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A Thesis Submitted to the Yale University School of Medicine in partial fulfillment of the requirements for the Degree of Doctor of Medicine

by

Stephen John Ferzoco

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COUNTER-REGULATION OF ILEAL MOTILITY IN RABBIT SMALL INTESTINE. Stephen J. Ferzoco. Department of Surgery, Yale University School of Medicine, New Haven, CT.

Disorders of intestinal motility continue to plague surgeons of the twentieth century. Unfortunately, the regulation of intestinal motility remains incompletely understood. Utilizing the isolated whole organ perfusion system, segments of rabbit terminal ileum were infused with a variety of known gastrointestinal hormones, peptides and neurotransmitters. In the first series of experiments, prokinetic agents such as carbachol (an acetylcholine analogue), cholecystokinin, motilin and were tested. All three agents caused a concentration-dependent increase in measured motor activity. In the second series of experiments, agents which increase intracellular levels of cAMP, vasoactive intestinal peptide (VIP), forskolin and norepinephrine, were tested against prokinetic-stimulated segments of ileum. All three agents caused a concentration-dependent inhibition of motility. In the third series of experiments, neuropeptide Y (NPY) and peptide YY (PYY), agents which block intracellular cAMP, reversed the inhibitory action of VIP. In the final series of experiments, various NPY/PYY analogues with specific Y receptor affinity were tested. The Y_1 receptor analogue

[Leu^{31}, Pro^{34}]NPY demonstrated similar ability to reverse VIP's effect. In conclusion, peristalsis can be divided into two distinct phases. Ascending contraction caused by prokinetic agents is orad to a food bolus. In addition, VIP is responsible for the descending inhibitory reflex distally. NPY released within neurons within the gut wall causes reversal of the VIP-mediated inhibition leading to a wave of ascending contraction. This reversal is mediated via a Y1 receptor mechanism.

LIST OF ABBREVIATIONS

CARB:	CARBACHOL
cAMP:	CYCLIC AMP
CCK:	CHOLECYSTOKININ
H & E:	HEMATOXYLIN AND EOSIN
FK:	FORSKOLIN
LPNPY:	[Leu^{31},Pro^{34}]NEUROPEPTIDE Y
MOT:	MOTILIN
NE:	NOREPINEPHRINE
NPY:	NEUROPEPTIDE Y
NPY(13-36):	NEUROPEPTIDE Y FRAGMENT 13 THROUGH 36
PP:	PANCREATIC POLYPEPTIDE
PYY:	PEPTIDE YY
TTX:	TETRODOTOXIN
VIP:	VASOACTIVE INTESTINAL PEPTIDE

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BACKGROUND

20th Century man is afflicted with the ravages of many newly-recognized diseases. Many of these are based on motility disorders of the gut. Indeed, the irritable bowel syndrome, diverticular disease, constipation and perhaps even the genesis of gastrointestinal malignancy can be traced to pertubations of normal intestinal peristalsis.

Consequently, exploration of regulatory systems which modulate small bowel and colon motility may lead to new therapeutic modalities for diseases of 20th century man. Indeed, this approach was the basis of classical medicine. Greek, Roman and medieval medicine realized the importance in restoring normal intestinal motility in various states of disease. Ancient physicians utilized a variety of cathartics and clysters to purge disease from the intestines and regulate motility.

In this thesis I have focused on the small bowel. Small bowel motility has been studied extensively <u>in vivo</u> but this has not allowed identification of specific regulatory systems. Examination of gut motility in an <u>in vitro</u> system eliminates exogenous neural and humoral influences and facilitates characterization of specific mechanisms. It is the identification of the intrinsic mechansism of the gut which will lead to new avenues of therapeutic intervention in diseases such as irritable bowel syndrome.

HYPOTHESIS

The regulation of intestinal motility remains incompletely understood. Peristalsis can be best defined as alternating zones of high and low pressure which sweep food down the intestines. Proximal to a food bolus, prokinetic agents cause smooth muscle contraction leading to generation of a high pressure zone. This high pressure zone is responsible for ascending contraction. Distally, it is postulated that vasoactive intestinal peptide mediates the descending inhibitory reflex. In addition there is a third zone, or transition zone, between the high and low pressure zones. We hypothesized that there must exist some counter-regulatory agent which reverses the descending inhibitory reflex caused by vasoactive intestinal peptide and allow the generation of the high pressure zone by prokinetic agents.

STATEMENT OF THE SPECIFIC AIMS

The specific aims of the study were:

1. to test the effects of various prokinetic agents on isolated segments of rabbit terminal ileum.
2. to test the effects of agents which increase intracellular cAMP levels on stimulated segments of ileum.
3. to demonstrate the effects of agents which inhibit cAMP accumulation on vasoactive intestinal peptide-mediated inhibition of motility.
4. to demonstrate the effects of various NPY receptor analogues on VIP-mediated inhibition of motility.

HISTORICAL BACKGROUND

The Role of Gut Motility Modulation in Classical Medical Therapy

Classical physicians based their treatment of disease upon concepts of physiology and pathology which were quite different than current concepts. Consequently, their approach to therapeutics seems alien to the modern practice of medicine. These views, however, persisted well into the 19th Century and many still survive among folk remedies for various conditions. Whereas modern therapeutics is based upon experimental science, classical practice was founded upon millenia of careful and meticulous observation. As a result, medical writings from previous centuries offer an immense wealth of clinical observations on the natural history of diseases. In addition, modern therapeutics is based on pharmacology while in classical therapeutics the physician attempted to guide and support their patients through illnessess by alterations of diet, bloodletting, and stimulation of changes in bowel function and urination.

The gastrointestinal tract played a prominent role in the therapeutics of classical medicine. The classical physician believed that the maintenance of normal bowel function was a prerequisite for continued good health (Celsus

1979). He considered that alterations of bowel function played a role in the genesis of some diseases. Moreover, the classical physician used manipulation of gastrointestinal motility as one of their primary modalities for the treatment of both acute and chronic diseases.

The definition of disease was quite different for the classical physician than that which is generally accepted at the present time. As a result, the goals of classical therapeutics were entirely disparate from those of modern medical practice. In this section, we will review the classical definition of disease and then examine how this definition led to the use of emetics, cathartics, clysters, purgatives and enemas in the treatment of acute and chronic illnesses by physicians until relatively recent times.

The Definition of Disease.

The approach of classical physicians to disease evolved from their assumptions of the economy of the human body and the genesis of disease. The role of the physician and his choice of therapies derived from these postulates. Hippocrates describes the development of medical practice in Ancient Medicine. The role of diet in health and in disease was evidently always a central concern of the physician. Hippocrates writes:

> "For the art of Medicine would not have been invented at first, nor would it have been made a subject of investigation (for their would have been no need of

> it,) if when men are indisposed, the same food and other articles of regimen which they eat and drink when in good health were proper for them, and if others were preferable to these. But now necessity itself made medicine to be sought out and discovered by men, since the same things when administered to the sick, which agreed with them in good health, neither did nor do agree with them (Hippocrates)."

Hippocrates indicated that the earliest role of the physician was the study of diet in health and disease and that therapeutics was initially based on changes in diet. Furthermore, Hippocrates stressed that the natural faculties functioned differently in health and in disease. We will return to this issue in the next section.

The teachings of Hippocrates remained a central theme in Western medicine until recent times. Thomas Sydenham, the leading practioner of medicine in the 17th Century, pleaded for a greater return to these Hippocratic ideals. A distinct definition of classical concepts of disease is offered by Thomas Sydenham in his Medical Observations Concerning the History and Cure of Acute Diseases:

> "A disease, however much its cause may be adverse to the human body, is nothing more than an effort of Nature, who strives with might and main to restore the health of the patient by the elimination of the morbific matter. For, since it is the will of God, the Supreme Arbiter and Regulator of all things, that the human frame be, by nature adapted to the reception of impressions from without, it follows that it must also be liable to a variety of maladies. These arise partly from the particles of the atmosphere, partly from the different fermentations and putrefactions of the humours. The first insinuate themselves amongst the juices of the body, disagree with them, mix themselves up with the blood; and, finally, taint the whole frame with the contagion of disease. The second are confined within the body longer than they ought to be, its powers

> having proved incompetent, first to their digestion, afterwards to their excretion. This may arise from either their bulk, or the incongruity of their qualities (Sydenham 1847)."

The contrast of this definition to modern concepts was pointed out by Benjamin Rush in his introduction to an American Edition of The Works of Thomas Sydenham, M.D. published early in the 19th Century (Sydenham 1809). Rush wrote:

> "I consider our author's (Thomas Sydenham) definition of a disease to be erroneous, viz. that it is 'a vigorous effort of nature to throw off morbific matter, and thus to recover the patient,' instead of which I believe a disease, to use the definition he has rejected, to consist 'in the confused and irregular operations of disordered and debilitated nature'. (Sydenham 1809, p. iv)

This contrast of views in the cause of symptoms is central to the differences between the classical practice of medicine and current therapeutics. For the classical physician, the symptoms of disease were the response of the patient's constitution to an illness and thus, something which should be promoted. In sharp contrast, modern physicians tend to think of symptoms as the consequence of disease and that suppression of the symptoms will ameliorate the disease.

Sydenham's definition highlights two fundamental postulates of classical medicine. The first is the belief that the symptoms of disease are produced by the response of the natural faculties to the disease. The second is that the genesis of disease is through the putrefaction of humors

within the body. Both of these points must be grasped to appreciate the use of prokinetic agents in the treatment of disease.

Vis Medicatrix Naturae

The classical physician studied attentively the progression of symptoms in acute and chronic illnesses. In particular, he focused on the divergence of symptoms between patients that recovered and those that succumbed from the disease. It was the role of the physician to promote symptoms such as vomiting and diarrhea when the response of the patient was judged inadequate and to temper symptoms when they became too extreme. This approach to therapeutics derived from the concept that symptoms were the response of the patient's constitution to the illness and represented the attempts of the patient's body to throw off the irritating causes of his illness. The role of the physician, then, was to assits nature in the fight against disease (Guthrie 1946, Kutumbiah 1971).

The importance of Nature in the battle against disease explicitly remained the central postulate of therapeutics until the 19th Century. This doctrine was deemed **Vis Medicatrix Naturae**. William Cullen, Professor of Medicine at the University of Edinburgh in the late 18th and early 19th Century, defined this important concept. Cullen wrote while addressing the Phaenomena of Fevers:

> "How the state of debility produces some of the symptoms of the cold stage, we cannot particularly explain, but refer it to a general law of the animal oeconomy, whereby it happens, that powers, which have a tendency to hurt and destroy the system, often excite such motions as are suited to obviate the effects of the noxious power. This is the VIS MEDICATRIX NATURAE, so famous in the school of physic; and it is probable, that many of the motions excited in fever are the effects of this power (Cullen 1777)."

Thus, the classical physician did not believe that he could directly treat a disease. Instead, his role was to support and to assist the natural response of his patient to his illness. The physician guided the powers of nature in the struggle against disease.

Classical Concepts of Digestion.

Classical physicians were forbidden to dissect the human body because of prevalent religious doctrines regarding the sanctity of man. Consequently, knowledge of human anatomy was limited. Similarly, the function of various organs could only be inferred. Nonetheless, concepts of the animal economy developed. In order to understand the goals of physicians in the use of cathartics and purgatives in the treatment of disease a brief description of classical concepts of digestion is required.

Classical concepts of digestive physiology and on the functions of the intestine have descended to us primarily through the writings of Galen. Classical perception of digestion is outlined in <u>On The Natural Faculties</u> (Galen

1927). The Natural Faculty of Digestion was accomplished by 1. **Presentation** (prosthesis) of the nutrients to the alimentary tract, 2. **Adhesion** (prosphysis) of the nutrients to the wall of the organs, 3. **Assimilation** (alteration) of the nutrients into the lacteals and vessels, 4. **Retention** of the nutrients until assimilation occurred, and 5. **Expulsion** of the unabsorbed substances (superfluidities). All discourses on digestion adhered to this general theory for the next fifteen centuries. It must be emphasized, however, that these processes were not confined to the gastrointestinal tract.

Role of Digestion in the Genesis and Treatment of Disease

The natural faculty of nutrition and the processes of digestion could lead to the generation of disease and could also be used to treat diseases. The manner by which alterations of digestion could lead to disease was descibed by Sydenham in his discussion On Acute Diseases in General. Sydenham wrote that diseases "arise partly from the particles of the atmosphere, partly from the different fermentations and putrefactions of the humors (Sydenham 1847)." Further, he detailed the alterations in digestion which caused illness:

> "The (different fermentations and putrefactions of the humours) are confined within the body longer than they ought to be, its powers having proved incompetent, first to their digestion, afterwards to their excretion.

> This may arise from either their bulk, or the incongruity of their qualities (Sydenham 1847)."

Thus, it was supposed that an accumulation of superfluidities and putrefactions within the body produced many of the acute and chronic illnesses.

Fortunately, Nature had forseen this eventuality and provided mechanisms for the body to cleanse itself of these offending substances. Sydenham again offers a succinct description of these processes:

> "Hence Nature, in the concatenation and exclusion of the peccant and foreign matter, which otherwise, would undo the whole fabric of our frame...This undertaking Nature performs at different rates; quickly or slowly, according to the different processes by which she strives to expel the morbid influence...As often she calls in the aid of fevers for the isolation of the tainted particles from the remainder of the blood; and when, by a further process, either by diaphoresis or diarrhoea, by eruptions, or some other evacuations, she expels the particles thus isolate (Sydenham 1847)."

Nature fights off these diseases through the elimination of the offending substances. One of the principle ways of cleansing the system is through evacuations of the bowels. Thus, manipulation of bowel function became an important focus of attention for the classical physician. Unfortunately, the response of Nature to these imbalances of the system were inappropriate. Sydenham points out: "When left, indeed, to herself, (Nature) may do too much or too little, and, in either case, kill the patient (Sydenham 1847)." The role of the physician, then, was to observe the

progress of Nature in the elimination of putrefactions and, if need be, either to promote or retard this process.

We will now examine more closely the writings of various medical authorituies over the last several millenia. We will focus on their use of the gastrointestinal tract in their treatment of disease.

Greek Medicine - Hippocrates

Unquestionably, the foundation of Greek Medicine is based on the writings of Hippocrates (Figure 1). Hippocrates, a native of the island of Cos, was born about 460 B.C. Although little is known about the man himself, he is said to have been taught medicine by his father and traveled extensively within the Greek Empire lecturing on medicine and surgery. After his death, his followers propagated his teaching to new generations of physicians and established the Library of the Hippocratic School at Cos (Osler 1921).

Osler wrote that "empiricism, experience, the collection of facts, the evidence of senses, the avoidance of philosophical speculations, were the distinguishing features of Hippocratic medicine. One of the most striking contributions of Hippocrates is the recognition that diseases are only part of the processes of nature, that there is nothing divine or sacred about them." Indeed, Hippocrates

based his treatment of disease on the *vis medicatrix naturae*, the power of nature (Osler 1921).

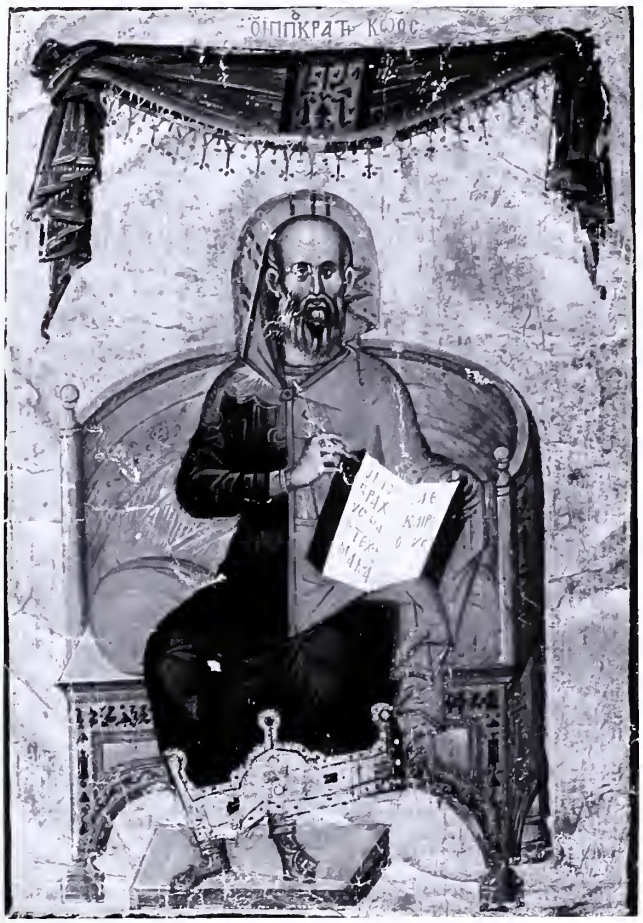

Figure 1: Reproduction of a fourteenth-century Byzantine portrait of Hippocrates, courtesy of the Bibliotheque Nationale, Paris.

Hippocrates devoted much attention to gut function. He carefully observed the quantity and appearance of stool in health and believed that maintenance of normal defecation was a fundamental requirement for continued good health. In illness, Hippocrates studied the changes in the quantity and character of vomitus or stool which was expelled from his patients. He believed that this gave him direct information about the processes of concoction and putrefaction which were occurring in his patient. Further, by following the changes in the character of the vomitus and stool he could draw conclusions about the course of the disease in his patient. If the patient stopped vomiting and the character of the stool returned to normal, the patient had passed through the crisis of the disease and would recover. If on the other hand, the vomitus became feculent and the stool remain filled with putrefaction or even worse blood, the patient would perish.

Hippocrates described the natural course of bowel movements in his Prognostics:

> "The excrement is best which is soft and consistent, is passed at the hour which was customary to the patient when is health, in quantity proportionate to the ingesta; for when the passages are such, the lower belly is in a healthy state. But if the discharges be fluid, it is favorable that they are not accompanied with a noise, nor are frequent, nor in great quantity; for the man being oppressed by frequently getting up, must be deprived of sleep...But in proportion to the ingesta he should have evacuations twice or thrice a day, once at night and more copious in the morning, as is customary with a person in health. The faeces should become thicker when the disease is tending to a crisis;

> they ought to be yellowish and not very fetid. It is favorable that round worms be passed with the discharges when the disease is tending to a crisis. The belly, too, through the whole disease, should be soft and moderately distended; but excrements that are very watery, or white, or green, or very red, or frothy, are all bad. It is also bad when the discharge is small and viscid, and white, and greenish, and smooth; but still more deadly appearances are the black, or fatty, or livid, or verdigris-green, or fetid. Such as are of varied characters indicate greater duration of the complaint, but are no less dangerous; such as those which resemble scrapings, those which are bilious, those resembling leeks, and the black; these being sometimes passed together and sometimes singly (Hippocrates 1849).

Hippocrates realized the importance of the intestines as an organ of "concoction" or digestion. It was important that the bowel perform this function in order that health be preserved. Hippocrates argued that it was necessary for digested material to be expelled by the body. In Aphorisms XXI of Section I, "Those things which require to be evacuated should be evacuated, wherever they most tend, by the proper outlets (Hippocrates 1849)." Otherwise, the accumulation of the superfluidities would lead to the genesis of disease. Similarly, in Aphorisms XXII of Section I, "We must purge and move such humours as are concocted, not such as are unconcocted, unless they are struggling to get out, which is mostly not the case (Hippocrates 1849)."

When the use of purgatives is indicated, it is necessary to augment them through their natural course. In Aphorisms II of Section IV, "In a purging we should bring away such matters from the body as it would be advantageous had they come away spontaneously, but those of an opposite character

should be stopped (Hippocrates 1849)." The physician assists nature in the regulation of digestion.

Hippocrates also used evacuation of the bowels as a central weapon in his arsenal for the therapy of diseases. In Aphorisms LXVIII of Section VII, Hippocrates wrote that in acute diseases: "When the dejections are allowed to stand and not shaken, and a sediment is formed like scraping (of the bowels), in such a case it is proper to purge the bowels; (Hippocrates 1849)." Further, in the Appendix to the works On the Regimen in Acute Diseases, Section XV, he adds: "All diseases are resolved either by the mouth, the bowels, the bladder, or some other such organ. Sweat is a common form of resolution in all these cases (Hippocrates 1849)."

Hippocrates felt that the use of purgatives, although necessary for the elimination of disease, had to be used judiciously. In Aphorisms XXIV of Section I, he states, "Use purgative medicines sparingly in acute diseases, and at the commencement, and not without proper circumspection (Hippocrates 1849)."

Hippocrates prescribed many regimens in the treatment of disease. The majority of them dealt with abnormalities of the gastrointestinal tract. The majority of his treatment modalities can be found in his <u>On Regimen in Acute Diseases</u>:

> Section V: "Ptisans are to be made of the very best barley, and are to be well boiled, more especially if you do not intend to use them strained. For, besides

> the other virtues of ptisan, its lubricant quality prevents the barley that is swallowed from proving injurious...for that which is well boiled is very lubricant...of very easy digestion...If then one do not pay proper attention to the mode of administering the ptisan, much harm may be done; for when the food is shut up in the bowels, unless one procure some evacuation speedily, before administering the draught, the pain, if present, will be exasperated (Hippocrates 1849).
>
> Section VII: But if the pain be below the diaphragm, and do not point to the clavicle, we must open the belly either with black hellebore or peplium, mixing the black hellebore with carrot or seseli, or cumin, or anise, or any of the other fragrant herbs; and with the peplium the juice of the sulphium (assafoetida), for these substances when mixed together, are of similar nature. The black hellebore acts more pleasantly and effectually than the peplium, while, on the other hand, the peplium expels wind much more effectually than the black hellebore (Hippocrates 1849).
>
> Section XV: But unmixed hydromel, rather than the diluted, produces frothy evacuations, such as are unseasonably and intensely bilious, and too hot; but such an evacuation occasions other great mischiefs, for it neither extinguishes the heat in the hypochondria, but rouses it, induces inquietude, and jactitation of the limbs, and ulcerates the intestines and anus (Hippocrates 1849)."

In describing treatment for "ardent fever (causus)", Hippocrates advocates administration of an emetic and clyster; "and if these things do not loosen the bowels, purge with the boiled milk of asses (Hippocrates 1849)."

In the Appendix to the works On the Regimen of Acute Diseases, Section XXI, he adds:

> "Those who have the inferior intestines hot, and who pass acrid and irregular stools of a colliquative nature, if they can bear it, should procure revulsion by vomiting with hellebore; but if not should get a thick decoction of summer wheat in a cold state, lentil soup, bread cooked with cinders, and fish, which should be taken boiled if they have the fever, but roasted if not feverish; and also dark coloured-wine if free of fever;

> but otherwise they should take the water from medlars, myrtles, apples, services, dates or wild vine. If there be no fever...the patient should drink hot asses' milk in small quantity at first, and gradually increase it, and linseed, and wheaten flour, and having removed the bitter part of Egyptian beans, and ground them, sprinkle on the milk and drink; and let him eat eggs half-roasted, and fine flour, and millet, and perl-spelt (chondrus) boiled in milk (Hippocrates 1849)."

In the Appendix to the works On the Regimen of Acute Diseases, Section XXXVIII, Hippocrates states: "A medicine for opening the bowels. Pour upon figs the juice of spurge, in the proportion of seven to one: then put into a new vessel and lay past when properly mixed. Give before food (Hippocrates 1849)."

Hippocrates felt that purgatives were only beneficial when the undigested material passed to the distal bowel. In the Appendix to the works On the Regimen of Acute Diseases, Section VII, he comments: "When fever seizes a person who has lately taken food, and whose bowels are loaded with faeces which have been long retained, whether it be attended with pain of the side or not, he ought to lie quiet until the food descend to the lower region of the bowels, and use oxymel for drink; but when the load descends to the loins, a clyster should be administered (Hippocrates 1849)."

Hippocrates attributed some states of diarrhea to an excess of black bile in the body. In the Appendix to the works On the Regimen of Acute Diseases, Section VIII, Hippocrates states:

> "In those cases of fever in which the bowels are loose, and the mind is disordered...such attacks appear to me to be connected with black bile. When in these cases there is a colliquative diarrhea, I am of the opinion that we ought to give the colder and thicker ptisans, and that the drinks ought to be binding, of a vinous nature, and rather astringent. (Hippocrates 1849)."

Disorders of intestinal motility could even be traced to dietary changes. In the Appendix to the works On the Regimen of Acute Diseases, Section XVIII, Hippocrates writes: "Disorders connected with regimen, for the most part, make their attack accordingly as any one has changed his habitual mode of diet...but if the bowels are not opened, he should get his body rubbed with hot oil...Cheese produces flatulence and constipation...The stalk and the juice of silphium (assafoetida), pass through some people's bowels very readily (Hippocrates 1849),"

Roman Medicine

While the name of Hippocrates is associated with Greek Medicine, Galen is recognized as the father of Roman Medicine (Figure 2). Born at Pergamos in 133 A.D., Galen united the concepts of observer, experimenter and philosopher (Osler 1921). Unlike Hippocrates, we know a great deal about Galen's life, principally from his own writings. After taking up medicine at age 17, he eventually gave public

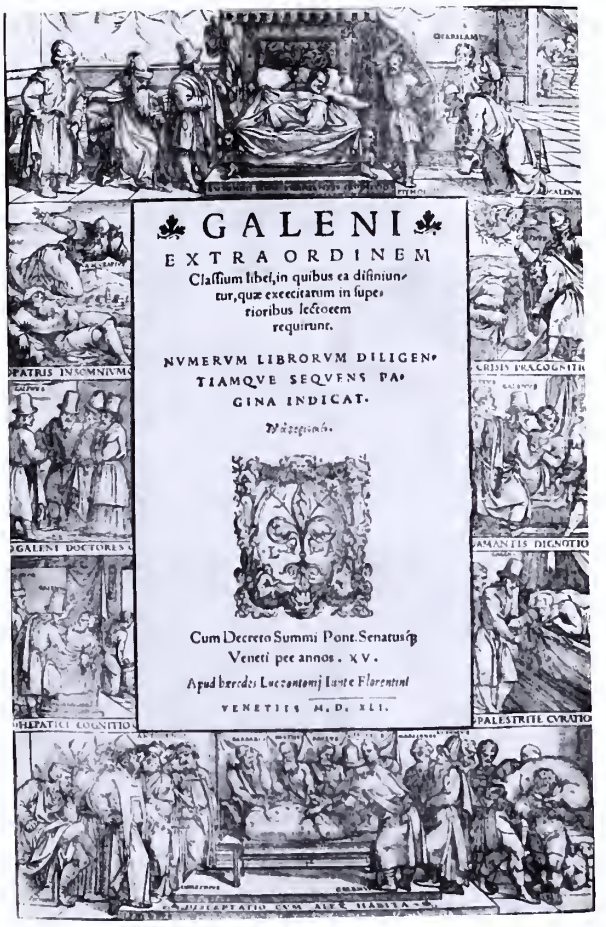

Figure 2: The frontispiece of the Latin translation of Galen published by the Venetian printing house of Giunta in 1541. Reproduced from Sherwin Nuland's Doctor's, the Biography, Vantage Books, New York, 1988.

lectures on anatomy in Rome in 162 A.D. Galen soon found himself the principle physician to Emporor Marcus Aurelius after successfully treating the Emperor's stomachache after

eating too much cheese. Aurelius is said to have remarked: "I have but one physician and he is a gentleman." (Osler 1921).

Perhaps the most prolific writer of the ancient physicians, Galen did not ally himself with any particular school of medicine, but regarded himself as a disciple of Hippocrates. Galen does not seem ever to have had the opportunity to dissect the human body but he did carry out large scale experiments which added to the basic corpus of medical information (Osler 1921).

In his Hygiene (De Sanitate Tuenda), Galen further develops his ideas on digestion and disease. In his third chapter, he describes the production and elimination of excrements. "Nature not only has provided organs for their excretion but has endowed them with powers whereby some attract the excrements, some propel them, and some eliminate them. And it is necessary that these should neither be obstructed by anything nor impaired in their functions to keep the body clean and free from impurities (Galen 1951)."

Galen also proposed that various organs of the intestinal tract had specific functions and roles in the maintenance of health. "She (Nature) provided the animals with many organs, some purging and separating the excrements, some propelling them, others collecting, and others eliminating them...For in the first place the excrement is

separated and gradually propelled through all the intestines into a large cavity called the rectum (Galen 1951)."

In chapter XVIII, Galen discusses the causes and prevention of excrementary retardation:

> "Now retardation of the excrements from the stomach may arise from fault in the food and drink taken, or from the stomach and intestines themselves. From fault of food or drink, retardation may occur on account of their quality or quantity or the order and manner in which they are taken. On account of their quality, if they are bitter, sour, or of a dry nature. On account of their quantity, if there is more or less than is proper...
>
> The causes of retardation of the excrements due to the stomach and intestines may be intrinsic or acquired. The intrinsic causes arise from faulty constitutions of the body...There are eight different forms of acquired disorders affecting the abdomen, each a dyscrasia of individual sort. Four of these are simple -- heat and dryness, heat and moisture, cold and dryness, cold and moisture.
>
> Dyscrasias from internal causes arise when there is in the food or drink something of a pharmacologic nature which either warms or cools or dries or moistens, or warm and dries, or produces any other combination of these qualitites...From these causes the excrement of the abdomen is suppressed (Galen 1951)."

Like Hippocrates, Galen noted the importance of evacuation of retained excrements. In Chapter XIV: "It is a universal docrine for all excrements, to employ the opposite to the cause of their retention." Similarly in chapter IX, he discusses the preparation and use of cathartics and enemas: "But if after being constipated for two days, they do not move on the third day, then a mercurial herb is sufficient...(as is) sea-cabbage, calomel in barley (Galen 1951)."

Galen further describes the importance of diet in the maintenance of health in chapter V, Diet and Venesection: "And since also the hypochondrium in all such patients becomes swollen and distended, and whatever they take turns easily to gas, it would be better to give some long pepper with the food; for this dissolves the thickness of the flatulent gas, and also pushes towards the lower abdomen what is sluggishly arrested in the hypochondrium, and contributes to the digestion of food...white pepper...so-called drug of Diospolis...equal parts cummin and pepper and springwort and nitre (Galen 1951)."

Classical physicians attentively observed the character of the urine and stools since this gave them insight into the processes of digestion and concoction that were occurring in their patients. It was believed that alterations in these processes contributed to disease. Much of the therapy of classical physicians was directed towards re-establishing the normal balance of gastrointestinal function through the use of medicated syrups.

DARK AGES

Following the death of Galen, virtually no new information was gained regarding anatomy or physiology until the Renaissance. The teachings of Galen survived, however, through three lines of descent (Osler 1921). A continuous series of physicians practiced medicine in the Greek

tradition. Many manuscripts of the original Greek texts remained extant in the Eastern Empire but apparently were unavailable to physicians of the West until the fall of Constantinople in 1453 (Nuland 1988). A second source of Greek learning was the South of Italy where Greek remained the spoken language until the thirteenth century. The schools of southern Italy and Sicily translated scientific manuscripts directly from the Greek to Latin as early as the eleventh century (Singer 1928). Alphanus, the Archbishop of Salerno (d. 1085), for example, translated a work by Nemesius into Latin. These translations increased in numbers over the following centuries. No anatomical works by Hippocrates, Aristotle or Galen are thought to have been available in western Europe before the 12th Century (Corner 1927). The major source of Galenic teachings during the late Dark Ages in the West was from Arabian medicine.

The major stream through which Greek medicine reached western Europe following the Eleventh Century was Arabian medicine. After the period of conquest, the Arabs settled down to the arts of peace. Baghdad and Cordova became great centers of learning. Unfortunately, the Greek teachings were second or third hand by the time they were translated into Latin. Much of Galenic anatomy was translated into Arabic not from the original texts but rather from synopses of Galen such as Collecta medicinalia which was compiled by Oribasius at the request of the Emperor Julian (McMurrich 1930).

Similarly, Islamic knowledge of Galenic medicine derived largely from translations of The Seven Books of Paulus Aegineta written in Alexandria in the seventh century (Nuland 1988).

Within a hundred years of the First Crusade, many Arabic texts were translated into Latin and became available in western Europe (Campbell 1926). Constantine the African between about 1070 and 1087 A.D. produced about 15 medical manuscripts at Salernum (Corner 1927). Stephen of Antioch produced in 1127 A.D. a translation of Hali Abbas which contained an important anatomical section (Singer 1928). Archbishop Raymond of Toledo established a school of translation called "The House of Wisdom". Gerard of Cremona (1115-1185 A.D.) translated at least 92 manuscripts from Arabic to Latin. The medical text which proved the most influential in western Europe was the Canon of Medicine of Avicenna which was largely based on Arabic translations of Galen. Thus, in a brief time period beginning in the 12th Century a prodigious volume of medical knowlege was reintroduced into western Europe through Arabic sources. These Latin translations contained much of Galenic anatomy and physiology but the texts were derived from Arabic translations of Byzantine synopses of Galen's works. The intervening centuries and nuances of different languages had introduced many vagaries and errors into the Galenic tradition.

The flood of knowledge into western Europe from Arabian sources also introduced Islamic customs. Arabian medicine divorced the practice of medicine from surgery. The Arabic authorities maintained that under certain conditions the body was unclean. This belief led to the Edict of Tours in 1163 which stated *"Ecclesia abhuret a sanguine."* As a result of this edict, surgery was relegated to barbers and mountebanks (Cambell 1926). This distinction was warmly received by the scholastic philosophy prevalent among the western scholars since medicine lent itself better to logical argument as to causes, principles and treatment while surgical conditions required prompt intervention (McMurrich 1930). This led to the banishment of surgery from the universities during their period of development. Similarly, anatomy was of little value in the practice of the Arabian version of Greek medicine. The study of anatomy and function was conventionalized into the reading of Latin translations of Arabic summaries of Galen. Cadaveric dissections were deemed irrelevant since Galen was accepted as the authoratative source of all anatomic information. Unfortunately, during the descent of Galenic anatomy from the second century A.D., it was forgotten that Galen rarely studied human anatomy but rather carried out dissections in various animals particularly monkeys (McMurrich 1930). The differences between Galenic teachings and actual human anatomy were only

discovered in the Sixteenth Century when cadaveric dissections became more prevalent.

Within a hundred years of the First Crusade, many Arabic texts were translated into Latin and became available in western Europe. The medical text which proved to be the most influential in western Europe was the Canon of Medicine of Avicenna which was largely based on Arabic translations of Galen. Avicenna (930-1037 A.D.), an Arabic philosopher, delineated the processes of digestion in The Canon on Medicine. Avicenna (Abu al-Hussain Ibn Abdullah Ibn Sina) was born in 980 A.D. in the village of Afshana in the province of Bukkara, Persia. "The Prince of Physicians" according to Persians, his textbook, The Canon on Medicine became the textbook of medical schools in many European medical schools. Even today, many consider the text an excellent amalgamation of all the medical doctrines of Hippocrates and Galen with the biological concepts of Aristotle (Lewis 1965).

Avicenna differed from Hippocrates on his definition of disease and the significance of symptoms. Avicenna defined disease as "an abnormal state of the human body, in virtue of which injurious effects result (Gruner 1930)." He no longer judged a symptom a response to an illness but rather a result of the disease. He wrote: "Symptom...a phenomenon consequent upon this non-natural state of the body (Gruner 1930)." Indeed, he had veered so far away from the views of

Hippocrates that Avicenna taught that a symptom could even be the primary cause of the affliction: "A symptom may be the cause of a disorder (Gruner 1930)." Thus, Arabian medicine was not just a restatement of classical teachings but had evolved away from some of the fundamental postulates of Hippocrates and Galen.

Despite these differences in the definition of disease and the importance of syptoms, many of the views of Avicenna were firmly founded on the teaching of the Greek tradition. Avicenna, like Hippocrates, felt that disease could be explained by an imbalance in the natural state of the body. Health derived from an equilibrium between what the body takes in and assimilates and what the body excretes. Observation of the characteristics of stool could render important information about the condition of the individual and the balance of his natural faculties. Avicenna wrote:

> "The following are the characters (of stool) to note: the quantity; the consistence; the colour; the form or shape; and the time occupied in the passage of food through the bowel.
>
> 1. Quantity. If greater than the amount of food taken, the reason lies in abundance of humours; if smaller in amount, the reason lies in deficient amount of humours, or in a retention of the food in the caecum or colon...The reason may also be that the expulsive power is insufficient.
>
> 2. Consistence. Moist excretion denoted defective digestion or obstruction of some form; weakness of the mesentery, so that it does not absorb sufficient water from the food; fluxion from the head; some constituent of the diet which causes the dejection to be moist. If the faecal matter is both moist and viscid, this shows that there is colliquation in the tissues. Fetor is then present...Dry stool results from...a long delay in the intestines.

> When fecal matter is both moist and hard, the dryness is due to undue delay in the intestines due to moisture which cannot escape.
>
> When the feces are passed out too rapidly it is a bad sign; it shows...that there is a weakness of the retentive power. A delay in the passage of fecal matter through the body denotes a feeble digestion, coldness of the intestines, abundant moisture (Gruner 1930)."

Avicenna described various regimens for the regulation of intestinal motility. Health could be maintained by expulsion of the humors associated with disease. The practioner, however, needed to take into account such variables as the age of the patient and the season of the year. "Articles of food which have a laxative action, appropriate for elderly persons.--For summer: Figs and prunes; for winter: dried figs cooked in water and in honey. They must be taken before food, to have a laxative effect (Gruner 1930)." The physician herded over his patient both in health and in illness. By proper attention to bowel function, the physician could hope to prevent the development of various maladies in his patients.

The physician could tailor his intervention to the specifics of the situation. He could stimulate evacuation of the rectum as outlined above or he could direct his attention to the more proximal gut. He described, for example, additional cathartics for the proximal gastrointestinal tract. "A medicine often leaves its odour behind in the stomach, making it appear to be still there. The remedy for this is to partake of a barley ptisan or barley-meal cake,

for this will have the effect of cleansing the stomach (Gruner 1930)." The selection of various remedies required meticulous evaluation of the clinical situation. Based on the character, color and quantity of the stool, the physician selected his remedy for the patient. Millenia of observation ensured the precipitation of the expected result if the physician carefully followed these guidelines which had been first developed among the Greeks and passed down by the Arabs.

15th Century

Physicians in western Europe gained access to the original manuscripts of Hippocrates and Galen following the crusades. Careful study of these works revealed the differences between Arabian medicine and the older Greek traditions. Many scholars of this period turned their attention to the cleansing of Galenic medicine from Arabian "heresies". In the same spirit of the Protestant Reformation which was sweeping western Europe at this time, physicians demanded the reinstitution of the "true" teachings of Galen. This is best illustrated by the writings of Michael Servetus (1511-1553), "theological reformer, scholar, geographer, astrologist, lawyer, mathematician, scholar, and spiritual founder of the modern Unitarian movement", who was condemned as a heretic by the Roman Church in Vienne and burned at the stake by Calvin in Geneva (Servetus 1989). Servetus viewed

the Arabs as the enemy of truth. His treatise The Syrups, which summarizes gastrointestinal function, attempts to free the "sacred authority of Galen" of Arabic inaccurancies (Servetus 1989). In his preface to The Syrups, Servetus writes:

> "In our happy age, (Galen) once shamefully misunderstood is reborn and re-establishes himself to shine in his former lustre; so that like one returning home he has delivered the citadel which has been held by the forces of the Arabs, and he has cleansed those things which had been bespattered by the sordid corruptions of the barbarians (Servetus 1989)."

As a result of this effort to restore the true teachings of Galen, the attention of physicians was directed towards Greek manuscripts of Galen rather than the investigation of function of the parts of the body.

Michael Servetus extensively described the teachings of Galen on the processes of digestion and concoction and the genesis of disease in The Syrups. Servetus also explained the use of these syrups in the treatment of disease.

Servetus demonstrated the importance of "digestive syrups" in the maintainence of health. "A great many disagree on the question of digestive syrups...They consider them as doing nothing other than to digest or concoct, and content that bilious humors are not to be evacuated without awaiting coction (Servetus 1989)."

Like his predecessors, Servetus postulated that imbalances in intestinal motility led to disease.

> "Therefore it is necessary that excrement, whether it be hot or whether it be cold, either be driven out or at least altered. Not all excrement receives alteration from nature, since not all food is concocted in the stomach of every living thing, but there must be a certain relationship of that which is concocted and that which concocts. Therefore what is entirely foreign can in no way be made so that it may receive the service of the nature, but as soon as possible it must be endeavored to evacuate it; equally surely those things which have been truly corrupted in the stomach had best be driven out by vomiting or by purging (Servetus 1989)."

Serevetus stressed the importance of the return of normal bowel function: "If expulsion is not achieved, obstructions can occur in which not the concocting faculty but the expulsion must be aided (Servetus 1989)."

> "Attempted purgation by disturbing the nature will impede future concoction which will be aided by quiet...In addition, not only must the crude humors then be expelled, but others which are unprepared for expulsion if they are blocking the passages of the crude through which the others must be eliminated (Servetus 1989)."

Servetus employed a variety of "syrups" in the treatment of intestinal disorders. These syrups were extracts derived from plants or animals, as well as oils. By expelling the bad humors, restoration of health would occur.

> "For a strong concocting force employing retention surrenders nothing until it expels by thickening it to a considerable consistency. A weak one concocts, retaining weakly, and discharges a thin liquid, as though filtered out; or overburdened, it expels prematurely. Therefore from a weal concoction attenuated excrements occur.
>
> And so the reason for the prohibition of purgation is that the crude humor, because of thickness and coldness, is of slow movement and does not respond to drugs; whence bad symptoms result and become worse in the degree that the drug is more powerful in attraction and the humor, because of the greater thickness,

> stronger in resistance or more fixed in the narrower passages (Servetus 1989)."

Not only was it necessary for the removal of the crude or bad humors from the body, but the method was also critical. Slow humors needed to be removed quickly and by the speediest route. "The reason of our aphorism forbids elimination of the crude humors from the stomach through the bowel. For the crude humor is slow in movement, the transit of the pylorus narrow, and the twists of the intestine many in which the humor may be delayed and especially in the supporting mesenteric veins (Servetus 1989)."

> "For what Hippocrates and Galen fear is the attractive strength of the drugs which, with no humor to expel, usually arose bad symptoms.
>
> "But if you assuage the belly with light drugs and ease it from excrements, very often as we have already said, that evacuation is not forbidden by Hippocrates because it is not evacuation of crude humors; indeed, evacuation of the bowel is permitted when venesection is not permitted, since the belly having been freed and the passages opened, the nature also more easily expels some of the noxious humor. But if by more powerful drugs you attempt to expel the crude humors downward through the bowel, you will adduce bad symptoms (Servetus 1989)."

Servetus stressed the importance of recognizing sickness and instituting treatment immediately. Recognizing that patients responded better to treatment the sooner therapy was administered, Servetus commented: "It is preferable to eliminate by the bowel in the beginning, by a suppository, clyster or a gentle drug, so that the intestines are relieved of the burden of excrements and rendered better prepared for the elimination of other juices (Servetus 1989)."

According to Servetus, the uses of syrups are many and varied.

> "There are many and various uses of syrups...first, as an aid to concoction...second, for aiding concoctions in all parts Galen causes those things which extenuate and comminute to precede...for they prepare the body, open the passages, dissipate obstructions and remove the thickness and stickiness of the humor, and as we shall say, they eliminate something...Third, emision of blood also occurs more easily with this aid where there is abundance of crude humors (Servetus 1989)."

Later during his discourse, Servetus lists a variety of syrups, each with their own purpose and indication: "If in the beginning of the sickness the bowel must be moved it is safer to employ sweet potions of this sort...than by other drugs harmful to it and which may destroy the nature already weakened by the fever." pp 154.

> "Of this class (of syrups) are those common potions generally evacuating through the bowel, sweats and urines, such as ptisan, mead and decoction of oxymel, apomel and parsley.
>
> "Those which at the same time cut up and control the bowel are honey of roses, syrups of lavender, hyssop, squill, cyclamen, or sowbread; decoctions of cabbage, nettle and *gallus decrepitus*...Finally there may be syrups which at the same time extenuate and loosen the bowel such as dodder of thyme, polypody and a fumatory of grass, or the same herbs with whey and a fumatory of grass or of hops (Servetus 1989)."

Servetus also commented on the need for multiple regimens to restore one's health. If one uses a particular medication too frequently, tachyphalaxis could occur.

> "A second matter for consideration which must not be overlooked is that regarding the accustoming of the nature to defecation...it ought to be accomplished with not one but various medicaments...so that the bowel ought to be loosened in turn by dog's-mercury, sea-cabbage, which is called soldanella, safflower, and

> turpentine. For...if it becomes accustomed to only one, it will (in time) disregard it (Servetus 1989)."

Sixteenth Century

In the Sixteenth Century, Leonardo da Vinci's studies of the alimentary tract provided new insights on current knowledge of the intestines. In 1508, Leonardo met an elderly patient at the Hospital of Santa Maria Nuova in Florence, Italy. Hours after his death, da Vinci began an autopsy, "in order to ascertain the cause of so peaceful a death" (Keele 1972).

After removing the omentum, Leonardo displayed the pattern of the small and large intestines in approximately their correct relationships (Figures 3,4). This had been the first time that the exact anatomical position had been achieved. In addition, da Vinci discovered the appendix. This so impressed the artist that he included a special sketch of the region. The force which propelled the intestinal contents was supplied by wind, according to da Vinci. He proposed that the appendix would provide a reservoir for excessive wind in the intestines (Kelle 1972).

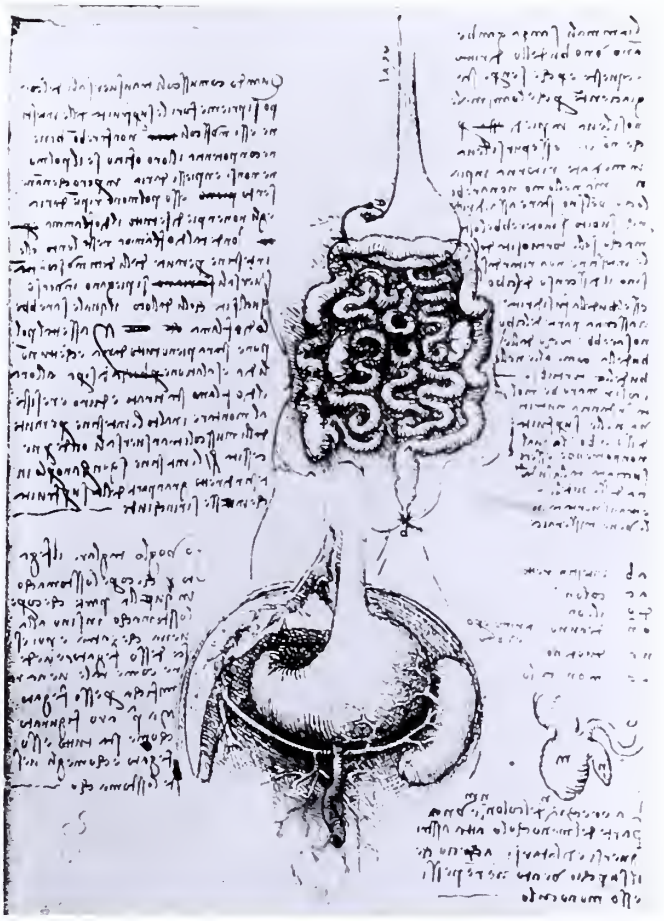

Figure 3: Drawing from Leonardo da Vinci showing an arrangement of the intestines. DaVinci felt that there was considerable variation in the arrangement of the loops of small bowel. From the stomach, the intestine bends downward (duodenum), courses upward (jejunum) and finally descends once again (ileum) before becoming the large intestine.

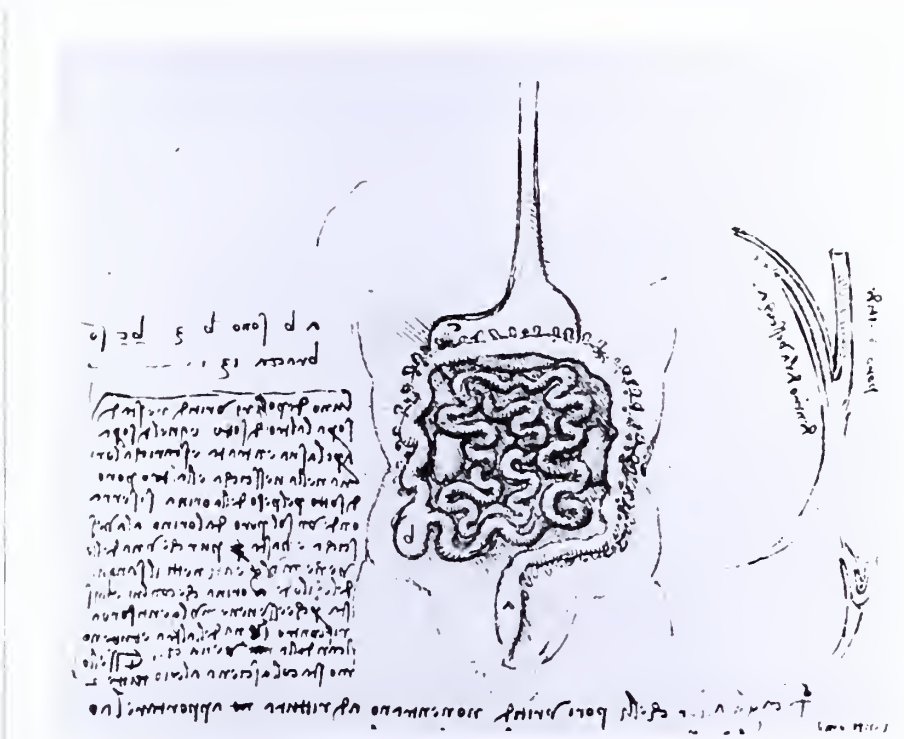

Figure 4: A second arrangement of the intestines. In this varient, daVinci demonstrates that the terminal ileum transverses the body before joining the ascending colon.

Although da Vinci's drawing of the alimentary tract were unsurpassed, his ideas of the physiology of digestion were less impressive. His one major error of observation was the failure to note the peristaltic action of the intestines. The reason for this is a simple one. Da Vinci held deep respect for life and deliberately excluded vivisection as a method of physiologic study; it has also been remarked that da Vinci was a vegetarian. Although he did recognize the muscles which lined the intestines, he felt that their sole purpose was to prevent rupture of the tube:

> "If you should say that the longitudinal muscles of the stomach are for drawing down the food and the transverse muscles for retaining it I shall reply that that the whole intestine and everything adapted to dilatation and contraction has transverse and longitudinal fibers, as is seen in the texture of cloth. And this is done in order that no force or power...shall be able to break it (Kelle 1972)."

Da Vinci believed that propulsion of food down the intestines was the result of action of the diaphragm and the transverse abdominal muscles. The opposing movements of these two muscles were called "Motors of the food and air within the human body" (Kelle 1972).

> "The flux and reflux of the two powers created by the diaphragm and the abdominal wall are those which compress the stomach and produce interrupted expulsion...during which food is alternately expelled and retained by the stomach (Kelle 1972)."

Seventeenth Century

Andreas Vesalius (1514-1564, Figure 5) and then the comparative anatomists deduced the function of organs by their gross appearance and eventually by experiments in live animals. Vesalius based his anatomic descriptions upon cadaveric dissections (Figures 6,7). An alternative approach evolved from the alchemists of the Renaissance. Basil Valentine, a Benedictine monk, pursued both the philosopher's stone (the substance which catalyzed the transmutation of things such as lead into gold) and also the nature of drugs (Foster 1901)." He pursued the role of vegetables and minerals in the cure of disease. He introduced many new chemical compounds such as hydrochloric acid. He also developed a new unifying concept of nature which rested upon three elements rather than the classical four. These elements represented the general qualities of all matter not individual substances as we use this term: sulphur represented all things which were combustible; mercury embodied things which temporarily disappeared but could be recovered; and salt indicated things that were fixed such as the ash which remains after combustion. In addition, he postulated an "archaeus" which was the force or forces by which God brought about events. This philosophy was taught to Paracelsus (Theophrastus Bombast von Hohenheim 1493-1541) by Bishop Trithemius. The great contribution of Paracelsus

was that he introduced into medicine and surgery a chemical approach to disease (Pachter 1951)."

Figure 5: Woodcut portrait of Andreas Vesalius (1514-1564) by Jan Stephan van Calcar.

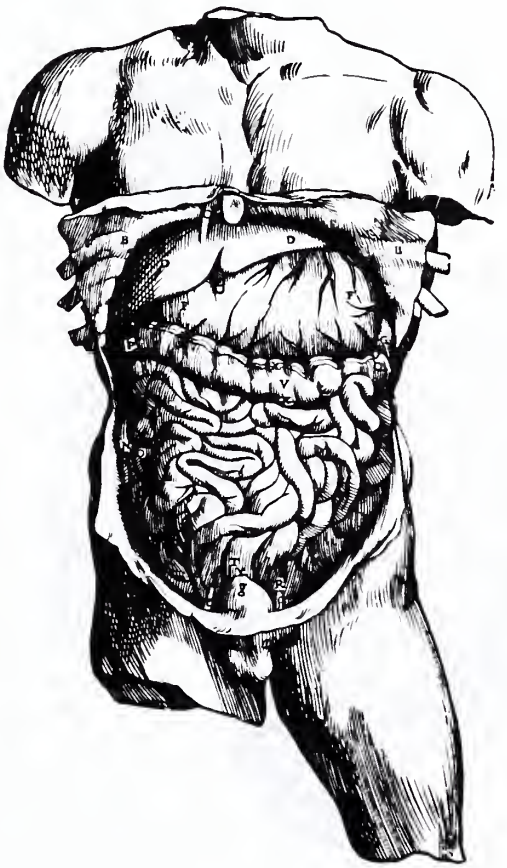

Figure 6: The sixth figure of the fifth book from Andreas Vesalius' *De Humani Corporis Fabrica* (1543) demonstrating the relationship of the liver, stomach and intestines in their true position.

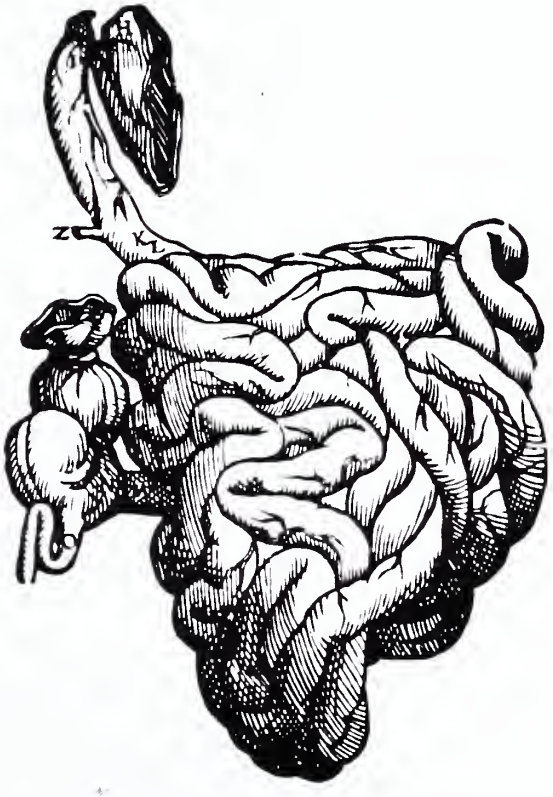

Figure 7: The seventh figure of the fifth book from *De Humani Corporis Fabrica*. Here, Vesalius provides a detailed look at the small intestine and in particular the terminal ileum and the ileocecal junction.

Paracelsus attacked the Galenic teachings of medicine. He was a staunch empiricist who believed in his own power of observation. Paracelsus wrote that "Every physician must be rich in knowledge, and not only of that which is written in books, his patients should be his book, they will never mislead him... and by them he will never be deceived (Jacobi 1979)." Paracelsus was an ardent advocate of experiment and scorned a dogmatic approach to medicine. "From his own head a man cannot learn the theory of medicine, but only from that which his eyes see and his fingers touch (Jacobi 1979)." He did not base his concept of disease upon anatomy but rather on the effect of drugs on disease. Thus, he suggested that diseases should be named by the drugs by which they are cured (Foster 1901). The impact of Paracelsus on the practice of medicine is suggested by Robert Boyle (1627-1691), the father of modern chemistry.

> "Chymists have put some men in hope of greater cures than formerly could be thought possible. Before men were awakened by the many promises and some cures of Arnaldus de Villanova and Paracelsus... many physicians used to pronouce a disease incurable. They would rather discredit the art and detract nature than confess the two could do what ordinary physick could not (Pachter 1951)."

The doctrines of Paracelsus persisted and contributed to the growth of chemical physiology in the Seventeenth Century. Ultimately, these concepts of treating disease with chemicals displaced the therapeutics of Hippocrates and Galen and became the basis of modern therapeutics.

In the Seventeenth Century, Jean Baptiste Verduc wrote on the importance of peristalsis and the circular valves of the intestine to the separation of the chyle from the excrements. He wrote that:

> "The intestines have a particular motion, in a manner, like that of the Earth Worm, which is called Peristaltick or Progressive...altogether necessary for clearing the Chyle from the Excrements, and for promoting its passage into the Milky Vessels. The Valves, which, at a certain distance from each other are plac'd in the Intestines, contribute very much to the retarding of the descent of the Chyle...all this brings the Chyle nearer to the inside of the Pipe (Verduc 1704)."

Eighteenth Century

In 1761, Giovanni Battista Morgangni wrote *De sedibus et causis morborum* -- The seats and causes of disease (Figure 8). The publication of this text marked the turning point from an ancient conception of disease to the modern one. Here we see the first combination of clinical history and underlying pathology of most of the diseases that we recognize today.

Figure 8: A contemporary portrait of Giovanni Battista Morgagni by the French engraver Jean Renard. Reproduced from Sherwin Nuland's Doctor's, the Biography, Vantage Books, New York, 1988.

Morgagni recognized the importance of peristalsis in maintaining health in the gastrointestinal system. He realized that digested food travelled down the intestine and all waste passed through the colon before exiting the body via the anus. However, in several diseased states, Morgagni observed what he termed "antiperistalsis" or antegrade motion of the intestines.

> "Why, therefore, must we altogether, and at all times, reject this cause, and suffer it to have no part in the performance? Is it because the peristaltic motion is perhaps scarcely to be acknowledged any longer? How is it then? Is it possible for the nature of animals to be so chang'd, that in our age the circumstance scarcely appears any more, which those very ancient observers have seen, in consequence of whose opinion Cicero has expresly written, "that the intestines both constringe and relax themselves alternately," either to agitate and prepare the food, or to drive the remains of it, after concoction, downwards? But left it should happen to any one of thise whom I have refer'd to, in the preface to the second Adversaria, near the latter end, that this passage of Cicero, also, may seem, "to be quoted" by me, "by way of severe reproach," I choose rather to neglect what may be replied on this occassion, and to come down from the ancients, to the more modern observers. Shall I then forget the great number of observations...of my own on dogs, sheep and rabbits...a motion alternately antiperistaltic. (Morgagni 1769) Book III, Letter XXXIV, Article 32."

As with others, Morgagni recognized the ability of certain foods or clysters to alter intestinal motility and function to help alleviate symptoms of disease.

> "So he also prescribed various remedies to be taken internally, and among thise the turpentine-resin, after which was to be drunk a water, medicated with vulnerary

> herbs...in the winter he recommended wine at the table, and that of the domestic kind, in which, at the autumnal season, when it fermented in the cask, such roots, woods, and leaves...had been macerated (Morgagni 1769) Book III, Letter XXXII, Article 9."

One of the major figures in surgery of the Eighteenth century was John Hunter (1728-1793). Hunter introduced the concept of experimental technique into surgery. His tireless work on comparative anatomy involving over five hundred dissections led to many discoveries. Hunter was the first to combine the disciplines of anatomy, pathology and surgery. His discourses on physiology were considered "so far in advance of his times that it was not comprehended (Dennis 1895)." In addition, Hunter's pupils such as John Abernathy would continue his philosophy of learning into the Nineteenth century.

Despite the immense amount of anatomic discoveries during this century, treatment modalities for various gastrointestinal disease continued to be based on centuries old Galenic teaching. William Cullen, professor at the University of Edinburgh, wrote on the Method of Cure in Fevers, "We form three general indications in the cure of continued fevers. The first is to moderate the violence of re-action. The second is, to remove the causes, or obviate the effects of debility. And, the third is to obviate or correct the tendency of the fluids to putrefaction (Cullen 1777)."

Nineteenth Century

John Abernathy, a student of John Hunter and surgeon at St. Bartholomew's Hospital in London provided new insight into the changes that occurred in the contents of the intestines. Abernathy focused his attention to the function of the intestines because he believed that he could treat specific diseases by altering gastrointestinal function. He states: "By correcting the obvious errors in the state of the digestive organs, the local disease, which had baffled all attempts to cure by local means, has speedily been removed (Abernathy 1809)."

Abernathy devoted an entire treatise to the remote connection between the brain and the gut. He wrote that "the reciprocal sympathy which exists between the brain and the digestive organs, is generally admitted (Abernathy 1809)." In addition, he commented "It is the remote sympathies, according to (Hunter's) division, of which I am now speaking (Abernathy 1809)." He reported that afflictions of the colon and rectum might adversely affect the stomach.

> "When digestion is imperfectly performed, the functions of the intestinal canal will soon participate in the disorders of the stomach...Should the disease commence in the large bowel, it disturbs the functions of the stomach, and secretion of the liver, and becomes augmented in its turn by its sympathy with these parts (Abernathy 1809)."

Thus, Abernathy conceived that humoral actions mediated this remote sympathy of the intestines on the stomach and liver.

More than one hundred years would pass before further experimental studies began to elucidate humoral factors (hormones) which might account for these observations.

Sir Astley Cooper, an eminent surgeon in London in the early nineteenth century, demonstrated that clinical practice was still Galenic. "A deficiency of secretion from the alimentary canal is the cause of a great number of the diseases which human beings are subject (Cooper 1839)."

With Rush's change of the classical definition of disease in 1809, (see earlier section of thesis) there followed a loss of the classical approach to the treatment of disease.

The later half of the nineteenth century saw a dramatic explosion in the field of medicine and, in particular, surgery. Gut motility now became an area of active interest. In 1857, Pfluger noted that splanchnic nerve stimulation inhibited intestinal movements. Intestinal peristalsis was further investigated by Ludwig. In 1861, he described the swaying motions of the intestines between the intervals of peristalsis. He termed these movements *Pendelbewegungen*. Auerbach and Meissner provided additional proof of an intrinsic control mechanism of intestinal peristalsis with the identification of intrinsic nerve plexuses in 1862. Mall demonstrated that the peristaltic wave occured in one direction -- proximal to distal. In his experiment of 1896, Mall excised loops of small bowel and reversed it <u>in situ</u>,

producing intestinal obstruction proximal to the reversed section (Garrison 1913).

Perhaps the most classic experiment of the 19th century was performed by Bayliss and Starling in 1899 who confirmed the hypothesis that intestinal peristalsis was a reflex through the intrinsic ganglia. The conclusion of 19th century investigation of intestinal motility was that the intestines were an autonomic mechanism which is regulated by, but not dependent upon, extrinsic nerves (Garrison 1913).

Twentieth Century

Cannon observed peristalsis or "segmentation" of the intestines by means of Roentgen rays in dogs and cats in 1902. Ten years later in 1912, Glenard made cinematographic studies of the intestinal movements under normal and purged states. These studies were performed using rabbit bowel which was isolated and constantly perfused with Locke's solution (Garrison 1913)

In 1902, Hemmeter attempted to elucidate the effects of various agents on the regulation of intestinal peristalsis. Carbon dioxide, hydrogen sulfide and methane were determined to cause an increase in motility. Exposure of bowel to oxygen, such as during a surgery, resulted in a paralysis of bowel function. Hemmeter further evaluated the effects of various drugs, chemicals and toxins. Belladonna and atropine administration led to reduced irritability of Auerbach's

plexus. Opium and morphine had biphasic effects. Low doses caused an increase in contractility while high doses inhibited peristalsis. Finally caffeine, muscarin and nicotine all resulted in increased motility (Hemmeter 1902).

Hertz expanded on the regulation of motility in 1909. After administration of a bismuth meal, transit time studies revealed that the meal could be localized to the cecum after an average of four hours. The effect of intestinal motility caused by food entering the stomach was due to a reflex action. Hertz also tested the effects of certain meals on transit time.

> "Much of the activity of intestines depends on the chemical stimulation produced by certain constituents of the food and of the products of digestion. Sugar...stimulates peristalsis in the small intestine but not the colon. Organic acids such as formic, acetic, butyric, tartaric, citric and lactic acid stimulate peristalsis in the small more than the large intestine...Carbon dioxide and marsh gas produced by fermentation of carbohydrates and sulpheretted hydrogen produced by putrefacation of proteins actively stimulate peristalsis in all parts of the intestine (Hertz 1909)."

Hertz also proposed treatment modalities for constipation in much the same way as the ancient physicians -- by means of altered diet.

> "Treatment of constipation by diet is one of the most effective methods of treatment...Mechanical stimulation of intestinal movements depends on direct irritant action of cellulose and distention produced by the food. The chief chemical stimulants of intestinal activity are the sugars, organic acids and their salts, which are present in vegetable food (Hertz 1909)."

Hertz divided his treatment modalities into four pharmacotherapy groups. In the first group he described the

use of alkaloids. Strychnine caused an increase reflex excitability of the nervous system as well as increasing muscular tone. Atropine was classified in his second group. Administration of this drug led to relief of "spastic constipation." The third group of drugs included opium, morphine and codeine. This group relieved constipation due to pain leading to biliary and renal colic. Finally the fourth group of drugs included pilocarpine and physostigmine. According to Hertz, this group caused direct stimulation of the motor and secretory nerve endings of the intestines (Hertz 1909).

Gastrointestinal function is considered fundamentally important for continued health. Abnormalities in gastrointestinal function led to disease. Physicians would then use the gastrointestinal tract to rid the patient of any morbidic matters by purging them from the body. Classical concepts continued as the basis of medical therapeutics well into the 20th century. As a point of speculation, many of the prevalent diseases of the gastrointestinal system such as diverticulitis, irritable bowel syndrome and colon cancer may be produced by alterations in motility. Therapy might better benefit from a more complete understanding of peristalsis and the subsequent development of pharmacotherapeutic probes.

MOTILITY

Review of Current Concepts

Galenic teachings dictated the concepts of disease and intestinal function for hundreds of years. In the eighteenth century John Hunter's experiments led to new insight into the mechanism of intestinal motility, and since that time more and more precise knowledge has accrued on regulation of motility. Disorders of intestinal motility continue to be a major concern to mankind during the twentieth century. The irritable bowel syndrome, diverticular disease and perhaps even the genesis of colon cancer can be traced to alterations of intestinal peristalsis.

Function

In 1899, the first major insight into the mechanism and regulation of intestinal motility was proposed by Bayliss and Starling: "The peristaltic contractions are true coordinated reflexes, started by mechanical stimulation of the intestine, and carried out by the local nervous mechanism...They travel only in one direction, from above downwards, and are abolished on paralysing the local nervous apparatus (Bayliss 1899)."

The main function of the small intestine is digestion and absorption of nutrients. The intestines accomplish this role, in part, by the process of motility in which food products mix with the digestive enzymes and the contact of chyme with the absorptive cells over a sufficient length of bowel, and finally to propel remnants into the colon.

Motility can be broken down into two components, peristalsis and segmentation. Peristalsis consists of waves of one or more contractions of circular muscle that is propagated along the bowel. This action has been described as a moving ring of contraction. The primary role of peristalsis is propulsion of food boluses down the intestine. Segmentation consists of two or more standing contractions, separated by a short distance. The purpose of these standing contractions is to form an occluded segment thereby allowing maximal mixing of luminal contents and absorption of digested nutrients to the mucosal lumen (Wingate 1983).

Smooth Muscle

The inner muscle layer of the small bowel is the muscularis mucosae, a thin layer of smooth muscle underlying the mucosa itself. The muscularis mucosae defines the boundary between the mucosa and submucosa. This layer is not believed to play a significant role in the gross movements of the small intestines but rather a role in mixing chyme adjacent to the mucosal surface and possibly in modulating the permeability of the mucosal layer.

Circular smooth muscle invests the entire small intestine. The circular muscle of the small bowel is thicker than the longitudinal muscle layer and is the major source of contractile activity. Contraction of the circular muscle layer will constrict the lumen. Muscle cells in this layer may play a role in coordinating propagation of slow waves down the intestine with the longitudinal layer (Weisbrodt 1987, Bortoff 1983).

The outer coat of muscle is the longitudinal muscle layer. Contraction of this muscle mass will shorten the intestine (Wingate 1983). Although thinner than the circular layer, it plays a major role in antegrade propagation of intestinal slow waves. The longitudinal arrangement of the smooth muscles facilitates rapid electrical conduction, compared to the perpendicular arrangement of the muscle cells in the circular layer.

The cell membranes of small intestinal smooth muscle exhibit a rhythmic depolarization (Bortoff 1969, Christensen 1971). This effect was first described by Alvarez and Mahoney in 1922 (Alvarez 1922) and has been referred to as slow wave, basic electrical rhythm, or electrical control activity (Cohen 1979). This rate of rhythmic depolarization decreases distally down the small intestine (Christensen 1964, Christensen 1966). This is due to the inherent pacemaker of the duodenum in initiating small intestinal motility (Herman-Taylor 1971).

Smooth muscle cells undergo cyclical depolarizations. Contraction only occurs when action potentials are superimposed on the depolarization plateau. The occurrence of action potentials is signalled by the appearance of spike bursts superimposed on the slow wave. The timing of these spike bursts is dictated by the slow wave and may be considered to be "phase-locked" events (Wingate 1983). This spike response is calcium dependent. The primary role of these slow-wave associated spike potentials is the mixing of intestinal chyme by intestinal segmentation (Cohen 1979).

Contractions of the wall of the small intestine are a consequence of changes in lengths of the smooth muscle cells that make up the tunica muscularis. The temporal and spatial patterns of intestinal contractions depend on factors that influence these smooth muscle cells. These factors include the intrinsic properties of the smooth muscle cells themselves, the activities of nerves that constitute the intrinsic nerve plexuses such as the myenteric plexus, the influence of the extrinsic sympathetic and parasympathetic nerves that are distributed to the muscle and intrinsic nerves, and the influence of the various chemicals that reach the nerves and muscles of the gut by endocrine and paracrine pathways (Cohen 1979).

Intrinsic Nervous Control

The major control system of the small intestine appears to reside within the intrinsic nervous system. Numerous neurons, nerve endings, and receptors lie in the intestinal wall. These nerve elements tend to be concentrated in nerve plexuses, the most prominent of which is the myenteric plexus of Auerbach between the longitudinal and the circular muscle layers. The neurons in the plexus receive input from several sources, including receptors in the mucosa and in the muscle wall, from other neurons in the plexus, and from extrinsic nerves. They form an important neural control center acting in effect as a "little brain" of the gut (Vantrappen 1975).

The regulatory capacity for the myenteric plexus has been difficult to evaluate. Research has focused on the inhibition and activation of these nerves in various intestinal preparations. Various neuronal agonists and antagonists have been utilized in hoping to elucidate the regulatory mechanisms of neural control. When an isolated segment of small intestine from a cat is placed in an organ bath, intermittent contraction is evident. When tetrodotoxin, a potent neuronal antagonist, is added to the bath, the segment contracts at the rate of the frequency of the slow wave (Biber 1973, Bortoff 1975). Recordings from neurons within the myenteric plexus showed that many of these neurons were active during those periods when the bowel was not contracting and that tetrodotoxin blocked activity of

those neurons (Ohkawa 1972). Thus, it appears that there are tonic inhibitory nerves in the plexus that actively suppress contractions of the circular smooth muscle. Additional studies supporting this hypothesis come from investigations of aganglionic segments of bowel. Wood demonstrated that these aganglionic segments lack a normal myenteric plexuses and are tonically contracted (Wood 1972). Electrical or chemical stimulation of the nerves within the myenteric plexus also affects contractions of the muscle (Hidaka 1969, Wood 1975, Wood 1979, Weisbrodt 1987).

Bennett postulated that from a neuropharmacologic viewpoint four types of efferent nerves are involved in the control of intestinal motility: cholinergic excitatory; adrenergic inhibitory; nonadrenergic, noncholinergic inhibitory; and probably, noncholinergic excitatory nerves (Bennett 1975).

Peristalsis consists of two simultaneous events: ascending contraction, proximal to a food bolus, and secondly, descending inhibition with smooth muscle relaxation distal to the bolus. These pressure zones allow food to be pushed down the lumen (Makhlouf 1989, 1990).

Ascending contraction: The present model for the regulation of peristalsis has been proposed by Makhlouf (Figure 9). Interneurons projecting cephalad in the myenteric plexus coordinate a wave of contraction proximal to

a bolus. This is referred to as ascending contraction. Stretch receptors in the intestinal wall synapse with

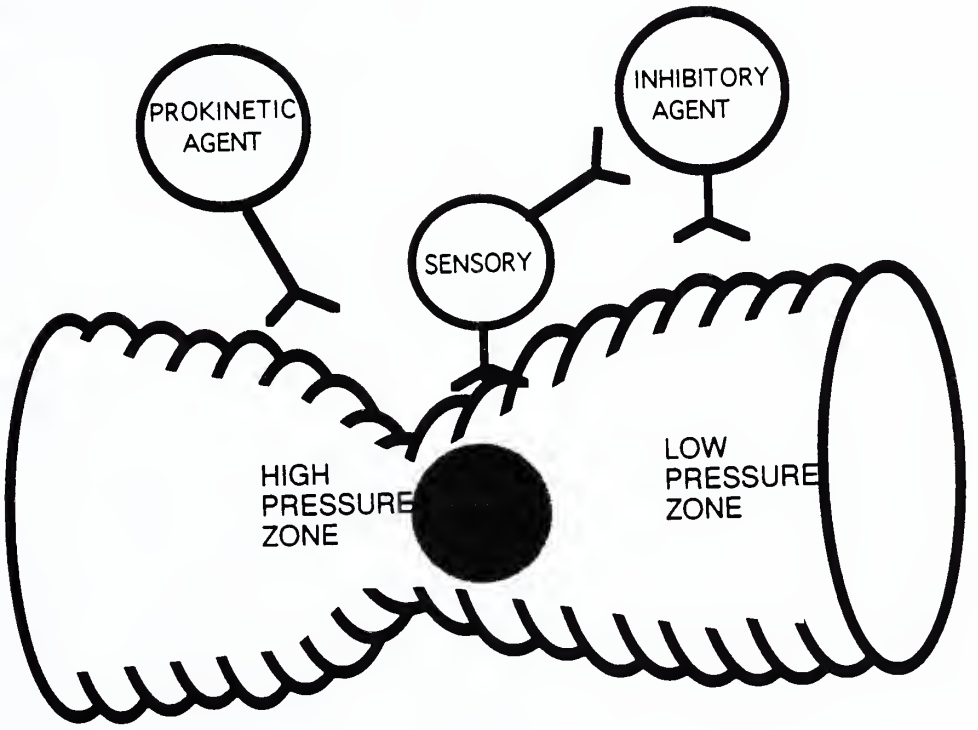

Figure 9: Mechanism of intestinal peristalsis. Prokinetic agents (here shown being released from a neuron) cause smooth muscle contraction. This in turn leads to generation of a high pressure zone proximal to a food bolus. Stretch receptors, sensing the bolus within the lumen, cause release of inhibitory agents such as vasoactive intestinal peptide distally. This release leads to smooth muscle relaxation and generation of a low pressure zone distal to the food bolus.

interneurons in the myenteric plexus. These project proximally and synapse with prokinetic cholinergic neurons, orad to the bolus. The cholinergic neurons cause contraction

in the circular muscle layer which propels the bolus distally.

Descending Inhibition: Synchronous with ascending contraction, descending inhibition is initiated by sensory receptors in the intestinal wall. These convey stretch from a food bolus in the lumen, to the network of ganglia in the myenteric plexus. The impulse is transmitted down a relay of three inhibitory neurons, whose transmitters are somatostatin, opioid and vasoactive intestinal peptide, in sequence.

Stimulation of somatostatin neurons causes inhibition of opioid neurons. The opioid neurons exert a continuous inhibitory restraint on VIP neurons. Release of somatostatin therefore inhibits release of the opioid mediator and the VIP neurons are no longer inhibited. The final inhibitory VIP neurons project into the circular muscle layer. Release of VIP into the muscle coat causes smooth muscle relaxation, and descending inhibition results. Intestinal muscle distal to the bolus then relaxes, enabling it to be propelled further down the intestine (Makhlouf 1990). This system produces synchronous contraction orad to the bolus, and relaxation distal to the bolus, enabling it to be propelled down the lumen.

Research has focused on the "transition zone" between the high and low pressure zones which are well characterized. Researchers suggest there must be some counter-regulatory

mechanism between the high pressure zone created by prokinetic agents and the low-pressure zone due to vasoactive intestinal peptide release (Figure 10).

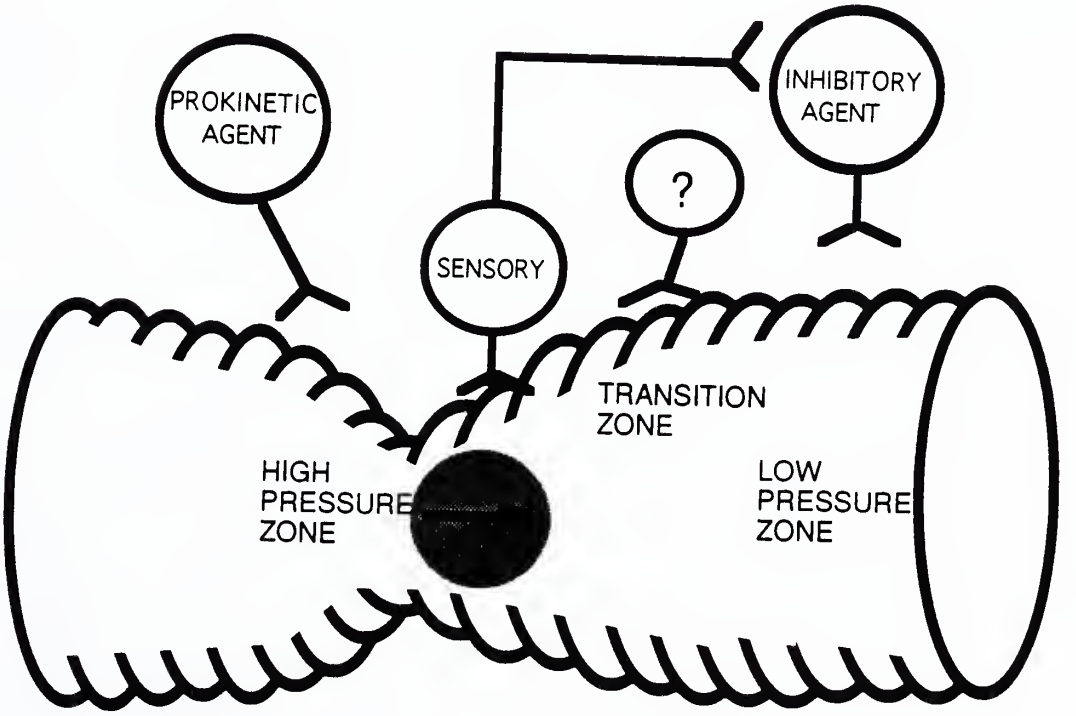

Figure 10: Research has focused on counter-regulatory agents involved in intestinal peristalsis. Between the high and low pressure zones generated by prokinetic and inhibitory agents, there must exist some mechanism which reverses the low pressure zone and allow generation of a high pressure zone by prokinetic agents.

Extrinsic Nervous Control

The small intestine receives an extrinsic innervation from both divisions of the autonomic nervous system, parasympathetic and sympathetic. The extrinsic innervation to the small bowel is provided by the vagal and splanchnic nerves (Gonella 1978, Gershon 1981). The exact role played by these extrinsic nerves remains incompletely understood. Small intestinal motor activity is essentially unaffected by either vagotomy or splanchnicectomy (Vantrappen 1985).

Parasympathetic input to the small bowel arises from the vagus which innervates the bowel to the mid-transverse colon. Pre- and post-synaptic neurons synapse in the vagal nucleus in the midbrain, and the left and right vagi pass down the esophagus as anterior and posterior vagi respectively.

Vagal input to the small bowel comes predominantly from the posterior vagus, which passes through the celiac and superior mesenteric plexi, without synapsing. The vagal fibers, like the sympathetic counterparts, reach the small bowel via the arterial tree (Longo 1989). The vagus is connected to the muscle through the myenteric plexus which then interfaces with intestinal smooth muscle cells (Wingate 1983).

Sympathetic (thoracolumbar) input arises from the intermedio-lateral cell column in the spinal cord and pre-synaptic fibers pass through the sympathetic chain without

synapsing. The fibers pass to the celiac and superior mesenteric ganglia, where they synapse with postganglionic fibers. The post-synaptic fibers travel together with vagal fibers via the arterial tree to the small bowel. Sympathetic fibers convey not only efferent signals, but also noxious stimuli from the gut to the central nervous system.

Early studies on the effects of nerve stimulation and transection on intestinal contractions demonstrated, in general, that activation of the parasympathetic nerves increased contractions and activation of the sympathetic nerves decreased contractions (Kewenter 1965, Kewenter 1970, Kosterlitz 1968). Researchers have also demonstrated that certain reflexes depended on integrity of extrinsic innervation (Gernandt 1946, Gregory 1947, Johansson 1967). The primary reflex studied was the intestinal inhibitory reflex, which is characterized by inhibition of intestinal contractions at all adjacent loci during marked distension of an area of bowel. This reflex appeared to depend on integrity of the sympathetic nerves. Some studies implicated participation of the brain and spinal cord, since sectioning of the splanchnic nerves abolished the reflex. Other studies, however, suggested that the reflex involved only the prevertebral ganglia, since the reflex persisted after splanchnic section but not after ganglionectomy (Weisbrodt 1987).

Several studies have been designed to determine the neural pathways between the intestine and the prevertebral ganglia (Kreulen 1979A, Szurszewski 1976). Both afferent and efferent fibers have been demonstrated electrophysiologically. Also, an intestinointestineal reflex has been observed in a preparation in vitro that contained only the colon, the prevertebral ganglia, and interconnecting nerves (Kreulen 1979B). Thus reflex arcs contained solely within the intrinsic and prevertebral ganglia do exist and they are functional. These studies were performed on preparations of colon. Whether or not they exist for the small intestine remains to be determined (Weisbrodt 1987).

Kosterlitz delineated the spinal and supraspinal influences on the intestine (Kosterlitz 1968). Although there have been studies which indicate that certain reflexes and patterns of motility can be expressed in preparations lacking central nervous connections, one should not conclude that the central nervous system is not needed or does not influence patterns of motility. There are areas within the brain that when stimulated cause either an increase or a decrease in intestinal contractions (Roman 1987). Thus higher centers can alter activity of other neural and muscular tissue. Also, endogenous peptides, autocoids and pharmacologic agents injected into the cerebral ventricles alter contractions and intestinal transit (Bueno 1985). Such activation of structures within the brain is thought to

elicit a response by way of the autonomic nervous system. However, there are data to indicate that in some instances a humoral substance may be involved (Bardon 1984).

The site of action of the extrinsic nerves could be either on the smooth muscle cells themselves or on the nerves of the myenteric plexus (Gabella 1972, Youmans 1972). Structural and physiologic studies indicate that extrinsic nerves end at the level of the myenteric plexus. This is true for both sympathetic and parasympathetic nerves. A few adrenergic nerve terminals can be found within the muscle layers themselves, but their function is not clear. Therefore, extrinsic nerves probably function to regulate and modulate activity of the intrinsic nerves, which in turn affect the intrinsic activity of the intestinal smooth muscle.

Cholinergic neurons

Acetylcholine is the transmitter of many, if not all, of the preganglionic vagal and sacral fibers that reach the intestines. Researchers have demonstrated that cholinergic preganglionics innervate both excitatory and inhibitory neurons (Kosterlitz 1964). Kosterlitz also demonstrated that intrinsic excitatory ganglion neurons are also cholinergic (Kosterlitz 1968). Segments of guinea pig ileum maintained *in vitro* spontaneously release large amounts of acetylcholine (Chujyo 1953). This spontaneous release reflects the spontaneous activity of the enteric nervous system.

Researchers reported that there is a larger release of acetylcholine when the gut is stimulated electrically (Gershon 1981). This extra store of acetylcholine probably arising from neurons within the enteric nervous system (Paton 1968).

HORMONAL CONTROL

CHOLECYSTOKININ

In 1928, Ivy and Oldberg described the release of a mediator from small bowel mucosa by infusing fat into the duodenum. The mediator caused contraction of the gallbladder when reinfused into the animal and was thus named cholecystokinin (Thompson 1984). Cholecystokinin (CCK) was isolated from hog intestine as a 33-amino-acid peptide. It possessed, in addition to gallbladder-contracting activity, pancreatic-enzyme-stimulating activity (Mutt 1968).

In the intestine, CCK is found in open-type endocrine cells, most abundant in the duodenum and proximal jejunum. It has also been isolated in neurones in the myenteric and submucosal plexi of both the small and large intestine (Gutierrez 1974, Stewart 1977, Amer 1972).

The chemical structure of CCK is complicated by the natural occurence of multiple molecular forms. In addition to the 33-amino acid form (CCK-33), intestinal extracts contain roughly equivalent amounts of a larger form composed of 39 amino acids (CCK-39) and an even larger form containing

58 amino acids (CCK-58) (Eysselein 1982, Mutt 1968). The principle smaller form is the carboxy-terminal octapeptide (CCK-8) that has been isolated from sheep and human brain (Dockray 1977, Reeve 1984, Walsh 1987).

CCK and gastrin have several common features. The carboxyl-terminal pentapeptide amide sequence, identical in the two peptides, includes the biologically active region for both. The six amino acid extensions that immediately follow the pentapeptide sequence are the same in the human CCK and gastrin precursors (Gly-Arg-Arg-Ser-Ala-Glu). Carboxyl-terminal amidation of CCK and gastrin involves the action of an enzyme that converts glycine-extended phenylalanine to carboxyl-amidated phenylalanine (Bradbury 1982, Eipper 1985). The principal structural difference related to biological activity between gastrin and CCK is the invariant presence of a tyrosine O-sulfate group in the seventh position from the C-terminus in CCK, while the tyrosine residue located in the sixth position from the C-terminus in gastrin may be either sulfated or non-sulfated (Walsh 1987).

Bertaccini and Levant have both reported that CCK analogs markedly decrease transit time of contrast material through the human intestine (Bertaccini 1971, Levant 1974). CCK causes disruption of the fasting pattern of myolectric activity in canine intestine, but the stimulated spike potentials are not identical to those that occur after a meal (Mukhopadhyay 1977). CCK-8 produced contraction of circular

muscle and relaxation of longitudinal muscle of dog small intestine that were antagonized by atropine, tetrodotoxin, and depolarizing concentrations of nicotine, suggesting that CCK-8 interacts with a nonnicotinic receptor on postganglionic cholinergic neural elements in intestine (Stewart 1977, Walsh 1987).

MOTILIN

Motilin, purified from hog upper intestine, was characterized by its ability to stimulate gastric motor activity in antral and fundic pouches in dogs and by stimulation of gastric pepsin but not acid secretion (Brown 1971).

Motilin is located in the endocrine-paracrine cells of the duodenal and jejunal mucosae (Polak 1976, Pearse 1976, Smith 1981). It is a linear peptide containing 22 amino acids and has a molecular weight of 2,700. Canine and porcine motilin differ in amino acid residues at positions 7, 8, 12, and 14, accounting for differences in immunoreactivity found with some antibodies (Poitras 1983, Reeve 1985). Researchers have reported that synthetic motilin, 13-N-Leu, and natural porcine motilin caused contractions of isolated segments of rabbit intestine (Shimizu 1976, Wunsch 1976). In addition, these contractions were not associated with excitation of cholinergic receptors nor with the release of acetylcholine from nerves.

Motilin is a hormone of the fasting state unlike most gut hormones which are released postprandially. Several physiological phenomena can induce rises in plasma motilin. Mitznegg demonstrated that duodenal acidification increased motilin levels 90% over baseline. He additionally discovered that fat ingestion increased motilin levels 65% whereas protein and glucose ingestion failed to change observed levels (Mitznegg 1976).

Motilin has significant effects on gatrointestinal smooth muscle. Intravenous infusion of motilin in conscious dogs during the interdigestive period initiates myoelectric complexes in the antroduodenal region that are propagated distally in the small intestine and appear identical to the myoelectric complexes that appear spontaneously at 80- to 90-minute intervals during fasting (Wingate 1975). However, it has little effects on the postprandial pattern of intestinal activity (Itoh 1976).

Motilin caused contraction of gastric and intestinal muscle strips from humans and rabbits in vitro, and this stimulation appeared to be direct rather than by neural mediation (Strunz 1975). The stimulation was abolished by the calcium antagonist verapamil. Similar results have been reported with synthetic motilin (Strunz 1975, Walsh 1987).

GASTRIN

Gastrin was the first gastrointestinal peptide for which the structure was determined. Subsequently, several

molecular forms have been identified. Noyes and Yoo identified the gene that encodes gastrin by the use of mRNA isolated from porcine antrum (Noyes 1979, Yoo 1982). Wiborg isolated the human gene from a human genomic DNA library (Wiborg 1984).

Lamers localized various forms of gastrin in the gastrointestinal tract. He discovered that the most abundant form of gastrin found in the antrum is the heptadecapeptide C-17. The 34-amino acid peptide, G-34, was found to be more abundant than G-17 in the human duodenum (Lamers 1982).

The effects of gastrin on smooth muscle in vitro include stimulation of muscle contractions and electrical activity. Morgan reported that pentagastrin is about as potent as G-17 and G-34 on canine antral muscle (Morgan 1978). In many systems, gastrin appears to act directly on smooth muscle cells to elicit contraction. In dog antrum, pentagastrin stimulates longitudinal muscle partially by release of acetylcholine from nerves, whereas it stimulates circular muscle directly (Szurzewski 1975). In some preparations, there is evidence that it exerts its effect entirely by release of acetylcholine or substance P from nervous elements. This is especially evident in guinea pig ileum where contractile responses to both gastrin and CCK peptides are abolished by tetrodotoxin and reduced by atropine and by substance P desensitization (Hutchinson 1981, Vizi 1974, Walsh 1987).

ERYTHROMYCIN

Erythromycin, a macrolide antibiotic, stimulates motility throughout the gastrointestinal tract. It promotes gastric emptying after post-vagotomy gastroparesis (Mozwecz 1990) and stimulates antral and duodenal peristalsis (DiLorenzo 1990). In the small bowel, it stimulates both anterograde and retrograde propulsion (Otterson 1990, Inatomi 1989), accounting for the diarrhea and abdominal cramps often seen as a consequence of erythromycin ingestion. In the large bowel, the drug may have a therapeutic role in colonic pseudoobstruction. Intravenous infusions of erythromycin at doses far below those needed for its antimicrobial activity induce a pattern of propagative gastrointestinal motility in the fasting state strongly resembling the interdigestive migrating motor complex (Itoh 1984 and 1985).

The prokinetic actions of erythromycin in the small bowel mimic the actions of endogenous motilin administration, since motility is stimulated only in the interdigestive phase (Inatomi 1989, Itoh 1984, Strunz 1975, Itoh 1985, Adachi 1981, Wingate 1976, Lang 1986, Itoh 1976, Depoortere 1990). In addition, Peeters demonstrated that erythromycin binds *in vitro* to motilin receptors, located on the smooth muscle cell surface (Peeters 1989). These receptors are distinct from the traditional cholinergic receptors, although activation of each results in smooth muscle contraction.

Armstrong demonstrated that erythromycin-stimulated ileal motor activity was not inhibited by neuronal blockade with tetrodotoxin, muscarinic blockade with atropine, or opiate antagonism with naloxone (Armstrong 1992). These results indicated that erythromycin acted by the direct stimulation of smooth muscle motor activity. Further studies indicated that the prokinetic effect of erythromycin was calcium channel-dependent since the nonspecific calcium channel blocker verapamil reversibly inhibited this effect. Finally, Armstrong demonstrated that the specific calcium channel blockade with dihydropyridine also reversibly inhibited erythromycin-stimulated ileal motor activity. These results suggested that erythromycin stimulated ileal motor activity by a direct effect on smooth muscle cells which was dependent upon both dihydropyridine-sensitive calcium channels (Armstrong 1992).

Armstrong additionally showed that erythromycin stimulated a concentration-dependent increase in small bowel motility which was not inhibited by atropine and the opiate antagonist naloxone. Furthermore, pre-treatment with tetrodotoxin had no effect on the erythromycin-induced activity. The prokinetic actions of erythromycin were therefore not mediated via the classical cholinergic receptor, nor via inhibition of the inhibitory opiate receptors. The lack of any inhibition with tetrodotoxin indicated that the receptor for erythromycin was located on

the smooth muscle cell surface, rather than within a neural pathway (Armstrong 1992). These findings were consistent with previous studies that indicated that target receptor of erythromycin was in fact the smooth muscle motilin receptor (Peeters 1989).

Contraction of smooth muscle involves calcium entry into the cell and Ca^{2+}-calmodulin complex formation. This in turn activates myosin light chain kinase and subsequent cross-bridge formation (Yoshino 1989). Entry of calcium into the cell is regulated by receptor-linked calcium channels. Currently, three subtypes of calcium channels are described, "t," "l," and "n," although the classification is still evolving (Nowycky 1985, Tsien 1987, Bean 1989).

VASOACTIVE INTESTINAL PEPTIDE

In 1970, Said and Mutt isolated VIP from porcine gut mucosa. When this was re-injected intravenously, arteriolar and venous dilatation resulted. They therefore named the mediator vasoactive intestinal peptide (Buchanan 1979, Thompson 1984).

Vasoactive intestinal peptide is not present in mammalian endocrine cells of the gut. It is found in neurones of the enteric nervous system, as well as the central nervous system, urological tract, cardiovascular system and lungs. In the gut, VIP is found in all the

component layers but is most abundant in the neuronal plexi (Korman 1989).

Vasoactive intestinal peptide release is seen after ingestion of a meal, but the specific stimulus is not known. Vagal stimulation releases VIP, and this can be reproduced by acetylcholine administration (Chijiiwa 1986). The ganglion blocker hexamethonium inhibits this, suggesting that preganglionic cholinergic nerves mediate VIP release.

In the gut, the actions of VIP have been fairly well characterized. It is a potent secretagogue, stimulating chloride secretion and inhibiting sodium absorption. It appears to act via adenylate cyclase mediated increase in intracellular cAMP (Grider 1988). Its action on gut motility involve relaxation of circular smooth muscle, again by increasing intracellular levels of cAMP. VIP plays an important role in descending inhibition of intestinal peristalsis. Descending inhibition distal to a food bolus allows anterograde propulsion down the intestine (Makhlouf 1989, 1990).

DISORDERS OF INTESTINAL MOTILITY

IRRITABLE BOWEL SYNDROME

The irritable bowel syndrome (IBS) is one the leading diseases of gastrointestinal function in the Western world. The irritable bowel syndrome refers to a well-characterized complex arising from interactions among the digestive tract,

the psyche, and luminal factors (Camilleri 1992). Studies have demonstrated that 15%-20% of the population suffer from IBS and that most do not seek medical attention for their symptoms (Drossman 1992).

An international working panel has provided a definition for this troubling disorder: "The irritable bowel syndrome is a functional gastrointestinal disorder attributed to the intestines and associated with symptoms of pain and disturbed defecation and/or symptoms of bloatedness and distension (Drossman 1990).

First recognized in 1849, Cumming commented on how constipation and diarrhea could occur in the same patient (Cumming 1849). Indeed, for nearly 150 years, the complete etiology and treatment of patients with the irritable bowel syndrome still eludes physicians.

What is known about patients suffering from IBS is that pain symptoms are produced by motor hyperreactivity. Alvarez noted abnormal peristalsis in a patient with the irritable bowel syndrome (Alvarez 1943). Horowitz and Farrar were the first to observe clustered contractions during periods of abdominal colic (Horowitz 1962). Cann demonstrated that patients with IBS and diarrhea had accelerated whole-gut transit times and, in some patients, fast orocecal transit was noted (Cann 1983). Vassallo showed accelerated transit in the ascending and transverse colon in patients with diarrhea-predominating IBS (Vassallo 1992).

Treatment modalities for IBS have been as varied as the symptoms themselves. Physicians have tried a variety of pharmacotherapy, hypnosis, and behavior modification -- all with limited success. Several drugs such as anticholinergics, opioids, calcium channel blockers, cholecystokinin antagonists, and selective serotonin antagonists have failed to provide symptomatic relief of patient symptoms.

NEUROENDOCRINE REGULATION OF GUT MOTILITY

Neuropeptide Y and Peptide YY

John Abernathy was the first to describe the neuro-gut axis (Abernathy 1809). Bayliss and Starling founded the discipline of endocrinology with the publication of "The Mechanisms of Pancreatic Secretion" in 1902. They described the substance "secretin" and defined the term "hormone." Since that time, a plethora of gut peptides have been identified. In particular, vasoactive intestinal peptide, peptide YY and neuropeptide Y appear to play a prominent role in gastrointestinal motility.

ISOLATION AND CHARACTERIZATION

Peptide YY (PYY) and neuropeptide Y (NPY) are members of a family of peptides which are structurally related to pancreatic polypeptide. Tatemoto first isolated PYY from porcine small intestine in 1980 (Tatemoto 1980). A characteristic of many biologically active peptides is the C-terminal amide structure. By employing a technique which isolated peptides with C-terminal amides, Tatemoto isolated a 36-amino acid peptide and discovered that it contained an N-

terminal tyrosine as well as a C-terminal amide. The peptide was called peptide YY (PYY) due to these terminal tyrosine residues (tyrosine = Y). It was this same technique that enabled Tatemoto in 1982 to isolate neuropeptide Y (NPY) from porcine brain (Tatemoto 1982).

The sequences of PYY and NPY were determined to be quite similar not only to each other but also to pancreatic polypeptide (PP) which Chance had isolated (Chance 1974) and sequenced. Thus, PYY, NPY and PP constituted a new family of peptides. Their sequences are depicted in Figure 11. PYY and NPY share nearly 70% homology while PYY and PP share 50% homology.

PYY: YPAKPEAPGEDASPEELSRYYASLRHYLNLVTRQRY

NPY: YPSKPDNPGEDAPAEDLARYYSALRHYINLITRQRY

PP: APLEPVYPGDDATPEQMAQYAAELRRYINMLTRPRY

Figure 11: The amino acid sequence of PYY, NPY and PP. Peptide YY shares nearly 70% homology with NPY and 50% homology with PP.

DISTRIBUTION OF PYY

Although PYY and NPY share structural homology, the two peptides exhibit distinct anatomical distributions. Immunohistochemistry provided the first clues to the localization of PYY containing cells. Utilizing tissue from five species, Lundberg demonstrated that PYY-like immunoreactivity occured almost exclusively in endocrine cells of the gastrointestinal mucosa. PYY immunoreactivity was also noted to be 100-fold higher in the colon than the duodenum in the rat (Lundburg 1982A). Further work revealed that PYY immunoreactivity occured in a variety of species including toad and grass lizard (El-Salhy 1982A); domestic fowl (El-Salhy 1982B); guinea pig and cat (El-Salhy 1982A); rat (Böttcher 1984); dog (Taylor 1985); rhesus monkey (El-Salhy 1983A); and man (El-Salhy 1983B). PYY-containing cells were shown to be present in the gastrointestinal tract from the stomach down to the rectum. Böttcher described PYY immunoreactivity in the intestine and more specifically in the basal portion of the crypts of Lieberkuhn (Böttcher 1984).

Researchers next focused on the morphology of the PYY-producing cell. Böttcher, utilizing protein A-gold labelling, described PYY-containing secretory granules in a population of L-type endocrine cells in feline colon and human rectum (Böttcher 1986). El-Salhy described these cells

as being of the open type, extending from the basal lamina to the gut lumen (El-Salhy 1983A). He noted that at the ultrastructural level, PYY-immunoreactivity was localized in a basal granulated cell containing round or slightly oval electron-dense granules with an average diameter of 190 nm (range 110-390). Studies have shown that PYY-immunoreactive cells emit cytoplasmic processes to the neighboring goblet cells (El-Salhy 1983B). This fact suggests the possibility of a paracrine function for these cells (Kishimoto 1985).

PYY has been shown to co-localize with enteroglucagon within the L-cells of the colon (Böttcher 1984 and 1986, Ali-Rachedi 1984). Ali-Rachedi demonstrated co-localization of PYY and enteroglucagon immunoreactivity in both rat and human tissue (Ali-Rachedi 1984). El-Sahly reported that PYY and enteroglucagon were co-stored in only some cells in the monkey (El-Salhy 1983A) and did not co-localize in human (El-Salhy 1983B). Böttcher demonstarted coexistence of PYY and enteroglucagon in sequential staining of tissue from rat, pig and man (Böttcher 1984). Since the two peptides were released from the same cell, it was hypothesized that perhaps PYY was contained in the proglucagon molecule. This proved not to be true and it was suggested that these endocrine cells are capable of expressing two separate gene products (Böttcher 1984 and 1986).

Nilsson demonstrated that L-cells were the sole source of PYY and enteroglucagon in the rabbit colon and that L-

cells contain different populations of secretory granules. He proposed that the existence of different secretory granules in L-cells may explain the selective release of PYY and enteroglucagon observed in the rabbit colon (Nilsson 1991).

Ali-Rachedi reported that radioimmunoassay of human fetal pancreatic extracts revealed the presence of PYY immunoreactivity. PYY concentrations declined with age in contrast to the amount of glucagon which remained statistically constant throughout the same fetal period (Ali-Rachedi 1984). These fetal pancreatic A cells, although morphologically primitive, exhibit similar characteristics as L-cells. Changes in cellular gene expression could explain the declining concentrations of PYY in these cells but more importantly it suggests that one cell type is capable of simultaneously translating two gene products (PYY and enteroglucagon).

Adrian mapped the distribution of PYY in the gastrointestinal tract of the pig utilizing radioimmunoassay (Adrian 1987C). PYY content was noted to be low in the foregut and higher in the distal colon. Peptide concentrations were 3.4 pmol/g in the antrum; 10.9 pmol/g in the mid-duodenum; and significantly higher in the ileum, 100.8 pmol/g. Similarly, PYY concentrations increased from the proximal to distal colon (pmol/g): cecum 14.0; ascending

colon 24.8; transverse colon 135.1; descending colon 270.2; sigmoid 435.0; rectum 406.5.

PYY distribution follows a similar pattern in other mammalian species. Results have shown that PYY concentrations are highest in the ileum, colon and rectum. Peak concentrations have been reported in the distal colon while trace amounts have been measured in the stomach, duodenum and jejunum (Adrian 1987B, Taylor 1985, Adrian 1982). Miyachi identified the distribution of PYY-like immunoreactivity in rat tissue utilizing specific RIA (Miyachi 1986). The highest concentration of PYY was in the colon (298.7-449.5 pmol/g). These levels were approximately 100-200 times more than that measured in the duodenum. The concentration of PYY in the mucosa was higher than that in the muscular layer in the small bowel, cecum, colon and rectum. Taylor measured higher levels of PYY in his study with dogs (Taylor 1985). The highest concentration of PYY was present in the ileum and colon, 1610 and 1607 ng/g respectively, while lower levels were measured in the proximal small bowel.

Adrian addressed the question of PYY distribution in human tissues (Adrian 1985B). In tissue taken from surgical specimens, PYY was found throughout the small intestines; duodenum 6 pmol/g; jejunum 5.0 pmol/g; but highest in the ileum, 84 pmol/g. Higher concentrations were measured in the

colon; ascending 82 pmol/g, sigmoid 196 pmol/g. PYY was found exclusively in the mucosal epithelium (Figure 12).

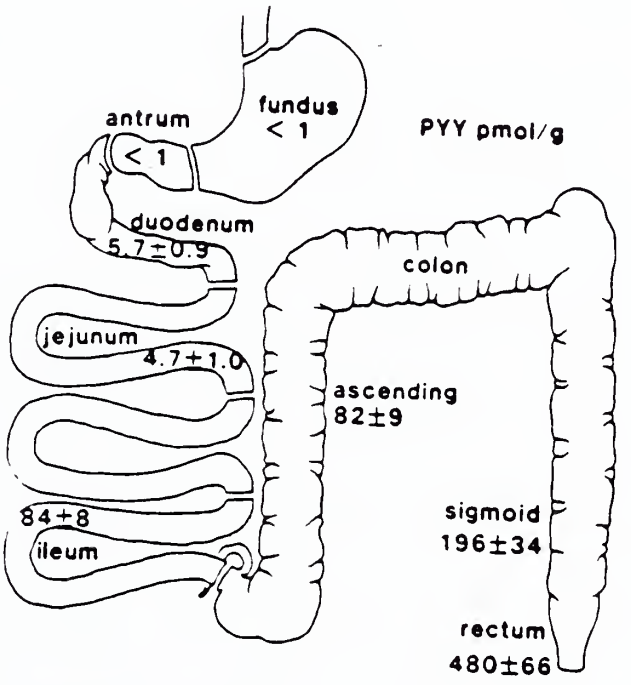

Figure 12: Distribution of peptide YY (PYY) in the human gastrointestinal tract (picomoles per gram wet weight of tissue). From Adrian TE. Gastroenterol 1985; 89:1070-1077.

DISTRIBUTION OF NPY

The localization of NPY has been studied using histochemistry and and radioimmunasssay techniques. Studies have shown that NPY has a wide distribution throughout the body with very high concentrations in the central nervous system (Adrian 1983), (Busch-Sorensen 1989). Allen demonstrated presense of NPY in the brain (Allen 1983) while Sasek described NPY-immunoreactivity in the spinal cord (Sasek 1985). Others have reported NPY in blood vessels (Lundberg 1982B), heart (Gu 1984), intestine, stomach, pancreas, genital tract (Lundberg 1982B), lung (Shepard 1983), spleen (Lundberg 1985), adrenal medulla (Varndell 1984) and other organs (Lundberg 1982B). Ding showed that NPY-containing nerves were identified in the liver, gallbladder and pancreas (Ding 1991). Neurologically, NPY is found to coexist with other neurotransmitters both centrally and peripherally (Carlei 1985, Everitt 1984). Emson demonstrated colocalization of NPY with somatostatin (Emson 1984) in the telencephalon while O'Donohue reported NPY colocalization with catecholamines in the brain stem (O'Donohue 1985). In the peripheral nervous system, NPY almost exclusively is colocalized with norepinephrine (Ekblad 1984A). NPY nerves densely innervate the circular and longitudinal muscle and myenteric plexus of many organs in the human digestive tract (Ekblad 1984B, Furness 1983). Although NPY has been shown to colocalize with norepinephrine in the periphery, Sundler

demonstrated in the gastrointestinal tract that nerve fibers which contained NPY lacked norepinephrine and that these nerves originated in intramural ganglia (Sundler 1983). The distribution of NPY throughout the gastrointestinal tract suggests an important role in the modulation of gastrointestinal functioning.

RELEASE OF PYY INTO CIRCULATION

The first studies which addressed the release of PYY into circulation focused on post-prandial release of the peptide. Adrian studied the levels of porcine plasma PYY after the ingestion of a meal (Adrian 1987B). Venous levels of PYY began to rise within the first thirty minutes after eating and peaking at two hours. Adrian also demonstrated some evidence for the metabolism of PYY during its pass through the liver. Greeley proposed that cholecystokinin may mediate the postprandial release of PYY (Greeley 1989A).

Taylor, in canine studies, demonstrated a 205 pmol/liter increase of plasma PYY levels after intragastric infusion of a liver extract meal (Taylor 1985). Serum PYY concentrations increased progressively over the two hour study period. Taylor hypothesized that the delayed peak PYY response to food could reflect small bowel transit time. In his second series of experiments, Taylor noted that there was no significant PYY response to insulin hypoglycemia. This

observation demonstrated that the parasympathetic system does not play a major role in the release of PYY.

Pappas observed an 86 pmol/liter increase over fasting levels of plasma PYY following ingestion of a meat meal in canines (Pappas 1985). More interesting however was the significantly larger rise in plasma PYY levels after infusion of oleic acid, 403 pmol/liter. Aponte expanded on the role of fatty acids and the release of PYY (Aponte 1985). Utilizing dogs with chronic gastric, duodenal and jejunal fistulas, fatty acids of varying length, oleate (C_{18}) and dodecanoate (C_{12}), were administered. The fatty acids were suspended in a taurocholate solution. He demonstrated that PYY release was not dependent on length of fatty acids. However, Aponte failed to evoke an increase in PYY when fatty acids were infused into the stomach or duodenum. Only by instilling the fatty acids into the distal gut did he demonstrate release of PYY. It is therefore apparent that there are at least two mechanisms involved in the release of PYY from the gut: a moderate post-prandial response as well as a second more potent release following the direct instillation of fatty acids and bile salt into the colon. Longo similarly reported a release of PYY in the isolated rabbit colon after infusion of short chain fatty acids (Longo 1991).

Adrian documented the release of PYY in human subjects (Adrian 1985D). Utilizing 25 healthy human subjects, PYY

levels were recorded during a fasted state as well as after various caloric meals. Fasting plasma PYY levels were 8.5 pmol/liter. After the administration of a 530 kcal meal, this level rose 3.7 pmol/liter. Similarly, an 870 and 4500 kcal meal caused significant increases in plasma PYY levels, 16.2 and 45.0 pmol/liter respectively.

Greeley compared the effects of various nutrients (fats, proteins, amino acids, and carbohydrates) given directly into the duodenum or the colon on the release of PYY in conscious dogs (Greeley 1989B). Although intraduodenal administration of an amino acid mixture, glucose or liver extract failed to elevate plasma levels of PYY, intracolonic administration of these nutrients significantly stimulated PYY release. The study suggested that unabsorbed nutrients can release PYY by direct contact with the PYY-containing cells of the terminal ileum, colon and rectum.

Ballantyne demostrated that deoxycholic acid is a potent agent for stimulating the release of PYY from the isolated perfused rabbit left colon. These findings suggest that the arrival of bile salts into the colon may play an important part in a feedback system whereby the colon participates in regulating proximal gastrointestinal function (Ballantyne 1989).

These studies have therefore demonstrated that PYY is released into the systemic circulation in response to normal feeding patterns in humans and mammalian systems. It has

also been demonstrated that there is an even greater release of PYY following instillation of fatty acids suspended in bile salts directly into the distal bowl. In addition, although the mechanism of release of PYY is incompletely understood, it does not appear to be vagally mediated.

NPY RELEASE

NPY is released with norepinephrine in response to sympathetic nerve stimulation (Lundberg 1982D). Sheikh demonstrated electrical stimulation of the splanchnic nerve supply to the isolated perfused pig pancreas resulted in the co-release of NPY and noradrenaline into the venous effluent (Sheikh 1988). Stimulation of the vagus nerve caused a substantial release of NPY without altering levels of noradrenaline. This release of NPY can be blocked by guanethidine in the cat spleen and is facilitated by alpha-2-adrenoceptor blocking agents in the dog spleen (Lundberg 1984, Schoups 1988).

PYY IN GASTROINTESTINAL PHYSIOLOGY

The effects of PYY on gastrointestinal physiology have been extensively studied. Although PYY is found in highest concentrations in the terminal ileum, colon and rectum, the major effects of the peptide include inhibition of gastric acid and pancreatic secretion, gastric and intestinal motility, and the release of insulin and glucagon.

Stomach In human studies, Adrian demonstrated that PYY inhibits gastric acid secretion (Adrian 1985A). Human subjects were given a continuous infusion of PYY, 0.2 pmol/kg/min, sufficient to cause an increase in plasma PYY of 27 pmol/liter. Pentagastrin stimulated gastric acid and pepsinogen output were decreased by 77% and 96%, respectively. The incremental gastric volume response decreased by 90%. Pappas evaluated the effect of PYY on meal-stimulated gastric and pancreatic secretion in dogs (Pappas 1985). Pancreatic protein secretion and gastric acid secretion were significantly inhibited by PYY infusion at a rate of 400 pmol/kg/hr.

Pancreas: The role of PYY on pancreatic exocrine function has also been evaluated. Tatemoto demonstrated in the anesthetized cat that a bolus injection of 100-200 pmol/kg PYY caused a 70-80% reduction of pancreatic secretion of fluid and bicarbonate following both secretin and cholecystokinin stimulation of the pancreas (Tatemoto 1982B). Adrian performed studies in which PYY failed to inhibit pancreatic secretions in human (Adrian 1985D). Although Adrian demonstrated that low dose PYY inhibited gastric acid and pepsin secretion, there was no effect on the secretion of pancreatic bicarbonate or trypsin, or duodenal juice following low-dose secretin and cholecystokinin-8 stimulation.

Szecowa demonstrated that PYY did not have any effects on pancreatic endocrine function (Szecowa 1983). In anesthetized rats, PYY was infused at a dose of 100 pmol/kg/min to elevate plasma levels well above normal physiologic levels. At this high dose, there was no effect on basal insulin and glucagon levels. Similarly, there was no observed change in glucose-induced insulin release from pancreatic islet cells.

Several reports stated that PYY exerts its inhibitory effect of pancreatic secretion via an activation of an adrenergic pathway. Konturek demonstrated that PYY inhibits pancreatic secretion only in vivo but not in vitro isolated pancreatic acini after administration of the alpha-receptor antagonist phentolamine and the beta-receptor antagonist propranolol (Konturek 1986). Pawlik reported that in a series of 22 anesthetized canines, phentolamine and propranolol blocked the the action of PYY on secretin-induced pancreatic secretion. In addition, the antisecretory activity of PYY was partially blocked by total bilateral adrenalectomy (Pawlik 1986).

Gallbladder: Further studies evaluated the role of PYY in gallbladder function. Grace demonstrated that PYY significantly inhibited CCK-stimulated sphincter of Oddi phasic wave frequency and motility index (Grace 1988). However, gallbladder contractions were unaffected. In a canine model, the common bile duct was canulated and PYY

infused in graded doses from 100-400 pmol/kg/hr. When intraduodenal administration of hydrochloric acid was performed, the bile flow rate increased but the total output of the bile salts remained unchanged (Gomez 1986). In human studies, Adrian demonstrated that low-dose PYY, 0.62 pmol/kg/min, had no effect on bilirubin output in the duodenum, following low dose secretin and cholecystokinin-8 stimulation (Adrian 1985D).

Vasculature: PYY is also known to demonstrate vasoconstricting properties. Utilizing an anesthetized cat model, Lundberg demonstrated that electrical stimulation of the cervical trunk resulted in submandibular salivary secretion and vasoconstriction (Lundberg 1982C). The vasoconstricting properties of PYY were still present after alpha-receptor blockade as well as in sympathectomized animals. Infusions of PYY, neuropeptide Y (NPY) as well as pancreatic polypeptide (PP) resulted in a vasoconstriction similar in nature to that of the adrenergically blocked electrically stimulated response, slow in onset and long in duration (Lundberg 1982D). These results suggest that noradrenaline (fast response) coupled with a PP-related peptide (slow response) might be the transmitters involved in these vascular nerves. In addition, PYY and these peptides elevated systemic blood pressure during these experiments. This is presumed to be a result of systemic vasoconstriction.

Thus, significantly elevated levels of PYY and NPY might act directly on the vascular smooth muscle (Vukasin 1989).

Lundberg demonstrated that PYY had marked vascular effects in the gastrointestinal tract (Lundberg 1982A). Close intraarterial administration of PYY (25-150 pmol/kg) in cats caused an intestinal vasoconstriction as well as an increase in systemic blood pressure. These effects were reproducable even with pretreatment with an adrenergic blocking agent. Adrian studied the hemodynamic effects of infused PYY into human volunteers (Adrian 1986B). During infusion periods, plasma PYY levels increased 73 pmol/liter more than basal levels. There was a significant increase in both systolic and diastolic blood pressure, 8.6 mmHg and 10.9 mmHg respectively. These studies further support the hypothesis that significantly elevated levels of PYY may act directly on vascular smooth muscle leading to contraction and elevation of systemic blood pressure (Vukasin 1989).

Motility: PYY has significant and profound effects on gastric emptying and bowel motility. Lundberg demonstrated that both jejunal and colonic motility were inhibited by intraarterial infusion of PYY (Lundberg 1982A). The inhibition of motility was most pronounced in the colon, lasting for nearly one hour after cessation of PYY infusion. Suzuki investigated the effect of PYY on gastric contractile activity in Heidenhain pouch dogs (Suzuki 1983). PYY was given in an intravenous bolus with doses ranging between 12.5

and 100 pmol/kg. PYY had no effect on contractile activity in the stomach during the digestive state. However, in the interdigestive state, PYY, in a dose-dependent manner, inhibited the interdigestive migrating contractions in the innervated main stomach but did not affect the motor activity in the pouch. This supports the hypothesis that PYY exerts its effects through the extrinsic nerves. Al-Saffar evaluated the effect of PYY on the myoelectric activity of the small intestine in conscious rats (Al-Saffar 1985). Following intravenous infusion, PYY (50 pmol/kg/min) had no effect on the occurence of the migrating myoelectric complex in the duodenum but interrupted its distal propagation and almost abolished total spike activity in the jejunum. The rats were additionally given a ^{51}Cr labelled marker and it was observed that its transit was significantly delayed in those given PYY. Al-Saffar suggested that inhibition of the activity front by PYY may account for the delay in small bowel transit in those rats receiving PYY (Vukasin 1989).

Several studies reported the effects of PYY on gastrointestinal motility in humans. Allen administered intravenous PYY (2 pmol/kg/min) to achieve a plasma level of 59 pmol/liter, which significantly delayed gastric emptying (Allen 1984). Savage evaluated the effects of peptide YY (PYY) on mouth-to-cecum intestinal transit time and on the rate of gastric emptying in healthy adults (Savage 1987). Subjects were given two doses of PYY, the lower dose 0.18

pmol/kg/min, mimicking the post prandial state while the higher dose, 0.51 pmol/kg/min, representing a malabsorptive state. Gastric emptying was prolonged from 37 minutes to 63 minutes for the low dose and to 130 minutes for the high dose of PYY. Mouth-to-cecum transit time was delayed from 67 minutes to 94 minutes for the low dose and to 192 minutes for the high dose of PYY.

Secretion: PYY may also play an important role in the regulation of chloride transport in the small bowel. Serosal application of PYY decreases basal short circuit current in rat jejunal and colonic mucosa. ^{36}Cl-flux studies indicate that this drop in short circuit current results from the inhibition of chloride secretion (Cox 1988A). Playford reported that PYY also inhibited VIP-stimulated secretion in healthy human volunteers (Playford 1990).

NPY IN GASTROINTESTINAL PHYSIOLOGY

Many NPY-containing enteric neurons of the gut innervate the epithelial cells of the intestinal mucosa (Furness 1983). Friel first reported that NPY may play an important role as a powerful modulator of epithelial ion transport (Friel 1986). Researchers have demonstrated that NPY inhibits carbachol, VIP, substance-P, and prostaglandin E_2-stimulated chloride secretion in rat jejunum (Cox 1988B, Saria 1985). Hubel, in a study with rabbits, demonstrated that NPY increases ileal chloride absorption (Hubel 1986).

The colocalization of NPY and vasoactive intestinal peptide (VIP) in neurons that innervate intestinal epithelia led researchers to believe that NPY down-regulates VIP-stimulated ion transport through direct effects on the colonic enterocytes. Indeed, Flint demonstrated that when NPY was administered to the basolateral membrane, there was inhibition of VIP-stimulated short-circuit current changes by a tetrodotoxin-insensitive mechanism (Flint 1990).

Hellstrom demonstrated that intraarterial infusion of NPY in the anesthetized rat caused a decrease in measured colonic motility (Hellstrom 1985). Allen reported that NPY inhibited contraction of longitudinal ileal nonvascular smooth muscle in vitro (Allen 1987). In the study, NPY inhibited both spontaneous contractions and also reduced amplitude of neurally mediated contraction of longitudinal smooth muscle elicited by electrical stimulation of the intramural nerve plexuses. Wiley reported that NPY caused a dose-dependent relaxation of guinea pig colon longitudinal muscle (Wiley 1987). This relaxation occured via release of norepinephrine with subsequent inhibition of acetylcholine release. Norepinephrine, located in sympathetic nerves in the myenteric plexus, inhibits cholinergic transmission via alpha-2-receptors located on postganglionic cholinergic nerves.

Researchers have also focused on the role of NPY on mammalian sphincter control. Ferri demonstrated the presense

of NPY in the intramural neurons of the internal anal sphincter of humans (Ferri 1988). NPY was shown to colocalize with VIP-containing nerve fibers in this region (Wattchow 1988). Nurko examined the role of NPY in the opossum internal anal sphincter (Nurko 1990). He determined that NPY acted directly on internal anal sphincter smooth muscle to increase sphincter pressure. It also inhibits relaxation of the sphincter produced by the rectoanal reflex.

CHANGES IN PLASMA LEVELS OF PYY IN STATES OF PATHOPHYSIOLOGY

Researchers have demonstrated altered plasma levels of PYY in various pathophysiologic states in addition to several post-surgical states of the gastrointestinal tract. Adrian examined the release of PYY following ingestion of glucose in patients with the dumping syndrome, a complication of gastrectomy and gastrojejunostomy (Adrian 1985C). The dumping syndrome is thought to be caused by the rapid transit of hyperosmolar chyme into the jejunum, followed by an osmotically driven fluid rush into the small bowel depleting intravascular volume, and hence, the cause of much of its symptomatology. Vasoactive intestinal peptide or neurotensin may be involved in the etiology of the disorder (Blachburn 1980, Sagor 1981). Adrian administered 100 grams of glucose to patients and measured plasma PYY levels. Plasma PYY levels in the control patients increased by 3.4 pmol/liter, while those in patients with dumping syndrome increased by 65

pmol/liter. The effect of PYY was completely abolished by the intravenous administration of somatostatin, a paracrine regulator present in endocrine cells throughout the gastrointestinal tract (Vukasin 1989).

Plasma PYY levels are also altered after various gastrointestinal surgical procedures (Ballantyne 1990). Savage demonstrated that plasma PYY levels were increased in rats who underwent 75% proximal small bowel resection (Savage 1985). Preoperatively, plasma levels averaged 28 pmol/liter and levels rose to 85 pmol/liter six days postoperatively. Levels were consistently elevated for 48 days. Adrian reported plasma PYY levels in a series of human subjects who had undergone resection of small bowel, colon or pancreas (Adrian 1987A). In 18 patients who had undergone partial ileal resection, basal PYY levels were greatly elevated as compared to control. However, in 16 patients who underwent colonic resection and ileostomy, PYY levels were significantly lower. In eight patients who had undergone pancreatectomies, the plasma levels of PYY were only moderately increased. The elevated levels of PYY in patients with pancreatectomy and ileal resection could be attributed to a release of PYY in response to increased bile salts, fats, or other nutrients reaching the distal bowel.

The "ileal brake" mechanism of small intestinal motility refers to the slowing of gastric emptying and small bowel transit following infusion of nutrients into the ileum.

Small intestinal resection prompts adaptive changes which slow intestinal motility and increase mucosal absorptive capacity. Circulating levels of PYY increase following small intestinal resection. This slowing effect of gut transit following meals has been termed the "ileal brake" effect (Spiller 1984, Macfarlane 1983, Hill 1974). Armstrong reported an adaptive increase in PYY after proctocolectomy and pelvic ileal reservoir construction in dogs (Armstrong 1991).

Large bowel resection leads to a reduction of plasma levels of PYY. This lack of hormonal adaptation may be due to the loss of hormone secreting cell mass (Adrian 1987C) and has been implicated in the etiology of post-colectomy diarrhea. In addition, combined large bowel resection and ileal resection results in watery diarrhea which is disporportionally profuse considering the short lengths of ileum resected (Neal 1984). This may result from the loss of the "ileal brake."

Similarly, PYY levels are significantly augmented in various gastrointestinal disorders. Adrian reported plasma PYY levels in patients with various gastrointestinal diseases (Adrian 1986A). Patients with steatorrhea due to tropic sprue had significantly elevated plasma PYY levels after a meal. Basal levels in these patients were reported to be 10-times higher than in their control population. Similarly, patients with steatorrhea due to chronic destructive

pancreatitis had increased basal and postprandial levels of PYY. In patients with inflammatory bowel disease, there was a moderate elevation of PYY levels. Sjölund reported that plasma PYY levels were increased in patients with celiac disease. In addition, he demonstrated that these abnormal levels were inversely proportional to the concentration of plasma folic acid concentrations. Adrian proposed that PYY levels were elevated in patients with disoders of malabsorption. The presence of abnormal bowel contents reaching the distal colon may cause release of PYY. PYY would likely assuage the symptoms of these disorders by delaying gastric emptying as well as small bowel motility leading to increased proximal absorption.

PYY AND NPY RECEPTORS

Cell surface receptors for PYY have been characterized but the number, distribution and classification of these receptors remain under intense investigation. To date, there exists at least four distinct NPY/PYY receptors, designated Y1, Y2, Y3 and Y4. High affinity receptors for PYY were first identified on rat jejunal epithelial cells (Laburthe 1986). The receptor bound both NPY and PYY but had a five-fold higher affinity for PYY. The distribution of PYY is not uniform within the intestinal mucosa. Studies have shown that the PYY-preferring receptor is found in greater number on the crypt cells than on the mature villus cells (Voisin

1990). Mannon demonstrated that the greatest density of PYY receptors in the rabbit was in the descending colon (Mannon 1991).

PYY and NPY may bind the same receptors. Sheikh reported distinct receptors for NPY and PYY, designated Y1 and Y2 (Sheikh 1989A). These receptors are pharmacologically distinct. Wahlestedt reported evidence for different pre- and post-junctional receptors for NPY and PYY (Wahlestedt 1986, Wahlestedt 1990). In various animal studies, he demonstrated that NPY and PYY exerted three distinct effects at the level of the sympathetic neuroeffector junction. NPY and PYY had a direct post-junctional effect, leading to constriction of certain blood vessels. Secondly, they potentiated the response to various vasoconstrictors. Finally, NPY and PYY were demonstrated to act pre-junctionally to suppress the release of noradrenaline from sympathetic nerve endings in stimulated rat vas deferens.

The distinction between Y1 and Y2 is based on the C-terminal fragment of these peptides. Sheikh demonstrated that the Y2 receptor binds the long COOH-terminal fragment of NPY or PYY (amino acids 13-36) (Sheikh 1989B). This fragment is unable to bind to the Y1 receptor. Fuhlendorff, in a study utilizing human neuroblastoma cell lines, classified a specific Y1 receptor in which two critical amino acid substitutions have been made (Fuhlendorff 1990A). This compound, [Leu^{31}, Pro^{34}]NPY displaced radiolabelled NPY only

from cells that expressed Y1 receptors and not those expressing Y2 receptors (Figure 13). Further work by Sheikh structurally characterized these receptors for NPY and PYY by affinity cross-linking (Sheikh 1990). Their results indicated that the Y1 receptor is a glycoprotein with an M_r = 70,000 binding subunit, whereas the Y2 receptor is a glycoprotein with a M_r = 50,000 binding subunit. The study demonstrated that the Y1 and Y2 receptor subtypes are structurally distinct glycoproteins, not disulfide-liked to other subunits. Fuhlendorff further determined that position 34 on the peptide was critical for specific binding (Fuhlendorff 1990B). The antiparallel pancreatic-polypeptide fold present in NPY and PYY is of structural importance for the receptor binding. This fold allows presentation of the carboxyl- and amino-terminal segments of the peptide to the receptor.

Y3 receptors can be activated by NPY but not PYY and may mediate cardiovascular responses (Baranowska 1987, Grundemar 1991, Sheriff 1990). Other receptor subtypes are still being investigated (Wahlenstedt 1992). A receptor with equal affinity for PYY, NPY and PP was detected on rat pancreatic islet and acinar cells, hepatocytes and epithelial cells of the stomach, duodenum and small intestine (Nata 1990). The Y4 receptor has only recently been characterized. Ballantyne has demonstrated a receptor which shows the same affinity for NPY, PYY and PP as well as the same efficacy. Activation of

this receptor blocks vasoactive intestinal peptide-stimulated chloride secretion from the colon (Ballantyne 1993).

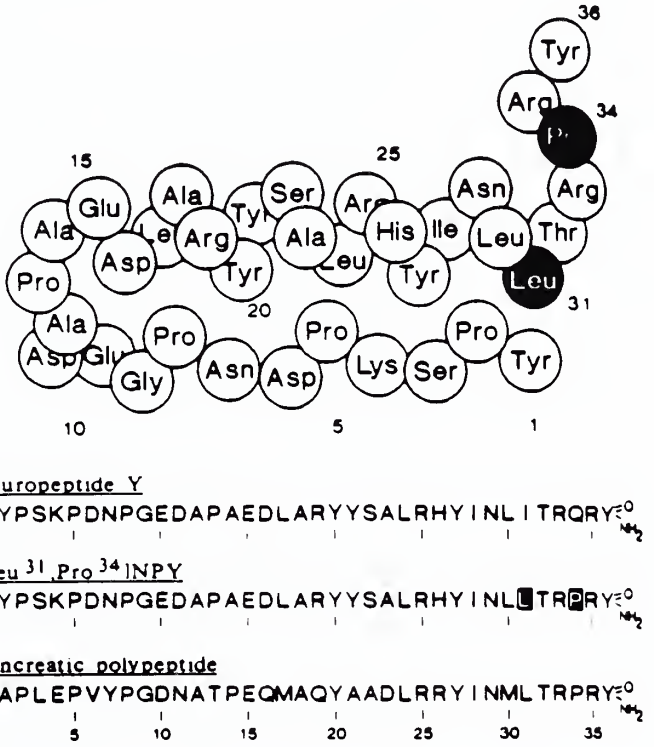

Figure 13: Diagram of the structure of [Leu31,Pro34]NPY and the aligned sequences of NPY, [Leu31,Pro34]NPY, and PP. The schematic structure of the NPY analog shown at the top was based on the x-ray structure of avian PP. From Fuhlendorff J. PNAS (USA) 1990; 87:182-186.

Initially, it was believed that the Y2 receptor was localized presynaptically while Y1 was confined postsynaptically but studies have demonstrated the presence of Y2 receptors on renal proximal tubule cells suggesting that this scheme may be overly simplified.

MECHANISMS OF ACTION OF PYY AND NPY

Although the exact mechanisms of action of both PYY and NPY are incompletely understood in gastrointestinal physiology, there are three possible actions of these peptides: 1)inhibition of cAMP formation; 2)increase of cytosolic levels of calcium; and 3)mobilization of intracellular calcium.

Research has demonstrated that both PYY and NPY cause hydroelectrolytic absorption and inhibit vasoactive intestinal peptide (VIP) and prostaglandin E_2-induced secretion in the small bowel *in vivo* and *in vitro* (Saria 1985, MacFadyen 1986, Friel 1986, Cox 1988A). VIP and prostaglandin E_2 are both potent secretagogues that act via increase in cAMP levels. Servin demonstrated that PYY and NPY inhibited VIP-stimulated cAMP production in epithelial cells isolated from rat intestines (Servin 1989). NPY and PYY also inhibited prostaglandin E_1-, prostaglandin E_2-, and foskolin-stimulated cAMP production and reduced basal cAMP levels. These effects were confined to the small bowel.

Further studies have demonstrated that NPY and PYY inhibited basal and forskolin stimulated adenylate cyclase activity (Westlind-Danielsson 1988).

Studies suggest that PYY may act through a pertussis toxin-sensitive G protein (Kassis 1987, Motulsky 1988). Valet demonstrated that PYY and NPY inhibited cAMP dependent lipolysis in both canine and human adipocytes through specific receptors coupled negatively with adenylate cyclase by a pertussis toxin-sensitive protein (Valet 1990). Additionally, Lobaugh showed that NPY specifically bound to a G protein-linked receptor that inhibits adenylate cyclase in human neuroepithelioma cells (Lobaugh 1990).

The role of calcium mediated processes in the actions of NPY and PYY is still under investigation. The effects of NPY on intracellular calcium levels has been partially defined whereas knowledge of PYY's effects remain incompletely understood. The mobilization of calcium can occur via influx of extracellular calcium or through mobilization on intracellular stores. NPY stimulates vasoconstriction as well as enhances the release of calcium channels (L-channels) (Pernow 1987, Lundberg 1988, Crowley 1990). NPY decreases the release of acetylcholine from rat nodose neurons by inhibiting gadolinium sensitive calcium channels (N calcium channels) through a pertussis toxin-sensitive mechanism (Wiley 1990). Effects of NPY on voltage dependent calcium channels may also be indirect. Shangold proposed that NPY

may inhibit calcium entry through L channels by enhancing a hyperpolarization K^+ conductance (Shangold 1989). Present studies support the hypothesis that NPY may modulate influx of extracellular calcium via actions on either L-, or N-type calcium channels in different cell types.

Other actions of NPY appear independent of extracellular calcium (Sabatino 1989). In human neuroblastoma cells which only express the Y1 receptor, [Leu^{31}, Pro^{34}]NPY but not NPY(16-36) causes a transient elevation of intracellular Ca^{++} (Aakerlund 1990). This effect is independent of extracellular calcium, is blocked by thapsigargin which selectively depletes calcium stores in the endoplasmic reticulum (Thastrup 1989), and is pertussis toxin-sensitive. Jackson demonstrated that the calcium pool affected by thapsigargin is inositol 1,4,5-triphosphate-sensitive (Jackson 1988). These studies suggest that when NPY binds Y1 receptors a transient calcium signal is produced through mobilization of calcium from stores in the endoplasmic reticulum and that this effect is mediated by a G protein and a generation of inositol 1,4,5-triphosphate.

Some of the actions of PYY may result from the inhibition of the release of other regulatory peptides. PYY may inhibit sham feeding-stimulated acid secretion by blocking acetylcholine release from vagal fibers (Pappas 1986). The effects of PYY on pancreatic secretion may derive from the inhibition of cholecystokinin release (Lluis 1988,

Hosotani 1989). PYY inhibits acetylcholine release from nerves in the guinea pig stomach (Wiley 1991). Also, PYY inhibition of cholecystokinin stimulated contractions of ileal muscle strips may stem from the inhibition of acetylcholine release from cholinergic nerves (Baba 1990). Thus the effects of PYY in vivo are complex and result from a cascade of direct and indirect actions.

METHODS

ISOLATED PERFUSED RABBIT ILEUM New Zealand white rabbits (3-4 kg) were anesthetized with subcutaneous ketamine HCl (50 mg/kg) and xylazine (20 mg/kg). Intravenous fluids (0.9% NaCl) were administered through a lateral ear vein to maintain stable heart rate, blood pressure and urine output. Preoperatively, heparin (2000 units) was injected intravenously. The abdomen was entered via a midline incision. The ileocolic artery and vein were then identified as well as the terminal ileum. The ileocolic artery was then cannulated with a standard 20 guage intravenous catheter (20G Cathlon, Critikon, Tampa, Florida). The artery was immediately perfused with warmed (37°C), oxygenated (95% O_2/5% CO_2) Krebs Ringer's Bicarbonate solution (NaCl 118.4 mM, KCl 4.7 mM, $CaCl_2$ 2.5 mM, $MgCl_2$ 1.3 mM, $NaHCO_3$ 23.4 mM, NaH_2PO_4 0.12 mM, dextrose 5.6 mM, bovine serum albumin 30 μM) at a rate of 3 ml/min. Perfusion was initiated before the vessels are ligated. Consequently, there was no period of ischemia. A 10 cm segment of terminal ileum based on the cannulated vasculature was harvested. The margins of the mesentery were ligated and divided so as to avoid loss of perfusate. The organ was then placed on a warmed humidified organ plate (Nigel Cox, Yale Instrumentation Shops, New Haven, CT). At the end of the operation, the rabbits were euthanized with an

additional ketamine bolus (200 mg/kg IV) followed by a saturated KCl solution (5 ml IV).

ORGAN VIABILITY Organs were examined by light microscopy to ensure structural integrity. At the termination of experiments tissue samples from the perfused segments of small bowel were preserved in formalin and subsequently embedded in paraffin, sectioned and stained with hematoxylin and eosin. In addition, serial arterial and venous blood gases were obtained at hourly intervals for the duration of the experiments. pH, pCO_2, and pO_2 were measured with a clinical grade Corning 168 pH/Blood Gas Analyzer (Corning Medical & Scientific, Medfield, MA).

MOTILITY Motility of the perfused segment of ileum was measured by insertion of a multilumen (4 port) manometry catheter (J.S. Biomedicals, Ventura, CA.) into the gut lumen. The manometer ports were spaced at 2.5 cm intervals and perfused at a constant pressure of 15 cm H_2O with distilled water (a total volume of 3 cc/min). The manometer catheter was linked to pressure transducers which were interfaced to a polygraph (Narco Biosystems MMS 200, Houston, TX.) The analog signal was then digitalized at a rate of 8 data points per second and stored on a computer hard disc (IBM PC System 2, Model 70, 386) for subsequent statistical analysis. Motility was quantified by the integration of the area under the digitalized pressure-time curve for one minute intervals using computer software (Narco MMS, Houston, TX.) Mean

activity in all four channels was used to quantitate motility. Results were expressed in units of mmHg.min.

STATISTICAL ANALYSIS For each test agent, the integrated response during the stimulated period was compared to the resting period and the recovery period using Student's t-test for paired samples. Integrated responses of groups of test agents were compared by testing the homogeneity of variances using Bartlett's test. When a significant F value results from the analysis of variance ($p<0.05$), the Tukey test was applied on the means of the integrated response of the test agents ranked in order of magnitude. All data analysis was performed using a computer software package.

EXPERIMENTAL DESIGN Each concentration of test agent or control was tested in four individual colon preparations (N = 4). In each preparation, the isolated ileum was allowed a ten minute recovery period, during which it was maintained on the organ perfuser with no intraluminal stimulation. Intraluminal test agents were then infused through the arterial side at a rate of 0.3 ml/min. Infusions of various test agents ranged from 5 to 90 minutes. Prokinetic agents were infused at a constant rate and concentration for ninety minute time periods. Inhibitory agents were infused from 5-10 minutes. Experiments with pancreatic polypeptide-fold family analogs (NPY, PYY or PP) required pretreatment of the appropriate test agent for five minutes prior to administration of the inhibitory agent (Figure 14).

MATERIALS The following chemicals and agents were obtained from Sigma, St. Louis, MO: NaCl, KCl, $CaCl_2$, $MgCl_2$, $NaHCO_3$, NaH_2PO_4, dextrose, bovine serum albumin, ketamine HCl, xylazine, formalin, paraffin, hematoxylin, eosin, tetrodotoxin, naloxone, atropine, carbachol, norepinephrine and forskolin. Cholecystokinin, the cholecystokinin antagonist L364,718, motilin, vasoactive intestinal peptide, peptide YY, neuropeptide Y, pancreatic polypeptide, [Leu^{31},Pro^{34}]NPY and NPY (13-36) were obtained from Peninsula Laboratories, Inc., Belmont, CA.

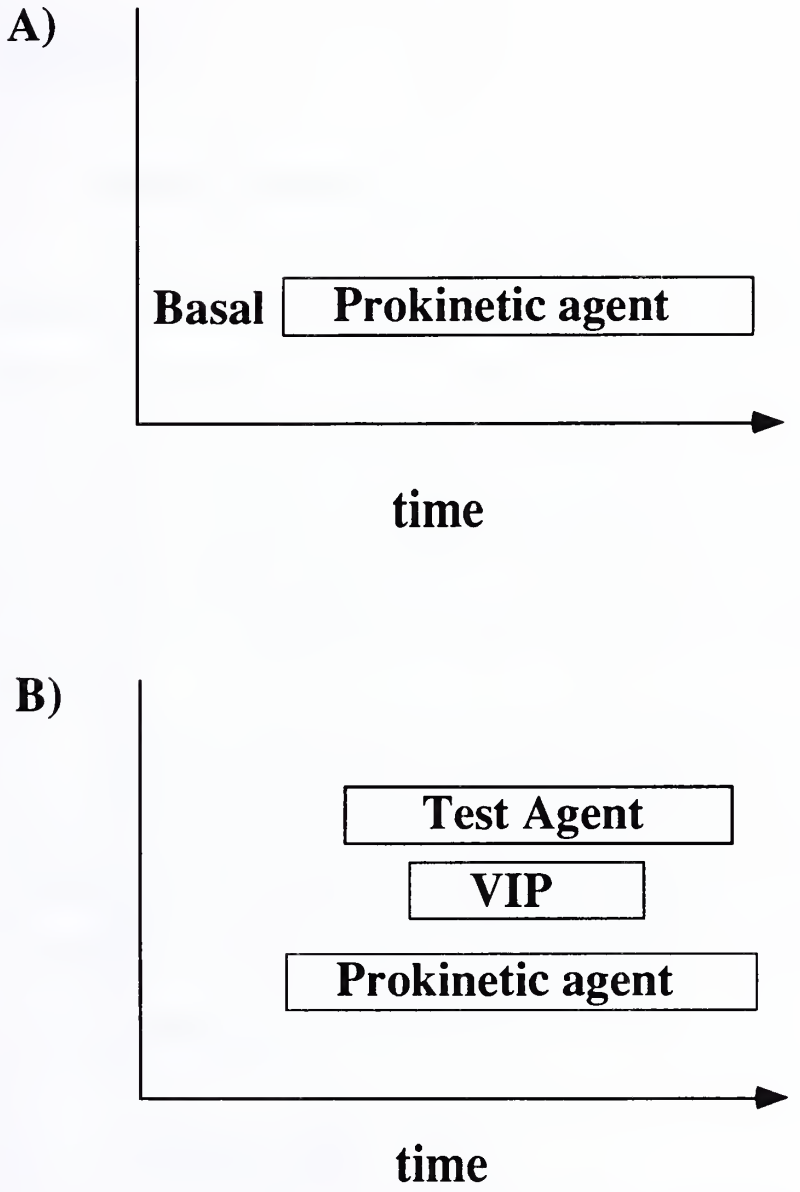

Figure 14: These figures represent the experimental protocol. After isolation of the segment of terminal ileum, the organ is allowed a brief recovery period prior to measurement of a baseline level of motor activity. The specific prokinetic agent tested is then infused at a constant concentration and rate (A). In those experiments in which a test agent is used, infusion of the appropriate test agent begins five minutes pri to administration of the prokinetic antagonist, in this case VIP (B).

RESULTS

ORGAN VIABILITY

After 90 minutes of perfusion on the whole organ apparatus, sections of tissue from the experimental ileum were collected for histological evaluation. Subsequently, these tissues were stained with hematoxylin and eosin for light microscopy. Cellular architecture and integrity of the ultrastructural features remained intact during this period (Figure 15). However, there was some interstitial edema present.

Oxygen and carbon dioxide tensions in the arterial and venous fluids were consistent with normal metabolic activity within the isolated organ preparation. At no time did the arterial PO_2 fall below 100 mm Hg or the venous CO_2 exceed 35 mm Hg (Table 1).

ILEAL MOTILITY

Unstimulated motility in the isolated ileum was evaluated. After a brief latency period, resting ileal motility reached a stable plateau: 41.3±2 mmHg/min. There were no statistically significant changes of resting ileal motility for 90 minutes of recording (Fig 16). All subsequent experiments were therefore limited to ninety minute test periods.

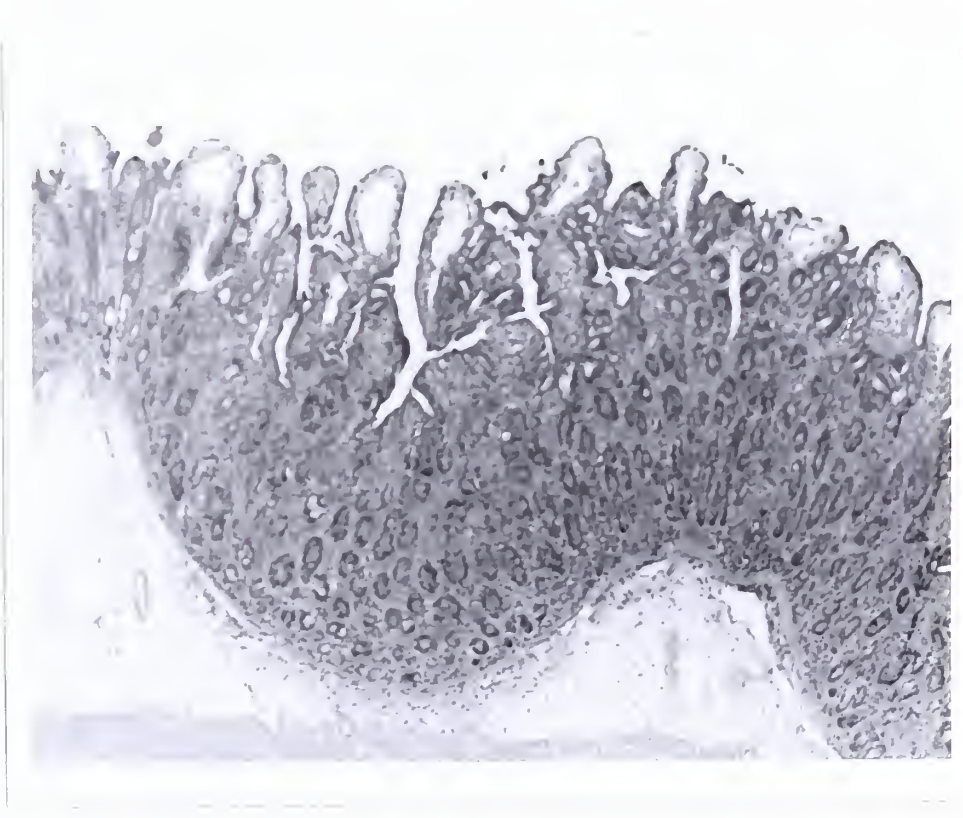

Figure 15: Hematoxylin and eosin section (x 20) of rabbit terminal ileum after 90 min of perfusion on an isolated organ platform. The smooth muscle and mucosa appear viable. Some interstitial edema is present.

TABLE 1. ARTERIAL AND VENOUS BLOOD GAS MEASUREMENTS

		ARTERIAL	VENOUS
1 HOUR	pH	7.46 ± 0.024	7.29 ± 0.05
	CO2	17.6 ± 1.1	17.4 ± 1.4
	O2	320 ± 34	131 ± 13
2 HOURS	pH	7.43 ± 0.06	7.44 ± 0.02
	CO2	19.1 ± 2.3	23.0 ± 3.2
	O2	223 ± 37.4	170 ± 19.8
3 HOURS	pH	7.46 ± 0.041	7.46 ± 0.056
	CO2	22.3 ± 4.5	22.2 ± 1.8
	O2	191.6 ± 19.8	106 ± 3.7

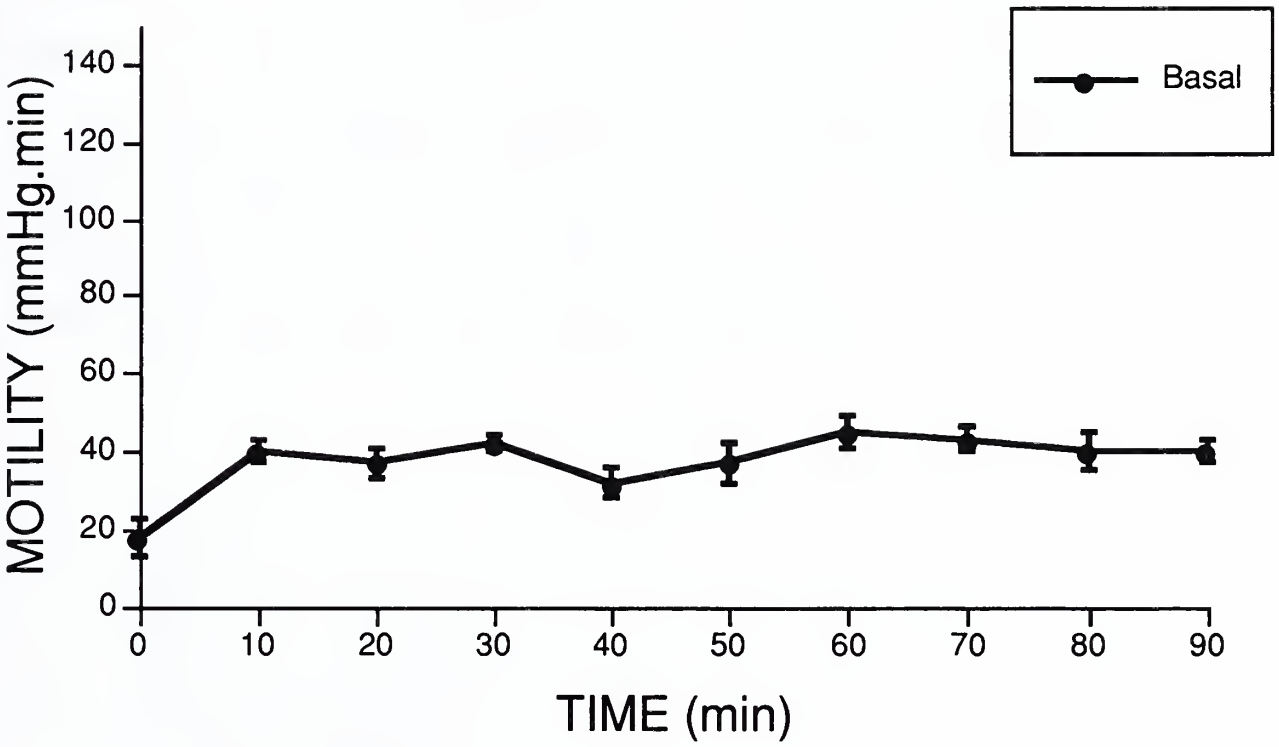

Figure 16: Baseline motor activity of unstimulated segment of terminal ileum after 90 minutes of perfusion.

PROKINETIC AGENTS

CARBACHOL

The effect of carbachol on ileal motility was tested. Carbachol (10^{-8}-10^{-5}M) infusion caused a concentration-dependent increase in phasic motor activity with an ED_{50} of 10^{-7}M (Figure 17) Carbachol-induced motility values remained stable for more than 90 minutes (Figure 18). All other experiments which utilized a continuous infusion of carbachol were limited to less than 90 minutes (Table 2).

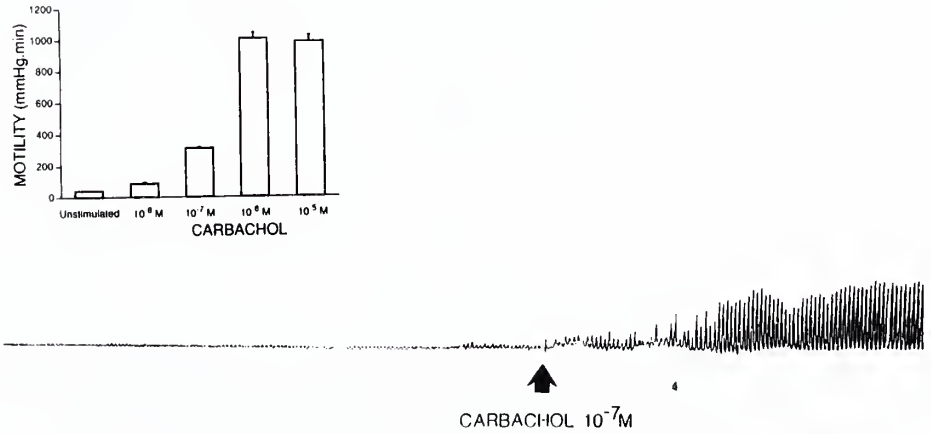

Figure 17: Action of carbachol on isolated, perfused ileum. Carbachol infusion resulted in phasic ileal contractions. A concentration-dependent increase in motor activity was seen with an ED_{50} = 10^{-7} M (inset). n = 4 for each group.

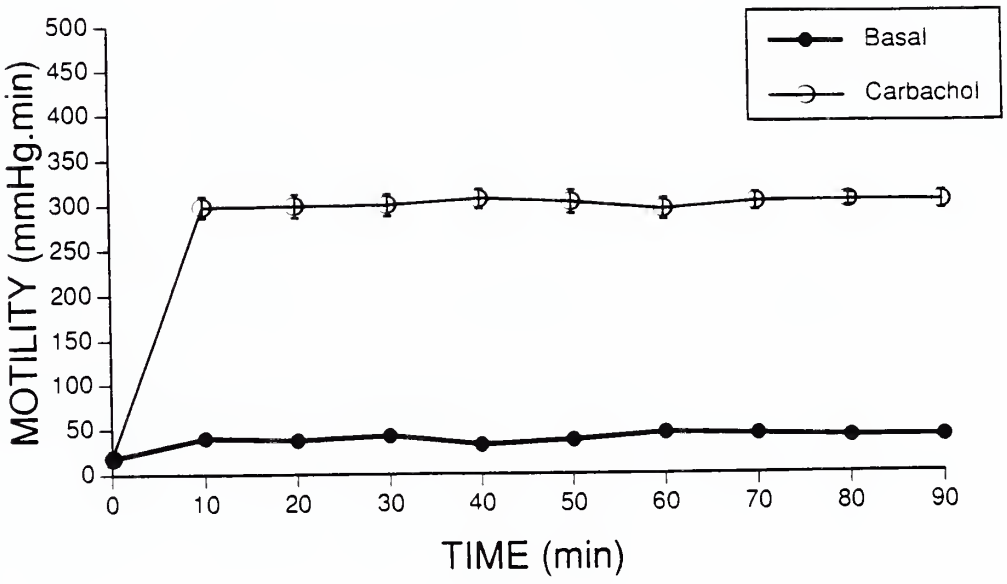

Figure 18: Pressure recording of motor activity for unstimulated and carbachol (10^{-7} M) treated ileum over a 90-minute time period. After a 10-minute equilibrium period, motor activity for both unstimulated and carbachol-stimulated preparations remained essentially unchanged for the duration of the experiment. n = 4 for each group.

TABLE 2. CARBACHOL-STIMULATED INCREASE OF MOTOR ACTIVITY IN ISOLATED PERFUSED SEGMENTS OF RABBIT TERMINAL ILEUM

	CARBACHOL			
BASELINE	10^{-8} M	10^{-7} M	10^{-6} M	10^{-7} M
41±2	88±8*	312±6*	1006±38*	984±40*

* $P < 0.05$ COMPARED TO BASELINE VALUES.

CHOLECYSTOKININ

CCK (10^{-9}-10^{-7} M) infusion resulted in an increase in motility (Figure 19). This phasic pattern of motor activity was unaffected by tetrodotoxin (10^{-6} M) or naloxone (10^{-5} M) but was inhibited by atropine (10^{-5} M) and the CCK antagonist L-365,718 (10^{-9} M) (Table 3). CCK-induced motility remained stable for greater than 90 minutes (Figure 20). All other experiments which utilized a continuous infusion of CCK were limited to less than 90 minutes.

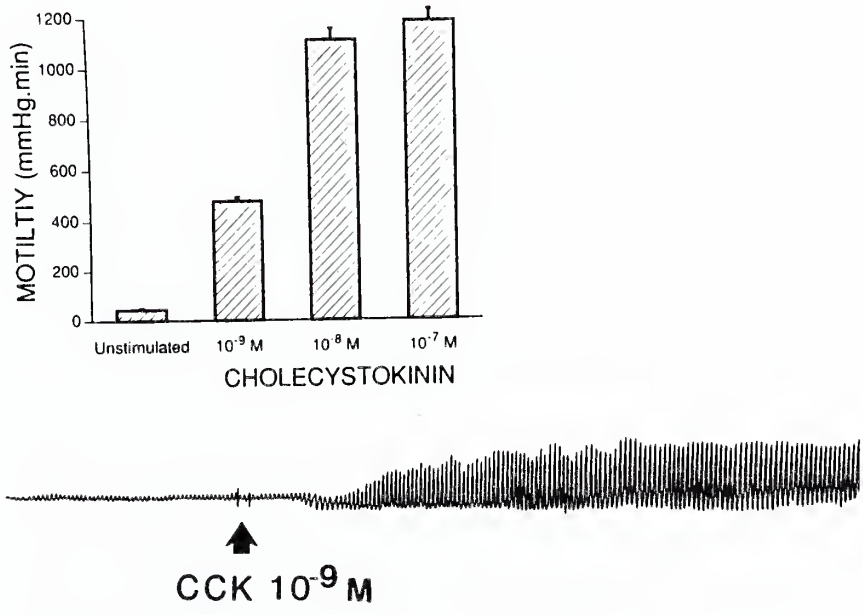

Figure 19: Effect of cholecystokinin on isolated segment of rabbit terminal ileum. Cholecystokinin, a naturally occuring peptide in the gastrointestinal system, resulted in phasic ileal contractions. A concentration-dependent increase in motility was seen with an ED_{50} of 10^{-9} M (inset). n = 4 for each group.

TABLE 3. EFFECTS OF TETRODOTOXIN, ATROPINE AND L364,718 ON CCK STIMULTED MOTILITY

	10^{-7} M	10^{-8} M	10^{-9} M
CCK ALONE	1187±44	1113±44	479±14
TETRODOTOXIN (10^{-6}M, n=5)			524±43
NALOXONE (10^{-5}M, n=4)			484±18
ATROPINE (10^{-5}M, n=4)			243±121*
L364,718 (10^{-9}M, n=3)			114±40*

* $P < 0.05$ COMPARED TO CCK (10^{-9}M) ALONE

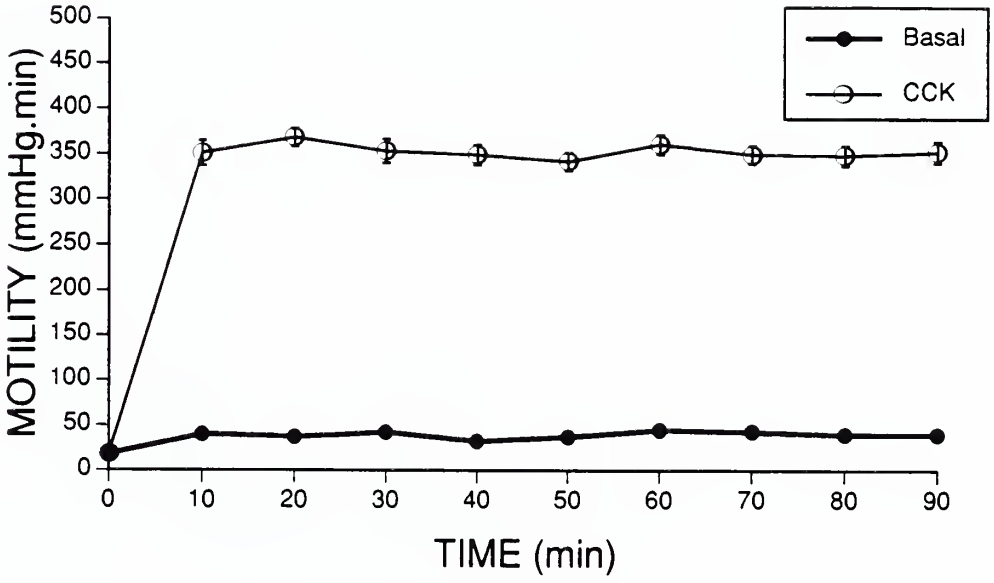

Figure 20: Pressure recording of motor activity for unstimulated and cholecystokinin (10^{-9} M) treated ileum over a 90-minute time period. After a 10-minute equilibrium period, motor activity for both unstimulated and cholecystokinin-stimulated preparations remained essentially unchanged for the duration of the experiment. n = 4 for each group.

MOTILIN

Motilin (10^{-10}-10^{-7} M) caused a concentration-dependent increase in measured motor activity in isolated segments of perfused terminal ileum (Figure 21). Phasic motor activity was similar to those of other prokinetic agents. Motor activity remained stable for the duration of infusion and returned to preinfusion levels with the cessation of motilin administration.

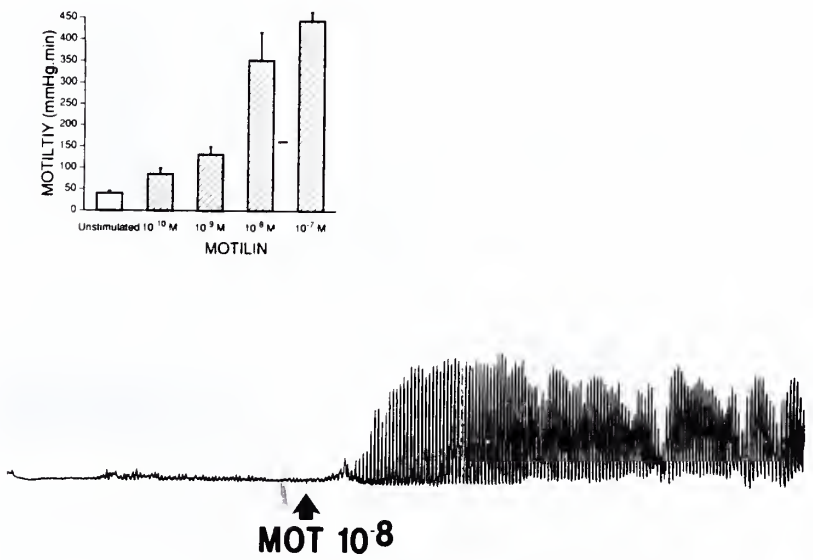

Figure 21: Action of motilin on isolated, perfused terminal ileum. Motilin infusion resulted in phasic ileal contractions. A concentration-dependent increase in motility was seen with an ED_{50} of 10^{-8} M (inset). n = 4 for each group.

cAMP DEPENDENT AGENTS

VIP INHIBITION

To determine the effects of cAMP dependent agents on intestinal motility, vasoactive intestinal peptide was tested against various prokinetic agents. VIP (10^{-9}-10^{-7}M) caused a concentration dependent inhibition of carbachol (10^{-7} M)-stimulated motility after a brief latency (Figure 22). This inhibition was reversible after the cessation of VIP administration. The half-effective dose (ED_{50}) for VIP was 10^{-8}M.

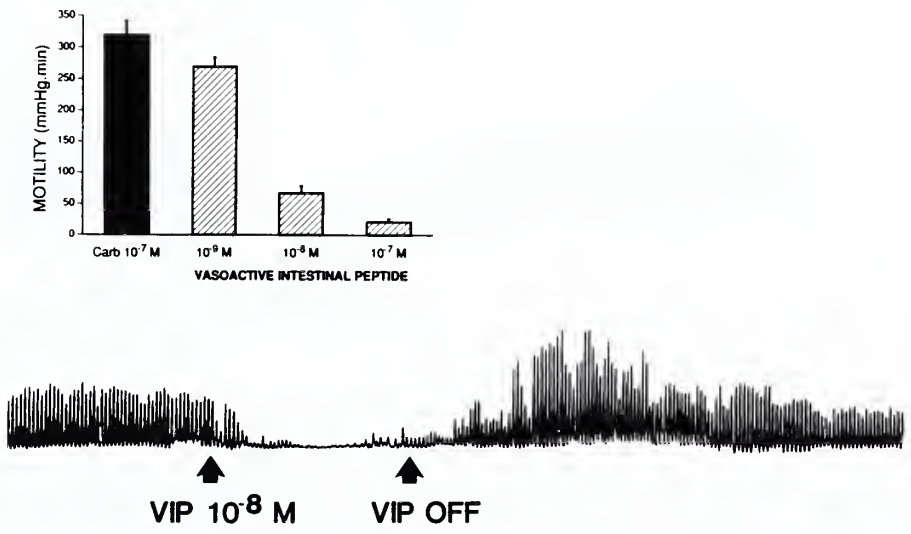

Figure 22: Effect of vasoactive intestinal peptide (VIP) on carbachol-stimulated ileum. The cAMP-mediated agent VIP caused a concentration-dependent inhibition of carbachol-stimulated ileal motility with an ED_{50} = 10^{-8} M (inset). This effect was reversible after cessation of VIP administration. n = 4 for each group.

VIP (10^{-7} - 10^{-9}M) similarly caused a dose-dependant inhibition of CCK (10^{-9}M)-stimulated motility after a latency of 15 - 20 seconds (Figure 23). Motility returned to previous levels after stopping the VIP infusion.

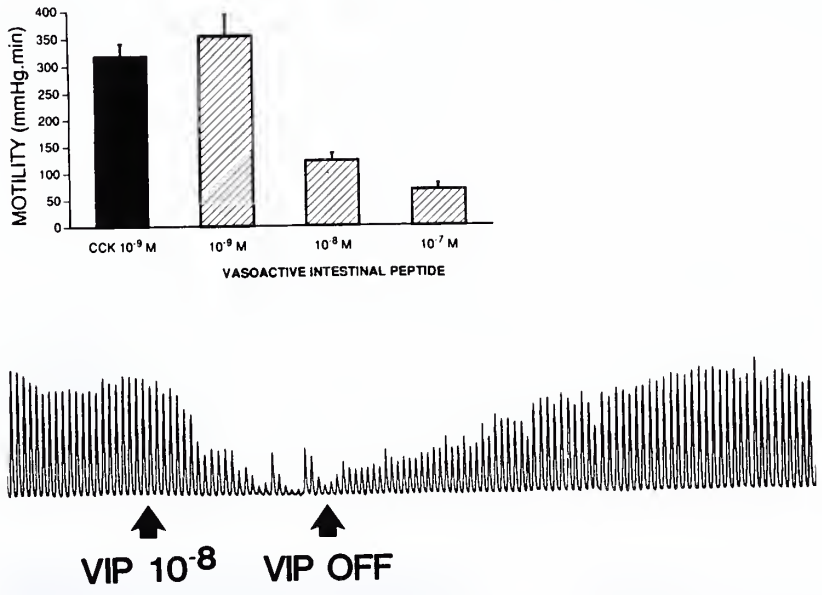

Figure 23: Effect of vasoactive intestinal peptide (VIP) on cholecystokinin-stimulated ileum. The cAMP-mediated agent VIP caused a concentration-dependent inhibition of cholecystokinin-stimulated ileal motility with an ED_{50} = 10^{-8} M (inset). This effect was reversible after cessation of VIP administration. n = 4 for each group.

NOREPINEPHRINE INHIBITION

Norepinephrine (10^{-8}-10^{-6}M) caused a concentration-dependent decrease in measure motility in carbachol-stimulated ileum (Figure 24). The inhibition was reversible upon the cessation of infusion. Preinfusion of tetrodotoxin (10^{-6}M) for five minutes, followed by administration of norepinephrine (10^{-6}M), failed to reverse norepinephrine's inhibition of motility (Figure 25).

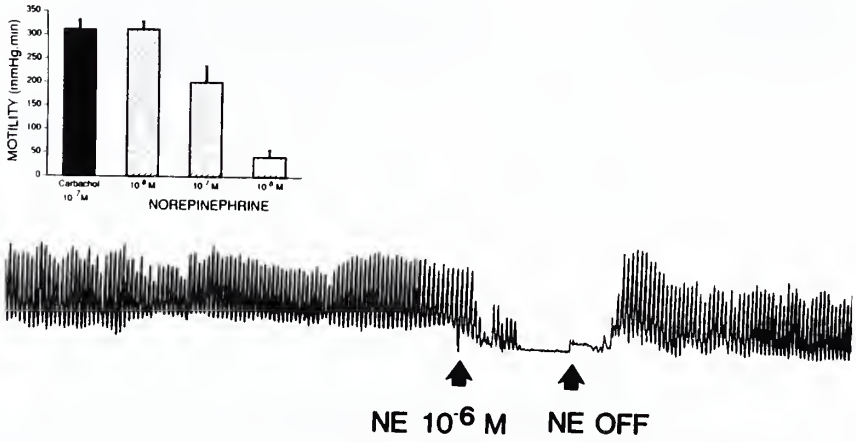

Figure 24: Effect of norepinephrine on carbachol-stimulated ileal motility. Norepinephrine caused a complete but reversible inhibition of carbachol-stimulated ileal motility. n = 4 for each group.

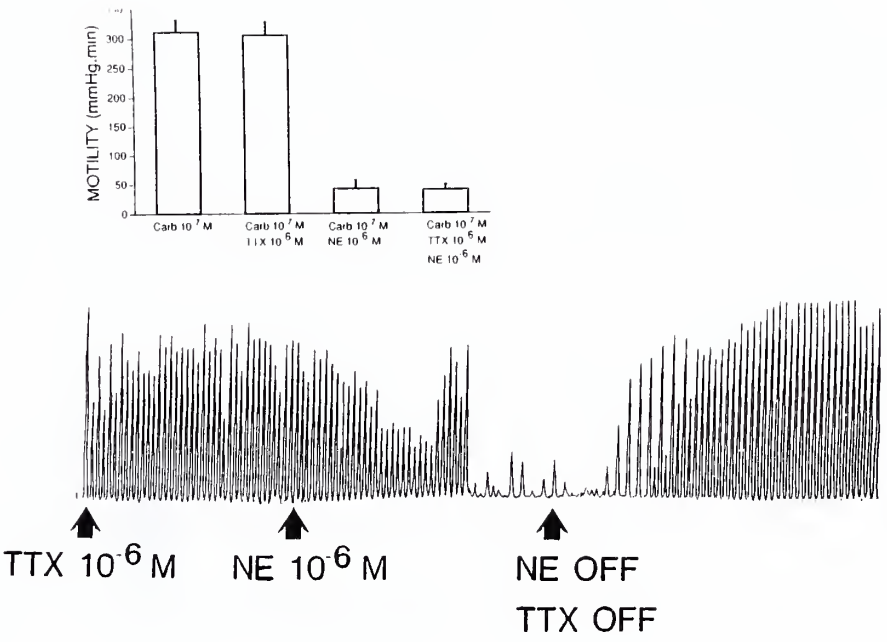

Figure 25: Effect of pretreatment with tetrodotoxin (10^{-6} M) on norepinephrine inhibition of carbachol-stimulated motor activity. Tetrodotoxin was preinfused for 3 min prior to the administration of norepinephrine. Norepinephrine completely but reversibly abolished measured motor activity in the carbachol-stimulated preparation. n = 4 for each group.

FORSKOLIN INHIBITION

Forskolin (10^{-7}-10^{-5}M) similarly caused a concentration-dependent decrease in measured motility in carbachol stimulated ileum (Figure 26). Like VIP and norepinephrine, forskolin's inhibitory effect was reversible. Norepinephrine appeared to be 10 times more potent in reversing carbachol stimulated motility than forskolin.

Results of the actions of cAMP dependent agents on carbachol-stimulated ileal motility is summarized in Table 4.

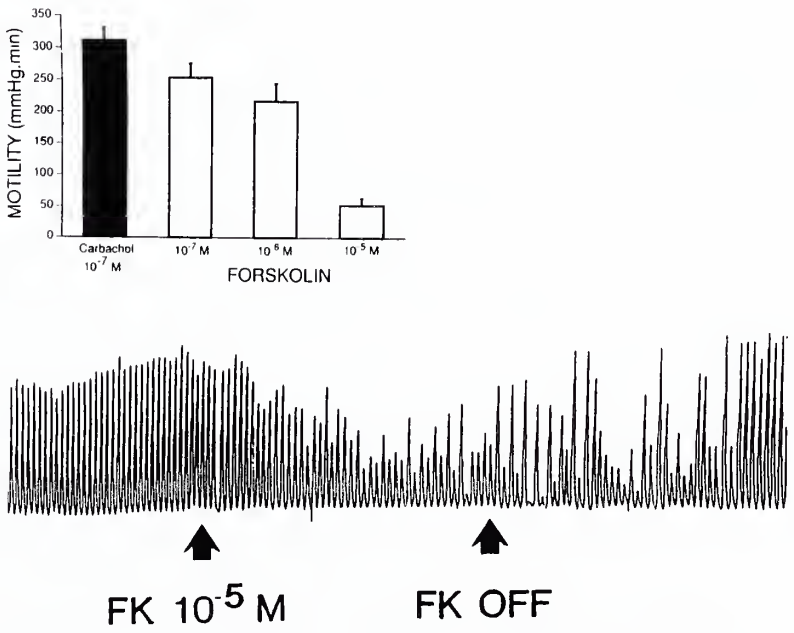

Figure 26: Effect of forskolin on carbachol-stimulated ileal motility. Forskolin, a diterpene compound which increases intracellular cAMP, reversibly inhibited carbachol-stimulation of phasic contractions. n = 4 for each group.

TABLE 4. EFFECT OF cAMP-MEDIATING AGENTS ON CARBACHOL-INDUCED MOTOR ACTIVITY

CARBACHOL (10^{-7} M)	VIP (10^{-8} M)	NOREPINEPHRINE (10^{-6} M)	FORSKOLIN (10^{-5} M)
312±6	67±11*	42±15*	53±11*

* $P < 0.05$ COMPARED TO CARBACHOL (10^{-7} M)-STIMULATED MOTOR ACTIVITY

AGENTS WHICH INHIBIT INTRACELLULAR cAMP FORMATION

NPY/PYY REVERSAL

We then attempted to define the role of agents which inhibit intracellular cAMP formation in intestinal motility. PYY and NPY had no effect on CCK-stimulated motility (Table 5). Preinfusion of PYY (10^{-9} - 10^{-8}M) for 5 minutes, followed by VIP (10^{-8}M) administration, did however reverse VIP's inhibition of motility (Figure 27). Preinfusion of lower doses of PYY (10^{-10}M) did not reverse the inhibitory action of VIP. Preinfusion of NPY (10^{-8}M) also reversed VIP's inhibitory activity (Figure 28), whereas lower concentrations of NPY (10^{-9}M) had no effect. PYY is therefore approximately 10 times more potent in reversing VIP's actions than NPY (Table 6).

TABLE 5. EFFECTS OF VIP, PYY AND NPY ON CCK-STIMULTED MOTILITY

PEPTIDES	10^{-7} M	10^{-8} M	10^{-9} M	10^{-10}M
CCK 10^{-9} M			480±52	
CCK 10^{-9} M + VIP	122±17*	162±14*	455±58	
CCK 10^{-9} M + NPY		598±62	554±58	384±82
CCK 10^{-9} M + PYY		416±16	518±46	470±42

* $P < 0.05$ COMPARED TO CCK (10^{-9}M)

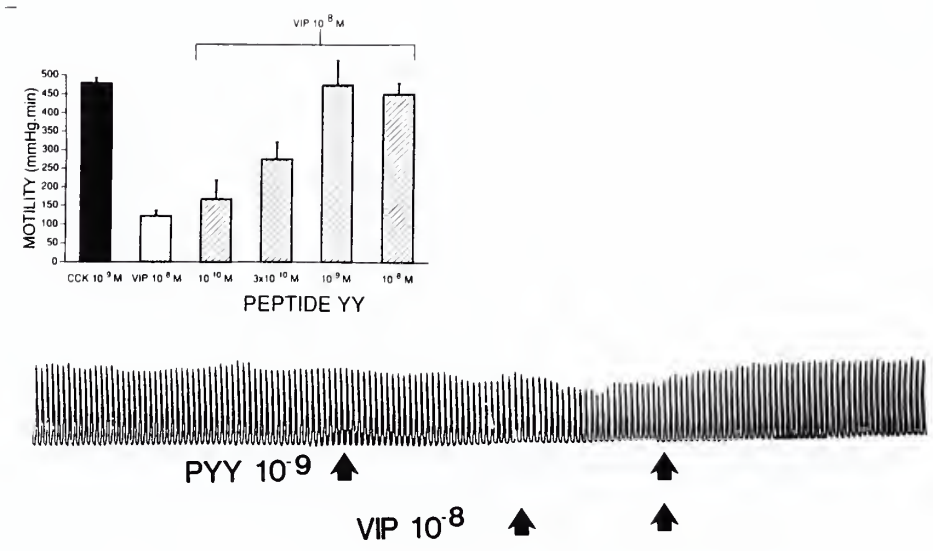

Figure 27: Effect of peptide YY (PYY) on VIP-mediated inhibition of cholecystokinin-stimulated ileal motility. PYY, when pretreated prior to VIP administration, abolished VIP's inhibition of motor activity. A concentration-dependent inhibition of motility was seen with an ED_{50} of 3 x 10^{-10} M (inset). This effect was 10 times more potent than NPY. n = 4 for each group.

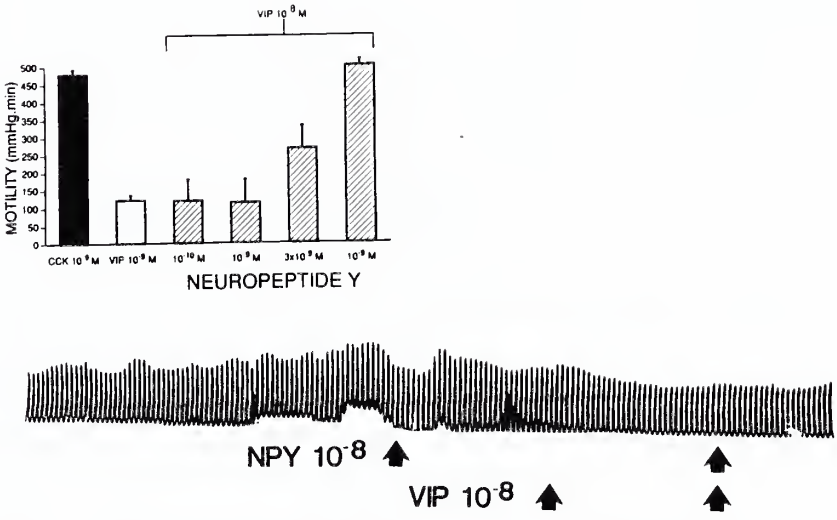

Figure 28: Effect of neuropeptide Y (NPY) on VIP-mediated inhibition of cholecystokinin-stimulated ileal motility. NPY, when pretreated prior to VIP administration, abolished VIP's inhibition of motor activity. A concentration-dependent inhibition of motility was seen with an ED_{50} of 3 x 10^{-9} M (inset). n = 4 for each group.

TABLE 6. EFFECTS OF PYY AND NPY ON VIP MEDIATED INHIBITION OF CCK STIMULTED MOTILITY

PEPTIDES	10^{-8} M	10^{-9} M	10^{-10}M
CCK		475±43	
CCK 10^{-9} M + VIP 10^{-8} M + NPY	500±17	114±66*	120±60*
CCK 10^{-9} M + VIP 10^{-8} M + PYY	452±29	476±66	167±51*

* $P < 0.05$ COMPARED TO CCK (10^{-9}M)

SPECIFIC Y AGONISTS

To determine which Y receptor may be involved in regulating intestinal motility, various Y receptor agonists were tested. When the ileal preparation was pretreated with the specific Y_1 agonist [Leu^{31},Pro^{34}]NPY, motility recordings were similar to those of NPY/PYY's reversal of VIP mediated inhibition. This inhibition of motility was active at the same concentration range as PYY (Figure 29). The ED_{50} of [Leu^{31},Pro^{34}]NPY was 3×10^{-11} M.

The specific Y_2 receptor agonist NPY(13-36) was similarly added to the preparation prior to the addition of the motility antagonist VIP. However, NPY(13-36) failed to reverse VIP-mediated inhibition up to concentrations of 10^{-7} M (Figure 30).

Similarly, pancreatic polypeptide, which recognizes the Y3 receptor, failed to reverse VIP-mediated inhibition up to concentration of 10^{-8}M (Figure 31).

The results of the NPY/PYY/PP receptor analogues are summarized in Table 7.

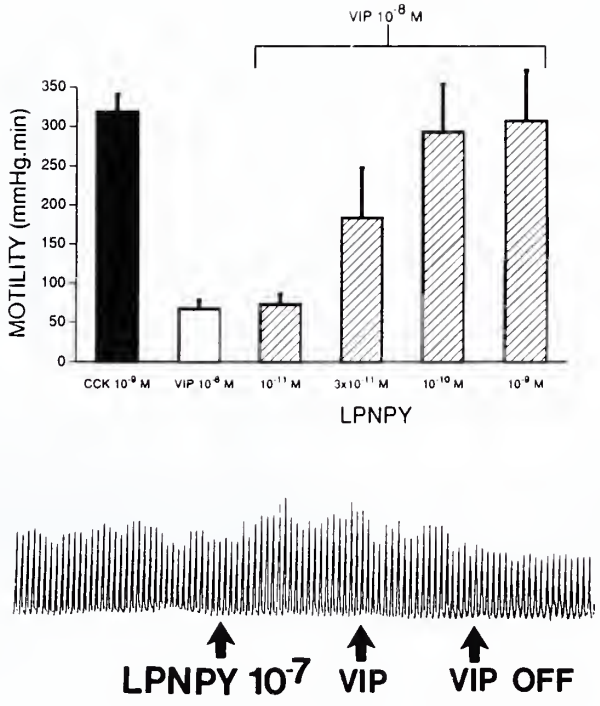

Figure 29: Effect of [Leu^{31}, Pro^{34}]NPY, (LPNPY), on VIP-mediated inhibition of cholecystokinin-stimulated ileal motility. LPNPY, when pretreated prior to VIP administration, abolished VIP's inhibition of motor activity. A concentration-dependent inhibition of motility was seen with an ED_{50} of 3 x 10^{-11} M (inset). n = 4 for each group.

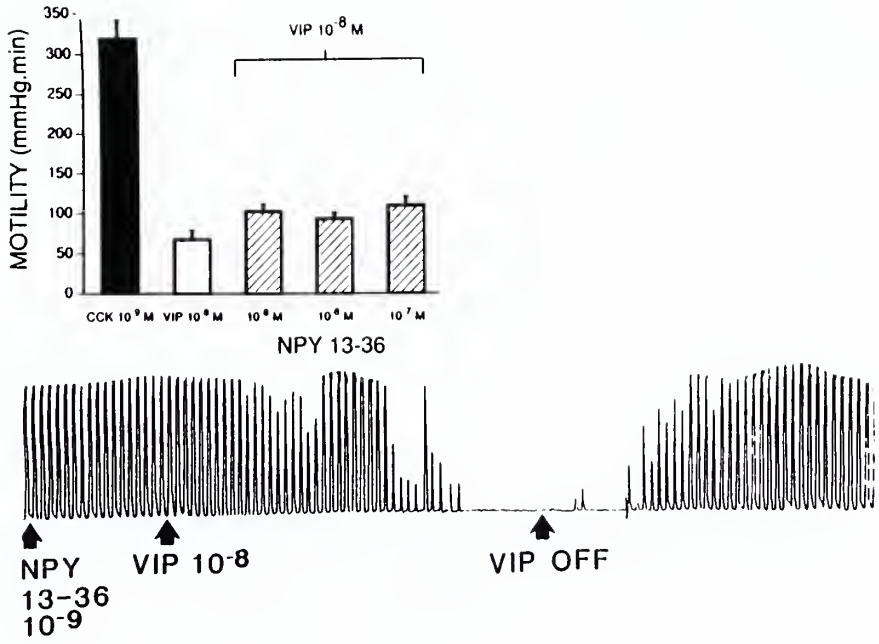

Figure 30: Effect of the long C-terminal fragment of NPY, NPY 13-36, on VIP-mediated inhibition of cholecystokinin-stimulated ileal motility. NPY 13-36, when pretreated prior to VIP administration, failed to abolish VIP's inhibition of motor activity in concentrations as high as 10^{-7} M. n = 4 for each group.

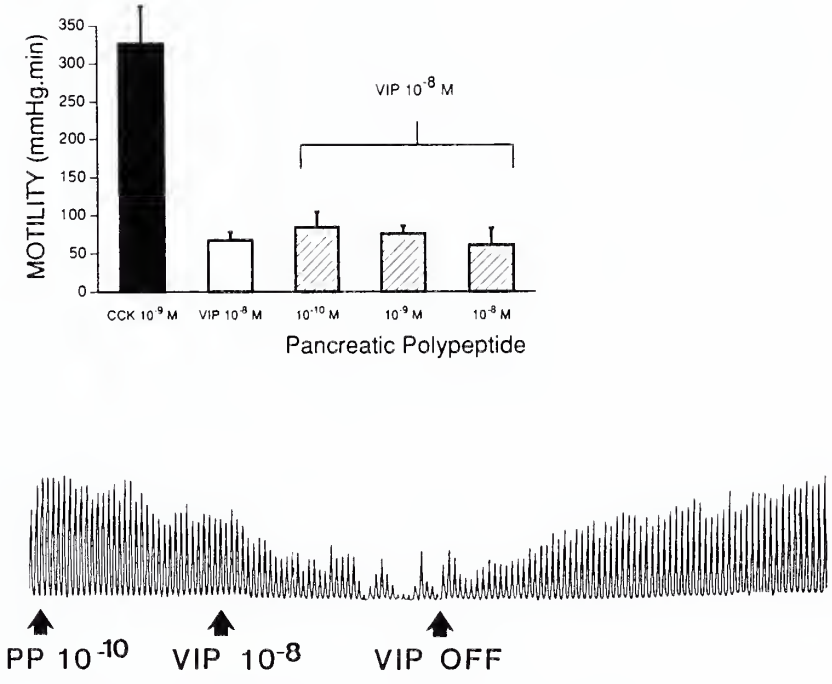

Figure 31: Effect of pancreatic polypeptide (PP) on VIP-mediated inhibition of cholecystokinin-stimulated ileal motility. PP, when pretreated prior to VIP administration, failed to abolish VIP's inhibition of motor activity in concentrations as high as 10^{-7} M. n = 4 for each group.

TABLE 7. EFFEECTS OF LPNPY, NPY 13-36, AND PANCREATIC POLYPEPTIDE ON VIP-MEDIATED INHIBITION OF CCK STIMULATED MOTILITY

PEPTIDES	10^{-8} M	10^{-9} M	10^{-10}M
CCK		316±32	
CCK 10^{-9} M + VIP 10^{-8} M + LPNPY	325±25	307±65	293±61
CCK 10^{-9} M + VIP 10^{-8} M + NPY 13-36	94±7*	103±8*	97±11*
CCK 10^{-9} M + VIP 10^{-8} M + PP	61±22*	76±10*	84±20*

* $P < 0.05$ COMPARED TO CCK (10^{-9}M)

DISCUSSION

Validation of model

The motility patterns derived using this model of gut motility were similar to those obtained in other isolated intestinal models and indistinguishable from those seen in vivo in man (Fox 1985, Sarna 1989, Summers 1983). Viability of the preparation was confirmed by analyzing oxygen tensions in the arterial perfusate and venous effluents. In addition, hematoxylin and eosin stained sections of ileum after ninety minutes of perfusion demonstrated viable bowel. A direct correlation between intestinal transit time and contractile activity, as measured in the present study, cannot be made. Using this model system, one is able to quantitate intestinal smooth muscle contractility and to investigate its regulation by gut peptides in an intact model which preserves the relationship between myenteric plexus and intestinal smooth muscle.

Prokinetic Agents

CARBACHOL

The present study demonstrated the prokinetic actions of carbachol in isolated perfused segments of terminal ileum. Carbachol, an agent which acts through Ca^{++}-dependent mechanisms, generated a concentration-dependent increase in ileal motility.

In this model, we utilized a Ca^{++} agonist to stimulate phasic motor activity. Prokinetic agents produce contraction in smooth muscle cells by increasing intracellular levels of Ca^{++} from stores within the cell (Makhlouf 1987). Smooth muscle contraction is brought about when Mg^{2+}-ATPase activity of actomyosin is activated in the presense of Ca^{++}. Cross-bridging between actin and myosin chains thus results in smooth muscle cell contraction (Hartshorne 1987).

CHOLECYSTOKININ

CCK is a well characterized motility agonist in the ileum of several species (Amer 1972A,B). Depending on the species involved, it exerts its prokinetic action by either acetylcholine release from the myenteric plexus, a purely neurogenic (tetrodotoxin sensitive) mechanism. Alternatively, in other species CCK acts via a combination of neurogenic and direct, non-neurogenic (tetrodotoxin-insensitive) stimulation of smooth muscle receptors (Stewart 1977). CCK caused a concentration-dependent increase in measured motor activity. In this model, tetrodotoxin did not inhibit motility and even resulted in a slight increase in contractile activity. This suggests that CCK activates rabbit ileal smooth muscle by a direct stimulation of cell surface receptors.

MOTILIN

In our study, motilin was demonstrated to possess significant prokinetic actions on isolated perfused segments of rabbit ileum. Intravenous administration of motilin was reported to produce contraction of the lower esophageal sphincter in dog (Jennewein 1973) and opossum (Gutierrez 1977) and dog stomach (Brown 1971, Brown 1972, Cook 1974). Motilin has also been shown to increase action potentials of the antrum and duodenum in dogs (Wingate 1976).

The primary role of motilin appears to be the coordination of the migrating myoelectric complex in both the stomach and duodenum. Phase III contractile patterns appear in the duodenum immediately after a similar, but not identical pattern, in the stomach (Tanaka 1989). This observation accounts for the coordination in both duodenal Phase III activity and gastric phase III activity in propelling digesta distally through the intestine. Peaks in plasma motilin levels have been demonstrated with the occurence of MMC in the stomach and duodenum (Lee 1978, Vantrappen 1979). In addition, administration of anti-motilin antibody resulted in abolished MMC activity in both the stomach and duodenum.

Motilin appears to act directly on the smooth muscle cells of the duodenum and cause propagation of the MMC distally through the small intestine in a calcium-dependent process (Adachi 1981). The effect of motilin is greatest in

the duodenum where it is reported to be 100 times more potent than acetylcholine and in addition, more pronounced in duodenum than ileum (Adachi 1981). This further supports the concept that the duodenum is an important pacemaker in regulating intestinal motility.

cAMP DEPENDENT AGENTS

In this study we explored the role of cAMP dependent agents in the regulation of intestinal motility. We used for a model carbachol-stimulated motility in isolated perfused segments of rabbit terminal ileum. In this experimental system, carbachol, an agent which acts through Ca++-dependent mechanisms, generated a concentration-dependent increase in ileal motility. Both VIP and norepinephrine are neurotransmitters which are found in high concentrations in enteric neurons and increase intracellular levels of cAMP. These two agents inhibited in a concentration dependent manner motility stimulated by carbachol. In addition, forskolin which also acts through cAMP mediated mechansisms inhibited carbachol stimulated motility. Neuronal blockade with tetrodotoxin failed to block the inhibition of carbachol stimulated motility by norepinephrine.

VASOACTIVE INTESTINAL PEPTIDE

Vasoactive intestinal peptide (VIP) is a neurotransmitter found within the myenteric and submucosal

plexuses of the gut (Bitar 1982). VIP containing neurons project into the circular muscle layer and run parallel with the smooth muscle cells. VIP can hyperpolarize the smooth muscle cell membrane and relax tension in gastrointestinal circular muscle (Bishop 1983, Mahklouf 1982). In addition, VIP causes an increase in intracellular levels of cAMP. These elevated levels of cAMP cause activation of cAMP-dependent protein kinases which are involved in smooth muscle cell relaxation (Willenbucher 1992). Indeed, our results support previous models since VIP caused a concentration-dependent reversal of carbachol-stimulated phasic contractions.

VIP's inhibitory action on intestinal motility is well characterized in the intestinal tract of many species. VIP neurones project into the muscle layers of the intestinal wall and are subject to inhibitory tone of opioid neurones. These in turn are inhibited by somatostatin neurones in the myenteric plexus. During the descending relaxation phase of peristalsis, somatostatin is released and this inhibits the opioid neurons, so releasing the restraint on VIP neurones. As a result VIP is released and smooth muscle relaxation results. This relaxation of smooth muscle is accompanied by an increase in cAMP levels. VIP is also a potent secretogogue in intestinal mucosa, its action likewise being mediated by the second messenger cAMP. Inhibition of motility demonstrated in the present study was dose dependent and

reversible after stopping the VIP infusion, when motility returned to previous levels. These findings point to a physiological inhibition of smooth muscle contraction.

NOREPINEPHRINE

In the gut, norepinephrine is found in high concentrations in postganglionic sympathetic neurons. It is thought to act on intramural ganglia of the gut and blood vessels (Burks 1987). Adrenergic fibers which contain norepinephrine are also known to synapse directly with smooth muscle cells. Binding of beta-adrenergic receptors by norepinephrine activates adenylate cyclase with subsequent increases in intracellular concentrations of cAMP. In our study, norepinephrine caused a concentration-dependent decrease in carbachol-stimulated motor activity. This observation supports the classical model of tonic opposition of the parasympathetic system by the sympathetic nervous system.

FORSKOLIN

Forskolin, a diterpene from the roots of Coleus forskohlii, (Bhatt 1977) is a known activator of adenylate cyclase with subsequent activation of cAMP-generating systems within the cell (Seamon 1981). Forskolin does not appear to activate adenylate cyclase by interaction with cell-surface receptors (Seamon 1981) but rather through direct activation

of the catalytic subunit of the adenylate cyclase enzyme complex (Rodbell 1980). As with norepinephrine and VIP, forskolin caused a concentration-dependent inhibition of carbachol-stimulated phasic contraction. These results suggest that inhibition of motility may be a characteristic shared by a broad class of agents which stimulate adenylate cyclase activity.

More than one mechanism may be involved in relaxation of smooth muscle which is mediated by cAMP. Among those invoked is mobilization of Ca^{++} into intracellular stores and/or inhibition of the activity of Ca^{++}/calmodulin-dependent myosin light chain kinase (Hartshorne 1987). Additionally, it has been suggested that cAMP modulates the sensitivity of the contractile apparatus to existing concentrations of calcium (Kamm 1989) as well as inhibition of phosphoinositol hydrolysis (Hall 1989, Kim 1989). Scheid proposed that cAMP enhanced Na^{+}/K^{+} transport and induce relaxation. Stimulation of this transport induces relaxation through enhanced Na^{+}/Ca^{++} exchange (Scheid 1979).

In conclusion, this study indicates that regulatory agents which act through cAMP dependent mechanisms inhibit carbachol stimulated motility in isolated segments of terminal ileum and suggests that these agents may play a role in the counter-regulation of calcium dependent gut motility. Furthermore, our results imply that pharmacologic agents which stimulate adenylate cyclase or lead to increased

intracellular levels of cAMP may be recruited to subserve this same role. These observations may point towards new avenues for the development of pharmacotherapeutic probes aimed at the treatment of the many disturbances of gastrointestinal motility which plaque Western society.

AGENTS THAT INHIBIT INTRACELLULAR cAMP FORMATION

PYY AND NPY

PYY is stored in mucosal "l" cells, principally in the colon, and is released in response to a variety of stimuli including bile salts and short chain fatty acids. Blood and tissue levels of PYY are increased in many diarrheal states and short bowel syndrome. In vivo, PYY inhibits myoelectrical and peristaltic activity in the small intestine. Evidence exists to support the concept that PYY causes a compensatory slowing of gut motility in rapid transit states such as celiac disease and short bowel syndrome. PYY shares 70% sequence homology with neurotransmitter, Neuropeptide Y (NPY). Both NPY and PYY have essentially the same biological activities although PYY is more potent in many biological systems. Centrally, NPY is involved with control of circadian rhythm, memory and food ingestion. Peripherally, NPY is a potent modulator of gut epithelial ion transport. Based on findings in Human Erythroleukemic cell Lines (HEL), two receptor subtypes have been proposed for NPY: Y_1 and Y_2. Y_2 represents the predominant receptor in the central nervous

system and is the major presynaptic receptor. Y_1 and Y_2 receptors occur in both pre- and post- synaptic locations. Specific agonists have been synthesised for Y_1 and Y_2 receptors; The specific agonist at Y_1 receptors is the analog [Leu^{31},Pro^{34}]NPY, and Y_2 agonist is the C-terminal fragment of NPY, NPY(13-36).

In addition, scientists have recently identified two new receptors, designated Y_3 and Y_4 (Wahlestedt 1992, Ballantyne 1993). The Y_3 receptor shows affinity for NPY but not PYY. The Y4 receptor is characterized by having equal affinity for NPY, PYY and pancreatic polypeptide.

Several previous studies have described an inhibitory role of PYY on CCK stimulated gut motility in the rat. This has been put forward as an explanation of PYY's slowing action on gut transit in diarrheal states. In the present rabbit model, neither PYY nor NPY inhibited CCK stimulated motility. In separate studies we were unable to demonstrate inhibition of carbachol stimulated motility by PYY at physiological concentrations in the isolated perfused rabbit model.

The present findings of PYY and NPY's reversal of VIP mediated inhibition of intestinal motility indicate that PYY and NPY may inhibit intestinal transit by abolishing VIP mediated descending inhibition. The results reported here indicate that this is mediated by post synaptic (Y_1)

receptors. It is of interest that both VIP and CCK (in the present model) also act at a post synaptic location.

In colonic mucosa, VIP-stimulated ion transport is inhibited by both PYY and NPY. This is due to a lowering of VIP-stimulated increase in cAMP levels. In the same isolated perfused colon model we have previously demonstrated PYY-stimulated release of VIP, which may represent an example of feed-back inhibition of VIP-stimulated mucosal ion transport. Other studies have demonstrated CCK-stimulated release of VIP from intestinal mucosa. These studies, together with the present findings demonstrate a close resemblance between regulation of gut secretion and control of gut motility. Stimulants of mucosal secretion (VIP) inhibit gut motility and conversely, antagonists of secretion (PYY and NPY) reverse VIP's inhibition of motility.

The integration of PYY and NPY's inhibition of VIP into the current model of gut motility is a new concept. A wave of relaxation (descending inhibition), mediated by VIP release, precedes a bolus traveling along the gut. Orad to the bolus, a wave of contraction (ascending contraction) is mediated by cholinergic neurones. The present study demonstrates PYY and NPY inhibit VIP mediated relaxation. Prevention of descending relaxation may inhibit the coordinated distal propagation of gut contents. This provides a mechanism for PYY's known action of slowing intestinal transit. Two important points emerge from the present study; first the demonstration of the

parallel control of gut secretion and motility and; second, a possible mechanism to explain PYY's slowing of intestinal transit.

REFERENCES

Aakerlund L, Gether U, Fuhlendorff J, Schwartz TW, Thastrup O. Y1 receptors for neuropeptide Y are coupled to mobilization of intracellular calcium and inhibition of adenylate cyclase. FEBS Letters 1990; 260:73-78.

Abernathy J. Surgical Observations on the Constitutional Origin and Treatment of Local Diseases; and on Aneurisms. London, Longman, Hurst, Rees, Orme, 1809.

Adachi H, Toda N, Hayashi S, Noguschi M, Suzuki T, Torizuka K, Yajima H, Koyama K. Mechanism of the excitory action of motilin on isolated rabbit intestine. Gastroenterol 1981; 80:783-788.

Adrian TE, Bacarese-Hamilton AJ, Allen JM, Tatemoto K, Ferri G-L, Polak JM, Bloom SR. Distribution of PYY in the porcine and human gastrointestinal tract. Reg Peptide 1982; 4:355.

Adrian TE, Allen JM, Bloom SR, Ghatei MA, Rossor MN, Roberts GW, Crow TJ, Tatemoto K, Polak JM. Neuropeptide Y distribution in human brain. Nature Lond 1983; 306:584-586.

Adrian TE, Savage AP, Sagor GR, Allen JM, Bacarese-Hamilton AJ, Tatemoto K, Polak JM, Bloom SR. Effect of peptide YY of gastric, pancreatic, and biliary function in humans. Gastroenterol 1985; 89:494-499.

Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR. Human distribution and release of a putative new gut hormone, peptide YY. Gastroenterol 1985; 89:1070-1077.

Adrian TE, Long RG, Fuessl HS, Bloom SR. Plasma peptide YY (PYY) in dumping syndrome. Dig Dis Sci 1985; 30:1145-1148.

Adrian TE, Ferri G-L, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR. Human distribution and release of a putative new gut hormone, peptide YY. Gastroenterol 1985; 89:1070-1077.

Adrian TE, Savage AP, Bacarese-Hamilton AJ, Wolfe K, Besterman HS, Bloom SR. Peptide YY abnormalities in gastrointestinal diseases. Gastroenterol 1986; 90:379-384.

Adrian TE, Sagor GR, Savage AP, Bacarese-Hamilton AJ, Hall GM, Bloom SR. Peptide YY kinetics and effects on blood

pressure and circulating pancreatic and gastrointestinal hormones and metabolites in man. Jour Clin End and Met 1986; 63:803-807.

Adrian TE, Savage AP, Fuessl HS, Wolfe K, Besterman HS, Bloom SR. Release of peptide YY (PYY) after resection of small bowel, colon, or pancreas in man. Surgery 1987; 101:715-719.

Adrian TE, Bacarese-Hamilton AJ, Smith HA, Chohan P, Manolas K, Bloom SR. Distribution and postprandial release of porcine peptide YY. J Endocr 1987; 113:11-14.

Adrian TE, Savage AP, Fuessel HS, Wolfe K, Besterman HS, Bloom SR. Release of peptide YY (PYY) after resection of small bowel, colon or pancreas in man, Surgery 1987; 101:715-719.

Ali-Rachedi A, Varndell IM, Adrian TE, Gapp DA, Van Noorden S, Bloom SR, Polak JM, Peptide YY (PYY) immunoreactivity is co-stored with glucagon-related immunoreactants in endocrine cells of the gut and pancreas. Histochemistry 1984; 80:487-491.

Allen YS, Adrian TE, Allen JM, Tatemoto K, Crow TJ, Bloom SR, Polak JM. Neuropeptide Y distribution in the rat brain. Science 1983; 221:877-879.

Allen JM, Adrian TE, Fitzpatrick ML, Yeats JC, Bloom SR. Gastric emptying in man: response to peptide YY and neuropeptide Y. Dig Dis Sci 1984; 29(8S):4S.

Allen JM, Hughes J, Bloom SR. Presence, distribution and pharmacological effects of neuropeptide Y in mammalian gastrointestinal tract. Dig Dis and Sci 1987; 32:506-512.

Al-Saffar A, Hellstrom PM, Nylander G. Correlation between peptide YY-induced myoelectric activity and transit of small-intestinal contents in rats. Scand J Gastro 1985; 20:577-582.

Alvarez WC, Mahoney LJ. Action current in stomach and intestine. Am J Physiol 1922; 58:476-493.

Alvarez WC. Helps in sizing up the patient. In: Nervousness, Indigestion and Pain. New York: Paul B. Hoeber; 1943:83-99.

Amer MS. Studies with cholecystokinin in vitro. III. Mechanism of the effect on the isolated rabbit gallbladder strips. J Pharm and Exp Therap 1972; 183:527-534.

Aponte GW, Fink AS, Meyer JH, Tatemoto K, Taylor IL. Regional distribution and release of peptide YY with fatty acids of different chain lengths. Am J Phys 1985; 248:G745-750.

Armstrong DN, Ballantyne GH, Adrian TE, Bilchik AJ, McMillen MA, Modlin IM. Adaptive increase in peptide YY and enteroglucagon after proctocolectomy and pelvic ileal reservoir construction. Dis Colon and Rectum 1991; 34:119-125.

Armstrong DN, Ballantyne GH, Modlin IM. Erythromycin stimulates ileal motility by activation of dihydropyridine-sensitive calcium channels. J Surg Res 1992; 52:140-146.

Baba H, Fujimura M, Toda N. Mechanism of inhibitory action of peptide YY on cholecystokinin-induced contractions of isolated dog ileum. Reg Peptides 1990; 27:227-235.

Ballantyne GH, Longo WE, Savoca PE, Adrian TE, Vukasin AP, Bilchik AJ, Sussman J, Modlin IM. Deoxycholate-stimulated release of peptide YY from the isolated perfused rabbit left colon. Am J Physiol 1989; 257:G715-724.

Ballantyne GH. Enteroglucagon and peptide YY response after construction of a pelvic reservoir in humans. Editorial Comment. Dis Colon and Rectum 1990; 33:969-970.

Ballantyne GH, Goldenring JR, Rush S, Modlin IM. Am J Physiol 1993 (In Press).

Baranowska B. Gutowska J, Lemire A, Cantin M, Genest J. Opposite effects of neuropeptide Y (NPY) and polypeptide YY (PYY) on plasma immunoreactive atrial natriuretic factor (IR-ANF) in rats. Biochem Biophys Res Comm 1987; 145:680-685.

Bardon T, Ruckebusch M. Changes in 5-HIAA and 5-HT levels in lumbar CSF following morphine administration to conscious dogs. Neurosci Lett 1984; 49:147-151.

Bayliss WM, Starling EH. The movements and innervation of the small intestine. J Physiol (London) 1899; 24:99-143.

Bean BP. Classes of calcium channels in vertebrate cells. Annu Rev Physiol 1989; 51:367-384.

Bennett A, Stockley HL. The intrinsic innervation of the human alimentary tract and its relation to function. Gut 1975; 16:443-453.

Bertaccini G and Agosti A. Action of caerulein on intestinal motility in man. Gastroenter 1971; 60:55-63.

Bhat SV, Bajwa BS, Dornauer H, deSouza NJ and Fehlhaber HW. Structures and stereochemistry of new labolane diterpenoids from coleus forskohlii briq. Tetrahedron Lett 1977; 19:1669-1672.

Biber B, Fara J. Intestinal motility increased by tetrodotoxin, lidocaine and procaine. Experientia 1973; 29:551-552.

Bishop AE, Blank MA, Christophides ND, Bloom SR, Tatemoto K and Polak JM. Colocalization of VIP and PHI in human gut. Reg Pept 1983; 6:287.

Bitar KN, Makhlouf GM. Relaxation of isolated gastric smooth muscle cells by vasoactive intestinal peptide. Science 1982; 216:531-533.

Blachburn AM, Christofides ND, Ghatei MA, Sarson DL, Ebeid FH, Ralphs DNL, Bloom SR. Elevation of plasma neurotensin in the dumping syndrome. Clin Sci 1980; 59:237-243.

Bortoff A. Current Concepts--Intestinal Motility. N Engl J Med 1969; 280:1335-1337.

Bortoff A, Muller R. Stimulation of intestinal smooth muscle by atropine, procaine, and tetrodotoxin. Am J Physiol 1975; 229:1609-1613.

Bortoff A. Smooth muscle of the gastrointestinal tract. Chapter 2. In: A guide to gastrointestinal motility. Christenson J, Wingate PSG (eds), Bristol 1983.

Böttcher G, Sjolund K, Ekblad E, Hakanson R, Schwartz TW, Sundler F. Coexistence of peptide YY and glicentin immunoreactivity in endocrine cells of the gut. Reg Pep 1984; 8:261-266.

Böttcher G, Alumets J, Hakanson R, Sundler F. Co-existence of glicentin and peptide YY in colorectal L-cells in cat and man. An electron microscopic study. Reg Pep 1986; 13:283-291.

Bradbury A, Finnie M, Smyth D. Mechanism of C-terminal amide formation by pituitary enzymes. Nature 1982; 298:686-688.

Brown JC, Mutt V, Dryburgh JR. The further purification of motilin, a gastric motor activity stimulating polypeptide

from the mucosa of the small intestine of hogs. Can J Physiol 1971; 49:399-405.

Brown JC, Cook MA, Dryburgh JR. Motilin, a gastric motor activity stimulating polypeptide; final purification, amino acid composition, and C-terminal residues. Gastroenterol 1972; 62:401-404.

Buchanan KD. The Gastrointestinal Hormones. Clinics in Endocrinology and Metabolism 1979; 8:247-447.

Bueno L, Fargeas MJ, Fioramonti J, Primi MP. Central control of intestinal motility by prostaglandins: A mediator of the actions of several peptides in rats and dogs. Gastroenterol 1985; 88:1888-1894.

Burks TF. Actions of drugs on gastrointestinal motility. In Johnson LR (Ed) Physiology of the Gastrointestinal Tract Vol 1. New York: Raven Press, 1987, pp 731.

Busch-Sorensen MS, Sheikh SP, O'Hare M, Tortora O, Schwartz TW and Gammeltoft S. Regional distribution of neuropeptide Y and its receptor in the porcine central nervous system. J Neurochem 1989; 52:1545-1552.

Camilleri M, Prather CM. The irritable bowel syndrome: Mechanisms and a practical approach to management. Ann Int Med 1992; 116:1001-1008.

Campbell D. Arabian Medicine and its Influence on the Middle Ages. London. Kegan Paul, Trench, Trubner & Co., 1926.

Cann PA, Read NW, Brown C, Hobson N, Holdsworth CD. Irritable bowel syndrome: relationship of disorders in the transit of a single solid meal to symptom patterns. Gut 1983; 24:405-411.

Carlei F, Allen JM, Bishop AE, Bloom SR, Polak JM. Occurence, distribution and nature of neuropeptide Y in the rat pancreas. Experientia 1985; 41: 1554-1557.

Celsus (Spencer WG, trans). De Medicina. Harvard University Press. London 1979.

Chance RE, Jones WE. Polypeptide from bovine, ovine, human and porcine pancreas. US Pat No. 3, 842,063:1974.

Chijiiwa Y, Misawa T, Ibayashi. Evidence of local mechanism involvement in vasoactive intestinal peptide release from canine small intestine. Gastroenterol 1986; 90:1877-1881.

Christensen J. The controls of gastrointestinal movements: some old and new views. N Engl J Med 1971; 285:85-98.

Christensen J, Schedl HP, Clifton JA. The basic electrical rhythm of the duodenum in normal human subjects and in patients with thyroid disease. J Clin Invest 1964; 43:1659-1667.

Christensen J, Clifton JA, Schedl HP. Variations in the frequency of the human duodenal basic electrical rhythm in health and disease. Gastroenterol 1966; 51:200-206.

Chujyo N. Site of acetylcholine production in the wall of the intestine. Am J Physiol 1953; 174:196-198.

Cohen S, Long WB, Snape WJ, Jr. Gastrointestinal motility. In International Review of Physiology, Gastrointestinal Physiology III, Vol 19, Ed. Crane RK. University Park Press, Baltimore, pp. 107-149, 1979.

Cook MA, Kowalewski K, Daniel EE. Electrical and mechanical activity recorded from isolated, perfused canine stomach: the effects of some GI polypeptides. In Daniel EE, ed. Proceedings, Fourth International Symposium on Gastrointestinal Motility. Vancouver: Mitchell Press, 1974, pp 223-242.

Cooper A. The Lectures on the Principles and Practice of Surgery. Haswell, Barrington and Haswell, Philadelphia, 1839.

Corner GW. Anatomical texts of the earlier Middle Ages. A study in the transmission of culture with a revised latin texts of Anatomia Cophonis and translations of four texts. Washington, D.C., Carnegie Institution of Washington, 1927.

Cox HM, Cuthbert AW. Neuropeptide Y antagonizes secretagogue evoked chloride transport in rat jejunal epithelium. Pflugers Arch 1988; 413:38-42.

Cox HM, Cuthbert AW, Hakanson R, Wahlestedt C. The effect of neuropeptide Y and peptide YY on electrogenic ion transport in rat intestinal epithelia. J Physiology 1988; 398:65-80.

Crowley WR, Shah GV, Carroll BL, Kennedy D, Dockter ME, Kalra SP. Neuropeptide-Y enhances leutinizing hormone (LH)-releasing hormone-induced LH release and elevations in cytosolic Ca++ in rat anterior pituitary cells: evidence for involvement of extracellular Ca++ influx through voltage-sensitive channels. Endocrinology 1990; 127:1487-1494.

Cullen W. First Lines of the Practice of Physic. Edinburgh, William Creech, 1777.

Cumming W. Electro-galvanism in a peculiar affliction of the mucous membrane of the bowels. Lond Med Gazette 1849; NS9:969-973.

Dennis FS. A System of Surgery. Philadelphia, Lea Brothers and Co., 1895

Depoortere I, Peeters TL, Vantrappen G. Development of motilin receptors and of motilin- and erythromycin-induced contractility in rabbits. Gastroenterol 1990; 99(3):652-658.

DiLorenzo C, Lachman R, Hyman PE. Intravenous erythromycin for postpyloric intubation. J Ped Gastroenterol Nutr 1990; 11(1):45-47.

Ding WG, Fujimura M, Mori A, Tooyama I, Kimura H. Light and electron microscopy of neuropeptide Y-containing nerves in human liver, gallbladder, and pancreas. Gastroenterol 1991; 101:1054-1059.

Dockray G. Immunoreactive component resembling cholecystokinin octapeptide in intestine. Nature 1977; 270:359-361.

Drossman DA, Funch-Jensen P, Janssens J, Talley NJ, Thompson WG, Whitehead WE. Identification of subgroups of functional bowel disorders. Gastroenterol Int 1990; 3:159-172.

Drossman DA, Thompson WG. The irritable bowel syndrome: Review and a graduated multicomponent treatment approach. Ann Int Med 1992; 116:1009-1016.

Eipper B, Myers A, Mains R. Peptidyl-glycine alpha-amidation activity in tissues and serum of the adult rat. Endocrinology 1985; 116:2497-2504.

Ekblad EL, Edvinsson VE, Wahlestedt C, Uddmann R, Hakanson R, Sundler F. Neuropeptide Y co-exists and cooperates with noradrenaline in perivascular nerve fibers. Reg Peptides 1984; 10:225-235.

Ekblad E, Hakanson R, Sundler F, VIP and PHI coexist with an NPY-like peptide in intramural neurones of the small intestine. Regul Peptides 1984; 8:47-55.

El-Salhy M, Grimelius L, Lundberg JM, Tatemoto K, Terenius L. Immunocytochemical evidence for the occurence of PYY, a newly

isolated gut polypeptide, in endocrine cells in the gut of amphibians and reptiles. Biomed Research 1982; 3:303-306.

El-Salhy M, Wilander E, Grimelius L, Terenius L, Lundberg JM, Tatemoto K. The distribution of polypeptide YY (PYY)-and pancreatic polypeptide (PP)-immunoreactive cells in the domestic fowl. Histochem 1982; 75(1):25-30.

El-Salhy M, Grimelius L. Immunocytochemical demonstration of polypeptide YY (PYY) in the gastrointestinal tract of the monkey, Macaca rhesus: light and electron microscopic study. Biomed Res 1983; 4:289-294.

El-Salhy M, Grimelius , Wilander E, Ryberg B, Terenius L, Lundberg JM, Tatemoto K. Immunocytochemical identification of polypeptide YY (PYY) cells in the human gastrointestinal tract. Histochem 1983; 77:15-23.

Emson PC, DeQuidt ME. NPY: a new member of the pancreatic polypeptide family. Trend Neurosci 1984; 7:31-35.

Everitt BJ, Hokfelt T, Terenius L, tatemoto K, Mutt V, Goldstein M. Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. Neuroscience 1984; 11:443-462.

Eysselein V, Reeve J, Jr, Shively J, Miller C and Walsh J. Partial structure of a large cholecystokinin (CCK58): Amino acid sequence. Peptides 1982; 3:687-691.

Ferri GL, Adrian TE, Allen JM, Soimero L, Cancellieri A, Yeats J, Blank M, Polak JM, Bloom SR. Intramural distribution of regulatory peptides in the sigmoid-rectoanal region of the human gut. Gut 1988; 29:762-768.

Fiocca R, Rindi G, Capella C, Grimelius L, Polak JM, Schwartz TW, Yanaihara N, Solcia E. Glucagon, glicentin, proglucagon, PYY, PP and proPP-icosapeptide immunoreactivities of rectal carcinoid tumors and related non-tumor cells. Regul Pept 1987; 17:9-29.

Flint JS, Ballantyne GH, Goldenring JR, Fielding LP, Modlin IM. Neuropeptide Y inhibition of vasoactive intestinal polypeptide-stimulated ion transport in the rabbit distal colon. Arch Surg 1990; 125:1561-1563.

Foster M. Lectures on the History of Physiology during the Sixteenth, Seventeeth and Eighteenth Centuries. Cambridge. The University Press, 1901.

Fox JET, Daniel EE, Jury J, Robotham H. Muscarinic inhibition of canine small intestinal motility in vivo. Am J Physiol 1985; 248:G526-531.

Fox AP, Nowycky MC and Tsien RW. Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. J Physiol 1987; 394:149-172.

Friel DD, Miller JM, Walker MW. Neuropeptide Y: a powerful modulator of epithelial ion transport. Br J Pharmacol 1986; 88:425-431.

Fuhlendorff J, Gether U, Aakerlund L, Langeland-Johansen N, Thogerson H, Melberg SG, Olsen UB, Thastrup O, Schwartz TW. [Leu^{31}, Pro^{34}]Neuropeptide Y: A specific Y1 receptor agonist. PNAS (USA) 1990; 87:182-186.

Fuhlendorff J, Johansen NL, Melberg SG, Thogersen H, Schwartz TW. The antiparallel pancreatic polypeptide fold in the binding of neuropeptide Y to Y1 and Y2 receptors. J Biolog Chem 1990; 265:11706-11712.

Furness JB, Costa M, Emson PC, Hakanson R, Moghimzadeh E, Sundler F, Taylor IL, Chance RE. Distribution, pathways and reactions to drug treatment of nerves with neuropeptide Y- and pancreatic polypeptide-like immunoreactivity in the guinea pig digestive tract. Cell Tissue Res 1983; 234:71-92.

Gabella G. Innervation of the intestinal muscular coat. J Neurocytol 1972; 1:341-362.

Galen. (Brock AJ, trans). On the Natural Faculties, Harvard University Press, Cambridge, Massachusetts, 1927.

Galen. (Green RM, trans). De Sanitate Tuenda. Charles C. Thomas, Springfield, Illinois, 1951.

Garrison FH. An Introduction to the History of Medicine. Philadelphia: Saunders, 1913.

Gernandt B, Zotterman Y. Intestinal pain: An electrophysiological investigation on mesenteric nerves. Acta Physiol Scand 1946; 12:56-72.

Gershon MD, Erde SM. The nervous system of the gut. Gastroenterol 1981; 80:1571-1594.

Gomez G, Lluis F, Draviam E, Greeley GH, Thompson JC. Peptide YY stimulates bile flow in dogs. Gastroent 1986; 90:1431.

Gonella J. La motilitie digestive et sa regulation nerveuse. J Physiol (Paris) 1978; 74:131-140.

Grace PA, Couse NF, Taylor IL, Pitt HA. Peptide YY inhibits cholecystokinin-stimulated sphincter of Oddi activity in the prairie dog. Surgery 1988; 104:546-552.

Greeley GH Jr, Jeng YJ, Gomez G, et al. Evidence for regulation of peptide-YY release by the proximal gut. Endocrinology 1989; 124:1438-1443.

Greeley GH, Hashimoto T, Izukura M, Gomez G, Jeng J, Hill FLC, Lluis F, Thompson JC. A comparison of intraduodenally and intracolonically administered nutrients on the release of peptide-YY in the dog. Endocrinology 1989; 125:1761-1765.

Gregory RA. The nervous pathways of intestinal reflexes associated with nausea and vomiting. J Physiol (Lond) 1947; 106:95-103.

Grider JR, Makhlouf GM. Vasoactive intestinal peptide: Transmitter of inhibitory motor neurons of the gut. Ann NY Acad Sci 1988; 527:369-377.

Grundemar L, Wahlestedt C, Reis DJ. Neuropeptide Y acts at an atypical receptor to evoke cardiovascular depression and to inhibit glutamate responsiveness in the brainstem. J Pharmacol Exp Ther 1991; 258:633-638.

Gruner OC. A Treatise on the Canon of Medicine of Avicenna. London, Luzac and Co., 1930.

Gu J, Polak JM, Adrian TE, Allen JM, Tatemoto K, Bloom SR. Neuropeptide tyrosine (NPY) - a major cardiac neuropeptide. Lancet 1983; 1:1008-1010.

Guthrie D. A History of Medicine. Philadelphia, JB Lippincott Co, 1946.

Gutierrez JG, Chey WY, Dinoso VP. Actions of cholecystokinin and secretin on the motor activity of the small intestine in man. Gastroenterol 1974; 67:35-41.

Gutierrez JG, Thanik KD, Chey WY, et al. The effect of motilin on the lower esophageal sphincter of the opossum. Am J Dig Dis 1977; 22:402-405.

Hall IP, Donaldson J and Hill SJ. Inhibition of histamine-stimulated inositol phospholipid hydrolysis by agents which increase cyclic AMP levels in bovine tracheal smooth muscle. Br J Pharmacol 1989; 97:603-613.

Hartshorne DJ. Biochemistry of the contractile process in smooth muscle. In Johnson LR (Ed) Physiology of the Gastrointestinal Tract Vol 1. New York: Raven Press, 1987. pp 423-482.

Hellstrom PM, Olerup O, Tatemoto K. Neuropeptide Y may mediate effects of sympathetic nerve stimulations on colonic motility and blood flow in the cat. Acta Physiol Scand 1985; 124:613-624.

Hemmeter JC. Diseases of the intestines. P. Blakiston's Son, Philadelphia, 1902.

Hermon-Taylor J, Code CF. Localisation of the duodenal pacemaker and its role in the organization of the duodenal myoelectric activity. Gut 1971; 12:40-47.

Hertz A. Constipation and allied intestinal disorders. Oxford Medical Publishers, London 1909.

Hidaka T, Kuriyama H. Response of the smooth muscle membrane of guinea pig jejunum elicited by field stimulation. J Gen Physiol 1969; 53:471-486.

Hill GL, Mair WSJ, Goligher JC. Impairment of "ileostomy adaptation" in patients after ileal resection. Gut 1974; 15:982-987.

Hippocrates. (Adams F, trans). The Genuine Works of Hippocrates. London, Sydenham Society, 1849.

Horowitz L, Farrar JT. Intraluminal small intestinal pressure in normal patients and in patients with functional gastrointestinal disorders. Gastroenterol 1962; 42:455-464.

Hosotani R, Inoue K, Kogire M, et al. Effect of natural peptide YY on pancreatic secretion and cholecystokinin release in conscience dogs. Dig Dis Sci 1989; 34:468-473.

Hubel KA, Renquist KS. Effect of neuropeptide Y on ion transport by the rabbit ileum. J Pharmacol Exp Ther 1986; 238:167-169.

Hutchinson JB, Dockray GJ. Evidence that the actions of cholecystokinin octapeptide on the guinea-pig ileum longitudinal muscle is mediated in part by substance P release from the myenteric plexus. Eur J Pharmacol 1981; 69:87-93.

Inatomi N, Satoh H, Maki Y, Hashimoto N, Itoh Z, Omura S. An erythromycin derivative, EM-523, induces motilin-like gastrointestinal motility in dogs. J Pharmacol. Exp Therap 1989; 251(2):707-712.

Itoh Z, Honda R, Hiwatashi K, Takeuchi S, Aizawa I, Takayangi R, Couch E. Motilin-induced mechanical activity in the canine alimentary tract. Scand J Gastroenterol 1976; 39(Suppl.11):93-110.

Itoh K, Nakaya M, Suzuki T, Arai H, Wakabayashi K. Erythromycin mimics exogenous motilin in gastrointestinal contractile activity in the dog. Am J Physiol 1984; 247:G688-694.

Itoh Z, Suzuki T, Nakaya M, Inoue M, Arai H, Wakabayashi K. Structure-activity relation among macrolide antibiotics in initiation of the interdigestive migrating contractions in the canine gastrointestinal tract. Am J Physiol 1985; 248:G320.

Jackson TR, Patterson SI, Thastrup O, Hanley MR. A novel tumor promoter, thapsigargin, transiently increases cytoplasmic free Ca^{++} without generation of inositol phosphates in NG115-401L neuronal cells. Biochem J 1988; 253:81-86.

Jacobi J (N. Guterman trans). Paracelsus Selected Writings. Princeton, New Jersey, Princeton University Press, 1979.

Jennewein HM, Hummelt H, Siewert R. The motor stimulating effect of natural motilin on the lower esophageal sphincter, fundus, antrum and duodenum in dogs. Digestion 1973; 13:246-250.

Johansson B, Jonsonn D, Ljung B. Tonic supraspinal mechanisms influencing the intestino-intestinal inhibitory reflex. Acta Physiol Scand 1967; 72:200-204.

Kamm KE and Stull JT. Regulation of smooth muscle contractile elements by second messengers. Annu Rev Physiol 1989; 51:299-313.

Kassis S, Olasmaa M, Terenius L, Fishman PH. Neuropeptide Y inhibits cardiac adenylate cyclase through a pertussis toxin-sensitive G protein. J Biol Chem 1987; 262:3429-3431.

Keele KD. Leonardo da Vinci's studies of the alimentary tract. J Hist Med 1972; 27:133-144.

Kewenter J. The vagal control of the jejunal and ileal motility and blood flow. Acta Physiol Scand. (Suppl.) 1965; 251:3-68.

Kewenter J, Pahlin P-E, Strom B. The effects of periarterial nerve stimulation on the jejunal and ileal motility in cat. Acta Physiol Scand 1970; 80:353-359.

Kim U, Kim JW and Rhee SG. Phosphorylation of phospholipase C-gamma by cAMP-dependent protein. J Biol Chem 1989; 264:20167-20170.

Kishimoto S, Kato R, Mukai T, Kanbara A, Okamoto K, Shimizu S, Daitoku K, Kajiyama G. Distribution and endocrine morphology of polypeptide YY (PYY) containing cells in the human gut. Hiroshima J Med Sci 1985; 34:155-160.

Konturek SJ, Bilski J, Tasler J, Jaworek J, Kitler ME. Peptide YY (PYY) inhibits pancreatic secretion indirectly through the adrenergic pathway. Gastroent 1986; 90:1499.

Korman LY, Sayadi H, Bass B, Moody TW, Harmon. Distribution of vasoactive intestinal polypeptide and substance P receptors in human colon and small intestine. Dig Dis Sci 1989; 34:1100-1108.

Kosterlitz HW, Lees GM. Pharmacological analysis of intrinsic intestinal reflexes. Pharmacol Rev 1964; 16:301-339.

Kosterlitz HW. Intrinsic and extrinsic nervous control of motility of the stomach and the intestine. In: Handbook of Physiology, Section 6, Vol. 4: Motility, edited by C. F. Code, William and Wilkins, Baltimore, 1968, pp 2147-2171.

Kreulen DL, Szurszewski JH. Nerve pathways in celiac plexus of the guinea pig. Am J Physiol 1979; 237:E90-E97.

Kreulen DL, Szurszewski JH. Reflex pathways in the abdominal prevertebral ganglia: Evidence for a colo-colonic inhibitory reflex. J Physiol (London) 1979; 295:21-32.

Kutumbiah P. The Evolution of Scientific Medicine. Orient Longman, Madras, India, 1971.

Laburthe M, Chenut B, Rouyer-Fessard C, Tatemoto K, Couvineau A, Servin A, Amiranoff B. Interaction of peptide YY with rat intestinal epithelial plasma membranes: binding of the radioidinated peptide. Endocrinology 1986; 118:1910-1917.

Lamers C, Walsh J, Jansen J, Harrison A, Ippoliti A, Van Tongeren J. Evidence that gastrin 34 is preferentially released from the human duodenum. Gastroenterol 1982, 83:233-239.

Lang IM, Sarna SK, Condon RE. Myoelectrical and contractile effects of motilin on dog small intestine in vivo. Dig Dis Sci 1986; 31:1062-1072.

Lee KY, Chey WY, Tai HH. Radioimmunoassay of motilin, validation and studies on the relationship between plasma motilin and interdigestive myoelectric activity of the duodenum of dogs. Am J Dig Dis 1978; 23:789-795.

Levant J, Kun T, Jachna J, Sturdevant R, Isenberg J. The effects of graded doses of C-terminal octapeptide of cholecystokinin on small intestinal transit time in man. Dig Dis 1974; 19:207-209.

Lewis JL. Avicenna, the intellectual physician (980-1037 A.D.). Medical Arts and Sciences Journal 1965; 19(3):107-119.

Lluis F, Gomez G, Fujimura M, Greeley GH, Thompson JC. Peptide YY inhibits pancreatic secretion by inhibiting cholecystokinin release in the dog. Gastroent 1988; 94:137-144.

Lobaugh AL, Blackshear PJ. Neuropeptide Y binding and inhibition of cAMP accumulation in human neuroepithelioma cells. Am J Physiol 1990; 258:C913-C922.

Longo WE, Ballantyne GH, Modlin IM. The colon, anorectum and spinal cord patient: A review of the functional alterations of the denervated hindgut. Dis Col and Rectum 1989; 32:261-267.

Longo WE, Ballantyne GH, Savoca PE, Adrian TE, Bilchik AJ, Modlin IM. Short chain fatty acid release of peptide YY in the isolated rabbit colon. Scan J Gastroenterol 1991; 26:442-448.

Lundberg JM, Tatemoto K. Vascular effects of the peptides PYY and PHI: comparison with APP and VIP. Euro Jour Pharm 1982; 83:143-146.

Lundberg JM, Tatemoto K. Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistent to alpha-adrenergic blockade. Acta Physiol Scand 1982; 116:393-402.

Lundberg JM, Tatemoto K, Terenius L, Hellstrom PM, Mutt V, Kökfelt T, Hamberger B. Localization of peptide YY (PYY) in gastrointestinal endocrine cells and effects on intestinal blood flow and motility. Proc Natl Acad Sci USA 1982; 79:4471-4475.

Lundberg JM, Terenius L, Hokfelt T, Martling CR, Tatemoto K, Mutt V, Polak J, Bloom S, Goldstein M. Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. Acta Physiol Scand 1982; 116:477-480.

Lundberg JM, Anggard A, Theodorsson-Norheim E, Pernow J. Guanethidine sensitive release of NPY-like immunoreactivity in cat spleen by sympathetic nerve stimulation. Neuroscience 1984; 52:175-180.

Lundberg JM, Anggard A, Pernow J, Hokfelt T. Neuropeptide Y-, substance P- and VIP-immunoreactive nerves in cat spleen in relation to autonomic vascular and volume control. Cell Tissue Res 1985; 239:9-18.

Lundberg JM, Hemsen A, Larsson O, Rudehill A, Saria A, Fredholm BB. Neuropeptide Y receptor in pig spleen: binding characteristics, reduction of cyclic AMP formation and calcium antagonist inhibition of vasoconstriction. Euro J Pharmacol 1988; 145:21-29.

MacFadyen RJ, Allen JM, Bloom SR. NPY stimulates net absorption across rat intestinal mucosa in vivo. Neuropeptides 1986; 7:219-227.

Macfarlane A, Kinsman R, Read NW, Bloom SR. The ileal brake: ileal fat slows dowm small bowel transit and gastric emptying in man. Gut 1983; 24:A471-472.

Makhlouf GM. Role of VIP in the function of the gut. In: Vasoactive Intestinal Peptide, edited by SI Said , New York:Raven Press, 1982, pp 425-446.

Makhlouf GM. Isolated smooth muscle cells of the gut. In Johnson LR (Ed) Physiology of the Gastrointestinal Tract Vol 1. New York: Raven Press, 1987, pp 568.

Makhlouf GM. (Ed) Neural and endocrine biology of the gut. Vol II Handbook of Physiology: Section 6 The Gastrointestinal Tract, Oxford University Press, New York, 1989.

Makhlouf GM. Neural and hormonal regulation of function of the gut. Hospital Medicine 1990; 25:79-98.

Mannon PJ, Hernandez EJ, Vigna SR, Taylor IL. Localization of specific binding sites for peptide YY in the rabbit gastrointestinal tract. Gastroenterology 1991; 100:A654.

McMurrich JP. Leonardo da Vinci. The Anatomist (1452-1519). Baltimore. The William and Wilkins Company, 1930.

Mitznegg P, Bloom SR, Christofides N, Besterman H, Domschke W, Domschke S, Wunsch E, Demling L. Release of motilin in man. Scand J Gastroenterol 1976; 11 (Suppl 39):53-56.

Miyachi Y, Jitsuishi W, Miyoshi A, Fujita S, Mizuchi A, Tatemoto K. The distribution of polypeptide YY-like immunoreactivity in rat tissues. Endocrinology 1986; 118:2163-2167.

Morgagni JB. (Alexander B, trans). The Seats and Causes of Diseases. London, A. Miller, 1769.

Morgan K, Schmalz P, Go V, Szurszewski J. Effects of pentagastrin G17 and G34 on the electrical and mechanical activities of canine antral smooth muscle. Gastroenterol 1978; 75:405-412.

Motulsky HJ, Michel MC. Neuropeptide Y mobilizes intracellular Ca^{2+} and inhibits adenylate cyclase in human erythroleukemia cells. Am J Physiol 1988; 255:E880-885.

Mozwecz H, Pavel D, Pitrak D, Orellana P, Schlesinger PK, Layden TJ. Erythromycin stearate as prokinetic agent in postvagotomy gastroparesis. Dig Dis Sci 1990; 35(7):902-905.

Mutt V, Jorpes J. Structure of porcine cholecystokinin-pancreozymin. Eur J Biochem 1968; 6:156-162.

Mukhopadhyay A, Thor P, Copeland E, Johnson L, Weisbrodt N. Effect of cholecystokinin on myoelectric activity of small bowel of the dog. Am J Physiol 1977; 232(1):E44-E47.

Nata K, Yonekura H, Yamamoto H, Okamoto H. Identification of a novel 65 k-Da cell surface receptor common to pancreatic polypeptide, neuropeptide Y and peptide YY. Biochem Biophy Res Comm 1990; 171:330-335.

Neal DE, Williams NS, Barker MCJ, King RFGJ. The effect of resection of the distal ileum on gastric emptying, small bowel transit and absorption after proctocolectomy. Br J Surg 1984; 71:666-670.

Nilsson O, Bilchik AJ, Goldenring JR, Ballantyne GH, Adrian TE, Modlin IM. Distribution and immunocytochemical colocalization of peptide YY and enteroglucagon in endocrine cells of the rabbit colon. Endocrinology 1991; 129:139-148.

Nowycky MC, Fox AP and Tsien RW. Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature 1985; 316:440-443.

Noyes B, Mevarech M, Stein R, Agarwal K. Detection and partial sequence analysis of gastrin mRNA by using an oligodeoxynucleotide probe. Proc Natl Acad Sci USA 1979, 76:1770-1774.

Nuland SB. Doctors. The Biography of Medicine. New York, Vintage Books, 1988.

Nurko S, Rattan S. Role of neuropeptide Y in opossum internal anal sphincter. Am J Physiol 1990; 258:G59-G64.

O'Donohue TL, Cornwall BM, Pruss RM, Mezey E, Kiss J, Eiden LE, Massari VL, Tessel RE, Picket VM, DiMaggio DA, Hotchkiss AJ, Crowley WR, Zukowska-Grojec. Neuropeptide Y and peptide YY neuronal and endocrine systems. Peptides 1985; 6:755-768.

Ohkawa H, Prosser CL. Electrical activity in myenetric and submucosal plexuses of cat intestine. Am J Physiol 1972; 222:1412-1419.

Ohkawa H, Prosser CL. Functions of neurons in enteric plexuses of cat intestine. Am J Physiol 1972; 222:1420-1426.

Osler W. The Evolution of Modern Medicine. New Haven, Yale University Press, 1921.

Otterson MF, Sarna SK. Gastrointestinal motor effects of erythromycin. Am J Physiol 1990; 259(3 Pt 1):G355-363.

Pachter HM. Magic into Science. The story of Paracelsus. New York, Henry Schuman, 1951.

Pappas TN, Debas HT, Goto Y, Taylor IL. Peptide YY inhibits meal-stimulated pancreatic and gastric secretion. Am J Phys 1985; 248:G118-G123.

Pappas TN, Debas HT, Taylor IL. Enterogastrone-like effect of peptide YY is vagally mediated in the dog. J Clin Invest 1986; 77:49-53.

Paton WDM, Zar MA. Th origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. J Physiol (Lond) 1968; 194:13-33.

Pawlik WW, Gustaw P, Sendur R, Czarnobilski K, Domschke W, Konturek SJ. Effects of peptide YY (PYY) on pancreatic blood flow and exocrine secretion in the dog. Gastroent 1986; 90:1580.

Pearse AGE. The cellular origin of motilin in the gastrointestinal tract. Scand J Gastroenterol 1976; 11(Suppl 39):35-38.

Peeters T, Matthijs G, Depoortere I, Cachet T, Hoogmartens J and Vantrappen G. Erythromycin is a motilin agonist. Am J Physiol 1989; 257:G470-474.

Pernow J, Svenberg T, Lundberg JM. Actions of calcium antagonists on pre- and post-junctional effects of neuropeptide Y on human peripheral blood vessels in vitro. Eur J Pharmacol 1987; 136:207-218.

Playford RJ, Domin J, Beacham J, Parmar KB, Tatemoto K, Couvineau A, Servin A, Amiranoff B. Interaction of peptide YY in defence against diarrhoea. Lancet 1990; 335:1555-1557.

Poitras P, Reeve J, Jr, Hunkapiller M, Hood L, Walsh J. Purification and characterization of canine intestinal motilin. Reg Peptides 1983; 5:197-208.

Polak JM, Heitz P, Pearse AGE. Differential localization of substance P and motilin. Scand J Gastroenterol 1976; 11(Suppl 39):39-42.

Reeve J, Jr, Eysselein V, Walsh J, Sankaran J, Deveney C, Tourtellotte W, Miller C and Shively J. Isolation and characterization of biologically active and inactive cholecystokinin-octapeptides from human brain. Peptides 1984; 5:959-966.

Reeve J, Jr, Ho F, Walsh J, Ben-Avram C, Shively J. Rapid high-yield purification of canine intestinal motilin and its complete sequence determination. J Chromatog 1985; 321:421-432.

Rodbell M. The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature (London) 1980; 284:17-22.

Roddy DR, Koch TR, Reilly WM, Carney JA, Go VLW. Identification and distribution of immunoreactive peptide YY

in the human, canine, and murine gastrointestinal tracts: species-related antibody recognition differences. Reg Pep 1987; 18:201-212.

Roman C, Gonella J. Extrinsic control of digestive tract motility. In: Physiology of the gastrointestinal tract. Ed: Johnson LR. Raven Press, New York, 1987.

Sabatino, FD, Collins P, McDonald JK. Neuropeptide-Y stimulation of luteinizing-releasing hormone secretion from the median eminence in vitro by estrogen-dependent and extracellular Ca++ independent mechanisms. Endocrinology 1989; 124:2089-2098.

Sagor GR, Bryant MG, Ghatei MA, Kirk RM, Bloom SR. Release of vasoactive intestinal peptide in the dumping syndrome. Br Med J 1981; 282:507-510.

Saria A, Beubler E. Neuropeptide Y (NPY) and peptide Y (PYY) inhibit prostaglandin E2-induced intestinal fluid and electrolyte secretion in the rat jejunum in vivo. Eur J Pharmacol 1985; 119:47-52.

Sarna SK, Soergel KH, Harig JM, Loo FD, Wood CM, Donahue KM, Ryan RP, Arndorfer RC. Spatial and temporal patterns of human jejunal contractions. Am. J. Physiol 1989; 257(3 Pt 1):G423-432.

Sasek CA, Elde RP. Distribution of neuropeptide Y-like immunoreactivity and its relationship to FMRF-amide-like immunoreactivity in the sixth lumbar and first sacral spinal cord segments of the rat. J Neurosci 1985; 5:1729-1739.

Savage AP, Gornacz GE, Adrian TE, Ghatei MA, Goodlad RA, Wright NA, Bloom SR. Is raised plasma peptide YY after intestinal resection in the rat responsible for the trophic response? Gut 1985; 26:1353-1358.

Savage AP, Adrian TE, Carolan G, Chatterjee VK, Bloom SR. Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers. Gut 1987; 28:166-170.

Scheid CR, Honeyman TW and Fay FS. Mechanism of beta-adrenergic relaxation of smooth muscle. Nature 1979; 277:32-36.

Schoups AA, Saxena VK, Tombeur K, DePotter WP. Facilitation of the release of noradrenaline and neuropeptide Y by the alpha-2-adrenorecptor blocking agents idazoxan and hydergine in the dog spleen. Life Science 1988; 42:517-523.

Seamon KB, Padgett W and Daly JW. Forskolin: unique diterpene activator of adenylate cyclase in membranes and in intact cells. Proc Natl Acad Sci (USA) 1981; 78:3363-3367.

Servetus M (JF Fulton, trans.). Christianismi Restitutio and Other Writings. Birmingham, The Classics of Medicine Library, 1989.

Servin AL, Rouyer-Fessard C, Balasubramaniam A, Pierre SP, Laburthe M. Peptide-YY and neuropeptide-Y inhibit vasoactive intestinal peptide-stimulated adenosine 3',5'-monophosphate production in rat small intestine: structural requirements of peptides for interacting with peptide-YY-preferring receptors. Endocrinology 1989; 124:692-700.

Shangold GA, Miller RJ. Two different G-proteins mediate neuropeptide Y and bradykinin-stimulated phospholipid breakdown in cultured rat sensory neurons. J Biol Chemistry 1989; 264:7317-7327.

Sheikh SP, Holst JJ, Shak-Nielson T, Knigge U, Warberg J, Theodorsson-Norheim E, Hokfelt T, Lundberg JM, Schwartz TW. Release of NPY in pig pancreas: dual parasympathetic and sympathetic regulation. Am J Physiol 1988; 255:G46-G54.

Sheikh SP, Hakanson R, Schwartz TW. Y1 and Y2 receptors for neuropeptide Y. FEBS Lett 1989; 245:209-214.

Sheikh SP, Sheikh MI, Schwartz TW. Y2-type receptors for peptide YY on renal proximal tubular cells in the rabbit. Am J Physiol 1989; 257:F978-984.

Sheikh SP, Williams JA. Structural characterization of Y1 and Y2 receptors for neuropeptide and peptide YY by affinity cross-linking. J. Biologi Chem 1990; 265:8304-8310.

Sheppard MN, Christofides ND, Allen JM, Tatemoto K, Bloom SR, Polak JM. Two newly discovered active peptides, PHI and NPY, are found in the mammalian lung. J Pathol 1983; 141:522-533.

Sheriff S, Rigel DF, Fischer JE, Balasubramaniam A. Interaction of ^{125}I-Neuropeptide Y with rat cardiac membranes. Neuropeptides 1990; 15:157-160.

Shimizu F, Imagawa K, Mihara S, Yanaihara N. Synthesis of porcine motilin by fragment condensation using three protected peptide fragments. Bull Chem Sco Jpn 1976; 49:3594-3596.

Singer C. From Magic to Science. Essays on the Scientific Twilight. London, Ernest Benn Ltd, 1928.

Smith PH, Davis BJ, Seino Y, Yanaihara N. Localization of motilin-containing cells in the intestinal tract of mammals: A further comparison using region-specific motilin antisera. Gen Comp Endocrinol 1981; 44:288-291.

Spiller RC, Trotman IF, Higgins BE, Ghatei MA, Grimble GK, Lee YC, Bloom SR, Misiewicz JJ, Silk DBA. The ileal brake-inhibition of jejunal motility after ileal fat perfusion in man. Gut 1984; 25:365-374.

Strunz U, Domschke W, Mitznegg P, Domschke S, Schubert E, Wunsch E, Jaeger E, Demling L. Analysis of the motor effects of 13-norleucine motilin on the rabbit, guinea pig, rt, and human alimentary tract in vitro. Gastroenterol 1975; 68:1485-1491.

Stewart J, Burks T. Actions of cholecystokinin octapeptide on smooth muscle of isolated dog intestine. Am J Physiol 1977; 232:E306-E310.

Summers RW, Anuras S and Greene J. Jejunal manometery patterns in health, partial intestinal obstruction and pseudo-obstruction. Gastroenterol 1983; 85:1290-1300.

Sundler F, Moghimzadeh E, Hakanson R, Ekelund M, Emson P. Nerve fibers in the gut and pancreas of the rat displaying neuropeptide-Y immunoreactivity. Cell Tissue Res 1983; 230:487-493.

Suzuki T, Nakaya M, Itoh Z, Tatemoto K, Mutt V. Inhibition of interdigestive contractile activity in the stomach by peptide YY in Heidenhain pouch dogs. Gastroent 1983; 85:114-121.

Sydenham T. (B Rush, trans.) The works of Thomas Sydenham, M.D. Philadelphia. Benjamin & Thomas Kite. 1809.

Sydenham T. (RG Latham, trans.) The works of Thomas Sydenham. London. The Sydenham Society, 1847.

Szecowka J, Tatemoto K, Rajamäki G, Efendic S, Effects of PYY and PP on endocrine pancreas. Acta Physiol Scand 1983; 119:123-126.

Szurszewski J. Mechanism of action of pentagastrin and acetylcholine on the longitudinal muscle of the canine antrum. J Physiol (London) 1975; 252:335-361.

Szurszewski JH, Weems WW. Control of gastrointestinal motility by prevertebral ganglia. In: Physiology of Smooth Muscle, edited by E. Bulbring and M. F. Shuba, Raven Press, New York, 313-320, 1976.

Tatemoto K, Mutt V. Isolation of two novel candidate hormones using a chemical method for finding naturally occuring polypeptides. Nature 1980; 285:417-418.

Tatemoto K, Carlquist M, Mutt V. Neuropeptide Y -- a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 1982; 296:659-660.

Tatemoto K. Isolation and characterization of peptide YY (PYY), a candidate gut hormone that inhibits pancreatic exocrine function. Proc Natl Acad Sci (USA) 1982; 79:2514-2518.

Taylor IL. Distribution and release of peptide YY in dog measured by specific radioimmunoassay. Gastroenterol 1985; 88: 731-737.

Thastrup O, Dawson AP, Scharff O, et al. Thapsigargin, a novel molecular probe for studying intracellular calcium release and storage. Agents Actions 1989; 27:17-23.

Thompson JC. Marx M. Gastrointestinal Hormones. In: Current Problems in Surgery 1984; 21:1-80.

Tsien RW, Hess P and Nilius B. Cardiac calcium channels at the level of single channels. Experimentia 1987; 43:1169-1172.

Valet P, Berlan M, Beauville M, Crampes F, Montastruc JL, Lafontan M. Neuropeptide Y and peptide YY inhibit lipolysis in human and dog fat cells through a pertussis toxin-sensitive G protein. J Clin Invest 1990; 85:291-295.

Vantrappen G, Janssens J, Hellemans J. Motilin and the interdigestive migrating motor complex in man. Dig Dis Sci 1979; 24:497-500.

Vantrappen GR, Janssens JP. Small bowel motility. In Bockus Gastroenterology, Ed. Berk JE. Saunders Company, 1985, pp 1493-1503.

Varndell IM, Polak JM, Allen JM, Terenghi G, Bloom SR. Neuropeptide tyrosine (NPY) immunoreactivity in norepinephrine-containing cells and nerves of the mammalian adrenal gland. Endocrinology 1984; 114:1460-1462.

Vassallo M, Camilleri M, Phillips SF, Brown ML, Chapman NJ, Thomforde GM. Transit through the proximal colon influences stool weight in irritable bowel syndome. Gastroenterol 1992; 102:102-108.

Verduc JB. (Davis J, trans). A Treatise of the Parts of a Humane Body with their Respective Uses. London, William Turner, 1704.

Vizi E, Bertaccini G, Impicciatore M, Mantovani P, Zseli J, Knoll J. Structure-activity relationship of some analogues of gastrin and cholecystokinin on intestinal smooth muscle of the guinea pig. Naun van Schmiedeberg's Arch Pharmacol 1974; 284:233-243.

Voisin T, Rouyer-Fessard C, Laburthe M. Distribution of a common peptide YY-neuropeptide Y receptor along rat intestinal villus-crypt axis. Am J Physiol 1990; 258:G753-759.

Vukasin AP. Peptide YY and enteroglucagon release in the isolated perfused rabbit colon and following colonic resection in rats. Yale Medical School Thesis 1989.

Wahlenstedt C, Yanaihara N, Hakanson R. Evidence for different pre- and post-juntional receptors for neuropeptide Y and related peptides. Reg Pept 1986; 12:307-318.

Wahlestedt C, Grundemar L, Hakanson R, Heilig M, Shen G.H, Zukowa-Grojec Z, Reis D. Neuropeptide Y receptor subtypes, Y1 and Y2. Ann. N.Y. Acad. Sci. 611:7-26, 1990.

Wahlenstedt C, Regunathan S, Reis DJ. Identification of cultured cells selectively expressing Y1-, Y2-, or Y3-type receptors foe neuropeptide Y/peptide YY. Life Sciences 1992; 50(4):PL7-PL12.

Walsh JH. Gastrointestinal hormones. In: Physiology of the Gastrointestinal tract. Ed: Johnson LR. Raven Press, New York, 1987.

Wattchow DA, Furness JB, Costa M. Distribution and coexistence of peptides in nerve fibers of the external muscle of the human gastrointestinal tract. Gastroenterol 1988; 95:32-41.

Weisbrodt NW. Motility of the small intestine. In: Physiology of the Gastrointestinal tract. Chapter 20. Johnson LR. Vol 2, Raven Press, New York, 1987.

Westlind-Danielsson A, Abens S, Bartfai T. Neuropeptide Y and peptide YY inhibit adenylate cyclase activity in rat striatum. Acta Physiol Scand 1988; 132:425-430.

Wiborg O, Berglund L, Boel E, Norris F, Norris K, Rehfeld J, Marcher K, Vuust J. Structure of a human gastrin gene. Proc Natl Acad Sci (USA) 1984; 81:1067-1069.

Wiley J, Owyang C. Neuropeptide Y inhibits cholinergic transmission in the isolated guinea pig colon: Mediation through alpha-adrenergic receptors. Proc Natl Acad Sci (USA) 1987; 84:2047-2051.

Wiley JW, Gross RA, Lu Y, MacDonald RL. Neuropeptide Y reduces calcium current and inhibits acetylcholine release in nodose neurons via a pertussis toxin-sensitive mechanism. J Neurophysiology 1990; 63:1499-1507.

Wiley JW, Lu Y, Owyang C. Mechanism of action of peptide YY to inhibit gastric motility. Gastroenterol 1991; 100:865-872.

Willenbucher RF, Xie YN, Eysselein VE, Snape WJ, Jr. Mechanisms of cAMP-mediated relaxation of distal circular muscle in rabbit colon. Am J Physiol 1992; 262:G159-164.

Wingate D, Ruppin H, Thompson H, Green W, Domschke W, Wunsch E, Demling L, Ritchie H. 13-Norleucine motilin versus pentagastrin: Contrasting and competitive effects on gastrointestinal myoelectrical activity in the conscious dog. Acta Hepatogastroenterol (Stuttg) 1975; 22:409-410.

Wingate DL, Ruppin H, Green WER, Thompson HH, Domschke W, Wunsch E, Demling LHD. Motilin-induced electrical activity in the canine gastrointestinal tract. Scand J Gastroenterol 1976; 11(Suppl) 39:111-118.

Wingate DL. The Small Intestine. In: A Guide to Gastrointestinal Motility. Ed. Christien JC, Wingate DL. Wright.PSG, 1983, pp 128-156.

Wood JD. Excitation of intestinal muscle by atropine, tetrodotoxin and xylocaine. Am J Physiol 1972; 222:118-125.

Wood JD. Neurophysiology of Auerbach's plexus and control of intestinal motility. Physiol Rev 1975; 55:307-324.

Wood JD. Neurophysiology of the enteric nervous system. In: Integrative Functions of the Autonomic Nervous System, edited by CMC Brooks, pp 177-193, University of Tokyo Press, Tokyo, 1979.

Wunsch E. Synthesis of motilin analogues. Scand J. Gastroenterol 1976; 39 (Suppl. 11):19-24.

Yabu H, Yoshino M, Someya T and Totuska M. Two types of Ca channels in mammalian intestinal smooth muscle. Prog Clin Biol Res 1989; 315:469.

Yoo O, Powell C, Agarwal K. Molecular cloning and nucleotide sequence of full-length cDNA coding for porcine gastrin. Proc Natl Acad Sci USA 1982; 79:1049-1053.

Yoshino M, Someya T, Nishio A, Yazawa K, Usuki T and Yabu H. Multiple types of voltage-dependent Ca channels in mammalian intestinal smooth muscle cells. Pflugers Archiv Eur J Physiol 1989; 414(4):401-409.

Youmans WB. Do adrenergic nerves innervate smooth muscle in the intestinal wall? Gastroenterol 1972; 62:1278-1279.

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