Beyond Alexis St Martin: contemporary techniques for studying the functioning of the gastrointestinal tract

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Clinicians and researchers no longer need to blast patients with a shotgun to study the physiology of the digestive tract. Modern approaches are less destructive, far safer, and often non-invasive, advancing both scientific knowledge and clinical practice.

Introduction
Many students are fascinated by the story of Alexis St Martin and the fortuitous accident that provided a 'window' into his stomach. Studies of digestive physiology have advanced well beyond those original observations of the surgeon Beaumont, aided by an array of specialized techniques. Excluding the endocrinological and biochemical approaches and methods, modern techniques include long-tried staples such as comparative anatomy revealed by dissection (Martin et al., 1985), the use of both cannulas and fistulas (Bueno, Fioramonti, and Ruckebusch, 1977), a range of radiographic methods (Wood, 1985), measurement of the electrical activity at different sites along the tract (Ruckebusch, Bueno, and Fioramonti, 1981), using strain gauges and balloons to record pressure changes within an organ (Ruckebusch et al., 1981), and using tracers and markers to determine dietary passage rates (Christensen, 1971).

Most current studies of the alimentary tract and/or other components of the digestive system are centred on human health issues. This has been the case especially in Western society, where extremely large amounts of money have been put into the endeavour to understand the normal anatomy and physiology of the body, and correction of dysfunctions. This has manifested itself in two principal ways: an explosion in the use of medications, and the development of more and more technological sophistication in the study of the body and the diagnosis and treatment of disease. Apart from the applied medical aspects, major current research questions include how the digestive tracts of different species are adapted for particular diets and the mechanisms by which digestive processes are controlled.

In this paper we outline the range of methods now available to investigate these questions, discuss their potential and limitations, and give examples of advances in our understanding of the alimentary tract which they have facilitated. The theoretical discussion is supplemented with suggestions for both first-hand and second-hand investigations that students could complete in class.

Abstract
A range of developments in methodology for studying digestive physiology including comparative anatomy, fistulas and cannulas, imaging, electromyography, and tracers are reviewed. This gives a methodological background to the teaching of digestive physiology, with the opportunity for critical appraisal of the collection and analysis of second-hand data. Exercises for gathering first-hand data on the use of tracers, analysing second-hand data on tracers, and interpretation of comparative anatomy of bird stomachs are given as possible curriculum extensions in this field.

Dissection and comparative anatomical techniques
Subsequent to the pioneering work of Beaumont with Alexis St Martin, direct observation of the alimentary tract of human subjects has been possible in patients with large umbilical hernias, where loops of intestine are exteriorized during abdominal surgery, or through paper-thin grafts over an abdominal wall defect (Hightower, 1968). However, researchers in the late nineteenth century concentrated on direct observations of the alimentary tracts of domestic animals,
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often by opening the abdomen under a bath of warm saline to observe intestinal movements. Some surgery was done to investigate the consequences of total removal or less dramatic modification of organs, but this was hampered by poor aseptic techniques leading to significant mortality. Current ethical standards prevent such abuses. Using modern surgical techniques on humans, local cancers of the stomach and/or the intestine can readily be removed with minimal side effects (Maignot, 1974). Today's surgeons routinely undertake procedures such as the removal of large segments of the intestine which, even twenty years ago, were out of the question.

As well as controlled experimental approaches, contemporary researchers have collected systematic observations on the form and dimensions of the alimentary tracts of both mammalian and non-mammalian species in their search for knowledge of how the alimentary tract functions. Ecomorphological studies have taken this even further with the endeavour to determine the interrelationships between diet and the form of the alimentary tract and then to relate these to the zoogeography of the animals. One approach that has been fruitful has been the application of the concept of allometry, which scales individual characteristics to match body size in different species (Martin et al., 1985). Researchers apply the empirical allometric formula: \( y = bx^c \), where \( x \) is a measure of body size (usually mass) and \( y \) is the character under consideration. This equation is linear in its logarithmic form: \( \log y = \log x + \log b \), and lines are fitted to plots of \( \log y \) vs \( \log x \). When the line is fitted to real biological data, some points may be positioned above or below the line of best fit, reflecting special adaptations of particular species. Additionally, the overall trend of relationships is likely to reflect a basic scaling principle such as surface area: volume ratios. Allometric studies may therefore be used to elucidate general scaling principles as well as variations in proportion likely to reflect specific adaptations.

In practice, applications of the method may be subject to a number of artefacts. For instance, Martin et al. (1985) point out that, in the case of studies of digestive systems, it is wrong to assume that the measurements of different workers will always be comparable. They also emphasize the need for standardized procedures that cope with problems such as measuring the surface area of irregularly shaped organs and the elasticity of an organ's walls. Furthermore, organ dimensions may vary markedly in relation to diet within many species, and even seasonally as the diet undergoes dramatic changes (Al-Dabbagh, Ijad, and Waheed, 1987). Consequently, care must be taken when extrapolating laboratory data to the field situation.

Despite these cautions, allometric approaches have been useful in several ways. Some of the points which have emerged include: species with enlarged stomachs do not have consistently shorter large intestines, as was once believed (Martin et al., 1985); mammals with cellulose-rich diets may cope using either an enlarged stomach or an enlarged colon, but not both (Martin et al., 1985); and carnivores tend to have simpler stomachs and intestines than herbivores (Stevens, 1980). Detailed descriptions of variations in the form of the alimentary canal of different vertebrates are given in Romer and Parsons (1977).

Fistulas and cannulas

Dissection reveals the gross anatomy and relative proportions of the internal organs and may also provide contents for chemical or microscopic analysis from which function may be inferred (Maloj, Clemens, and Kamau, 1982). However, it is preferable and more relevant to draw such samples from living animals, thereby removing artefacts such as post-mortem digestion and avoiding the ethical problems of killing animals. In the latter case, such information as the state of digestion of particular dietary items at a specific location along the alimentary tract can be gained by exteriorization of the tract segment, often with simultaneous cannulation (insertion of a tube into the relevant duct or cavity). However, exteriorized organs are subject to problems such as desiccation and temperature fluctuations, with the result that the technique is no longer in common use. Fistulas (abnormal or experimental passages from a body passage to the exterior) also allow routine sampling of digesta from precise locations in the alimentary tract so that dietary composition at different levels of the tract may be determined. This approach is free of many of the problems of exteriorization, and has provided the knowledge of what is digested, where, and at what rate, along the entire length of the tract. An interesting historical profile of the many studies in the nineteenth century and early twentieth century is given by Ruckebusch (1980). From this an understanding of malabsorption syndromes and consequently rational regimens for treatment have developed.

Whilst fistulation of domestic animals has been and is undertaken routinely, such experiments are not done on humans. However, as technology has advanced new miniaturized tools have allowed the sampling of digesta from along the alimentary tract of humans (Malagelada, 1981). Routinely, a length of fine gauge polyvinyl tubing can be introduced into the nostril, then through the nasal cavity and into the pharynx. From here the tubing can be manipulated, using radiographic techniques to monitor progress, as far along the tract as required and then a sample may be taken. Sampling can be extremely precise, giving a normal physiological sample which can subsequently
be analysed. One arresting fact shown by such techniques is that freshly secreted human gastric juice contains about 0.1N hydrochloric acid.

Imaging
With the invention of the fibre-optic gastroscope in 1957 we have been able to view directly and routinely the inner surface (lumen) and, when needed, the outer surface (serosa) of the alimentary tract (Boyce and Palmer, 1975). To view the lumen of the tract the objective lens with its entrained fibre-optic bundle is introduced into the appropriate orifice and directed to the site, such as a tumour or ulcer, which is of interest. The outer surface of the tract is usually viewed by introducing the fibre-optic device through a small abdominal incision. This allows the examination of abnormalities such as adhesions causing strictures of the tract, vascular abnormalities, and cysts, all of which may interfere with the normal functioning of the organ.

Radiography as applied to humans and animals began when Cannon, in 1898 and 1902, reported the radiographic appearance of a cat's stomach and intestine as they moved after the cat had been fed. Interestingly, he reported not only that food was mixed, ground, and expelled, but also that anxiety, rage, and distress would inhibit stomach movements. Since then radiographic technology has advanced dramatically. Now we have 'TV' viewing (fluoroscopy) of the body and organ systems, or view it slice by slice (computer-assisted tomography = CAT scans) (Armstrong and Wastie, 1981).

However, nuclear magnetic resonance, where magnetic fields are used to modify proton spin in the body and thus create a computer-derived view of the body systems, has not been a great success in the visualization of the alimentary tract (Armstrong and Wastie, 1981).

Sound waves (ultrasound) have been used very effectively to study moving organs such as the heart but have not played a great role in investigations of the alimentary tract (Rollandi and Carriati, 1988).

Electromyography
The alimentary tract, other than functioning to digest the food chemically and to absorb nutrients, relies absolutely on its ability to move digesta so that mixing, grinding, and transport may occur. These are achieved by the coordinated movement of the outer muscle layers of the tract. The nature and extent of these movements has always been difficult to determine. Some studies have used pressure-sensing devices (electronic tablets, manometry tubes) which have been swallowed by the subject. These have given general movement patterns but have usually suffered from the problems of calibration and of knowing exactly what has been measured. However, miniaturized endoluminal transducers or very small-gauge perfusion catheters have allowed high quality recordings from within the lumen along most of the length of the alimentary tract. They reveal any abnormal motility patterns along the tract, but they are awkward and invasive, and their use is restricted to specialized centres (Stanghellini et al., 1988).

Invasive techniques such as the surgical implantation of electrodes or strain gauges to the outer surface of the tract have been highly successful. The former, in particular, act in a similar manner to an electrocardiogram, where the sensor device records the sum result of all the adjacent cell populations' depolarizations and polarizations over time and can, with the correct instrumentation, give a chronological readout of those changes. Basically, two types of electrical event are recorded along the tract: pacemaker potentials and action potentials. Pacemaker potentials can be recorded from along most of the alimentary tract. They are rhythmical events which occur at a frequency which is characteristic of the site along the organ and of the species being examined (Del Tacco and Blandizzi, 1988). Action potentials may occur between sequential pacemaker events. They vary in amplitude and number and determine the occurrence and the strength of contractions at any particular site. Thus, from a recording one can determine when, where, and the level of contractions at single and multiple sites along the tract. This has given great insight into the motor functions and dysfunctions of the tract and has been a major aid to human medicine (Bertaccini and Coruzzi, 1988; Zappatore et al., 1988).

Tracers
The rate of passage of digesta through an animal can be monitored by the use of tracers introduced into the food and ultimately retrieved in the faeces. A great variety of tracers has been used including charcoal, coloured foodstuffs, coloured pellets, radio-opaque pellets, and radioisotopes. However, they have limitations because factors such as the state of organ fill and the rate of emptying at various sites along the alimentary tract affect the passage of both the solid and the liquid phases. Some tracers measure only the transit of the fluid phase while others follow the solid phase. As well as this, the physical (volume, density) and the chemical (pH, reactivity) properties of the markers all affect their movement (Christensen, 1971). Branch and Cummings (1978) state that a good tracer should be inert, non-toxic, completely unabsorbed and non-metabolized, easily measured, lacking in any appreciable bulk, and readily mixable with the intestinal contents. No markers meet all these requirements, so compromises are always required.
Classroom exercises

Some of the applications of the techniques reported here can be adapted to exercises for senior secondary students, either as part of the basic curriculum or as extension exercises. The following suggestions may be useful.

Gathering first-hand data

Food transit times can be measured simply in a range of laboratory animals including mice, rats, guinea pigs, rabbits, and chickens. The commercial dry food which is the staple of the laboratory diet for these species can be coloured readily with food dyes which then serve as a marker to study transit time. The smaller pellets such as chick starter feed or guinea pig pellets can be dyed directly, while the larger rodent cubes are easier to mark if broken into pea-sized lumps with a rolling pin. Equal volumes of food and dye are sealed in a strong plastic bag and shaken vigorously for three to five minutes to ensure thorough mixing, after which the coloured pellets are spread out to dry on blotting paper. It may not be necessary to use the food dye at full strength and a 1:10 dilution of dye in water may be adequate. Some fluid will be left and can be used to dye more pellets.

Much flexibility is available in the experimental design the students can adopt. One simple approach is to withhold all food from the animals overnight while ensuring that water is freely available. This will not harm them, but ensure that they don’t have alternate food such as straw bedding in the cage! Next morning all droppings are cleared from the cage and the animals given the dyed food. Note when they first begin to eat, and record this as time zero. After an hour, replace the dyed food with the normal food and collect any fresh droppings into a labelled bag. Subsequent hourly collections can be made and studied for the presence of dye.

Dye may be visible directly, or it may be necessary to smear droppings on slides and examine them using a dissecting microscope. This will reveal the time interval until the first of the dyed remains are passed and how long after this the last of the meal is cleared. An alternative to dyes is pollen, and the techniques for use of this marker are given by Caution (1988).

Keen students may wish to design their own procedures to try to answer such questions as: how can the analysis be quantified? does the presence or absence of the dye influence transit time? does hunger influence transit time? what are the relative advantages/disadvantages of dye and pollen grains as markers? Of course, students should follow normal sanitary procedures when working with droppings.

Analysing second-hand data

Transition time graphs are often distinct in species with markedly different diets, and sometimes within a species when different foods are eaten. The following data sets present transition time data for three very different mammal species: a frugivorous bat (Cynopterus brachyotes), mass 25 g; the nectar feeding marsupial honey possum (Tanopetes rostratus), mass 12; and the Australian sea lion (Neophoca cinerea), mass 250 kg. Note that two sets show cumulative recovery, while the honey possum data do not.

Students could be asked to plot these relationships and to describe their similarities and differences. They may wish to hypothesize as to why different foods have different transition times within the same species, or try to predict the adaptations of gross anatomy likely to be found in the alimentary tracts of each species. These predictions could be checked as a library research exercise.

Data set 1

Cumulative recovery of Sephadex G-25 marker from the faeces of an Asian fruit-eating bat: (a) after feeding on papaya and (b) after feeding on bananas. Markers were incorporated in the diets. Time in hours. Ref. Richardson et al., 1987.

(a) t = 1, % = 5; t = 2, % = 40; t = 3, % = 60; t = 4, % = 70; t = 5, % = 82; t = 6, % = 86; t = 7, % = 91;

(b) t = 1, % = 0; t = 2, % = 5; t = 3, % = 25; t = 4, % = 45; t = 5, % = 64; t = 6, % = 78; t = 7, % = 91;

t = 8, % = 98; t = 9, % = 100; t = 12, % = 100.

Data set 2

Percentage of ingested marker pollen subsequently voided in faeces of the marsupial honey possum in relation to time (hours), after feeding on (a) a 25 per cent honey solution and (b) a 60 per cent honey solution. Ref. Richardson, Wooller, and Collins, 1986.

(a) t = 2, % = 0.5; t = 4, % = 2.5; t = 6, % = 5.0; t = 8, % = 4.0; t = 10, % = 1.0; t = 12, % = 1.3; t = 14, % = 0.7; t = 16, % = 0.3; t = 18, % = 0.2; t = 24, % = 0.

(b) t = 2, % = 0; t = 4, % = 0.3; t = 6, % = 4.5; t = 8, % = 4.5; t = 10, % = 4.2; t = 12, % = 3.3; t = 14, % = 2.7; t = 16, % = 1.0; t = 18, % = 0.7; t = 24, % = 0.

Data set 3

Cumulative recovery of markers voided in faeces of the Australian sea lion in relation to time (hours): (a) large markers and (b) small markers. Each point is the mean from three individuals. Ref. Richardson and Gales, 1987.

(a) t = 6, % = 20; t = 12, % = 35; t = 18, % = 45; t = 30, % = 48; t = 42, % = 50; t = 52, % = 52; t = 60, % = 52; t = 72, % = 50; t = 84, % = 52; t = 96, % = 52.

(b) t = 6, % = 48; t = 12, % = 64; t = 18, % = 75; t = 30, % = 84; t = 42, % = 92; t = 52, % = 96; t = 60, % = 98; t = 72, % = 100; t = 84, % = 100; t = 96, % = 100.

Comparative anatomy exercises can also be approached by examining diagrams of particular or-
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![Diagram](image)

**Figure 1** Longitudinal sections through the gizzards of three birds, each of approximately 10 g: (a) Brown Honeyeater, (b) Silvereye, (c) Splendid Fairy-wren. The stippled area shows the gizzard's muscular wall, and the clear central area is the lumen. The scale line is 2 mm.

**References**


*Journal of Biological Education* (1991) 25 (4)

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