were observed on the ridges and the side walls of the openings of the crypts, but their number was smaller than those of the colon. The mucosal folds of the rectum were very low in height and the crypts were short. The mucosa was lined with a simple columnar epithelium and goblet cells. The microvilli of the columnar cells were relatively sparse and not uniform in length (Fig. 13).

Throughout the entire length of the intestine, there were several types of endocrine cells in the villi and crypts and Paneth cells were present in the bottom of the crypts.





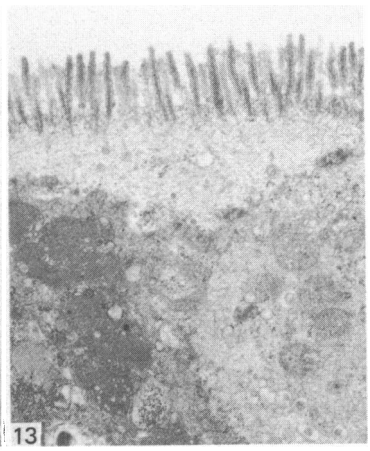


Fig. 11. Transmission electron micrograph of the apical part of the columnar cells on the side wall of the crypt in the colon. Well developed microvilli are also seen. A lumen of a crypt is indicated by arrows. × 10000.

Fig. 12. Scanning electron micrograph of the luminal surface of the rectum. The fissure-like openings (arrows) are surrounded by low ellipsoidal ridges. Mucous droplets are seen on the ridges and the side walls of the openings of the crypts. × 300.

Fig. 13. Transmission electron micrograph of the apical part of the columnar cells in the rectum. Microvilli are relatively sparse and not uniform in length. × 10000.

DISCUSSION

The length of the bat intestine is one third to one fifth of that in a mouse of similar weight (Klite, 1965). In particular, the intestine of the insect-feeder bat is shorter than that of the fruit-feeder bat (Park & Hall, 1951; Madkour, 1977; Okon, 1977; Madkour *et al.* 1982). Mathis (1928*a*) observed that the short length of the bat intestine contributes to the low body weight, possibly affecting the bat's flight. In the present study, the insect-feeder bat, *Myotis frater kaguae*, also has a short intestine of about 12 cm long.

One of the most characteristic features of the bat intestine, confirmed in the present study, is that the caecum is absent and hence the small and large intestines are indistinguishable externally (Park & Hall, 1951; Okon, 1977; Madkour *et al.* 1982). On the other hand, Forman (1974*a*, *b*) reported that many species of New World bats, including insect-feeder bats, have large Peyer's patches which are easily observed macroscopically at the ileo-colonic junction, which protrudes from the intestinal wall as an ampulla or vestibule. He considered that these Peyer's patches may represent the beginning or remnant of a caecal structure. In the present study, the intestine was almost uniform in calibre throughout its whole length, and did not show the usual features of the colon nor were Peyer's patches as seen by Forman (1974*a*, *b*) observed at the transition from ileo-colon to colon.

However, the mucosal surfaces of the small and large intestines were clearly distinguished by scanning electron microscopy in the present investigation. Numerous tongue shaped villi are present in the duodenum, jejuno-ileum and in the 'ileo-colon'. The portion with villi is considered to be the small intestine although the ileo-colon corresponds topographically to the ascending and mid-colon of most other mammals. Villi have been investigated with the light microscope in other species of bats (Okon,

1977; Madkour *et al.* 1982), but the mucosal folds reported by Mathis (1928*a*) have not been observed in the present study.

In the fruit-feeder bat, *Rousettus aegyptiacus*, glucose and fructose are absorbed three to four times faster than in the laboratory rat (Keegan, 1977), related to the threefold greater length of microvilli of the columnar cells in the bat small intestine, according to Keegan & Mödinger (1979). In the insect-feeder bats, the transit time through the intestine is very fast (Klite, 1965), even though they are heavy feeders (Gould, 1955). In *Myotis frater kaguae*, however, the microvilli do not differ greatly from those of the rat (Palay & Karlin, 1959; Millington & Finean, 1962). The rapid absorption of nutrients may be assisted by the numerous villi which are present in the greater part of the intestine in the insect-feeder bat.

In the insect-feeder bat, *Tadarida nigeriae*, Okon (1977) reported that the colon is completely absent, and that the large intestine, defined histologically, consisted of only the rectum, the mucosa of which was made up almost entirely of the goblet cells. Okon stated that a meal of insects requires a copious supply of mucus but not much liquid, hence, only the rectum is well developed. However, in the bats investigated in the present study, there are numerous goblet cells in the distal portion of the jejuno-ileum and in the ileo-colon, and the histological structures of the large intestine are different from those of the rectum in *Tadarida nigeriae*. The mucosa of the large intestine in *Myotis frater kaguae* has columnar cells with well developed microvilli although goblet cells are also plentiful. Therefore, it is suggested that a considerable length of the large intestine formed the colon in the bats studied here. This conclusion is supported by the finding of the arterial supply study discussed below.

It is said that the blood supply of the bat intestine is comparable to that of other mammals (Kallen, 1977). However, in *Myotis frater kaguae*, the arterial distribution is markedly different to that in other species of bats. The coeliac trunk and cranial mesenteric artery branch off from the abdominal aorta as a single common trunk and almost all the intestinal arteries branch off from the cranial mesenteric artery. The caudal mesenteric artery is distributed only to the short portion of the end of the large intestine. In most mammals, the inferior or caudal mesenteric artery distributed generally to the hindgut. In the bats of the present study, it is possible that the part of the large intestine derived from the hindgut is restricted to the very short portion immediately proximal to the anus, and that the colon, as defined in the present study, is derived from the midgut.

It is known that Paneth cells, which are commonly restricted to the small intestine in other mammals and birds (Sandow & Whitehead, 1979), are present in the entire length of the intestine of the bat (Hamperl, 1923; Mathis, 1928*b*). Moreover, neurotensin-, glucagon- and secretin-immunoreactive endocrine cells, which are present in the small intestine in most mammals (Solcia *et al.* 1981), are also present in the distal half of the intestine in the vampire bat (Yamada *et al.* 1984). These two facts also suggest that the part of the large intestine derived from the hindgut is underdeveloped in the bat, although further studies are necessary.

Thus, in *Myotis frater kaguae* it is concluded that the greater part of the intestine is small intestine containing numerous villi, and that the large intestine is relatively short. In particular, the rectum, which may be only the derivative of the hindgut, appears to be the very short portion immediately proximal to the anus.

SUMMARY

The intestine of the insect-feeder bat, *Myotis frater kaguae*, was studied macroscopically, with the light microscope, and with the scanning and transmission electron microscopes. Macroscopically, the intestine was a short and convoluted tubule without a caecum, and the small and large intestines were not distinguishable using external criteria. The cranial mesenteric artery supplied almost the entire length of the intestine except the very short portion immediately proximal to the anus, which was supplied by the caudal mesenteric artery. Microscopically, numerous tongue shaped villi were observed on the mucosal surface in the duodenum and the jejuno-ileum. Moreover, similar villi were also present in the ascending portion succeeding the jejuno-ileum although that portion was topographically equivalent to the ascending and transverse colon of other mammals. These villi were covered by typical columnar cells with microvilli, and the goblet cells were abundant in the distal part of the intestine. The large intestine, which did not possess villi, was about one tenth of the length of the whole intestine. Columnar cells with well developed microvilli and numerous goblet cells were observed on the mucosa of the large intestine. Therefore, in this type of bat, the greater portion of the intestine was considered to be a small intestine, and the large intestine was restricted to the short portion near its end, the colon, and the rectum immediately proximal to the anus.

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