Is there a functional large intestine in the ferret?

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Summary. The intestine of the ferret *(Putorius furo)* is unusual in that there is no external anatomical division between ileum and colon. Up to 8–10 cm from the anus the electrical activity was organized into migrating myoelectric complexes typical of the small intestine. At this point the pattern of electrical activity changed abruptly to that characteristic of the colon, namely short and long spike burst activity. Histological examination showed that at this point the muscular layers were interupted by a band of connective tissue sufficient to permit the functional autonomy of the last part of the intestine.

Some carnivores and insectivores such as the ferret *(Putorius furo)* have no caecum and have no externally visible anatomical division into ileum and colon, the intestine appearing as one continuous tube[2]. In many species the pattern of electrical activity and associated motility changes abruptly at the ileo-caecal junction[3]. It therefore seemed of interest to determine the nature of the electrical activity in the ferret where no division into small and large intestine is seen.

In the small intestine a typical pattern of activity consists of migrating myoelectric complexes (MMC) traversing the length of the small intestine[4,5]. Each MMC consists of irregular spiking activity (ISA) in which some of the slow waves have spike bursts superimposed on them; regular spiking activity (RSA) in which every slow wave has a spike burst associated with it and then a period of quiescence consisting of slow waves alone before ISA recurs. Each phase of the MMC recurs at any one point in the intestine at 20-80-min intervals in different species[4].

In the large intestine no migrating myoelectric complexes are seen and slow waves only occur at irregular intervals[6]. Electrical activity is characterized by 2 patterns of spike burst which are independent of slow wave activity: long spike bursts (LSB) are associated with onward propulsion of large intestinal contents while short spike bursts (SSB) are the electrical correlates of local mixing movemenrs[3,7].

It might be expected that the MMC pattern of activity would be propagated to the large intestine by electrotonic conduction through the smooth muscle. This does not seen to occur, at least in dogs, and the ileo-colonic junction appears to be an electrically silent zone which prevents the myogenic propagation of electrical events from ileum to the colon[8].

As has already been indicated, the ferret intestine appears to be a simple indifferentiated tube. This work was performed to investigate whether the MMC pattern of activity persisted throughout the ferret intestine, or whether there was a functional differentiation into small and large intestine for the type of electrical activity and hence the pattern of motility. If there were changes in electrical activity, it would then be of interest to find out whether there was any histological basis for this functional differentiation.

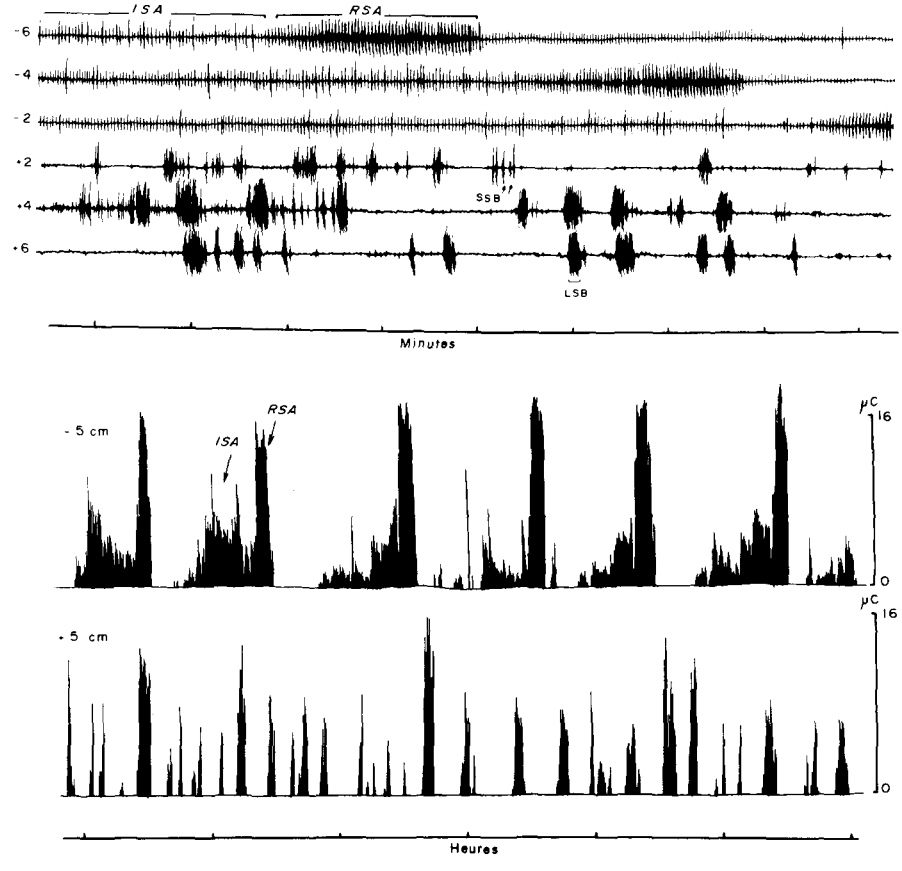

Fig. 1. Direct and integrated electromyograms from the terminal part of the intestine in ferret. The oral limit of the subserosal lymphoid formations was considered as the reference point and the distances were expressed in centimeters from this site. Note on the direct record the presence of rhythmic slow waves (28–32 c/min) and migrating myoelectric complexes (ISA and RSA) typical of the small intestine oral to this site. At electrode sites caudal to the reference points long (LSB) and short spike bursts (SSB) typical of the colon in other carnivores were observed. The integrated record clearly shows the different patterns of spiking activity recorded from these 2 areas.

Methods. 4 adult ferrets, weighing 400–600 g, were housed singly in wire-bottomed cages and received 100 g of reconstituted milk daily. Under halothane anaesthesia (Fluothane ND), 8 groups of Ni/Cr electrodes (0.12 mm in diameter and 1 m in length) were implanted at 20, 10, 5 and 2 cm orally and 2.5 and 10 cm aborally to the anterior limit of the subserosal lymphoid formations which are a characteristic of the caecal region in carnivores[9] and were taken as a reference point. The free ends of the electrodes were brought s.c. to the back of the neck.

The electromyograms were first recorded 5 days after surgery (Reega VIII - ALVAR) and for 10 consecutive hours each day. In addition, the electrical activity was continuously plotted at 20-sec intervals using a double linear integrator circuit connected to a potentiometric recorder. At the end of the recording period (15 days), the animals were killed with ethyl-ether and each electrode site was precisely measured. The terminal part (20 cm) of the gut was removed, a glass tube was inserted into its lumen before it was fixed in a 10% formalin solution. 2 days later, serial longitudinal sections (5 µm thick) of 1-mm portions of the region showing changes in the electromyographic records were made and the histological preparations were stained with hematoxylin-eosin.

Results. Electromyographic records. The electrical activity of the groups of electrodes oral to the subserosal lymphoid formations was characterized by continuous slow waves occurring at a frequency of 30.6±0.6 cycles per min (mean±SE for 4 animals) and 29.8±0.5 c/min (3 animals) at 5 and 2 cm oral to the sub-serosal lymphoid formations respectively. Slow waves were absent at 2 cm orally from this reference site in 1 animal (table).

The spiking activity recorded on the 4–5 electrode sites oral to the reference point was characterized by a recurring sequence of irregular (ISA) and regular (RSA) spiking activity and quiescence and was similar to the pattern of activity observed in the dog ileum. The ISA and RSA phases, the 2 components of the migrating myoelectric complexes, lasted 37.2±6.7 min and 5.9±0.4 min respectively. These 2 consecutive phases were propagated from the 1st to the 4th-5th group of electrodes and appeared on the ileum at 67±13-min intervals (figure 1).

The electrical activity observed with the groups of electrodes caudal to the reference point was markedly similar to that observed in the colon of other carnivores. The slow waves occurred irregularly, their frequency varying in the range of 9–13 c/min; they occupied only 7% of the recording time. In addition, electromyograms exhibited long and short spike bursts. At 5 cm from the reference point duration of LSB was 21.3±7.8 sec (mean±SE for 4 animals) and SSB was 4.6±2.5 sec. Spike amplitude was 100–150 µV for SSB and LSB. The LSB appeared at 2 or 5 cm from the junction and were propagated aborally at a velocity of 5–6 cm per min. In contrast, SSB occurred in series at one electrode site and were not propagated over more than 5 cm.

Histological observations. Longitudinal sections at the level of the theoretical junction between ileum and colon performed on 3 animals revealed a 110–260-µm-wide disruption in the continuity of the muscular layers. The region of this disruption varied in a range of 1–1.5 cm from each site of the reference point, i.e. the oral limit of the lymphoid formation. This electrical isolator strip consisted of a fibrous connective tissue which expanded from the submucosa to the serosa and was accompanied by an artery and vein (figure 2). Progressive longitudinal displacement of this submucosal-serosal fibrous tissue as shown in serial sections suggested a spiral configuration of this fibrous strip.

Discussion. This study demonstrates that the electrical activity of the distal part of the ferret intestine is different

Characteristics of the electromyogram of the terminal part of the intestine of the ferret (mean±SE of 4 animals)

	Distance from the oral limit of lymphoid formation (cm)			
	−5	−2*	+2	+5
Slow-waves				
Frequency (c/min)	30.6±0.6	29.8±0.5	9.2±2.7	10.1±3.4
Spikes				
MMC/12 h	20.2±3.4	-	-	-
Duration of LSB (sec)	-	-	16.5±6.4	21.3±7.8
Duration of SSB (sec)	-	-	4.3±1.8	4.6±2.5

* Mean for only 3 animals, no slow-wave activity being detected at this electrode site for the last animal.

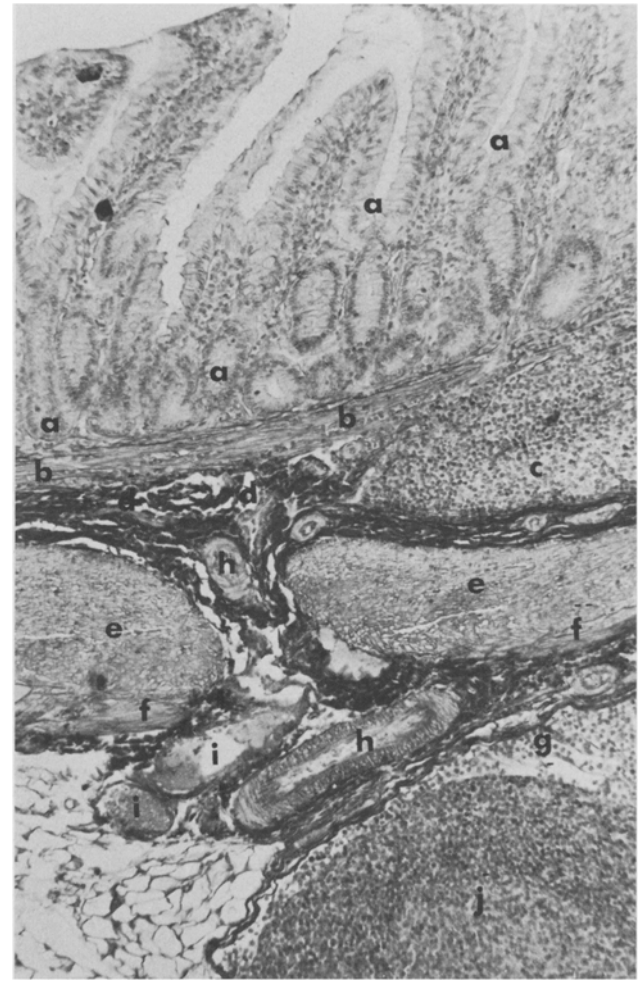

Fig. 2. Longitudinal section of the intestinal wall (anti-mesenteric border) at the supposed ileo-colonic junction in a ferret (hematoxylin and eosin; × 120). Glands of the ileum (a); muscularis mucosae (b); lymph node (c); submucosa (d) circular (e) and longitudinal (f) smooth muscle layer; serosa (g); artery (h); vein (i), mesenteric lymph node (j). Right and left parts correspond to ileum and colon respectively. Note the disruption of the 2 muscular layers by fibrous connective tissue, originating in the submucosa and the associated vessels coming from the posterior mesenteric artery and vein. This strip of connective tissue will prevent the myogenic spread of the MMC pattern of electrical activity to the terminal portion of intestine.

from the more proximal part even though there is no external anatomical differentiation between these 2 regions. The myoelectric complexes observed oral to the sub-serosal lymphoid formations had similar durations of the phases of the MMC and the frequency of the slow waves to those previously described for the ileum in carnivores[4,5]. The last part (about 10 cm) of the intestine in the ferret caudal to the lymphoid formations exhibited a pattern of colonic electrical activity similar to that previously described in the dog[3,6] and other species[10]. The oral limit of the serosal lymphoid formation thus appears to be a good indicator or the transition from ileum to colon.

This change in electrical activity was observed despite the absence of a caecum and an ileo-colonic sphincter in the ferret. The presence of a sphincter and the anatomical differentiation into small and large intestine does not appear to be necessary for the generation of typical small and large intestinal electrical activity patterns. This is confirmed by results showing the absence of both colonic motor changes and bowel habits after ileo-colonic sphincterectomy in dog (unpublished results).

a A histological change was observed at the point of functional transition from small to large intestine. At this point the continuity of the muscle layers was disrupted by a band of connective tissue which may be considered as an 'electrical insulator'. This insulator prevents slow waves and spike propagation from the ileum to the colon in a manner similar to that observed at the ileo-colonic sphincter on the dog[8]. There can be no electrotonic spread of the myoelectric complex to the last 10 cm of intestine in the ferret.

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Taste responsiveness of the transplanted supernumerary leg in the fleshfly *Sarcophaga bullata*[1]

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Summary. Ectopic legs were produced on the abdomen of the fleshfly *Sarcophaga bullata* by transplantation of prothoracic leg imaginal discs. Stimulation of the tarsal chemoreceptors of these ectopic legs with sucrose solution resulted in the extension of the host proboscis indicating the functional connectivity of the sensory axons.

During development of the nervous system, the growing axons navigate towards and find their target tissues; having reached a given target, they establish specific patterns of connectivity. Proper functioning of the nervous system depends upon the appropriateness of these connections. How axons actually navigate and establish specific connectivity is still far from clear[3,4]. Taste reponsiveness in flies seems to be a promising system for the analysis of 2 fundamental problems of developmental neurobiology, namely, axonal navigation and neuronal specificity. In flies, taste receptors (chemosensory hairs with water, sugar and salt receptors) are located on the tarsal segments of the legs. When a hungry or thirsty fly lands on a potential food or water source these sensilla send impulses to the central nervous system which results in the extension of the fly's proboscis to initiate feeding[5]. This is called the proboscis extension response (PER). A positive PER is an indication of the existence of sensory axons in the leg as well as their specific connectivity with the central neurons. To study the developmental aspects of neural connectivity supernumerary legs were produced by ectopic transplantation[6] of leg imaginal discs in the fleshfly *Sarcophaga bullata* and the taste responsiveness of the chemosensilla on these legs was examined. The results reported here indicate that the growing sensory axons have made appropriate connections with the central interneurons, at least to some extent.

Materials and methods. Prothoracic leg imaginal discs from donor larvae (red spiracle stage, about 4–6 h before pupariation) were dissected out in sterile *Drosophila* Ringer's[7] solution and transplanted onto 2–3-h-old prepupae[6]. The operated hosts were kept in the environmental chamber till adult emergence (about 12 days post operation). The newly emerged host flies had their wings clipped off. The dorsal thorax of each fly was glued on to the tip of a wooden stick and it was left overnight starving before testing the PER the next morning. The flies were tested for

Proboscis extension response of host flies upon stimulation of their tarsal chemosensilla of transplanted or in situ legs

Test solution	Physiological status	PER of in situ prothoracic leg of the host fly	PER of transplanted leg
Distilled water	Thirsty and hungry	6 (15)	3 (10)
Sucrose solution			
0.15 M	Hungry	6 (15)	2 (9)
0.5 M	Hungry	6 (15)	3 (11)
1.0 M	Hungry	6 (15)	3 (11)
1.0 M sodium chloride solution	Thirsty and hungry	0 (15)	0 (11)

PER is expressed on a scale of 0–6. The numbers in parentheses represent number of flies responding out of a test number of 15 flies. (2nd column from the right) or the number of flies having a fully developed ectopic leg which could be tested (extreme right-hand column). PER score is not average of tested flies. Each fly in the parentheses exhibited the same response.