THE ENTERIC NERVOUS SYSTEM IN THE RUMINANT STOMACH OF THE SHEEP (OVIS ARIES)

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PROEFSCHRIFT

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IN GRATEFUL DEDICATION TO

MY PARENTS

MY WIFE AND OUR CHILDREN

LIST OF ABBREVATIONS

A.	Arteria
ABO	Abomasum
AC	Adenylate Cyclase
ACh	Acetylcholine
AChE	Acetylcholinesterase
ADP	Adenosine Diphosphate
AH	After Hyperpolarization
AMP	Adenosine Monophosphate
ANS	Autonomic Nervous System
AP	Antrum Pyloricum
APP	Avian Pancreatic Polypeptide
APUD	Amine Precursor Uptake Decarboxylation
ATP	Adenosine-5'-Triphosphate
c-AMP	cyclic-Adenosine Monophosphate
CMC	Carboxy-Methyl-Cellulose
C-R	Crown-Rump
CA	Catecholamine(s)
Ca	Canis familiaris
CCK	Cholecystokinin
CGRP	Calcitonin Gene Related Peptide
ChAT	Choline Acetyltransferase
CNS	Central Nervous System
CoA	Coenzyme A
CSF	Cerebro-Spinal Fluid
DAB	3,3'-Diaminobenzidine tetrahydrochloride
DAG	Diacylglycerol
DBH	Dopamine-Beta-Hydroxilase
DNES	Diffuse Neuro-Endocrine System
DYN	Dynorphin
EC	Enterochromaffin
EDTA	Ethylenediamine Tetraacetic Acid
EEC	Entero-Endocrine Cell(s)
ENK	Enkephalin(s)
ENS	Enteric Nervous System
EPSP	Excitatory Post Synaptic Potential
Eq	Equus caballus (horse)
FAM	Fast-Adapting Mechanoreceptor(s)
FIF	Formaldehyde Induced Fluorescence
FITC	Fluorescein Isothiocyanate
FUN	Fundus
G.I.	Gastro-Intestinal
GABA	Gamma Amino Butyric Acid
ggl.	ganglion
GIP	Gastric Inhibitory Peptide
GRP	Gastrin Releasing Peptide
HPLC	High Pressure Liquid Chromatography
5-HT	5-Hydroxytriptamine or serotonin
HRP	Horseradish Peroxydase
IA	Intra-arterial
IgG	Immunoglobuline G
IP_3	Inositol triphosphate
IR	Immunoreactive, -reactivity
IV	Intravenous
iso-OMPA	iso-Octamethyl Pyrophosphoramide
LDH	Lactic Acid Dehydrogenase
LES	Lower Esophageal Sphincter
MAO	Monoamine Oxydase
mm	millimeter
MMC	Migrating Myoelectrical Complex
mV	millivolt (s)
NA	Noradrenaline
NANC	Non-Adrenergic, Non-Cholinergic
NGF	Nerve Growth Factor
NGS	Normal Goat Serum
nm	nanometer
NNE	Non-Neuronal Enolase
NPY	Neuropeptide Y
NSE	Neuron Specific Enolase
OMA	Omasum
ORO	Ostium Reticulo-Omasicum
pA	protein A
PAK	Pyruvic Acid Kinase
PAP	Peroxidase Anti-Peroxydase
PBS	Phosphate Buffer Saline
PHI	Peptide H I
PNS	Peripheral Nervous System
PYL	Pylorus
PYY	Peptide Y Y
RDS	Ruminal Dorsal Sac
RET	Reticulum
RG	Reticular Groove
RIA	Radioimmunoassay
RITC	Rhodamin B Isothiocyanate
RVS	Ruminal Ventral Sac
SAM	Slowly-Adapting Mechanoreceptor(s)
SN	Sodium Nitroprusside
SOM	Somatostatin
Su	Sus scrofa domestica (pig)
Sub. P	Substance P
TBS	Tris Buffer Saline
TTBS	Triton Tris Buffer Saline
TTEN	Tonic-Type Enteric Neuron
µg	microgram
µm	micrometer
V.	Vena
VFA	Volatile Fatty Acid(s)
VIP	Vasoactive Intestinal Polypeptide
WDHA	Watery Diarrhoea Hypokalemia Achloremia

PART I
THE ENTERIC NERVOUS SYSTEM (ENS)

TABLE OF CONTENTS

Chapter 1. SETTING OF THE STUDY

I. 1. 1. FORMULATION OF THE PROBLEM

Quantitatively and economically the ruminants represent, undoubtedly, the most important group of large domestic animals. Their enormous economical impact (over the 13 x 10^{12} Bfr/year) is already clearly demonstrated by a schedule simply based upon two aspects: milk and meat (see Addendum/Part I/ table 1). However, the real economical importance of the domestic ruminants encompasses much more since many other aspects, not mentioned in this discussion, play also a role e.g. animal food, textile, leather and the pharmaceutical industry; veterinary research; and the production of manure, power etc. Furthermore, one has to realize that hundreds of thousands of people depend entirely or to a very high degree for their subsistence on this group of animals. Consequently, diseases in ruminants not only implicate considerable economical losses but, even worse, they would threaten the existence of entire communities. So, it needs no arguing why in veterinary medicine ruminants are still a very important, probably the most important, group of domestic animals and why the ruminant has been -and still is- a major topic for physiologists, pharmacologists, clinicians and pathologists.

Ruminants subsist mainly on those structural parts of the plants, stem and leaves, that contain appreciable amounts of beta-linked glucose polymers, cellulose and other polysaccharides, incorporated into the cell wall. No multicellular animal is known to manufacture enzymes (e.g. cellulase) that can successfully break down these plant components. In consequence, ruminants are in fact deprived not only of the nutrient value of the cellulose itself, but also of the digestible cell contents bounded by the cellulose wall (16; 391). Moreover, no mammalian enzymes are secreted by the forestomach. Hence, all forestomach digestion (carbohydrate, protein, fat) is attributable to the action of microbial enzymes and to the microbial transformation of the ingested substrata. The microorganisms digest cellulose and other carbohydrates, ferment the end products to volatile fatty acids (VFA) and convert nitrogenous substances to ammonia and protein. Additionally, essential amino acids and water-soluble B vitamins are synthesized by them. Hence, microbial digestion is quite different from mammalian enzymatic digestion and supplies energy, protein, essential amino acids and vitamins to the body from sources that mammalian systems cannot utilize (36; see 36). So, it is clear that in herbivores digestion not only depends on the motor and secretory functions of the digestive tube but, to a large extent, on the activity of the microflora as well. In consequence, different circumstances e.g. failure to provide the correct diet, prolonged starvation or loss of appetite, hyperacidity (engorgement of grain) etc, that modify the activity of the flora lead to an abnormal or even a cessation of the digestion (81; 528; see 528).

As a result of such a digestion-strategy any animal, subsisting on a fibrous diet, must provide its digestive tract with a fermentation chambers in which bacteria can break down the fibrous parts of plants (16; 391). Since herbivores largely depend on the products of microbial activity a part of their alimentary tract is expanded to form an organ that harbors a microbial population valuable in the digestion of foods for which the animals themselves do not have the necessary complement of enzymes (16; 527). Therefore, the bowel in herbivores is voluminous, compartmentalized and able to store food extensively and transfer it slowly (38). In ruminants the beginning of the gastrointestinal tract is enlarged to form the major fermentation chamber, the forestomach, which represents approximately 25% of the body weight (38; 391; see 391). Ruminants depend for up to 80% or more for their daily energy requirements on microbial digestion and hence on the normal functioning of their complex stomach. For that purpose the ruminant stomach can perform complex propulsive and non-propulsive motility patterns (mixing and retaining of the ingesta, regurgitation, eructation, and the controlled and orderly transport of the ingesta within the forestomach complex) which are accomplished in complex but coordinated cycles of forestomach motility (36). This facilitates microbial fermentation, the exchange of water and electrolytes, the absorption of VFA, nitrogenous products and water-soluble vitamins (639). Furthermore, the complex

structure together with the multiple functions of the forestomach necessitate an adequate control of its muscle activity. Even minor changes in the normal motility patterns (largely regulated by the enteric nervous system) certainly lead to serious functional disorders and economical losses. So, one of the primary tasks of the enteric nervous system in the ruminant stomach is the initiation, coordination and control of these different motility patterns. Moreover, one has to realize that the different parts of the ruminant stomach are closely associated, both anatomically and functionally, with each other. As a consequence, damage to one part hampers the basic motility patterns of the others leading to a dissociation of the ordered sequencing and direction of electrical activity in the smooth muscle cells (639). As a result, any dysfunction of the enteric nervous system causes interference with the normal movements (mixing contractions, eructation contractions etc.) and induces the classical spectrum of ruminant stomach diseases (81).
In conclusion, it must be emphasized that the ruminant stomach is, morphologically and functionally, the most important and critical part of the gastrointestinal tract in ruminants. Its voluminous and complex structure as well as the critical physiological conditions (time, temperature, pH) required for microbial digestion, predisposes this organ to a broad spectrum of functional disorders (carbohydrate engorgement, acute and chronic ruminal tympany, vagus indigestion, abomasal displacements) resulting every year in serious economical losses.

I. 1. 2. OBJECTIVES OF THE STUDY

Notwithstanding the enormous importance of the pathology of the ruminant stomach in veterinary medicine (and hence in economy) and the fact that adequate functioning of this gastrointestinal segment largely depends upon the integrity of the enteric nervous system, it is rather surprising to ascertain that little or no basic information concerning the intramural innervation of the ruminant stomach is available in the literature. Nevertheless, if we are to have any chance of understanding and treating the abnormalities in the mechanisms underlying the various diseases of the stomach (chronic ruminal tympany, displacement of the abomasum etc.), it is imperative to have detailed information on its innervation.
In an attempt to fill this lacuna in our knowledge a morphological study of the intramural nervous system of the stomach of sheep was undertaken. This study had the following aims:

to demonstrate the intramural neuro-endocrine complex (Part II);

to obtain an overall picture of the intramural innervation of the different compartments of the stomach (Part II);

to study some substances which are known to be actively present in the ENS of other mammalian species;

and to study the effect of these substances on the smooth muscle tone of the different segments of the stomach.

To attain the above-mentioned objectives this study has been planned in three phases.
In the first phase (Part I) some introductory remarks concerning the morphology and functions of the ruminant stomach and the autonomic nervous system will be given. Thereafter, much attention will be paid to the morphology, the neurochemistry, the functional significance and to some clinical aspects of the ENS. The principal data arising from this review will function as the key-stones for the original part i.e. Part II and III of this study.
The aim of the second phase (Part II) is to gain an overall picture of the architecture of the intramural innervation of the ruminant stomach, using an antiserum for neuron specific enolase.
In the third and last phase of this study the results emerging from Part II will be further neurochemically differentiated. Finally, the effect of the immunohistochemically established neurotransmitters/modulators on the muscle tone of the ruminant stomach will be studied.

I. 1. 3. MORPHOLOGY OF THE RUMINANT STOMACH

Due to the great functional importance of the forestomach of the ruminant the development, the macro- and microstructure and, finally, the functioning of this gastrointestinal segment will be considered in the following paragraphs.

Embryology

It has often been presumed that the primordium of the ruminant stomach develops from the esophagus but the literature showed that there is no esophageal contribution to any part of the compartmentalized stomach (see 24). The whole ruminant stomach (i.e. rumen, reticulum, omasum and abomasum) originates from a primordium similar to that of the simple stomach. This primordium is, however, more flattened laterally while the lesser curvature is convex (3; 50; 184; see 321; 682). Early in the development the primordium undergoes an apparent rotation to the left so that its right side becomes dorsal and its left side ventral (24; 321; 682). In addition, the cranial part distends slightly and forms a fundus which becomes separated from the rest of the primordium.

The rumen develops as a dorsal outgrowth of the fundus. It extends at first dorsally, cranially and to the left. Later this ruminal primordium turns caudally and grows back dorsal to the rest of the stomach. Meanwhile the future rumen is subdivided into two sacs: dorsal and ventral sac (24; 50; 321; 682).

The reticulum develops as a left ventral bulge at the origin of the rumen (50; 184; 321; 682).

The development of the omasum occurs in two portions. The base, with the omasal groove, develops from the greater curvature of the "true" stomach. Hence the omasal base is considered to be a constricted portion of the greater curvature hidden between the reticulum and abomasum. The rest of the omasum originates from the original lesser curvature of the true stomach, accounting for the peculiar convexity of the omasal body (24; 50; 321).

The rest of the primordium, corresponding to the lower part of the corpus and the pyloric part of the simple stomach, forms the abomasum (3; 24; 50; 321; 682).

The four compartments of the ruminant stomach have been found to develop about a central axis: the gastric groove (sulcus ventriculi). This groove is a mucosal depression that starts at the esophagus and ends at the orifice between the omasum and abomasum (ostium omaso-abomasicum). Depending on its topography in the ruminant stomach complex the gastric groove is usually divided into three parts. The first part, running from the cardia to the reticulo-omasal orifice, is normally referred to as the "reticular or esophageal" groove and is open along its left side, facing in this way the cavity of the reticulum and rumen. The second part begins at the reticulo-omasal orifice and ends at the ostium omaso-abomasicum. The omasal groove is open along its right side facing the cavity of the omasum. The last part of the gastric groove extends along the lesser curvature of the abomasum and is smooth with no folding of the tunica mucosa (24; 321).

The histogenesis of the mucosal membrane of the ruminant stomach is relatively slow in onset and development. It begins at about one and a half months of gestation in the forestomach (proventriculus) at the borders of the gastric groove. From there it spreads into the forestomach (=proventricular) compartments. The differentiation of the glandular abomasal mucosa starts a few days later. After two months of gestation the mucosal characteristics of the different compartments are clearly visible, though they evolve substantially during the rest of foetal life (50; 321).

The development and relative size of the different compartments of the ruminant stomach is irregular and change remarkably during the foetal and neonatal periods.

In the foetus the rumen represents the largest compartment and it is only in the last month of gestation that the true stomach, the abomasum, rivals the rumen in such a way that at birth the abomasum takes up more than 50% of the whole ruminant stomach complex and even has reached about 60% of the adult organ. In comparison the other compartments are small and somewhat collapsed. Changes in the diet induce remarkable changes in the volume of the different subdivisions (the rumen and reticulum in particular) and accelerate the differentiation of the wall (184; 321). At birth the wall of the different subdivisions of the forestomach seems relatively "underdeveloped" but, due to the functioning of the organ, the tunica muscularis increases in length and thickness. Meanwhile the epithelium, even though the characteristic form can still be recognized, proliferates resulting in the formation of numerous mucosal protrusions (reticular folds, ruminal papillae, omasal leaves). Fermentation of the food produces large quantities of VFA and it has been shown that butyric acid strongly stimulates the mitosis. So, this fermentation, at least in part, is responsible for the considerable proliferation of the ruminal epithelium (36; 184).

However, based upon this widely accepted picture of the development of the ruminant stomach it remains difficult, if not impossible, to explain why all the forestomach compartments, if they indeed evolve from a primordium similar to that of the simple stomach, are lined with an *aglandular, multilayered, keratinized* epithelium. Some investigators therefore claimed that the forestomach did not originate from the gastric pri-

mordium but, most probably, from a part of the primitive foregut just in front of the primordium (P. Krediet, personal communication) (see Addendum/ Part I/ fig. 1). Indeed, due to the rise of the cephalic fold, the tubular foregut becomes isolated from the yolk sac, though it remains fixed to the buccopharyngeal membrane. Dorsal to the septum transversum the foregut curves in a ventral direction and continues as the descending part of the primitive gut. The outside bend of the curve grows faster than the inside bend, leading to the formation of a stomach-like dilatation. Moreover, in this region the foregut becomes sandwiched between the left and right parts of the coelom cavity. This means that the primitive gut is fixed to the dorsal body wall by the dorsal mesentery and to the septum transversum by the ventral mesentery.

In ruminants two recesses, dorsal and ventral, develop from a small part of the primitive foregut situated between the septum transversum (providing a part of the future diaphragm) and the cardia (i.e. the beginning of the stomach). The ventral recess (i.e. the future omasum) lies between the stomach (i.e. the future abomasum) and the dorsal outgrowth (i.e. the future reticulo-rumen). Furthermore, in both recesses the primitive foregut continues to exist as a groove situated in the ventral wall of the dorsal recess and the dorsal wall of the ventral recess. This groove forms the future reticular (esophageal) groove.

The dorsal recess has two parts: one directly connected with the primitive foregut i.e. the future reticulum, and a second, larger, part i.e. the future rumen. The latter divides into left and right halves. At the border of both halves is the attachment of the dorsal mesentery. The ventral recess evolves into the future omasum and joins the ventral mesentery.

The further development, by which the embryonic ruminant stomach will gain its adult form and position, is relatively complex. Hence, for didactical reasons, the development of the forestomach and glandular stomach will be considered separately. However, one has to realize that in the embryo itself both processes occur simultaneously.

The "true" stomach (abomasum) of the ruminant, as in other mammals, performs a rotation at 90° around its cranio-caudal axis to the left. So, its original dorsally orientated greater curvature takes now a lateral (left) position.

Meanwhile, the reticulo-rumen grows first dorsally but, due to the lack of space, this growth is further continued caudally and to the left. Due to this caudal outgrowth of the rumen the esophagus is in ruminants bent in a ventral direction so that the reticular groove becomes a vertical's position. Moreover, because of this movement the omasum leaves its original ventral position and, likewise, becomes vertical. The further outgrowth of the omasum is hindered by the septum transversum. Consequently, the omasum turns to the right and induces a twist in the reticular groove of more than 90°. Meanwhile, the rumen rotates, with respect to the reticulum, about 90° around its longitudinal axis to the right. This rotation, together with the substantial caudal outgrowth of the rumen, induce two important changes in the position of the embryonic stomach complex.

First, the abomasum is pushed ventrally and medially by the growing rumen and thereby rotates once again about 90° to the left. Both the above-mentioned rotations (now 180°) of the abomasum as well as the displacement of this segment to the ventro-medial plane, result in the definite position of the glandular stomach. Indeed, the original dorsally situated greater curvature (together with the dorsal mesentery) is now found ventral and to the right of the median plane, while the original right side (anatomically this is now the left side of the "true" stomach) of the abomasum bulges into the omental bursa.

As a consequence of the rotation of the rumen to the right, the original left ruminal sac takes a dorsal position whilst the original right sac takes a ventral position.

All the processes of growth and rotation discussed above are, finally, reflected in the rather complicated distribution of the greater omentum in ruminants (P. Krediet, personal communication). This is clearly illustrated in a transverse section through the abdominal region (see Addendum/Part I/ fig. 2).

Macroscopic Anatomy

It is beyond the scope of this study to discuss in detail the anatomy of the ruminant stomach. For that purpose the reader is referred to the classical text books of veterinary anatomy (3; 50; 321; 682; 685; etc). The basic anatomy of the stomach of the sheep is given in a scheme (see Addendum/Part I/ fig. 3).

The gastric complex in ruminants (cattle, sheep and goat) is large and in the adult occupies about three-quarters of the abdominal cavity, filling the left half (except a small space occupied by the spleen and a part of the small intestine) and extending well into the right half (3; 24; 50; 184; 321; 682). Embryology has dem-

onstrated that the ruminant stomach consists of four compartments i. e. the rumen (RDS & RVS), the reticulum (RET), the omasum (OMA) and the abomasum (ABO). The first three parts comprise the forestomach or proventriculus, while the ABO is homologous to the "true" stomach of monogastric animals. In the following paragraphs the anatomy of these different compartments will be very briefly reviewed.

Compartments

Rumen

In adult cattle, sheep and goats the rumen is the largest compartment of the forestomach (approximately 80% of the total capacity) and occupies the major portion of the abdominal cavity. It extends from the diaphragm to the pelvic inlet filling, almost completely, the left half of the abdominal cavity. Additionally, it extends to the right of the median plane ventrally and caudally. Externally a right and left longitudinal groove (sulcus longitudinalis dexter & sinister) indicate the division of this compartment into a dorsal and ventral sac (saccus ruminis dorsalis & ventralis). The caudal extremity reaches nearly the pelvic inlet and is divided, by means of a deep transverse groove (sulcus caudalis) connecting both longitudinal grooves, into a caudodorsal and caudoventral blind sac (saccus caecus caudodorsalis & caudoventralis). Internally the rumen is further subdivided by longitudinal and transverse infoldings of the wall called pillars (3; 24; 50; 321; 682).
Compared to the ox, the ventral ruminal sac in the sheep is relative larger and extends more to the right of the median plane. In addition the ventral blind sac in the sheep extends further caudally than the dorsal one.

Reticulum

The reticulum is the most cranial compartment. The greater part of it lies on the left of the median plane between the diaphragm and the rumen. From the latter it is internally separated by an U-shaped rumino-reticular pillar (3; 24; 50; 321; 682). Its diaphragmatic surface (facies diaphragmatica) lies against the diaphragm and liver. This topography has a very important clinical significance. Foreign bodies (nails and wires), often swallowed by the ruminant, commonly lodge in the reticulum and may, due to its forceful contractions, perforate the reticular wall. Since the diaphragm is in contact with the pericardium and lungs such perforation by foreign bodies may often cause a traumatic reticulo-pericarditis and/or liver abscesses (24; 50; 184; 321; 682).
In the sheep and goat the reticulum is relatively larger than in the ox (50; 321; 682).

Omasum

The omasum is ellipsoidal in form and its long axis is nearly vertical. It lies chiefly to the right of the median plane and is clearly marked off from the other compartments. The omasal cavity is, for the most part, occupied by longitudinal mucosal folds (laminae omasi) which spring from the dorsal curvature and the sides. In addition, there are second order (shorter), and third and fourth order (still shorter) laminae. Due to this anatomical arrangement the interior of the omasum has been described as having the appearance of the "pages of a book". On the surface of the laminae low, rounded papillae develop.
The omasum of the sheep and goat is much smaller than the reticulum. In the sheep the laminae omasi are, in addition, less numerous (3; 24; 50; 184; 321; 682).

Abomasum

The abomasum is the "true" stomach and thus homologous to the stomach of the other domestic animals (dog, pig and horse). In consequence, the classical subdivisions can be observed (24; 321; 682). Generally speaking, the abomasum is an elongated sac lying chiefly on the abdominal floor to the right of the median plane. A constriction, the angular incisure, divides the stomach into a proximal (fundic) portion that contains fundic glands and a distal (pyloric) part holding pyloric glands. (3; 24; 50; 321; 682). The fundus is in the xiphoid region and is related to the reticulum and the ventral ruminal sac. The body (corpus abomasi), lying more on the left than on the right side of the median plane, extends caudally. The pyloric part (pars pylorica) turns to the right, inclines dorsally and joins the duodenum at the pylorus. However, the form and position of the abomasum is chiefly determined by the contraction and the degree of fullness of the rumen and reticulum to which it is attached.
The abomasum in the sheep and goat is relatively larger and longer than in the ox. In the sheep the body runs

obliquely caudally and to the right. This arrangement is made possible because of the fact that the omasum is small and light and situated on the dorsal surface of the abomasum (3; 24; 50; 184; 321; 682)
As mentioned earlier, the ruminant stomach complex develops about a mucosal channel, the gastric groove, extending from the esophagus to the abomasum.
The first part of the gastric groove, the *reticular groove* (sulcus reticuli), starts at the caudal end of the esophagus, passes in a spiral fashion ventrally in the right wall of the reticulum and terminates at the reticulo-omasal orifice. The groove is formed by right and left lips (labium dextrum & sinistrum), named according to their relation to the esophageal ending, and by a floor (fundus sulci reticuli). The relative position of the lips is reversed at the ventral end so that the reticulo-omasal orifice is overlapped by the right lip.
At the level of the reticulo-omasal orifice there are peculiar horny, curved papillae resembling the claws of a bird. Hence their name "papillae unguiculiformes". They impede the passage of coarse ingesta to the abomasum (3; 24; 50; 184; 321; 682). The smooth muscle, surrounding this ostium, is well developed and is referred to as the reticulo-omasal "sphincter". The sphincter contracts in rumination (24; 36; 50).
The *omasal groove* (sulcus omasi) is the second part of the gastric groove and passes from the reticulum to the abomasum. The space between the groove and the free borders of the laminae omasi represents the omasal canal (canalis omasi) forming a direct passage for ingesta from the rumen and reticulum to the abomasum (3; 50; 321; 682).

Vascularization

(see Addendum/ Part I/ fig.4)

The arterial blood supply of the ruminant stomach is from the coeliac artery (A. celiaca). The origin of the three primary branches of this artery i.e. A. lienalis, A. gastrica sinistra and the A. hepatica is variable and distorted due to the great development of the arteries to the rumen and reticulum. Arteries to these compartments are homologous to the gastric branches of the A. lienalis in monogastric animals (321).
In the sheep the A. ruminalis dextra usually arises in common with the A. lienalis. It is the main and largest artery to the rumen. The vessel descends caudoventrally to the origin of the right longitudinal groove of the rumen, where it is accompanied by a branch of the dorsal vagal trunk. In the right longitudinal groove both (artery and nerve) course caudally and supply large portions of the dorsal and ventral sacs. The A. ruminalis dextra terminates by forming anastomotic connections with the A. ruminalis sinistra.
The A. ruminalis sinistra is likewise a large vessel originating either from the A. gastrica sinistra or (sometimes) from the A. ruminalis dextra. At first it runs ventrally on the visceral (right) surface of the rumen. In the rumino-reticular groove the artery turns to the left (parietal surface of the rumen) and courses in the left longitudinal groove, giving off dorsal and ventral branches. It regularly supplies the reticulum (A. reticularis) and has in addition esophageal branches.
The A. gastrica sinistra appears to be the continuation of the A. coeliaca following the origin of the A. lienalis, the ruminal arteries and the A. hepatica. This artery provides most of the blood supply to the abomasum. It runs ventrally and follows the lesser curvature of the stomach by anastomosing with other branches of the celiac trunk, especially the A. gastrica dextra (3; 50; 321; 682). The A. gastroepiploica sinistra originates from the A. gastrica sinistra, runs ventrally on the visceral surface of the omasum and follows the greater curvature of the abomasum.
The A. gastrica dextra arises from the A. hepatica and follows the lesser curvature of the abomasum, where the vessel anastomoses with the A. gastrica sinistra. The A. gastroepiploica dextra is a terminal branch of the A. gastroduodenalis and follows the greater curvature of the abomasum (3; 24; 50; 321; 682)
In monogastric animals the V. porta carries blood from the stomach, the intestine and the spleen to the liver. In these species the main tributaries of the V. porta are the V. mesenterica cranialis, V. lienalis, and the V. mesenterica caudalis. In ruminants there are, in addition, the V. ruminalis dextra and sinistra, the V. reticularis and the V. gastrica sinistra which run parallel with the corresponding arteries. The V. lienalis joins the V. ruminalis dextra, which in turn unites with the V. ruminalis sinistra to form a major venous tributary. This returns blood from the forestomach and abomasum to the liver by way of the V. porta. (3; 24; 50; 321; 682)

Innervation

(see Addendum/ Part I/ fig.5)

The ruminant stomach receives sympathetic and parasympathetic fibres.

Parasympathetic innervation

The vagal nerves are arranged similarly in ruminants and non-ruminants. Thus, there is a truncus vagalis dorsalis and ventralis (both containing afferent and efferent fibres) passing into the abdomen through the esophageal hiatus. In monogastric animals both vagal trunks run, from the cardia to the pars pylorica, along the lesser curvature of the stomach. The truncus vagalis dorsalis gives off branches to the visceral surface of the stomach, the truncus vagalis ventralis to the parietal surface. Since these areas of the simple stomach, from which the different compartments of the forestomach develop, are normally supplied by the dorsal trunk, this nerve bundle innervates a relatively large portion of the ruminant stomach (24; 321; 682). However, taking into account the principle that the reticulo-rumen originates from a dorsal outgrowth of the primitive foregut, the innervation of these compartments by the dorsal vagal trunk becomes even more clear and logical. Likewise, the nervous supply of the omasum, the ventral outgrowth of the primitive foregut, by the ventral trunk can be explained (P. Krediet, personal communication).
The truncus vagalis dorsalis, after receiving the communicating branch from the ventral trunk, gives off branches to the celiac plexus (via the dorsal mesentery), branches that accompany the right and left ruminal arteries and veins, and several branches to the visceral surface of the reticulum. The continuation of the trunk joins the left gastric artery and runs over the dorsal curvature of the omasum and the visceral side of the lesser curvature of the abomasum to the pars pylorica. It gives branches to both sides of the omasum and to the visceral side of the abomasum
The truncus vagalis ventralis is continued ventrally and to the right. The branches to the diaphragmatic surface of the reticulum are numerous. They also supply the region near the cardia. A long pyloric branch splits off and runs ventrally to the pylorus. The trunk continues close to the parietal surface of the base of the omasum and along the lesser curvature of the abomasum, giving branches to the parietal surface of both segments. A small branch reaches the pylorus and joins the long pyloric branch (3; 24; 50; 184; 321; 682).
The efferent vagal fibres synapse with perikarya in Auerbach's plexus and in the abomasum also with nerve cell bodies in the submucosal plexus (184).

Sympathetic innervation

The sympathetic innervation, which is particularly important in regulating the luminal diameters of arterioles (and to a lesser extent the veins) of the gastrointestinal tract, is chiefly from the greater splanchnic nerves (preganglionic fibres from the fifth and sixth as well as from a variable number of the more caudal segments of the thoracic sympathetic trunk). The greater splanchnic nerve passes caudally in the abdomen and synapses mainly in the celiac and cranial mesenteric ganglia. After synapsing, the resultant postganglionic fibres pass with branches of the celiac trunk and cranial mesenteric artery to all parts of the viscera (24; 321).

Microscopic anatomy

The basic microscopic structure of the ruminant stomach in adult sheep is summarized in a scheme (largely based upon 50; 184; 321; 447; 682; 721) (see Addendum/ Part I/ fig.6).
It consists of the four classical layers i. e. tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa.

Tunica Mucosa

Lamina Epithelialis

All parts of the forestomach are covered by a stratified, squamous, keratinized, aglandular epithelium, which has a characteristic appearance within the different compartments.
The epithelium of the rumen (RDS, RVS) is relatively thin. Nevertheless, numerous flat ruminal papillae of connective tissue covered by epithelium are present. In this way the functional absorptive surface of the rumen is considerably increased (see functional morphology). As a rule the papillae are "underdeveloped" in the RDS.
In the reticulum the mucosa is thrown into primary, secondary and tertiary folds. The primary folds divide the reticular mucosa into typical, small hexagonal compartments: the reticular cells. Within these cells the secondary and tertiary folds are formed. Normally the reticular epithelium compared with that in the rumen, is more highly keratinized. On the omasal side of the ostium reticulo-omasicum horny, claw-like papillae

(papillae unguiculiformes) occur.
Leaf-like primary, secondary tertiary and quaternary folds are characteristic of the OMA. As a rule the largest leaves alternate with the smallest ones in a typical sequence (I-IV; II-IV; III-IV etc.). Additionally, numerous keratinized papillae are present on either side of the leaves.
The abomasal mucosa is arranged in spiral folds (plicae spirales). The epithelium consists of tall columnar cells while the microarchitecture of the cardiac, fundic and pyloric zones corresponds to the classical picture.
At the level of the pylorus the torus pyloricus, a mushroom-like protrusion formed by a thickening of the submucosa and the circular muscle layer, bulges into the lumen.

Lamina Propria

Throughout the ruminant stomach the lamina propria is a dense connective tissue layer containing a capillary network and numerous elastic fibres. It normally forms the central axis of the different mucosal protrusions i.e. ruminal papillae, reticular folds, omasal leaves, plicae spirales abomasi.
In the rumen a band of condensed connective tissue demarcates the lamina propria from the tunica submucosa.
In the reticular folds and omasal leaves the lamina propria usually contains large lymphatic vessels.
The lamina propria of the abomasum is a loose connective tissue layer in which there are many elastic fibres.

Lamina muscularis mucosae

The development of this, rather thin, smooth muscle layer differs in the various regions of the ruminant stomach.
In the reticular groove there is no "true" lamina muscularis mucosae although the layer may be represented by a few scattered smooth muscle bundles.
In the rumen this muscle layer is normally absent, apart from occasional, single smooth muscle cells.
In the reticulum the lamina muscularis mucosae, contains numerous elastic fibres, and is usually observed in the apical portion of the primary and secondary folds.
In the omasum the muscularis mucosae is clearly developed and occurs as an uninterrupted layer extending within the leaves. At the top of the omasal laminae the muscularis mucosae fuses, except the smallest, with an extension of the circular muscle layer. Consequently, in a transverse section of most omasal leaves three smooth muscle layers can be observed i.e. the extension of the circular muscle layer centrally and on either side the lamina muscularis mucosae.
In general three muscle layers, perpendicular to each other, make up the muscularis mucosae in the abomasum. The inner and outer are circular whilst the middle is longitudinal.

Tunica Submucosa

The tunica submucosa is made up of a loose connective tissue. In those segments where the muscularis mucosae is absent or fragmentary (i.e. OG, RET, RDS and RVS) the submucosa can not be clearly separated from the lamina propria. As a rule all the other classical submucosal elements i. e. blood and lymphatic vessels as well as a submucosal nerve plexus have been described.
In the sheep simple branched tubulo-acinar mucous or mixed submucosal glands may be present at the level of the esophageal groove.
In the rumen a condensation of the lamina propria marks the border between the tunica mucosa and the submucosa. The ruminal submucosa is relatively thin.
Finally, in the reticulum this layer contains a considerable quantity of elastic fibres.

Tunica Muscularis

Taken as a whole, the microarchitecture of the tunica muscularis of the ruminant stomach agrees with the classical picture i.e. an inner circular and an outer longitudinal smooth muscle layer separated from each other by a thin connective tissue band and an extensive nervous network, the myenteric (Auerbach's) plexus. This plexus is distinctly developed in the groove between the reticulum and the rumen and in the wall of the OMA. Consequently, it has been assumed that the motility of the ruminant stomach is regulated from this region.
Some particularities are noteworthy.
A well developed longitudinal muscle band forms the inner muscle layer in the lips of the reticular groove. This layer continues in the reticulum as the internal circular muscle layer. The floor of the groove has similarly two muscle layers. The inner muscle layer is an extension of the longitudinal muscle layer of the

reticulum, while the outer muscle layer is formed by the prolongation of the outer (longitudinal) muscle layer of the esophagus. Hence, a few bundles of striated muscle fibres can be observed.
In the omasum small muscle bundles split off from the innermost part of the circular muscle layer and extend into the center of the leaves (except in the smallest ones), where they are sandwiched between the two layers of the lamina muscularis mucosae. At the free edge of the leaves these muscle layers fuse. The longitudinal muscle layer of the omasum is remarkably thin.
In the abomasum the tunica muscularis is often arranged in three layers i. e. inner oblique, middle circular and outer longitudinal layers.

Tunica Serosa

In all parts of the ruminant stomach the tunica serosa consists of a mesothelium (lamina epithelialis serosae) and a lamina subserosae. The latter is a loose connective tissue layer sometimes rich in adipose tissue, as in the rumen, and in blood vessels and nervous tissue.

I. 1. 4. FUNCTIONAL MORPHOLOGY OF THE RUMINANT STOMACH

The activity of the ruminant stomach is varied and, due to its extreme compartmentalization and functional importance, very complex.
Food enters the ruminant stomach through the reticular groove. In the newborn ruminant this groove has great functional significance since, during suckling, due to chemical stimulation of the pharyngeal wall by salts in the milk, the groove is partially closed and forms a canal that bypasses the reticulo-rumen complex and directs the milk through the omasal groove into the abomasum. In the adult this reflex can still be elicited by chemicals (copper sulfate and bicarbonate). However, under normal circumstances the groove conveys liquids into the abomasum and vegetable food to the reticulo-rumen (3; 24; 50; 184; 321; 682).
The rumen and reticulum constitute together a large reservoir in which the vegetable food is broken down by biological, chemical and mechanical processes. Availability of the substrata, a prolonged retention of the food, an adequate and a thorough mixing of the food in the fermentation chambers, coordinated transport of the ingesta within the forestomach complex, an optimal pH and, finally, anaerobic conditions are the different physiological prerequisites for normal microbial digestion in the stomach and thus for the normal production of energy in herbivores (24; 36; 37; see 37; 50; see 321; 682). Consequently, fermentation may be regarded as a critical and time consuming process that largely depends upon an adequate microenvironment (pH, temperature) and upon controlled and coordinated motility. Microorganisms ferment a wide range of carbohydrates (sugars, starches, cellulose, hemicelluloses and pectins) that may be either in the form of complex polysaccharides (starch, cellulose) or in the form of simple sugars (lactose, sucrose) (36; 38; 39; see 39). These carbohydrates are degraded by extracellular bacterial enzymes to hexoses and pentoses. The further metabolism of these sugars occurs inside the bacteria via the glycolysis or the pentose phosphate pathway. Pyruvate is formed and rapidly converted to the end products of anaerobic fermentation i.e. VFA, H^+, CO_2 and CH_4. Approximately 75% of the original energy content of the carbohydrate serves for the production of VFA, while the remaining 25% is used by the microflora for growth or is lost as gas (CO_2 and CH_4) (528). In ruminants substantial quantities of gas are produced in two ways i.e. by the neutralization of the acid products from microbial metabolism and by the fermentation process itself (36). The principal end products of fermentation are the three VFA, i.e. acetic, propionic and butyric acids. They form the major source of metabolic energy in ruminants (24; 36; 50; see 321; 682). In ruminants 70 to 80% of the daily energy requirement derives from the metabolism of VFA (36; see 39; see 628). In sheep VFA have been found to provide between 60 and 85% of the metabolizable energy (36; see 297; 528; see 528). In addition, the large intestine in ruminants is responsible for up to 13% of the animal's total VFA production (528; see 528). Consequently, it has been stated that ruminants are, anatomically, better adapted to the herbivorous mode of life because they subject their ingesta to two fermentations: one before and the other following the usual mammalian digestive processes (16). Small amounts of lactate and succinate are also produced (24; 36; 50; see 321; 682). The relative proportion of the three VFA is normally acetate> propionate> butyrate

but the ratios at which they are formed, depend on the diet (36; 39; see 39; 391). For example, ingestion of a diet abnormally high in starch results in a fulminating production of VFA within the rumen. The large quantities of VFA cause a marked lowering of the pH, leading to a more rapid absorption of VFA and an eventual replacement of the normal flora by lactobacilli which produce lactic acid. This situation may cause necrosis of the rumen epithelium, atony of the rumen muscle, systemic acidosis, dehydration and, finally, death (36; see 36. 38; see 38).

A pH of 5.5 - 7.0 is required for the maintenance of ruminal microorganisms and, hence, for normal energy production (see 15; 38). Consequently, under normal circumstances the pH of the rumen contents must remain fairly constant and is maintained above pH 5.5 in spite of the production of large quantities of VFA (39; see 39). This goal is reached in two ways: by the rapid absorption of the VFA and by the addition of large volumes of heavily buffered saliva (15; see 15; 36; see 36; 38; see 38; 39; see 39; 41; 391).

Microbial fermentation products are absorbed from the forestomach and metabolized (16; 527; 528; see 528). For that purpose the absorbing surface of the rumen is greatly increased by the formation of numerous ruminal papillae which, in addition, function to heat up the ruminal contents (24; 36; 50; see 321; 682). But, the development of a functional and absorptive surface area (papillae) in the forestomach depends on the diet. It is now well established that the end-products of the microbial carbohydrate digestion, especially butyrate and propionate, are responsible for this development. Therefore, an active microbial digestion must be established prior to maximal absorptive function (36, see 36). The forestomach epithelium absorbs a large proportion of the VFA, together with ions (Na^+ and Cl^-), and transforms them to ketone bodies, lactate and CO_2. In consequence, approximately 30 to 90% of the VFA, produced in the rumen, never reach the general circulation for use by the body tissues (36, see 36). The rate at which individual fatty acids, even though they are largely dissociated, are absorbed and transported across the forestomach epithelium is inversely related to their molecular weight, but linearly related to their initial concentration and determined by the rate at which they are metabolized. No difference has been found between the absorption rate of acetate, propionate or butyrate (39; see 39; 528; see 528).

Two environmental conditions, essential for microbial digestion, are produced by the saliva. *First*, since a fluid medium must be provided for the microflora, and since the forestomach has no secretory glands, this fluid comes, almost exclusively, from the salivary secretion. In ruminants, therefore, the parotid gland spontaneously secretes a fluid that is not under the control of secretory nerves. *Second*, because of the large quantities of VFA produced in rumen, the salivary glands must provide a substantial amount of highly buffered medium to maintain an optimal ruminal pH. The parotid secretions, therefore, have a sodium/potassium value similar to that of plasma, high concentrations of bicarbonate and phosphate and a pH of 8.2. Furthermore, the salivary glands of the cow secrete from 100 to 200 liters/day of a fluid rich in sodium bicarbonate and sodium phosphate. However, even this quantity has been found insufficient to buffer the total amount of acid produced. For example, it has been calculated that 10 liters of sheep saliva would bring 3.3 moles of VFA to a pH of only 4.5. In consequence, absorption of the VFA by the forestomach epithelium is of crucial importance for the maintenance of an adequate pH in the ruminant stomach. As a result of such a considerable production of saliva approximately two times the Na^+ content of the extracellular fluid volume passes to the rumen each day and, at any time, half of the Na^+ content of the extracellular fluid is sequestered in the forestomach (36, see 36). Besides the isotonicity the basal concentration of PO_4^{3-} is remarkably high in the saliva of ruminants. Further it has been found that the concentration of PO_4^{3-} decreases as the rate of secretion increases. This is reciprocated by an increase in HCO_3^- (not Cl^-) (36). Approximately 60% of the Na^+ and Cl^- entering the reticulo-rumen via the diet and saliva are absorbed directly from the forestomach. Na^+ and Cl^- are absorbed by active transport, while Cl^- transport appears to be partially accomplished by exchange for HCO_3^- providing an additional mechanism for the buffering of VFA (15; see 15; 36; 38; see 38; 39; see 39). In conclusion, it may be claimed that the normal functioning of the forestomach, as well as the internal electrolyte balance, critically depend on the salivary secretion (36; see 36).

Due to the peculiar anatomical arrangement of the gastrointestinal tract in ruminants soluble carbohydrates and proteins cannot avoid being fermented along with the cellulose before the animal has had an opportunity to absorb them (391; see 391). Extensive breakdown of the ingested protein, therefore, occurs in the forestomach. Approximately two-thirds of the intact dietary protein is degraded by the action of the proteolytic microbial enzymes. Peptides and amino acids, formed during this process, are in turn attacked by

deaminases to yield free ammonia. However, under normal dietary conditions only 30% of the dietary N is converted to NH_3, while the remaining 70% either passes intact to the intestine or is converted to peptides and amino acids, which are then directly used for the synthesis of microbial protein (36; see 36). Ammonia is either directly utilized as a protein source by the bacteria or absorbed and sent to the liver. There it is converted to urea and, subsequently, returned to the forestomach either by inclusion in the (parotid) saliva or by simple diffusion from the blood. Once in the rumen ureases, of microbial origin, convert urea to NH_3 that is then utilized by bacteria for their growth. Large numbers of these bacteria are continuously swept through to the abomasum where they are digested by proteases. In this way the ingested nitrogen finally becomes available to the animal as microbial protein. This complicated cycle uses all the available nitrogen and leads urea, from metabolic sources, into a cycle rather than into the urine. Moreover, excretion of urea is always accompanied by a certain amount of water and, as a consequence, this cycle may help ruminants to conserve water. The nitrogen cycle further protects the animal from amino acid imbalance in the diet. To end, some recycled urea functions to increase the cellulase activity of the bacteria (36; see 36; 391; see 391).

In the rumen fine food particles sift to the bottom of the fluid mass while the coarse unchewed or partially chewed food floats on the top. Parts of this floating mass enters the esophagus in regurgitation for rumination (mechanical break down). Regurgitation is a reflex initiated through a stimulation of the wall of the reticulo-rumen and the reticular groove by coarse ingesta. The reflex is coordinated with the ongoing reticulo-ruminal cycle. An extrareticular contraction immediately precedes the biphasic reticular contraction. Coinciding with the former contraction a negative intrathoracic esophageal pressure is accomplished by an inspiratory effort against a closed glottis. Aspiration of digesta into the caudal esophagus is followed by an antiperistaltic contraction wave of the esophagus. At the end of the cycle of rumination the bolus is reswallowed and immediately followed by regurgitation of another bolus. These closely integrated reflex events with the normal cyclic forestomach activity indicate a high degree of nervous control (24; 36; see 36; 50; see 321; 682).
During fermentation large quantities of gas are rapidly produced and have to be eliminated adequately. This is realized mainly by eructation and to some extent by absorption. Consequently, eructation is a vital function in ruminants. The reflex is elicited by pressure in the dorsal sac. Increase in the intraruminal pressure augments the number of secondary contractions associated with eructation. These contractions appear to originate in the caudodorsal blind sac and occur at a time when the reticulum has relaxed and the LES is exposed. The opening of this sphincter cannot take place until its vicinity is cleared of ingesta. Indeed, receptors in this area mediate an inhibition of the eructation reflex if the region is covered by fluid or foam. The reticulum empties itself into the rumen and relaxes. Then the eructation contraction of the dorsal sac forces the gas to the LES, which then opens, so that gas is expelled in the esophagus (24; 36; see 36; 50; see 321; 682).

The reticulum has been shown to be the segment in which forestomach motility is initiated. In fact, a biphasic reticular contraction is followed by a primary contraction of the ruminal dorsal sac. A secondary ruminal contraction also occurs but this is not associated with every cycle. This cyclic motor activity of both the reticulum and rumen is necessary to mix and circulate the ingested food in the rumen and to further prepare the contents for regurgitation, absorption or passage into the omasum (36, see 36).
Moreover, based upon its anatomical location, it is clear that the reticulum has a strategic position in the ruminant stomach complex as it lies at the junction of the esophagus, rumen and omasum. Due to this peculiar position the reticulum functions as a cyclic pump mixing and separating the food. Indeed, when it relaxes ruminal contents are sucked in and when it contracts the contents are partially pushed back into the rumen. As the reticulum and the atrium ruminis contract alternately, ingesta are tossed backwards and forwards over the reticuloruminal fold. Thus, during the consecutive reticulo-ruminal cycles food contents of a high specific gravity (i.e. fine particles) are trapped and collected by the reticular cells, while the coarser (lighter) material is sent back into the rumen for further breakdown. In this way ingesta, that have been sufficiently disintegrated by rumination and bacterial fermentation, are collected at the bottom of the reticulum and finally presented to the reticulo-omasal orifice. During the second reticular contraction, a negative pressure is recorded in the omasal canal. At the height of this reticular contraction the reticulo-omasal orifice opens and reticular contents are aspirated into the canal (24; 36; see 36; 184; see 321; 682).

The omasum is a two-stage pump (lift and force pump) necessary for the regulation of the transport of ingesta from the reticulum and rumen to the abomasum. Hence, reticular and omasal motility are normally coordinated. The first stage (lift) is coupled to the reticulo-ruminal cycle and consists of aspiration of reticular contents. When the reticulum contracts the omasum relaxes aspirating in this way part of the reticular ingesta. Coarse particles are retained partially by the papillae unguiculiformes in the sulcus omasi, and partially by the contractility of the reticulo-omasal orifice. Fluid and food particles, sufficiently disintegrated, pass directly into the abomasum via the sulcus omasi. Contraction of the omasal canal is associated with closure of the reticulo-omasal orifice and contents are forced into the body of the omasum between the omasal laminae (recessus interlaminares) (36). Thus, the ostium reticulo-omasicum, together with the papillae unguiculiformes, act as a sieve allowing passage of only those particles to the abomasum that have been reduced by the processes of ruminal fermentation to the required size. In this way the reticulo-omasal orifice is largely responsible for the retention of food in the reticulum and rumen and for the reduced passage of the ingesta to the true stomach. In consequence, on a high fibre diet the food remains in the rumen for a much longer period of time since it takes longer to reduce the particles to a size suitable to pass through this orifice. The animal's intake, therefore, is depressed since it largely depends on the fulness of the rumen. Thus, for a ruminant there is a definite cut-off point in the percentage of fibre in the diet that it can tolerate and beyond which it would be unable to support itself (391; see 391).
The second stage (force) is probably stimulated by distention of the omasum, though conditions in the abomasum most probably also affect the omasal motility. The omasum contracts (contractions of the omasal body are not synchronized with the reticulo-ruminal cycle but occur at random)and, the ingesta are compressed into thin layers and further refined. The fluid which is pressed out is drained off either to the reticulum or the abomasum. Moreover, the epithelium of the omasal leaves is covered by numerous papillae. Both structures, just like the ruminal papillae, considerably increase the absorbing surface of the omasum. Hence, one action of the laminae is thought to be rhythmic contractions effecting the refinement of the food and the extraction of fluids from the semisolid ingesta coming in from the reticulum and rumen (see 37; 5; 23; 87; 6, see 6). Finally, the "dehydrated" ingesta are pushed into the "true" stomach for further enzymatic digestion.
From the description above it is clear that the omasum, as a whole, selectively retains particles in the reticulum and rumen and rapidly propels liquid to the abomasum. As a consequence, the omasum may function as a filter. Finally, in this connection, as is well-established, paralysis of the omasum causes a fatal stasis of ingesta in the reticulum and rumen (24; 36; 184; see 321).

The abomasum undergoes independent contractions which are more frequent and forceful in the pars pylorica. The functions of the abomasum (true stomach) are almost comparable with those of the non-ruminant stomach which is mainly involved in protein digestion. The secretion of gastric juice in the abomasum resembles that of the simple stomach but is continued since in the ruminant ingesta are continually transferred in small amounts into the abomasum. Thus, abomasal secretion is not dependent upon the stimuli associated with feeding.
In order to prevent destruction of the colostral immunoglobulines (proteins) the secretion of protein-splitting enzymes starts a few days after birth. In the suckling calf the zymogenic cells produce an enzyme, rennin, which causes milk to flocculate delaying it in its passage through the abomasum (24; 321).

Vegetable food must be retained and mixed for a considerable period of time so that it can undergo the relatively slow process of microbial digestion and absorption. Some contents must be regurgitated, resalivated and reswallowed, whilst large amounts of gas must be eructated. Last but not least, the contents must leave the forestomach compartments in an orderly and controlled fashion. All these different motility patterns are accomplished by complex but coordinated cycles of forestomach motility and it may be assumed that the above-mentioned motility patterns are largely controlled by the ENS (36; see 36).
The vagal nerves are of crucial importance to the ruminant animal. They control and coordinate, by means of the intramural nervous system, the different contraction cycles of the stomach, they control regurgitation, resalivation, remastication and eructation, are involved in the closure of the reticular groove in the suckling ruminant and, finally, stimulate the contraction of the abomasum so that its contents may be transferred to the duodenum. Section of both vagal nerves abolishes, therefore, all motor activity of the forestomach, while section of only the dorsal trunk results in a nearly complete, but not always permanent, paralysis of the

rumen with a less marked effect on the other forestomach compartments (see anatomy). In contrast section of the ventral trunk usually has less effect on rumen motility.
The sympathetic fibres to the ruminant stomach are principally vasomotor, although it has been shown that they may inhibit to some extent gastric motility. Section of the splanchnic (sympathetic) nerves does not affect rumen motility. Thus, bilateral splanchnectomy has been shown to have no effect on gastric motility in sheep, nor does it prevent the serious distention of the forestomach which follows sectioning of both vagal trunks.
Thus, it may be concluded that, if both vagal nerves are incapable of transmitting impulses to the ruminant stomach, paralysis of the forestomach will occur even in the presence of normal sympathetic tone. The fatal gastric distention, resulting from this complete lack of vagal function, is, therefore, not due to a spasm of the pyloric sphincter caused by unopposed sympathetic impulses (24; 36; 321).

To conclude, all the available morphological and functional data make it clear that the forestomach is more than a simple storage device but is a fermentation chamber of vital importance for the preservation of the life of the ruminant. The multiple functions of this gastrointestinal segment are, to a high degree, correlated with the different and complex motility patterns, which are in turn principally controlled and coordinated by the ENS. In consequence, diseases of the "stomachs" of ruminants are often the cause and/or the consequence of a "diseased" ENS (16; 527; 528; see 528).

Chapter 2.
THE AUTONOMIC NERVOUS SYSTEM

I. 2. 1.
DEFINITION

The nervous system is usually divided into somatic and autonomic components. Since both systems have several features in common (similar embryological origin, similar neurons) and since their activities depend to a large extent upon each other, this classification is in fact artificial.

Originally, the concept "autonomic" "vegetative" or "visceral" system comprised only structures found outside the central nervous system i.e. the sympathetic trunks and the large nervous plexuses in the body cavities. In 1921 Langley defined the autonomic nervous system (ANS) as a purely visceral motor system consisting of " the nerve cells and nerve fibres by means of which efferent impulses pass to tissues other than multinuclear striated muscle" (89; see 116; 603). Later it was shown that, in most cases, visceral motor fibres were accompanied by sensory fibres, forming the afferent links of most of the visceral reflex arcs. The discovery that even the cerebral cortex can influence functions attributed to the ANS makes the delineation of this system even less clear and therefore, more accurate definitions of the autonomic system have been proposed (34; 89; 116) e.g.

"...The ANS is a division of the peripheral nervous system that is distributed to the smooth muscle cells and glands throughout the body ..." (128).

"...Portions of the central and peripheral nervous system primarily concerned with the regulation and control of visceral functions are termed collectively the visceral, vegetative or ANS ..." (116).

"...As ANS the entire mass of those nerve cells and fibres is designated which are concerned in the innervation of the internal organs, in so far as these are made up of smooth muscles or belong to glandular organs ..." (89).

In the opinion of the author the last definition is the most accurate and, consequently is adopted in this study.

I. 2. 2.
EMBRYOLOGY OF THE ANS

The brain and spinal cord are derived from the neural plate, the central parts of which deepen, forming the neural groove. The lateral parts of the groove proliferate and, form the neural folds, which fuse and transform the neural groove into the neural tube. The tube, finally, detaches itself from the dorsal ectoderm (see 603; 753). Before this the cells from the neural folds which close the tube proliferate and constitute a bilateral flat band, the neural crest, that runs along the entire length of the dorsolateral aspect of the neural tube (603; 753). From the neural crest the primordia of the peripheral autonomic (autonomic ganglia) and sensory (cerebro-spinal ganglia) nerve cells originate (459; see 459; 460; see 460; 603).

With respect to its participation in the formation of the ANS the neural crest can be divided into three regions: vagal, cervico-dorsal and lumbo-sacral.

The *vagal region* (corresponding to somites 1 to 5), is devoted to the formation of the enteric ganglia and, is the source of "para"sympathetic nerve cells and fibres.

The *cervico-dorsal region* (somites 8 to 28) gives rise to sensory and sympathetic ganglia in the cervical and thoracic parts of the body, the aortic and adrenal plexuses and the adrenal medulla. Sympathetic ganglia are found either at the side ("para") or in front ("pre") of the vertebral column. Hence, paravertebral and prevertebral sympathetic ganglia are distinguished.

The *lumbo-sacral region* (posterior to the 28th somite) gives rise to both parasympathetic (intramural ganglia, ganglion of Remak) and sympathetic derivatives (chains and plexuses) (459; 460).

I. 2. 3.
STRUCTURE OF THE ANS

(see Addendum/ Part I/ fig.7)

Based upon morphological (e.g. the location of the central perikarya and the outflow of the efferent fibres), chemical and physiological grounds the ANS is commonly subdivided into two major parts: the sympathetic (orthosympathetic) and the parasympathetic systems. However, one has to realize that in the periphery of the ANS fibres of both subdivisions run usually intermingled with each other, although they retain their functional independence. In this way the large autonomic plexuses found in the body cavities contain fibres of both kinds. In addition, several authors recognize the intramural nervous system as the third morphological and functional part of the ANS (3; 89; 116; 128; 682; 684; 753; 814; see chap. 3).
Essentially, effects mediated by the ANS are based on reflex activities. Consequently, the structure of the ANS will be briefly discussed below in the form of a reflex arc containing:

an afferent link
a central part and
an efferent link.

The afferent link

The afferent link consists of visceral receptors, afferent fibres and nerve cell bodies.

Visceral Receptors and Afferent Fibres

Originally, the apparent insensitivity of the viscera to cutting, pinching and burning led to the view that viscera and blood vessels do not posses afferents and hence visceral sensation. However, later it was demonstrated that distention rather than cutting, pinching or burning is the adequate stimulus for exciting true visceral afferents (340; see 340; see 603; 626; see 626).
Most likely there are numerous types of receptors in the viscera, represented by free nerve endings, particularly in the muscle coat and mucous membranes, though other more specialized endings (e.g. Pacinian corpuscles) are also found (89).
Receptors in the gut mucosa react to chemical stimuli. Others, mechanoreceptors, mainly occurring in the muscular layers, are activated by intestinal contractions and artificial stimuli such as distension (89; see 89; 221). Information on the volume or the degree of filling of a viscus collected by these "tension receptors" may be important in moving and/or expelling intestinal contents (89). Thermoreceptors and osmoreceptors, responding to changes in the tonicity of the intestinal contents, comprise other sensory information recorded from the intestine (221). In the peritoneum, particularly in the mesentery, Pacinian corpuscles have been demonstrated (89), reaching the central nervous system via the splanchnic nerves and it has been suggested that they participate in the regulation of blood flow to the intestine (221).
Visceral afferents are unmyelinated (C fibres) or thinly myelinated (Aδfibres). They travel centripetally by the somatic and/or autonomic nerves. The largest myelinated fibres come from Pacinian corpuscles, while smaller fibres (myelinated or unmyelinated) originate from the more numerous diffuse visceral receptors (89; see 89; 814). Sensory fibres from thoracic, abdominal and pelvic viscera pass uninterrupted through the sympathetic trunks in which they may ascend or descend for a considerable distance. This is especially the case in the cervical, lower lumbar and sacral regions. Finally, the visceral afferents join the spinal nerve via the white or gray rami communicantes (89; 603; see 603). However, some visceral afferent fibres, particularly in the sacral spinal cord, enter the cord by way of the ventral root (160; see 160; 793; see 793). Afferents arising from large blood vessels (the aorta and its major branches) most likely enter the sympathetic trunk directly (603).
Viscera can be exposed to stimuli such as burning or cutting without evoking pain. This apparent insensitivity is because the stimuli are inadequate. The main factors capable of inducing pain in visceral structures are: (1) abnormal distension and contraction of the muscle walls of hollow viscera, (2) rapid stretching of the capsule of solid visceral organs such as the liver, spleen, and pancreas, (3) abrupt anoxemia of visceral muscle, (4) the formation and accumulation of pain-producing substances, (5) the direct action of chemical stimuli (especially important in the oesophagus and stomach), (6) traction or compression of ligaments and vessels, (7) inflammatory conditions and, finally, (8) necrosis of some structures (myocardium, pancreas).

With regard to the contraction of hollow viscera, it must be noted that a strong contraction in approximately isometric conditions provokes a more severe pain than in approximately isotonic conditions. This may explain the strong pain of some diseases such as acute intestinal obstruction and biliary or ureteral colics. The different visceral structures show different pain sensitivity. Serosal membranes have the lowest pain threshold and are followed, in order of ascending threshold, by the walls of hollow viscera and the parenchymatous organs (340; see 340; 626; see 626). Three types of visceral pain can be distinguished: *pure visceral* pain (felt in the region of the affected organ); *visceral referred* pain (projected in the territory of the corresponding spinal nerves) and *viscero-somatic* pain (caused by the spread of visceral disease to somatic structures) (221). All the available experimental evidence supports the main postulates of the "viscero-somatic convergence-projection" theory of referred visceral pain. No pathway has been found carrying exclusively visceral sensory information. This indicates that "true" visceral pain is not due to the activation of specific visceral sensory channels but, more likely, is the consequence of the spread of the visceral lesion to regions innervated by somatic nerves, the stimulation of which leads to restricted and well-localized experiences of deep pain. Excitation, therefore, of visceral sensory receptors always evokes sensory experiences that are ill-localized, poorly discriminated and referred to somatic structures (120; see 120). Some visceral afferents may branch peripherally to supply receptors in different tissues. These afferent fibres may supply both visceral and somatic receptors and could serve as part of the mechanism for referred pain (793; see 793).

Visceral pain from most abdominal and pelvic organs is carried chiefly by the sympathetic nerves (89; 116; 682). The lower thoracic spinal cord receives the bulk of the visceral sensory input mediated by the sympathetic splanchnic nerves. These afferent fibres transmit nociceptive signals from many abdominal viscera to the spinal cord, where visceral and somatic sensory information are jointly processed (120; see 120). Visceral afferent fibres contained in the splanchnic nerves excite second-order neurons in the spinal cord, which in turn generate extensive divergence within the cord and brain stem, sometimes involving long supraspinal loops. Such a divergent input can activate many different systems, motor and autonomic as well as sensory, and thus trigger the general reactions that are characteristic of visceral nociception : a diffuse and ill-localized pain referred to somatic areas, viscero-visceral reflexes that alter the autonomic control of viscera and viscero-somatic reflexes and result in prolonged muscle spasms (120).

The organization of viscero-somatic convergence in the thoracic spinal cord can be compared to that of a trip-wire alarm mechanism. Such a system requires a few peripheral sensors capable of triggering extensive sensory-motor reactions when excited by visceral nociceptive stimulation. The low density of visceral sensory innervation and the extensive divergence of the visceral input within the central nervous system are probably responsible for the diffuse localization and poor discrimination of the sensations evoked by visceral nociceptive stimulation (120).

Myelinated fibres from Pacinian corpuscles travel (along the splanchnic nerves) into the sympathetic trunk and they are believed to transmit dull, deeply felt abdominal pain (see 603). Unmyelinated afferents from free nerve endings mediate or transmit the distinct sharp pain which is experienced when the mesentery is cut or torn (603). In man, however, fibres from the distal colon, rectum, the neck of the bladder, the prostate, uterus and cervix appear to be an exception to this rule since they travel with the parasympathetic nerves (pelvic nerves and the associated plexuses) (89).

Likewise, the parasympathetic nerves contain many visceral afferent fibres which are most probably involved in processing information such as appetite, hunger, thirst, saturation, urination, defecation and reproduction (682). In addition, vagal afferents seem to be concerned in the transmission of certain diffuse sensations such as nausea (89; 682). Contrary to the sympathetic system, there is no conclusive evidence of pain-conducting fibres in the vagal nerve (89; 603).

Afferent Nerve Cell Bodies

Visceral afferent cell bodies are gathered in cerebrospinal (i.e. cranial, spinal) or intramural ganglia (3; see 3; 89; 116; 221; 459; 460; 603; 682; 814).

Principally, the parasympathetic afferent perikarya are aggregated in two locations: (1) in the nodose ganglion, from where peripheral processes are distributed to the heart, lungs and other viscera, and (2) in the sacral spinal ganglia from where fibres are conveyed to the pelvic organs.

The sympathetic afferent nerve cell bodies are found in the thoraco-lumbar spinal ganglia. Two types of thoracic afferent spinal neurons can be distinguished: (a) "somatic" neurons, which can be excited by stimula-

tion of cutaneous or subcutaneous receptive fields, but do not receive inputs from visceral afferent fibres in the splanchnic nerve and (b) "viscero-somatic" cells which respond to stimulation of somatic structures, but in addition can be driven from the splanchnic nerve or by the natural stimulation of viscera such as the gallbladder. No evidence has been reported for the existence of significant numbers of spinal cord neurons which receive only visceral inputs. The sizes of the receptive fields differ between the somatic and viscero-somatic groups of neurons. Viscero-somatic neurons tend to have larger receptive fields than somatic cells. Most of them receive nociceptive inputs such as pinch or noxious heat (120; see 120). Their central process enters the spinal cord via the dorsal or ventral roots, but the level of their inflow is not necessarily identical to that from which the corresponding efferent neurons emerge (89; 603; see below). Although the number of visceral afferent fibres entering the thoracic cord is small in relation to the number of somatic afferents, a large proportion (56-75%) of thoracic spinal neurons respond to stimulation of the splanchnic nerve. This indicates extensive divergence of visceral inputs to the thoracic cord (120; see 120). Viscero-somatic cells are concentrated in laminae I, V, VII and VIII. This follows the pattern of termination of visceral afferents of which endings are absent from laminae II-IV (120; see 120). The exact central termination of the visceral afferents is insufficiently known. Contrary to the fine somatic afferent fibres (lam. I, II and V), visceral afferent fibres end largely in lam. I, V, VII and X suggesting that the processing of information from somatic and visceral structures in the dorsal horn is quite different (160; see 160; 793; see 793). In the gray matter they do not end directly on visceral efferent cell bodies present in the intermediolateral cell column, but end indirectly via interneurons. This can be concluded from the observation that no degenerating cells are observed in the intermediolateral cell column after transection of dorsal roots while, in contrast, retrograde changes are seen following thoracic or abdominal sympathectomy. Furthermore, most spinal cord interneurons with visceral input have a convergent input from somatic tissue (793). In the dorsal horn, in addition, visceral afferents synapse directly with the second order neurons of the spinothalamic and spinoreticular tracts, which ascend in the venterolateral funiculus of the cord, some possibly ultimately reaching the level of consciousness.(89; 221). This and other findings suggest that visceral reflex arcs are most probably polysynaptic (see 89).

The central part

Visceral motor neurons are located in visceral cell columns within the central nervous system (CNS) (brain stem, spinal cord).

Retrograde cellular changes, following thoracic or lumbar sympathectomy (monkey) and/or the labelling of cells following HRP injections into sympathetic ganglia, have confirmed that the great majority of the visceral motor neurons of the **sympathetic** division of the ANS arises from cell bodies located in the transitional zone between the dorsal and ventral horns i.e. the intermediolateral cell column also referred to as: the superior nucleus intermedio-lateralis, the lateral splanchnic motor cell column, the superior lateral sympathetic nucleus and thoracolumbar cell group. Additionally, smaller contributions come from some medially situated cell groups as well (3; 89; see 89; 116; 128; 603; 682; 814). The extent of the thoracolumbar cell column varies between species e.g. in ruminants Th_2-L_4; in the horse Th_2-L_2 and in man C_8-L_3 (3; 89; 116; 128; 603; 682; 814). Cells of the intermediolateral column are oval, spindle- or club-shaped, have few, fine Nissl granules, have peripheral nuclei and are described as bi- and multipolar. They are smaller than the cells in the ventral horn but larger than the dorsal horn cells (603). Preganglionic neurons have as a rule very thin axons (89; 603).

The central nerve cell bodies of the **parasympathetic** division of the ANS are found in the gray matter of the midbrain and brainstem (cranial division) and in the intermediolateral nucleus of the sacral spinal cord (S_2-S_5) (sacral division) (89; 116; 128; 682; 814).

The efferent link

Contrary to the somatic nervous system a "two neuron pathway" (there are *never* more than two) connects the central part of the ANS with the end organ. The first visceral motor neuron is localized in the visceral efferent cell column of the brain stem or spinal cord. The second visceral motor neuron is found outside the CNS in one of the autonomic ganglia (3; 89; 116; 128; 603; 682; 814). Consequently, the efferent link is made up of three different elements i.e.

a preganglionic (i.e.presynaptic) nerve cell body and fibre

a ganglion of second neurons which makes a synapse with the preganglionic fibres and

a postganglionic (i.e.postsynaptic) efferent fibre.

Preganglionic Cell Bodies and (efferent) Fibres

Principally, each nerve fibre leaving the CNS is myelinated. Hence, preganglionic (efferent) nerve fibres are (thinly) myelinated (3). After entry into the autonomic ganglion a preganglionic fibre will split up into several terminals in order to spread its discharge over a considerable number of postganglionic neurons (3). In the parasympathetic division this ratio is rather low; in the sympathetic system, in contrast, the ratio is much higher (see below) (89, see 89).

The position of the preganglionic (i.e.presynaptic) motor neurons and, as a consequence, the outflow of the preganglionic efferent fibres from the CNS represents one of the classical anatomical criteria which distinguishes two subdivisions of the ANS i.e. the sympathetic and parasympathetic systems. Chiefly, the outflow of the preganglionic fibres from the CNS occurs at three levels: cranial, thoraco-lumbar and sacral.

The *cranial outflow* starts from visceral cell groups in the brain stem. The preganglionic fibres terminate either in the autonomic ganglia of the head or in ganglia near/within the walls of thoracic (e.g. heart, lungs, oesophagus) and abdominal (stomach, intestine) viscera. These fibres are involved in parasympathetic functions.

The *thoracolumbar outflow* arises from cells localized in the intermediolateral nucleus of the gray matter. The presynaptic fibres leave the cord through the ventral roots of the corresponding spinal nerves and terminate in one of the autonomic sympathetic ganglia. These fibres belong to the sympathetic nervous system.

Finally, the *sacral outflow* originates from the intermediate zone of the mid-sacral (S_2-S_5) spinal cord. These preganglionic fibres emerge via the ventral roots of their corresponding spinal nerves and pass to terminal ganglia near or within the walls of pelvic viscera. These fibres are involved in parasympathetic functions (3; 89; 116; 128; 603; 682; 684; 814).

Thus, from this anatomical arrangement the synonymous names for the sympathetic and parasympathetic systems of 'thoracolumbar' and 'cranio-sacral' become clear (89). Both the cranio-sacral and thoracolumbar systems will now be discussed in some detail.

The cranial and sacral outflow: parasympathetic outflow.

Preganglionic nerve fibres leave the CNS along certain cranial (NIII, VII, IX and X) and sacral spinal nerves (603). In the cranial part the preganglionic nerves course to rather well defined groups of postganglionic neurons (the ciliary ganglion, submandibular ganglion etc.). In the vagal and sacral divisions, in contrast, the postganglionic cells lie in the walls of the target organs (e.g. in the intestinal plexuses) (89; 116). As a rule the preganglionic parasympathetic fibres are long since the parasympathetic ganglia lie near (juxtamural) or inside (intramural) the target organs. Consequently, postganglionic parasympathetic fibres are very short (89; 128; 814). Moreover, the preganglionic fibres terminate with few collaterals on postganglionic neurons (e.g. 1 preganglionic fibre synapses with 2 postganglionic neurons in in the ciliary ganglion of the cat) (603). This is in contrast to the sympathetic system in which the situation is reverse (see below). In their peripheral course preganglionic parasympathetic fibres are usually intermingled with sympathetic fibres. Consequently, the peripheral parasympathetic tracts are morphologically indistinct from the sympathetic nerve bundles and only a few peripheral autonomic nerves hold predominantly parasympathetic fibres (3; 684).

By far the most important contingent of preganglionic parasympathetic fibres is contained in the vagus (N X). It has been suggested that the vagal dorsal motor nucleus gives rise only to secretomotor fibres and that neurons responsible for vagal visceromotor activity are found in an area between the nucleus ambiguus and the spinal trigeminal nucleus (see 89).

Running along the esophagus the vagal nerves perforate the diaphragm. In monogastric animals the left vagus is distributed mainly to the ventral surface of the stomach, whilst the right vagus is distributed to the dorsal surface. In the gastric wall a plexus is formed. Both vagal nerve branches than take part in the formation of the celiac plexus (89; 684). Due to the complex morphology of the ruminant stomach the abdominal part of the vagus is, compared to other species (Ca, Eq, Su), distributed in a different way. In ruminants both vagal nerves branch into dorsal and ventral parts, the corresponding parts of both sides recombining into dorsal and ventral *trunks*. Both trunks are interconnected by a ramus communicans which is usually seen on the left side of the oesophagus (682). The dorsal vagal trunk gives off branches to the left side of the stomach, the ventral trunk to the right side (682) (fur-

ther description of the innervation of the ruminant stomach see chap. 1).

Preganglionic fibres, originating from the sacral division of the spinal cord, leave the cord via the ventral roots of the spinal nerves S_2-S_5. They follow these nerves (mainly the pudendal nerve) and leave them as the pelvic nerves (nn pelvini) on each side of the rectum. They then run independently to the postganglionic (second) neuron situated either in the pelvic ganglia or in the intramural ganglia of the pelvic viscera. In this way the pelvic nerves distribute (via the hypogastric plexus) parasympathetic fibres to the distal part of the large intestine, pelvic viscera and genitalia where they become intermingled with sympathetic fibres (3; 89; 128; 603; 682; 684).

The thoracolumbar outflow

Retrograde labelling (HRP) of the preganglionic sympathetic cells have shown that, in agreement with some physiological studies, preganglionic fibres leaving the cord via one ventral root are most probably derived from many segments and that some of these axons run for varying lengths in the spinal cord before leaving it (see 89). It has also been demonstrated that preganglionic axons projecting to thoracic sympathetic chain ganglia arise ipsilaterally, whilst those to lumbar ganglia arise bilaterally (89). The central sympathetic perikarya have short axons (preganglionic fibres) which terminate with many collaterals on the postganglionic cell bodies found in the sympathetic autonomic ganglia (e.g. 1 preganglionic fibre is associated with 32 postganglionic neurons in the cranial cervical ganglion of the cat) (see 603).
Preganglionic sympathetic fibres synapse in the autonomic ganglia lying either just beside the spine, i.e. paravertebral ganglia or at some distance in front of the vertebral column, i.e. the prevertebral ganglia (3; 116; 128; 603; 682; 684; 814).
Most preganglionic sympathetic fibres leaving the spinal nerves are bundled together and, since they are myelinated, are conducted via the white rami communicantes to the *paravertebral ganglia*. The preganglionic fibres either terminate in the ganglion which they first enter or pass up and down the sympathetic trunk for considerable distances giving off collaterals. The preganglionic fibres from the upper thoracic nerves pass mainly up the sympathetic trunk; those from the middle thoracic spinal segments pass up or down the trunks and those from the lowest thoracic and lumbar segments pass only down the trunks. As a consequence many of the fibres originating in the upper thoracic segments end in the cranial cervical ganglion, while the sacral vertebral ganglia receive their preganglionic fibres from the lower thoracic and upper two lumbar segments of the cord (89; 116; 128; 603). All paravertebral ganglia are connected by nerve strands, the internodal rami, which lead to the formation of the sympathetic trunk (truncus nervi sympathici). The sympathetic trunk is, therefore, made up of bilaterally symmetrical strands of preganglionic and postganglionic sympathetic fibres that run from the base of the skull to the lower end of the spine (3; 89; 603; 682; 684; 814).
From the paravertebral ganglia most postganglionic (unmyelinated) fibres rejoin as gray rami communicantes, the spinal nerves for further peripheral distribution. Thus, the divergence of the preganglionic sympathetic fibres on one hand, and the fact that these fibres may ascend and/or descend the sympathetic trunk for several segments on the other hand, enables the sympathetic system to be involved in diffuse reactions affecting the entire organism (3; 89; 603; 682; 684; 814).
Not all preganglionic sympathetic fibres synapse in the paravertebral ganglia. Some traverse the paravertebral ganglia without interruption and continue (as the nervus splanchnicus) to the *prevertebral ganglia* related to the viscera (e.g. celiac and cranial and caudal mesenteric ganglia). Consequently, the splanchnic nerves are made up of preganglionic axons and thus correspond to (long) white rami communicantes (89; 116; 128; 603; 684; 814).
Splanchnic nerves originating from the thoracic spinal cord segments Th5 to Th10 (individual variations!) form the **greater (major) splanchnic nerve** (n. splanchnicus major). This nerve is often considerably thicker than the sympathetic trunk itself and in its thoracic course is either very closely associated with the sympathetic trunk or exchanges fibres with the interganglionic branches of the trunk (3; 89; 116; 128; 603; 684; 814). In most domestic animals the separation of the greater splanchnic nerve from the trunk is normally evident about the the sixth thoracic ganglion (see 814). The nerve then passes into the abdominal cavity and terminates mainly on postganglionic neurons in the adrenal plexus, and in the celiac and cranial mesenteric ganglia (plexuses) (3; 89; 116; 128; 603; 682; 684; 814).
Splanchnic nerves, originating from the lumbar spinal cord segments, may be arranged into two groups, cranial and caudal. The *cranial group* or **lesser splanchnic nerve** (n. splanchnicus minor) is primarily made

up of preganglionic fibres from the last two or three thoracic ganglia and the first lumbar ganglion (89; 116; 128; 603; 814; see 814). In cattle, sheep and goats even the second lumbar segment may contribute to the formation of this nerve (individual variations) (3; 682; 684; 814). The lesser splanchnic nerve is macroscopically represented by one to three delicate filaments exact terminations of which are difficult to see grossly. The available information is that the nerve terminates in the adrenal gland and possibly in the celiaco-mesenteric plexus (ganglia) (89; 116; 128; 603; 684). The *caudal group* consists of fibres from the more caudal lumbar region and conveys them to the caudal mesenteric ganglion and the small ganglia associated with the hypogastric plexus (128). In this way the caudal lumbar segments also contribute to the sympathetic innervation of the abdominal and pelvic viscera (814).

Autonomic Ganglia

The second key-elements of the efferent link in the visceral reflex arc are the autonomic ganglia. Indeed, the second visceral motor neuron of the "two neuron pathway" is stationed, with certain exceptions, outside the brain or spinal cord in autonomic ganglia which have a wide distribution in the visceral. In the autonomic ganglia the preganglionic fibres synapse with the, illogically referred, postganglionic neurons (3; 89; 116; 128; 603; 682; 814). It appears that the number of ganglion cells in a particular ganglion shows species differences and increases with body weight as does also the ratio preganglionic fibres/postganglionic neurons (89; see 89). The classical handbooks of histology describe the autonomic ganglia as generally similar to cerebro-spinal ganglia. There are, however, certain differences.

Autonomic ganglion cells are usually multipolar and have numerous and long dendrites making it often very difficult to identify the axons. The axon is frequently very thin and may either spring from the perikaryon or from one of the dendrites (89; 682).

Several cell types may be distinguished among the autonomic ganglion cells. Numerous authors have attempted to classify them according to their size, dendritic arborizations, arrangement of Nissl granules and other morphological criteria (see 89).

Based upon light microscopic studies some authors have stated that in autonomic ganglia the nerve cells form a true syncytium. This has been fully confirmed by electron microscopy. Indeed between ganglion cells an extremely intricate feltwork of fine fibres is present. These fibres are either partly dendrites and partly axons or collaterals from preganglionic fibres terminating in the ganglion or preganglionic fibres which pass through the ganglion without interruption (89). In general, the synaptic structures in the autonomic ganglia correspond to those in the central nervous system although the great majority of the synapses are axo-dendritic (see 89).

Depending on their anatomical position and pharmacological properties the autonomic ganglia are usually classified in two groups i.e. sympathetic and parasympathetic ganglia.

Sympathetic ganglia

The site of the synaptic junctions (ganglia) in the sympathetic system is subject to variations. This can be explained on the basis of their embryological development. Sympathetic cells originate from the neural crest later join the spinal nerve trunks and form the peripheral sympathetic ganglia. During this migration the sympathetic cells remain in contact with the spinal cord through the preganglionic fibres. Some cells comes to a halt lateral to the spine forming the paravertebral ganglia (see 89). The paravertebral ganglia are interconnected with each other by longitudinal and transverse (left-right) fibres forming in this way the paired ganglionated cords, the **sympathetic trunks** (truncus sympathicus) (3; 116; 814). Other cells pass the paravertebral ganglia and become assembled as prevertebral ganglia (the celiac, the cranial and caudal mesenteric ganglia) (see 89). Hence, the prevertebral ganglia (and plexuses) reflect the ventral outgrowth of the primordial sympathetic trunks along the lateral aspects of the aorta (603; 753). Furthermore, sympathetic ganglion cells are not confined to the ganglia proper since they are constantly found, as "intermediate" ganglia, in large or small numbers along the white and gray rami communicantes. This is obviously the case in the cervical and lumbar regions (see 89). In the splanchnic nerves, for example, large numbers of sympathetic cells (as many as 40.000) are scattered or in the form of small distinct ganglia (see 603). Finally, a considerable number of postganglionic cell bodies can even migrate past the prevertebral ganglia and spread out widely along the peripheral sympathetic nerve plexuses which innervate the viscera (603; see 603).

A primordium of a paravertebral ganglion is originally

laid down paramedially on each side for each segment of the spinal cord (3; 814) and divides into cranial and caudal portions. The ultimate fate of these portions determines the number and position of paravertebral ganglia at various segmental levels. As a rule the original pattern is preserved in the upper thoracic part of the trunk since the two portions may fuse again, forming a single true segmental ganglion. In the cervical, lumbar and sacro-coccygeal region, however, this paradigm is partly lost during development. In the lower thoracic region the caudal half of the former primordial mass may fuse with the cranial half of the mass next lower, resulting in a ganglion with connections to two spinal nerves. In the cervical and lumbar region finally several primordial masses may fuse, forming ganglia which connect to three or more spinal nerves (see 603). So, along the *cervical* sympathetic trunk there are usually three ganglia: the cranial, the middle (sometimes absent in the horse and pig (3; 682; 684) and the caudal cervical ganglion. The latter is furthermore frequently fused with the first and second thoracic ganglia to form the so called stellate ganglion (ganglion stellatum) (3; 89; 116; 128; 603; 682; 684; 814). Theoretically *lumbar* ganglia are present in each lumbar segment, though they are either frequently split up, making a wide variation in their total number, or are too diffuse to be identified grossly. The lumbar ganglia are usually interconnected to one another by doubled rami interganglionares (814). Finally, in the pelvic part of the trunk five (cattle), three (horse) ganglia or one paravertebral ganglion, corresponding to the respective sacral segments, occur (682; 684). From these ganglia stem the rami communicantes to the sacral spinal nerves, the branches to the plexus mesentericus caudalis and branches forming the plexus hypogastricus that accompany the pelvic arteries (684). In the coccygeal region both trunks fuse forming the "ganglion impar" (3; 89; 116; 128; 603; 682; 684; 814).
As a consequence of the above mentioned variations in the development it is not surprising to regularly find considerable individual modifications in number, size, location and extent of fusion of the sympathetic ganglia (89; 603).

Prevertebral (collateral) ganglia are found as irregular ganglionic masses in plexuses which supply the abdominal and pelvic viscera and surround the visceral branches of the aorta (3; 89; 116; 603; 682; 684; 814). The paired celiac and cranial mesenteric ganglia are the most prominent, while the phrenic, the aortico-renal and the caudal mesenteric ganglia are less developed. All these ganglia are closely interconnected by numerous nerve fibres, forming large networks, which serve as areas of redistribution for the autonomic motor and sensory fibres which enter into their formation (116; 684).
The paired *celiac ganglia* are round and lie close to the origin of the celiac artery (3; 684; 814). The celiac plexus extends along the branches of the abdominal aorta, forming plexuses with other ganglia (e.g.. the cranial and caudal mesenteric plexus) (89; 116; 128; 603; 684).
The apparently "single" *cranial mesenteric ganglion is* closely related to the cranial mesenteric artery (682; 814). The plexus mesentericus cranialis communicates on the one hand with the celiac plexus, forming the celiaco-mesenteric plexus, and on the other hand, through the plexus aorticus abdominalis, with the caudal mesenteric plexus (3; 682). In addition the greater splanchnic nerve and the dorsal vagal trunks furnish preganglionic fibres to the cranial mesenteric plexus (3; 89; 116; 128; 682; 684; 814). Thus, numerous mixed sympathetic/parasympathetic nerve fibres radiate, just like the rays of sunshine, from the ganglion. mesentericum craniale. Hence, its original anatomical name "plexus solaris" ("Sonnengeflecht"). Nerve fibres, radiating from this plexus, follows the homonymous artery to the small intestine, pancreas, caecum, colon ascendens, colon transversum and the onset of the colon descendens. The nerves fibres terminate in the intestinal plexuses (3; 682; 684; 814).
The *caudal mesenteric ganglion* and plexus are situated just caudal to the corresponding artery. The ganglia of the left and right sides are usually fused (682; 684; see 814). It must be stressed that the origin of the caudal mesenteric artery shows considerable variations and, consequently, the formation of the plexus differs substantially between specimens (814). The visceral branches of the caudal mesenteric plexus follow the terminal branches of the corresponding artery (the left colic and cranial rectal) for their peripheral distribution.(3; 814)
The *intermesenteric plexus* is that part of the abdominal aortic plexus that extends between the celiaco-mesenteric and the caudal mesenteric ganglia/plexuses (682; 684; 814).
The *hypogastric plexus* finally, is located in front of the last lumbar vertebra and the promontorium. It receives sympathetic fibres from the aortic plexus, the lumbar trunk ganglia, the ganglion and plexus mesentericus caudalis, the ganglia sacralia and the parasympathetic fibres from the pelvic nerves (128; 684).

Parasympathetic ganglia

Ganglia of the cranio-sacral division of the ANS are as a rule located close to, within the structures they innervate and, hence, are named peripheral, terminal ganglia. These ganglia show extreme variations in size and compactness. Some are organized into anatomically distinct, encapsulated structures (e.g. autonomic ganglia of the head), whilst others form extensive intramural plexuses (e.g. intestinal plexuses). Finally, small ganglionic masses or scattered cell groups are found within or near the walls of visceral structures (e.g. the heart, bronchi, pancreas and urinary bladder). Terminal ganglia contain parasympathetic postganglionic cell bodies (89; 116; 603; 814).

Histochemical studies have made it clear that there are important differences between the sympathetic and the parasympathetic ganglia. Using the Falck-Hillarp fluorescence method most of the cell bodies in the para- and prevertebral sympathetic ganglia, as well as their axons and terminal branches in the organs they supply, have been shown to be noradrenergic. However, a minor proportion of these ganglion cells are cholinergic. These cholinergic sympathetic fibres supply the sweat glands and act as vasodilators in skeletal muscle and the tongue. Whether in the parasympathetic ganglia all neurons are cholinergic (e.g. the ciliary ganglion) is not clear (89). More recent findings have, however, demonstrated a large spectrum of putative neurotransmitters/modulators in postganglionic neurons and their terminals, as well as a co-storage/co-release of different transmitters /modulators in/from the same nerve terminals (q.v.). There are thus morphological reasons to believe that the autonomic ganglia are not simple relay stations serving only the transmission of impulses from preganglionic to postganglionic neurons (see 89).

Postganglionic Efferent Fibres

As a rule the postganglionic fibres are unmyelinated. Thus, sympathetic fibres, having synapsed in the paravertebral ganglia, may return to and join the spinal nerves as gray rami communicantes. Along the spinal nerves they travel to the blood vessels (vasoconstriction), sweat glands (sweat secretion), arrectores pilorum (pilo-arrection) and probably also to blood vessels of the striated muscles and bone (3; 89; 116; 128; 603; 682; 684; 814).
As the parasympathetic ganglia are located close to or within the target organ the postganglionic parasympathetic fibres are very short.
Along their course and at their ends the peripheral postganglionic nerves show small swellings (varicosities) in apposition to the target structures. In some organs (e.g. vas deferens) there is close contact, while in other organs (uterus and the gastrointestinal tract) there is a "great" distance between the swellings and the target structure (muscle fibre) (see 89). The varicosities contain vesicles of different types. These include small, clear vesicles which are cholinergic; small, dense-cored vesicles which are noradrenergic; and large, dense-cored vesicles which are peptidergic. In addition the varicosities are characterized by numerous mitochondria and in several respects resemble the terminal boutons and boutons "en passage" seen in the central nervous system. The common occurrence of different types of vesicles in the same terminal indicates that the situation is complex (89).
Depending on the nature of the chemical mediator elaborated at the junction of the postganglionic nerve endings with the effector(s) two kinds of postganglionic neurons are distinguished in the autonomic ganglia. Some postganglionic nerve fibres are cholinergic, because they act upon their effectors through the mediator acetylcholine; others produce NA for the stimulation of their effectors (603). The fine terminal branches in the plexuses of several organs have been found to contain NA, indicating that they are derived from postganglionic sympathetic perikarya (see 89). NA-ergic terminals have often been found, using the fluorescence (Falck-Hillarp) technique, to establish synaptic contact with postganglionic parasympathetic neurons in the plexus of the intestine and it has been suggested that this arrangement may be the basis of the sympathetic inhibition of intestinal motility (see 89). In addition, in the alimentary tract of the guinea-pig the proportion of NA-ergic fibres is much greater in the muscular layer of the sphincter regions than elsewhere (see 89). Recently, the existence of non-adrenergic non-cholinergic neurons in the autonomic sympathetic ganglia has been established.
Postganglionic autonomic nerves are predominantly unifunctional, since they innervate only one type of effector (blood vessels, sweat glands, smooth muscle in skin or viscera). The existence of multifunctional postganglionic unmyelinated fibres that innervate via their collaterals different types of effectors has not been fully confirmed (603).

I. 2. 4.
FUNCTIONAL SIGNIFICANCE OF THE ANS.

From the fore-mentioned anatomical arrangements in the sympathetic and parasympathetic systems it may be argued that the autonomic functions of a rather extensive terminal area may be controlled by relatively few central connections, that a single primary efferent neuron may serve to discharge a number of ganglion cells and that the sympathetic system must be involved predominantly in diffuse reactions affecting the entire organism (e.g. cutaneous vessels), whereas the structural organization of the parasympathetic system permits more restricted, localized effects (89; 128; 814). These anatomic-functional observations are in accord with a number of physiological findings which show that, in general, the parasympathetic system has considerable possibilities for influencing local functions, while the sympathetic system, by way of its more diffuse distribution, tends to have widespread and general bodily effects (89; 814)

Besides the actions of parasympathetic nerves in the head region (pituitary; pupillary sphincter; lens accommodation; lacrimal secretion) the parasympathetic component controls also the activity of the digestive system. Parasympathetic fibres to the gastrointestinal tract are secretory and motor. Thus, stimulation of the vagus produces increased peristalsis, relaxation of the sphincters and secretion of gastric and intestinal juices. The gastric secretion and peristalsis which occur after physical, gustatory, and olfactory stimuli are mediated through the vagus (89). Sympathetic stimulation is followed by an increase in the tone of the sphincters and a reduced peristalsis and secretion. Thus the sympathetic influence on the digestive tube is, broadly speaking, inhibitory (89). Nevertheless, considerable secretory and motor activity of the stomach is retained even if both vagal and splanchnic nerves are cut and in time such activity tends to become even more normal. It is assumed that this remaining activity is mediated through the nerve cells and plexuses in the wall (89). However, morphology is not the only criterion to classify the ANS into a parasympathetic and a sympathetic component. This distinction can also be made on functional grounds (603; 814).

The significance of the parasympathetic component of the ANS is *anabolic*, because it is directed toward the preservation, accumulation and storage of energies in the body (89; 603).

The sympathetic component, on the other hand, is mainly brought into action in emergency states (stress situations such as physical exertion, fear, rage etc.) when the bodily reserves are drawn upon. The general effect of a sympathetic discharge is thus *catabolic*, since it causes the expenditure of bodily energies and inhibits the intake and assimilation of nutrient matter. Thus, in rapidly adjusting the internal organization for efficiency in meeting sudden external demands on the body the sympathetic system is of critical importance. Nevertheless, in heavy stress or emergency states in man it is well known that unfavorable autonomic reactions may occur (slowing heart rate, loss of control of the sphincters, loss of consciousness and eventually death) (89; 603).

Based upon these actions it has been generally believed that the two divisions of the ANS have antagonistic functions and, as a consequence, exert an antagonistic influence on their common targets. This principle is undoubtedly valid for the innervation of the gastrointestinal tract, but it is not an absolute rule. Both anatomical and physiological data have supported evidence that the alleged antagonism between the sympathetic and the parasympathetic system is far less absolute than originally assumed. The distinction is especially difficult in the higher levels of the autonomic centers. Furthermore, it is important to realize that both divisions of the autonomic system, in spite of their partly antagonistic effect on various functions, in most instances collaborate in an integrated manner and participate in the intricate regulation of visceral functions. In this way they ensure a proper adjustment and functioning of the various organs. This influence is not only limited to adjusting them with regard to each other, but with regard to somatic functions also. A proper homeostatic balance between the activity of the parasympathetic system (the accumulator of reserves) and that of the sympathetic (the spender of energies) system is therefore a must for the preservation of life. If the body spends too much it goes bankrupt. If it is too frugal, incapable or afraid to spend it will be overwhelmed by external influences (another organism, extreme temperature or an excess of material retained in its own body). Adequate spending, therefore, helps to free an individual from the restrictions of environment and upholds the constancy of its "milieu intérieur" (89; 603; see 603).

Chapter 3.
THE ENTERIC NERVOUS SYSTEM (ENS)

It is beyond the scope of this study to extensively review the ENS. The only objective of this chapter is to give the reader, on behalf of Parts II and III of this work, a general picture of the ontogeny, the morphology, the functions and the clinical significance of the ENS. Consequently very detailed information concerning the above-mentioned aspects have been omitted and for that purpose the reader can be referred to some excellent reviews e.g. 239.

I. 3. 1.
INTRODUCTION

Anatomically and functionally the most important component in the neural control of the gut is the ENS. This system is characterized by structural peculiarities, as well as by the uneven distribution of its nervous elements. It may as thus be defined as the intrinsic innervation of the gastrointestinal tract (see 239; see 516; see 758). The ENS is a complex integrative system of neurons and their supporting cells embedded in the wall of the gut where it innervates every structure i.e. the smooth muscle layers, the submucosa, the mucosa and the blood vessels (see 239; see 605). Extending from the smooth muscle part of the esophagus to the internal anal sphincter, it programmes, controls and coordinates (independently of the CNS) the various motility patterns, the secretion/absorption process and the (intestinal) blood flow (106; 339; 473; 616). Recently, it has been established that all these functions are carried out by means of a large spectrum of neurotransmitters/modulators (203; see 605).
The morphology of the system (i.e. the complexity of the enteric ganglia and the diversity of neurons and their terminals), its chemistry (i.e. abundance of putative and established neurotransmitters) and its functioning (i.e. the relative independence of CNS control and presence of complete reflex pathways) are all fundamental characteristics that enable us to consider the ENS as a unique part of the ANS and to distinguish it from the sympathetic and parasympathetic divisions (35; see 239; 246; 256; see 273; 275; see 605; 638).

I. 3. 2.
EMBRYOLOGY OF THE ENS

The development of the ENS as well as the sympathetic innervation of the gut entirely depends on a transitory embryonic structure: the neural crest (see 239; see 796). After an extensive migration in the developing embryo the crest cells differentiate into a variety of cell types i.e. the cells of bones, cartilages and dermis in the face, endocrine and para-endocrine elements, all the pigment cells of the body (except in the retina) and practically all the cells of the peripheral nervous system, including the neurons and supportive cells of the autonomic nervous system (see 130).
It has been found that the intrinsic innervation of the gut originates from precise levels of the neural axis, while other levels give rise to the sympathetic chains and the adrenal medulla (130; for references see 764; see chapter 1).
"Emigrés" from the vagal region of the neural crest (opposite to somites 1 to 7) provide the main contribution to the formation of the enteric ganglia since esophageal, gastric and upper intestinal plexuses fail to develop in chick embryos after removal of the hindbrain. This indicates therefore that neuroblasts, originating from that region, form these plexuses. In the chick, crest cells from the hindbrain (vagal region) migrate caudally along the gut and give rise to all intramural ganglion cells (myenteric and submucosal) in the preumbilical intestine (up to the cloacal end) and to most ganglion cells in the postumbilical intestine. Evidence indicates that the neural crest cells migrate along pathways rich in fibronectin. Likewise since the ganglion coli fail to develop after removal of the caudal end of the neural tube, this indicates that the lower part of the digestive tube is supplied with neuroblasts from the sacral cord. Thus, a supplementary, although minor, contribution to the nervous structures in the postumbilical gut is made by the lumbosacral level of the crest (caudal to the 28 th somite). Crest cells from this region give rise essentially to the parasympathetic ganglia of Remak.
Furthermore transplantation, before the migration of the neural crest cells starts, of quail neuraxis from

various levels into developing chicks indicated that while the neural crest from the vagal level populates the entire bowel, a second contribution of sacral neural crest cells also reaches the postumbilical gut.

In conclusion, extirpation as well as transplantation experiments have clearly demonstrated that the intrinsic innervation of the gut arises from two different levels of the neural axis and that the parasympathetic ganglia of the gut are cranio-sacral in origin as well as in function (34; 130; see 273; 284; see 284; 401; 459; 647; see 647 649). Based upon the foregoing findings it has been argued that parts of the neural crest primordium, located between somites 7 to 28, do not provide the developing gut with ganglion cells (130; 459; 551). However, transplantation of quail neural crest cells from different levels into the vagal region of chicks has established that enteric ganglia can also develop from heterotopic transplants (130; 273; see 273; 459). Furthermore, crest cells from the adrenomedullary region, grafted at the vagal level, colonized the gut and gave rise to functional cholinergic enteric ganglia. In the same way presumptive enteric ganglioblasts from the vagal region, transplanted into the adrenomedullary area, are found to populate the adrenal gland and differentiate into adrenomedullary cells (see 774). Moreover, cholinergic and serotoninergic neurons develop in cultures of explanted gut before the appearance of morphologically recognizable neurons. As a consequence the original explants must have contained precursor cells and the final phenotypic characteristics of the mature neurons are most probably acquired by the precursor cells within the microenvironment of the gut itself (284). Thus, it has been concluded therefore that the fate of the precursor cells of the ENS is not irreversibly determined in the neural crest itself. Most probably the microenvironment of their final destination may have a decisive role in determining the ultimate phenotype of the precursor cells. Preferential migratory pathways, rather than an intrinsic commitment or predestination, characterize the adrenomedullary as well as the vagal level of the neural axis. They lead the cells to differentiate into adrenomedullary cells and enteric ganglia respectively. Finally, neither an extraintestinal migratory experience of neural crest cells nor the presence of central preganglionic fibres.seems to be required for the neural crest cells to form neurons in general or enteric neurons in particular (130; 273; see 273;459). The repertoire of the crest cells that have migrated to the gut seems limited since those which reach the bowel produce only the neurons and supporting cells for the ENS and not the endocrine, connective tissue or pigment cells. The latter can be produced by cells originating from some regions of the neural crest. (see 273). However, a plasticity in the phenotypic expression has been found to be retained late in development even after the ganglion cells have already started their differentiation (459).

"The vagal" crest cells migrate in a cranio-caudal direction in the splanchnopleural wall of the gut (130; see 273). At 2 1/2 days they have been seen within the mesenchyme of the foregut, at about 5 days the front of the migration was observed at the level of the umbilicus, while the colorectum was not fully colonized before 8 days of incubation. Thus a proximo-distal gradient occurs in the appearance of neurons in the avian gut (130; see 273; 284; see 284). This is apparently not the case in mammals. Indeed, the developing mouse gut is colonized by a wave of precursor neurons (cholinergic and serotoninergic) by the time that the entire bowel is fully closed. Both foregut and hindgut appear to be colonized simultaneously or nearly simultaneously. Thus, in mammals no proximo-distal gradient in the colonization of the bowel by neuronal precursors could be demonstrated. In human embryos and foetuses the myenteric plexus is formed by neuroblasts, which were distributed to the alimentary tract by cranio-caudal migration during the 5th to the 12th week of gestation. The vagal trunks appear to play an important role in conveying neuroblasts from the CNS and in the entry of neuroblasts into the alimentary tract. The sympathetics and pelvic parasympathetics do not appear to participate directly in this process. The submucosal plexus is formed by neuroblasts migrating from the myenteric plexus into the submucosal layer (572). Furthermore neuronal precursor cells reside in the primordial mammalian gut for a much longer period of time than they do in the avian bowel before their phenotypical expression is evident: in chicks for 12 hours, in the mouse foregut 3 days and in the mouse hindgut 5 days (647; see 647; 649).

The mature ENS is characterized by a remarkable degree of neuronal abundance and diversity (245; 284). Indeed the ENS not only contains a great number of intrinsic neurons (about the same order of magnitude as in the spinal cord) but it contains many types of neurons since, in addition to cholinergic neurons and NA-ergic axons, intrinsic serotoninergic (278), peptidergic (681) and even other (246) neurons and fibres have been found. It is unknown how this number and diversity of neurons develop?

The generation of large quantities of enteric neurons

may be linked to the finding that enteric neuroblasts continue to proliferate even after they have begun to express phenotypic markers and neurotransmitter-related properties. Thus "émigrés" from the neural crest remain plastic for an extended period of time and whilst the microenvironment in the embryonic gut powerfully influences their phenotypic expression. NA-ergic cells of the developing sympathetic system, for instance, continue to divide after their NA-ergic nature has become demonstrable. In their continued capacity to divide neural crest cells seem to differ from cells that originate in the neural tube. Moreover, the post-mitotic cells can change for a period their neurotransmitter (273; 284; see 284). Although in chickens the initial expression of the phenotypic character of enteric ganglioblasts has been detected early in development (130; see 273; 284; see 284) there is a difference in time between colonization of the bowel and the phenotypic expression by the precursors. In addition, this expression seems to be inconstant in different regions of the gut. Likewise in mice, the entire gut is colonized by neuronal precursors very early in development. Thus, precursor cells reside in the bowel for a relative long and regionally variable period. As development proceeds the enteric microenvironment changes. Consequently, subsets of proliferating precursor cells mature at different times and are thus subjected to different microenvironmental signals at critical moments in their development. Because of the continued capacity of the crest cells to divide, it seems reasonable to suppose that the influence of the microenvironment is even greater while they are still dividing. Most probably therefore both the sequential changes in the enteric microenvironment and its interactions with the persistent pool of neuronal precursors are responsible for the sequential appearance of the various types of enteric neurons during ontogeny and, in consequence, for the neuronal diversity found in the mature intestine. Moreover, there appears to be only a single wave of precursors that migrate to the gut. The phenotypic expression therefore is most likely not a straightforward reflection of the temporal sequence in the arrival of precursors in given segments of the bowel. Finally, the sequential development of enteric neurons, which seems to be similar in a variety of animals, opens the possibility that early developing neurons themselves can critically change the enteric microenvironment and influence the later pattern of differentiation of the ENS (273; see 273; 284; see 284). Based upon these results it has been argued that the proximo-distal pattern of neuronal appearance results only from a property of the gastrointestinal tract itself i.e. a proximo-distal change in the microenvironment, provided by the non-neural elements of the developing bowel. This proximo-distal appearance is, however, to some extent species dependent since it is, for example, not seen in rabbits and rats (273; see 273; 284). It now seems clear that the somitic mesenchyme and perhaps other cell types or hormones influence both migration and neurochemical differentiation of the neural crest cells (see 107; 460). Hindgut mesenchyme is the only tissue able to stimulate ACh synthesis in mesencephalic crest cells, while somitic mesenchyme is the most effective stimulator of adrenergic differentiation in truncal crest cultures (130). Nerve growth factor (NGF) might also be involved since during limited periods it is produced by target organs that attract appropriate nerves along a chemical gradient (see 107). Other conditioning and growth factors include those arising from glial cells, Schwann cells and other cell types as well as circulating factors such as adrenal corticoids (107). Manipulation of the fluid environment has revealed the existence of a soluble factor mediating the conversion of an adrenergic to a cholinergic phenotype. During the switch in transmitter metabolism in culture, neurons with both adrenergic and cholinergic functions have been identified in single-cell cultures (see 130). Horse serum was found to stimulate ACh synthesis, while foetal calf serum preferentially enhanced CA production. Thus the chemical differentiation of the autonomic neuroblasts is highly dependent upon the environment in which they grow. It therefore strongly suggests that direct intercellular contact with non-neural tissues might not be essential for regulating neurotransmitters synthesis (130).

In summary, it may be said that the phenotypic expression of the enteric neurons occurs within the gut, that precursors remain exposed to the enteric microenvironment for considerable periods before expressing their phenotype, that the proximo-distal gradient in phenotypic expression most likely reflects proximo-distal changes in the bowel environment. These findings suggest that an interaction between neuronal precursor cells and the enteric microenvironment is of crucial importance in determining the ultimate nature of the enteric neuron. Furthermore, it indicates that everything required to give rise to any of the neurons of the ENS is contained within the gut wall (273; see 273).

Little is known about factors controlling the growth of cholinergic and of NANC nerves (e.g. peptidergic, purinergic and aminergic) but indirect evidence, largely

from culture studies, suggests that these factors exist (see 107). Neither the migration that the neural crest cells undergo to reach the gut, nor the influence of central nerve fibres seems to have a significant impact on the orientation towards a cholinergic metabolism. The gut mesenchyme is most probably solely responsible for cholinergic differentiation. Choline acetyltransferase (ChAT) and acetylcholinestrase (AChE), the marker enzymes for cholinergic neurons, are detectable soon after the appearance of enteric ganglioblasts in a given region of the gut (6 and 7 days of incubation in chick duodenum and hindgut respectively). They recapitulate the proximo-distal migration of enteric neuron precursor cells within the gut. So, it seems that cholinergic traits appear early and develop very rapidly in the intramural ganglia (130). An early cholinergic mechanism has likewise been demonstrated in other parasympathetic ganglia and, in sympathetic and sensory ganglia. Recent observations finally, have indicated the presence of AChE in crest cells from all axial levels before and during their migration. Thus, a cholinergic mechanism appears to be a general characteristic of differentiating neuroblasts (130; see 130)

In contrast, catecholamine-containing cells have never been detected in associations of aneural intestinal segments with primordia from the vagal and trunk level. Furthermore, it was recently demonstrated that such an association showed NANC inhibitory activity in addition to the expected cholinergic excitatory responses and that crest cells, migrating into the gut, never expressed the adrenergic phenotype. Thus gut mesenchyme is probably unable to provide appropriate conditions for the adrenergic phenotypic expression (see 130). However, neural crest cells cultured in the virtual absence of other embryonic structures achieve a certain degree of biochemical differentiation characterized by the synthesis of ACh and CA. These findings confirm the bipotentiality of the crest cell population along the neural axis. Moreover, clones of neural crest cells can give rise to daughter cells expressing the phenotypes either of melanocytes or neurons. Thus some of the premigratory crest cells are pluripotent or at least bipotent. Furthermore, neuraxial precursor cells will differentiate into cholinergic neurons or intrinsic inhibitory neurons when implanted directly into explants of an aneural gut (see 130; see 273). Thus, it seems probable that neural crest precursors of the ENS have limited their options to neuronal or glial cell development by the time they have completed their migration to the bowel (273). Nevertheless, cells that can synthesize catecholamines have been found very early in the developing gut of the fetal mouse (days E10-E13) or rat (days E11-E15) but not in birds. Their morphology, location and time of appearance strongly suggests their neural crest origin. In contrast, in the bowel of the adult mouse or rat no catecholaminergic cells were observed. Thus, the appearance of adrenergic cells is most probably transient (130; see 130). These transitory cells were found to exhibit a high affinity uptake-mechanism for NA. Interestingly, cells with a similar transmitter uptake system were demonstrated in the gut at later stages, suggesting that the disappearance of the catecholaminergic cells was not due to their death but more likely to the loss of some of their adrenergic traits (see 130). The existence of a population of cells, expressing transiently a variety of adrenergic characters, further suggests that the mammalian gut lacks the appropriate stimulation for the maintenance of the CA neuron. As such the initial appearance and persistence of the adrenergic phenotype are regulated by different environmental factors (130; see 130; 284). It now seems clear that a stimulus produced by trunk axial tissues i. e. the notochord, neural tube and by the somitic mesenchyme is of decisive importance in the initiation of this process (130; 459). Association of the notochord with a primitive gut explant results in the appearance of groups of catecholaminergic cells along the developing circular muscle layer, suggesting a direct relationship between the occurrence of CA-containing cells and the amount of notochordal material. The fundamental role of the notochord is further demonstrated by its ability to promote the appearance of an adrenergic phenotype in an ectopic environment such as the gut mesenchyme (130).

The uptake of ^{3}H-5 hydroxytryptamine, demonstrating the presence of enteric serotoninergic neurons, cannot be observed in the chick duodenum before days 8-9 of incubation. Hence, the ontogeny of cholinergic neurons precedes that of 5-HT nerve cells (see 130). Both type of neurons develop before the gut receives its adrenergic innervation. Peptidergic neurons have been reported to develop even later (452; 453; 727). This pattern seems to be general as it is found in the chick (191; 192; 286), the mouse (see 284), the guinea-pig (284) and the rabbit (see 284; 291).

Peptidergic neurons and nerve fibres are intrinsic to the gut since they develop in cultures of gut of mammals (see 130) and since the distribution of VIP-ergic neurons is unchanged 1 to 4 months after jejunal autotransplantation in the pig. However, a small population of VIP-immunoreactive fibres has been identified in the vagus in man and other species (see 188; see neuro-

chemistry). As compared to the controls a greater number of VIP- and Sub. P -immunoreactive neurons appear in grafted guts. Consequently, the lack of a central innervation for enteric ganglia is most likely to be responsible for this accumulation. Moreover, the important number of peptide-containing neurons in the gut further reinforces the idea that cholinergic and peptidergic functions may coexist in certain neurons (see 647). Precursors of peptidergic neurons have been found to colonize the bowel prior to the appearance of recognizable neurons of any type, though the expression of peptidergic traits in enteric neurons occurs much later than that of the cholinergic metabolism (see 130; 647). In quail and chick embryos Sub. P and VIP immunoreactive fibres are first visible in the foregut and at 12 days they extent over the whole length of the gut (130). In mammals they appear in the gastroduodenal region prior to their appearance in the colon, while some investigators found no Sub. P -immunoreactivity in the mouse colon until 4 days after birth (see 647; 727). Thus, despite the absence of a proximo-distal pattern in the colonization of the mammalian bowel by neuronal precursors, peptidergic neurons, just like cholinergic and serotoninergic, display a rostro-caudal pattern in their phenotypic expression. Although this sequence in the appearance of Sub. P and VIP could reflect an intrinsic difference in the neural crest émigrés reaching the gastrointestinal tract it most probably reflects a similar pattern of maturation of non-neuronal elements in the gut wall. Furthermore, it supports the idea of an interaction between the enteric neuronal precursors and the intestinal microenvironment by which neuronal phenotypic expression is modulated. These observations may further indicate that the mammalian bowel is colonized by a single wave of precursor cells that also contains the primordia of peptidergic neurons (130; 647).

In conclusion, it seems likely that the initial set of neural crest émigrés, colonizing the gut, is a multipotential one and contains the primordia of cholinergic, serotoninergic and peptidergic neurons. The developing gut maintains an immature proliferating pool of neuronal precursors that may tentatively and transiently (cfr. catecholaminergic expression) express a given neuronal phenotype while still being part of an immature and proliferating population of cells (647).

Despite some insight in the process of generation of numerous enteric neurons and the formation of diverse neuronal types the question remains how synapses are established between appropriate neurons within the enteric plexuses and how the number of synaptic contacts between them are regulated?
The way in which synaptic contacts between appropriate partners are achieved, remains largely unsolved (34). Nevertheless, the survival of preganglionic neurons most probably depends on the presence of their postganglionic opponent (see 107).
The relationship between pre- and postsynaptic neuronal populations and the synaptic contacts between them is better known. Indeed, the population of neurons has been found to be well-matched to the capacity of the target structures innervated. Neurons are overproduced during development and compete for survival in early embryonic life. Presynaptic neurons are dependent on some activity of their targets. If, for example, an increase in target size is achieved experimentally, the survival and size of the innervating neurons is increased. Conversely, artificially decreasing the target size increases neuronal death. Evidence has also been found that a trophic factor is produced by the targets for which innervating neurons compete during development. Thus it may be argued that the number of neurons is adjusted to the functional demands and depends upon feedback mechanisms (34).

Neurons only express neurofilament proteins. Thus, expression of this protein is an early sign of commitment to the neuronal lineage and, as non-neural cells do not express the protein, it is also an excellent neuronal marker. It seems plausible that the commitment to a neuronal lineage by neural crest cells occurs before or during their migration to the gut since, at least in chicken, they already appear to produce this protein when colonizing the gut. The neuronal phenotype is, on the contrary, not determined when the "émigrés" arrive in the bowel (vide supra; 273; see 273). In the guinea-pig small intestine primitive neurons can be recognized by their occurrence as closely packed aggregates, surrounding a number of neurites and growth cones that constitute an early neuropil. After formation of the first neural islands, the circular muscle develops and divides the enteric mesenchyme into an inner (submucosal) and an outer (myenteric) compartment. The neural islands remain in the outer compartment and form the early myenteric plexus. At first there is an extensive interdigitation between the developing myenteric plexus and the growing smooth muscle cells around it. Then the neural tissue becomes ensheathed by processes of its supporting cells and by a basal lamina. As a consequence the nerve-smooth muscle interdigitation is lost. Many varicosities of terminal axons, however, pro-

trude through the sheath and retain at least one uncovered surface exposed to the connective tissue space that separates nerve and muscle. These surfaces may be points of transmitter release for neural control of the smooth muscle activity (284). Auerbach's plexus is surrounded by mesenchyme, but initially not covered by the longitudinal muscle. The submucosal plexus is then formed by a migration of cells from the myenteric plexus through the circular muscle layer (273; see 273; 284).

In summary, neurons and supportive cells of the enteric ganglia stem from precise levels of the neural crest. Crest cells from these regions migrate in a single wave to the gut and, in the chick, travel caudally along the gut leading to a proximo-distal gradient in the appearance of neurons. It seems likely that multipotential neural crest émigrés colonize the gut and reside there for a considerable time (chick 1/2 day, mammals several days) before they express their neuronal phenotype. They continue to divide even after they express their phenotype. In this way large quantities of enteric neurons are formed.

The intestinal microenvironment is of fundamental importance for the ultimate neurotransmitter phenotypic expression of the enteric ganglioblasts since they undergo a prolonged period of maturation during which changes in the intestinal microenvironment occur. Thus, proliferating precursor cells are subjected to different microenvironmental influences at the time they ultimately mature. In consequence, the sequential changes in the enteric microenvironment together with the persistence of a pool of relatively uncommitted neuronal precursors are most probably responsible for the diversity of the enteric neurons found in the adult species. The sequential appearance of enteric neurons is, moreover, consistent with this hypothesis. Gut and somitic mesenchyme, NGF, factors from glial cells, Schwann cells and other cell types, circulating factors, the composition of the culture medium etc. are all found to be, at least partially, involved in the migration and neurochemical differentiation of the crest cells. Until now little is know about the environmental factors controlling the growth of cholinergic and NANC nerves.

Despite the absence of a proximo-distal pattern in the colonization of the mammalian gut by neuronal precursor cells, a proximo-distal pattern in the phenotypic expression of the cholinergic, serotoninergic and NANC neurons has been found. This seems most probably related to a similar pattern in the "maturation" of the intestinal microenvironment. Crest cells, synthesizing catecholamines, are found very early in the developing mammalian gut (mouse, rat) but their appearance is transient. Stimuli produced by the notochord and neural tube are of decisive importance in the initiation of this process.

At least in the chicken enteric neuronal precursor cells produce neurofilament protein when they colonize the gut. So it seems probably that commitment to a neuronal lineage by crest cells occurs before or during their migration to the primitive gut.

I. 3. 3.
MORPHOLOGY OF THE ENS

Microscopic anatomy

Meissner in 1857, Billroth in 1858 and Auerbach in 1862 and 1864 respectively made detailed examinations of the submucous, mucous and muscle layers of the gut and discovered nerve plexuses, which were in many respects similar to neural elements elsewhere in the autonomic nervous system. Each plexus in the submucous and muscle layers of the gut was shown to contain aggregations of nerve cells (ganglia), linked by bundles of nerve fibres (interganglionic bundles, internodal strands) some of which were continuous with similar bundles of fibres in the mesentery of the gut (35; 148; 246; see 246; 275; 473; see 573; 673; 674; see 674; 741; 745; 758; see 758). After these first detailed descriptions it was gradually realized that, at least in mammals, the enteric plexuses, when compared to other autonomic ganglia, constituted a system of enormous complexity, since they comprise vast numbers of neurons (several millions) which were associated with many more glial cells (395). Additionally it was established that both the (ultra)structural and neurophysiological characteristics of the enteric ganglia resembled the CNS more closely than was the case for any other autonomic ganglion outside the alimentary tract (see 239; 261; 339; 516; 797; 799).

The two principal plexuses of the gut are the plexus myentericus (Auerbach) and plexus submucosus (Meissner). Each is made up of ganglion cells, interconnecting nerve fibres as well as several subsidiary groupings of fibres supplying the target organs i. e. smooth muscles, mucosa and blood vessels. The submucosal plexus lies within the irregularly arranged connective tissue of the submucosa, whereas the myenteric plexus is characteristically found between the circular and longitudinal smooth muscle layers. Ganglia form, together with their interconnecting nerve bundles, a continuous meshwork, the ganglia lying at the nodes of the meshwork. Hence the interganglionic nerve bundles are sometimes described as internodal strands. Synaptic connections are essentially formed between the ganglion cells and with the sympathetic and parasympathetic nervous systems, the vagal nerve in particular. Thus, axons of both parasympathetic and sympathetic origin, as well as the peripheral processes of sensory neurons, enter the plexuses and form connections with the intrinsic neurons. However, the majority of the axons in the intestine are of intrinsic origin (35; 148; see 239; 246; see 246; 275; 473; see 573; 638; 673; 674; see 674; 741; 745; 758; see 758).

The arrangement of the enteric plexuses has been examined in a wide variety of species (for references see 239; 473; 673; 758) and it has been found to follow an essentially similar pattern throughout the digestive tract. However, in different regions and/or species there are clear differences in the degree of development of the different parts of the plexuses and, going from the serosal to the mucosal side in a transverse section, in the size and shape of the ganglia (35; 246; see 473; see 573; 674). In the esophagus of the rat and cat, for example, no submucosal ganglia have been observed, while the submucosal plexus mainly consists of medium-sized nerve bundles. Finer nerves leave the parent bundles at varying depths and independently passed towards the mucous membrane where their terminal branches entered the basal layer of the epithelium (126; 674; 758). In the opossum, on the contrary, several ganglia have been observed in the submucosa (126).

The transmural topography, the typical form of the nervous network, and the proportional distribution of the nerve cells and their fibres are the classical morphological criteria used to subdivide the intramural nervous system into different plexuses (see 239; 546; see 546; 758). Starting from the serosa these are:

the plexus subserosus
the plexus myentericus (Auerbach's plexus)
the plexus muscularis
the plexus submucosus (Meissner's plexus)
and the plexus mucosus

These different plexuses are in fact joined by numerous nerve bundles composed of axons from intrinsic neurons as well as from extrinsic neurons (see 239). These plexuses will be discussed below.

The ENS contains a variety of nerve types distinguishable by their different morphological, histochemical, biochemical, pharmacological, electrical and functional characteristics (246). Morphologically they are classified into large (2-4 μm), medium-sized (1-2 μm) and small fibres (less than 1 μm). Fibres arising from enteric neurons (intrinsic fibres) are either small or medium-sized, while large nerves are extrinsic in origin. Some of them arise in spinal ganglia and terminate in the mucous membrane of the stomach and intestines. Many fibres in the enteric plexuses are irregular in out-

line. Large fibres are usually wavy throughout their length, while some of the medium-sized fibres are slightly varicose at irregular intervals (674). Most nerve fibres, originating from the subserous and myenteric plexuses and running to the submucosal plexus, travelled in fasciculi which accompany blood vessels. The fibres then usually pursue an independent course through the submucosal plexus. Medium-sized and small nerve bundles continue with the blood vessels into the mucous membrane. The pattern of their branching is usually similar to that of the vessels they accompany (638; 674).

Plexus Subserosus

The morphology of this plexus has been mentioned in only a few studies. Consequently, available information about its morphology is rather scanty.
Schabadasch described the subserous plexus as a plexus of fine bundles found in the connective tissue layer between the serosal mesothelium and the external muscle layer of the gut (see 239). These nerve bundles are the connections between the extrinsic nerves and the nerves of the deeper layers of the gut wall. Small ganglia were sometimes noticed, particularly on the surface of the stomach and rectum and near the attachment of the mesentery (see 239).
In the guinea-pig the subserosus plexus is found lying on the surface of the longitudinal muscle layer. The plexus in this species itself consists of a two layered dense network of fine unmyelinated fibres, connecting extrinsic nerves with the intrinsic plexuses. Few or no ganglia were identified (246; 546).

Plexus Myentericus
(Auerbach's plexus)

Auerbach's plexus or the myenteric plexus may be defined as a ganglionated nerve plexus, confined to the space between the circular and longitudinal muscle layers. Principally, it supplies the smooth muscle fibres of the muscular coat (see 239; 256; 382; 546; 758; see 758). The plexus appears in the embryo on the outer surface of the circular muscle layer before the longitudinal layer differentiates. The latter layer develops at a later stage and covers both the plexus and the circular muscle (256). As a consequence the plexus is, except in esophagus and stomach, adherent to the circular muscle layer (382).

In the connective tissue, separating the longitudinal and circular smooth muscle layer, the neurons of Auerbach's plexus are aggregated at irregular intervals into ganglia lying at the nodes of the nervous network. Because of this position the ganglia are greatly affected by the mechanical activity in the muscles and are therefore firmly anchored to the surrounding stroma. Indeed, ganglia most probably undergo enormous variations in size and shape during intestinal contractions. Hence the changeable shape of the myenteric ganglia is very likely correlated with the degree of contraction of the surrounding musculature. In a fully distended intestine, for example, the ganglia are spread out and can measure about 15 µm in thickness. Hence, the neurons are flattened and arranged into a single layer. Conversely, in the maximally contracted intestine the ganglia become 2-3 times thicker and, as a consequence, the neurons lie side by side and tend to form a palisade. These changes are probably also mirrored by equally extensive changes in the shape of the individual ganglion neurons. Whether or not this "message", produced by muscular contractions, affects the neuronal activity in the myenteric neurons remains unknown. Nevertheless, a comparison of some features such as frequency and thickness must take into account this deformation of the myenteric ganglia. As the submucosal ganglia lie in the loosely arranged submucosa, these above-mentioned changes are most probably less pronounced (see 239; 256; see 256; 261).
Passing from one ganglionic mass to another thick are (15-30 µm) nerve bundles, forming a rather open two-dimensional network. This nervous network extends without interruption, even in the transition zones between the different parts of the digestive tract, from the esophagus to the terminal part of the large intestine and has a characteristic pattern in each portion of the gut. Interganglionic trunks are, for the most part, made up of axons of intrinsic cells, although some extrinsic nerve fibres also enter into their formation (sympathetic fibres enter the plexus along with the blood vessels) (256; see 473; 546; 701; 758; see 758). However, many of the interganglionic nerve fibres do not enter the ganglia but pass through the ganglion to continue into another interganglionic bundle (see 239). Finer nerve strands, forming a secondary plexus, branch off from the interganglionic bundles and run, parallel to the circular muscle layer, between the primary plexus and the muscle. Furthermore, from the ganglia, the interganglionic bundles and the secondary nerve strands finer nerve bundles originate and form a tertiary plexus within the meshes of the primary plexus (see 239).

The shape of the meshwork, the size of the ganglia and the neuronal density per ganglion vary considerably from one species to another in the same part of the alimentary tract, from one part of the gut to another within any species, from the mesenteric to the antimesenteric side within a single intestinal segment, and from birth to maturity. The appearance of the plexus even depends on the orientation of the section (see 239; 258; see 473; 673; 758).

the meshwork

In spite of the of the considerable variations in the shape of the myenteric meshwork this is quite characteristic and readily identified in any one area from a particular species (see 239).
In the esophagus of the rat the main nerve bundles are thin (5-10 axons) while in the intestine Auerbach's plexus is principally characterized by thicker (15-30 axons) internodal strands from which finer nerve bundles split off. The thick nerve bundles form a wide-meshed (1000 x 1500 μm) rectangular network which with the exception of the esophagus, shows no notable differences along the gastrointestinal tract. From the thick bundles finer bundles (the finest contain 1-5 axons) split of and form a narrow-meshed (50 x 100 μm) network situated within the meshes of the former network (758). In this way secondary and tertiary nerve bundles and plexuses are formed. From the tertiary plexus, and to a lesser extent from the primary and secondary plexuses, very fine nerve bundles enter the adjacent tissue or pass with blood vessels inwards to the submucosa. The tertiary bundles consist of numerous small neurites enveloped singly or in groups by Schwann cells (see 239; 637; 758; see 758).
The interganglionic fibre bundles in the guinea-pig small intestine are short and nearly all arise at right angles from the ganglia. No variations in the plexus has been observed at the mesenteric attachment (382; 517).
In the rectum the bundles are very broad and frequently contain nerve cells (382). The width of the myenteric meshwork decreases gradually from the cardia to the pylorus, where the smallest meshes are found. Then it increases again distally towards the caecum, where the meshes reach their maximum dimensions.
On either side of the ileo-colic sphincter the number of neurons and fibres increase considerably and, consequently, the plexus becomes more dense. Finally, running from the colon to the rectum the meshes become smaller again (319; 546).
In the haustral regions of the rabbit's caecum Auerbach's plexus consists of a wide-meshed network. The meshes (11 to 21/ cm^2) have the shape of elongated triangles or irregular rectangles. Their size is much smaller in the region of the taeniae. In the colon the meshes show an uneven distribution and in the haustral region they are mainly rhomboid with their longitudinal axis almost perpendicular to the main axis of the gut. In the taenial region the plexus consists of narrow and dense oval-shaped meshes.
Finally in the rectum, the meshes are characterized by great variations in shape and size and by their large number as compared with the other regions of the intestine (from 72 to 81/cm^2). The internodal strands on the other hand are thinner (10-25 μm) (516).
From the caecum onwards to the colon descendens of the horse a gradual increase in the density of Auerbach's plexus has been observed. In the transitional segments (ostium caeco-colicum, flexura pelvina, colon transversum) the network is always densest (786; 787).

the ganglia

The surface of the ganglia is normally ensheathed by small spindle shaped cells (fibrocytes) and by collagen, reticular and elastin fibres. Thus ganglia became isolated from the surrounding connective tissue and from the musculature (56; 758; see 758). However, myenteric ganglia are not always well individualized. Where they are large and numerous (e. g. in the proximal colon of the guinea-pig) they form a sort of fenestrated neuronal sheet. Conversely, they can be small and scattered along nerve strands running parallel to the circular musculature (cfr rat). Moreover, single neurons have regularly been seen in the internodal strands (256; see 473; 546; 758).
Auerbach's ganglia shows notable variations in size and shape along the gastrointestinal tract within the same species and between species.
In the rat and guinea-pig the esophageal ganglia are small as compared to other portions of the gut (517; 758). At the cardiac orifice they are sparse, small and elongated with very loosely arranged nerve cells (382; 517; 758). In the midgastric region they gradually increase in number and size and become more compact. Likewise the number of nerve fibres gradually increases in the interganglionic tracts. From the mid-gastric zone onwards the ganglia change little in shape, though the nerve cell count per ganglion increases reaching a

maximum at the level of the pylorus (382; 517; 758). In the small intestine of the rat Auerbach's ganglia are more numerous and larger than in the stomach (758). In the guinea-pig the plexus is characteristic but, does not vary in essence from the duodenum down to the ileo-caecal junction. In the upper part of the duodenum few differences compared with the pyloric region have been observed, though the ganglia have fewer nerve cells. In the middle third of the duodenum the ganglia are somewhat elongated, while in the lower third the ganglia are much longer and narrower. In the rest of the small intestine the ganglia are very narrow, long (frequently 3 mm) and lie parallel to the circular muscle fibres. Principally, the nerve cells are arranged in such a way that their long axes are parallel to the long axis of the ganglia (382; 517). The number of ganglia is significantly smaller in the ileum (1.2/mm^2) as compared to the large intestine i. e. the caecum (2.21/mm^2) and colon (2.06/mm^2). No significant difference between the last-mentioned segments have been found (728).
In the small intestine of the sheep Auerbach's ganglia are larger and further apart from one another than in the mouse (813).
On either side of the ileo-caecal sphincter of the guinea-pig the myenteric plexus became more dense and a special type of association of neurons (multicellular glomeruli) has regularly been found. Glomeruli occur singly and usually at the intersections of the fibre tracts and comprised groups of about 6-12 neurons. The nerve cell nuclei are arranged towards the perimeter of the group and an intimate intermingling of the cell processes was seen (319; 382). This sort of association has also been observed in the feline colon close to the ileo-colic sphincter and at the distal end of the ileum in the pig where glomeruli may occur with similar frequency in young and old animals. Glomeruli have so far not been observed in the sheep, although close associations of two to three neurons, in which a part of one neuron actually becomes embedded in the other, have been demonstrated. In the sheep the ganglia are small and the myenteric plexus comparatively delicate (319).
In the large intestine of mammals and man Auerbach's plexus consists of irregularly shaped accumulations of large nerve cells (56). In the rat the ganglia are small and elongated (758).
In caecum of the guinea-pig compact spherical ganglia, like those at the level of the pylorus are connected by long interganglionic fibres. Beneath the taeniae the ganglia are large, circular , lie close together and are interconnected by short thick nerve strands. In the intertaenial area the ganglia are smaller and lie further apart, being joined by fairly thin interganglionic bundles (382). In the caecum of the rabbit the ganglia have numerous processes and the nerve strands are thicker than in the higher regions of the intestine (516). In the colon of the guinea-pig the ganglia are compact, irregular in size, lie close together and do not have any polarity. They are joined by thick interganglionic bundles. Ganglia, underlying the taeniae, are larger and contain more cells than in the intertaenial areas, though this difference is not as marked as in the caecum (382). In the colon of the rabbit the ganglia have a stellate form with usually 4-6 processes (516). In the feline colon the composition of Auerbach's plexus is not very different from that of the ileum except for the fact that the fibre tracts are thicker. As there is no evidence for an increased number of intrinsic neurons this increase in thickness of the interconnecting fibres tracts is most probably due to the presence of extrinsic fibres (postganglionic sympathetic fibres) (319). In the large intestine of the pig Auerbach's plexus is fairly dense with large ganglia. Their cellular composition is in marked contrast to the ganglia of the small intestine and most neurons appear to be unipolar or to have very few processes. In the sheep, finally, there is a striking difference between the colon and the small intestine. In the colon (a wide intestinal segment with fluid contents) the plexus is very dense with many neurons and thick interganglionic fibre tracts (319).
The rectum of the guinea-pig represents a striking picture. The ganglia here are large with the nerve cells loosely arranged. The plexus can be traced to the level of the internal sphincter, where it ends abruptly by sending efferent fibres to the muscle (382). In rabbits the ganglia are large, have numerous processes and a variable shape (516). Finally, in the rectal portion of the human gastrointestinal tract the myenteric plexus is very rich both in ganglia and fibres (35).

neuronal density per ganglion

The density of nerve cell bodies in the myenteric plexus varies from one part of the digestive tract to another, from the mesenteric to the antimesenteric zones in the same segment and from one species to another (highest in mouse, intermediate in guinea-pig, lowest in sheep). The technique which is used to study the neurons is also a significant factor (see 239; 258; 673; 813).
Although the total number of neurons is somewhat related to the total volume of the intestinal musculature neurons generally seem more densely packed in smaller animals (e.g. the mouse) than in larger species

(e. g. sheep and man) (see 256; 813). Furthermore, in each of the major subdivisions of the gut the number of neurons in Auerbach's plexus is greatly in excess of that in the submucosal plexus (see 239; see review 258; 473; 517; 674). Moreover, the data available are discrepant. Indeed, some authors claimed that in the guinea-pig small intestine the myenteric neurons outnumber those of the submucosal plexus by about 7 times, whereas others found a ratio of 2/3. In contrast, still other investigators have maintained that in the feline small intestine the submucosal neurons outnumber Auerbach's perikarya 3 to 1, while in the guinea-pig a ratio of 1.75/1 was found (239, see 239; 462).
In most areas and species the density of neurons ranges from about 1000 to about 20 000 neurons/cm^2 (see 239; see review 258; 473; 517; 674). Low values have been found in the esophagus and proximal stomach, intermediate values in the small intestine and high values in the distal stomach and large intestine (see 239). The vast neuronal population is heterogeneous in all respects (see 256). Using different techniques (methylene blue; toluidine blue; silver impregnation; tetrazolium etc.) several authors have quantified the myenteric neurons in different species. The results of these studies are summarized in the addendum (see Addendum/ Part I/ table 2). From these studies it may be concluded that:

- In most species the myenteric plexus holds several millions of neurons e.g. guinea-pig 2,75 x 10^6; sheep 31,5 x10^6 (see 239; 813).
- The mesenteric edge contains, as a rule, many more nerve cells than the antimesenteric zone (see table).
- In the guinea-pig the estimated average number of nerve cells/cm^2 of the myenteric plexus is remarkably uniform from duodenum to ileo-caecal junction (382; see 665). In the rabbit, on the contrary, the number of nerve cells decreases progressively towards the caecum (1760/cm^2), where the lowest values have been noted. The number of nerve cells then gradually increases in the colon (3375/cm^2) and decreases again in the rectum (2940/cm^2). A similar regular arrangement has been observed in the intestinal tract of monkeys (516; see 516).
- A loss of enteric ganglion cells probably occurs after birth. Indeed, young rats have more myenteric ganglion cells than older animals suggesting that myenteric ganglion cells may be lost with advancing age (see 796).

microarchitecture of the myenteric neurons

Enteric neurons are morphologically different from neurons in other autonomic ganglia and many studies have classified them on the basis of their staining properties and/or the morphology of their nucleoli, nuclei and processes (see 239; 258; see 473; see 673; see 758).
After injection of horseradish peroxidase (HRP) into the mesenteric nerve trunks, labelled nerve cells have been observed in both the myenteric and submucosal plexuses. The majority of the labelled neurons were medium-sized and multipolar with their long axis randomly orientated. These neurons were morphologically different from the unlabeled ones and they have synapses on their surfaces. Consequently, it is supposed that they collect information from other nerve cells in the vicinity. This information enters the local intramural circuitry and/or is then transported centripetally to higher centers (212).
In contrast with other autonomic ganglia the range of neuronal sizes in the myenteric plexus is extremely wide. In the alimentary tract of the rat, for example, the maximal surface area of the myenteric neurons varies from about 50 μm^2 in the small intestine to more than 975 μm^2 in the caecum (260; 261; see 261; 473). In the myenteric plexus of the guinea-pig the neuronal sizes vary characteristically along the length of the gut. Indeed, neurons on average are the largest in the stomach and duodenum, smallest in the ileum, and intermediate size in the colon and rectum. In general, the highest values were found in those regions where the musculature is thickest. Furthermore, it was discovered that the average neuronal size, the percentage volume of the neuropil and the relative number of glial cells ranks in the same order as the body weight of the species. The number of neurons per unit surface of intestine, on the contrary, ranks in the opposite order (261).
Depending on their size the enteric neurons have been divided in two main classes: i.e. large and small neurons. It has been hypothesized that the large neurons are adult and that the small ones are young (319; see 319; 758; see 758). In the intestine of the frog small neurons were found to be evenly distributed throughout the myenteric plexus but the larger ones were, almost without exception, distributed along the line of mesenteric attachment (319; see 319). In the rat smaller neurons are more abundant in the small intestine, while a wider range of sizes and more large neurons are found in the stomach and distal colon. The very large neurons are a

characteristic of the caecum (see 256). In the cat small and large neurons occur and are intimately mixed in the ganglia throughout the myenteric plexus. In the sheep and goat, finally, the clear distinction between both types is apparent, although the large neurons are less numerous (ganglia are often entirely composed of small neurons) . As in the cat, neurons of different size but similar type are often found in association with each other. In all the animals studied, small neurons have been found in all parts of the intestine (319; see 319).
On the basis of the nuclear morphology two types of cell are recognized in the ganglia. The first type are cells with medium-sized, round to void (sometimes triangular) nuclei. They are scattered among bundles of unmyelinated nerve fibres. Their karyoplasm appears as a network of fine granular material, partly condensed at the site of the nuclear envelope. Such nuclei are believed to belong to Schwann cells. The second cell type are nerve cell bodies characterized by a large, light nucleus with one, or often two, prominent nucleoli and by a homogeneously dark stained cytoplasm (56).
Enteric ganglion cells give off various processes that branch many times and principally terminate on other neurons. Thus, one nerve cell can communicate with many others (701).
The number and appearance of the neuronal processes represent a classical criterion to classify the enteric neurons. Dogiel (in 1896,1899) was the first to classify the neurons of the ENS into three different types (I, II, III). In contrast to the CNS, many autonomic nerve cells are multiaxonal, though bipolar and monopolar forms are also found. Whether a different morphology denotes a different function is as yet not fully elucidated (see 239; see 473; 701; see 707; see 709; see 711; 716; see 758).
Dogiel's type I cells have a stellate or angular shape. They have many (4-20 or more) short, irregular, flat dendrites (dendritic lamellae) and one long slender axon that can be followed into the internodal strands for up to four ganglia. These multidendritic (short-dendritic), uniaxonal neurons are, as a rule, organized in aggregates essentially in the peripheral and cranial parts of the ganglia. They receive synaptic nicotinic inputs from other neurons. Most axons run cranially forming a type specific ascending tract (711). Since the axons go to intestinal muscle Dogiel believed that these cells were motor neurons (see 239; 319; see 473). Recent studies, using an intracellular labelling-technique have demonstrated that in the guinea-pig Dogiel type I neurons in the myenteric plexus of the small intestine could be further subdivided into Ia and Ib neurons. Type Ia neurons have 6-11 short, broad processes which give off secondary processes. The short processes of type Ib neurons are mushroom-shaped and do not give rise to secondary processes. Both types have a single long process. In addition, a considerable chemical overlap of peptides has been found in the Dogiel type I neurons (239; see 239).
Dogiel's type II neurons are angular, star or spindle shaped, have a small number (3-10) of long processes and a short axon with branches within the ganglion of origin. However, some axons can be traced to other ganglia (see 239; 319; see 473). Type II neurons do not have prominent nicotinic inputs and, moreover, do not have nicotinic receptors for ACh. As the structure of all the processes of the Dogiel type II neurons strongly resembles the structure of an axon, type II neurons are defined as adendritic and multiaxonal. In the small intestine of pigs type II-cells are principally concentrated in aggregates of the periphery and outside the ganglia and can send their processes to the submucosal/subglandular layer. Thus, type II cells are thought to be sensory neurons (see 473). Contrary to the multidendritic, uniaxonal neurons (type I, III, IV and V), type II-axons form circular routes (besides vertical ones) in the secondary branches of Auerbach's plexus. They can be traced up to the intramuscular plexus and in consequence, type II-cells have been considered as adendritic, multiaxonal efferent neurons which it was argued may be associative (710; see 710). However, it has also been claimed that the intestinal mechanoreceptors correspond on the whole to the location of Dogiel type I cells, while chemoreceptors correspond to the arrangement of Dogiel type II cells (348; 349).
Dogiel's type III cells are multidendritic (2-10), uniaxonal neurons. The dendrites end in the ganglion of origin and are relatively short (intermediate length) compared with Dogiel's type II neurons. They are generally concentrated in aggregates that are localized in the central and aboral parts of the ganglia and form type-specific descending routes in the myenteric plexus. The majority of the axons terminate in the same or adjacent ganglia and do not appear to provide a major supply to the muscle. Recently, it was demonstrated that type III neurons have a strong NPY immunoreactivity (see 239; 319; 473; see 473; 516; 553; 707; 711).
Although most authors agreed that enteric ganglion cells have different morphologies and that cells, similar to Dogiel types I and II neurons, have been recognized in all animals studied so far, still other enteric nerve cell types have been described (see 239; 473). However, one has to realize that the shape of a neuron is undoubt-

edly influenced by its position in the ganglion (see above). Indeed, ganglia are deformable and change their overall shape with intestinal movements and, in consequence, are changed the relative dimensions of the enteric neurons. In addition, perikarya at the periphery of a ganglion tend to have an other profile at their outward surface compared with that on the surface that faces the central part of the ganglion (239; see 239).

Stach and his co-workers have studied the enteric plexuses (mainly guinea-pig and pig) in more detail. They revealed, on the basis of the light microscope, the existence of complementary neuronal types, classified as types IV, V and VI (706; 707; 708; 709; 715; 716; 815). Type IV cells are, according to Stach, polar, multidendritic, uniaxonal neurons with extremely eccentric nuclei. They are organized in aggregates and are topographically related to the communicating branches between the myenteric and submucosal plexus. Axons of the type IV cells run through these branches forming in this way type-specific vertical routes within the ENS (708). Type V neurons were often found in pairs and have some very long dendrites and aborally orientated axons. Type VI neurons are axo-dendritic cells that are normally localized in the aboral part of the ganglion. Their axon course orally (815).

Recently, some physiological correlates of Dogiel's classification have been found. The neurophysiological type 1/S and type 2/AH myenteric neurons (see the section on the functional significance of the ENS) showed clear morphological differences. Many type 1/S-neurons have a large soma and are provided with many short thick processes giving the soma a coarse silhouette. S-cells were seldom uni- or bipolar (see 239; 349). Thus, the majority of the S-cells have a Dogiel type I morphology, although some may refer to Dogiel type III. Moreover, it was discovered that the S/Dogiel type I neurons were immunoreactive for VIP and enkephalin (see 239). The morphology of the type 2/AH-neurons is quite variable. Being mainly multipolar, the soma of AH-cells is either smooth or endowed with fine, short processes and some (usually three) relatively long processes. These processes are mainly circumferentially disposed, run within the ganglia and are frequently branched, smooth or varicose (see 239; 348; 349). On these morphological grounds most AH cells appear to be Dogiel type II neurons (see 239). As might be expected considerable variations in the relative proportions of the different cell types were observed between the plexuses, between the regions of the gastrointestinal tract and between species (473; 516). Type I neurons were reported to predominate in the esophagus and stomach, both types I and II were seen in the small intestine (though most investigators claim that here type II neurons predominate), while in the large intestine both types were present in about equal numbers. As a general rule large ganglia contain all recognizable nerve fibres and cell types (see 239). In the guinea-pig each ganglion normally contains several type I cells, which are usually found on the outer edges of the ganglion. In the pig's small intestine, type I cells are present in large numbers. The proportion of type I/type II cells is far higher than in the cat, in which type I cells are present but difficult to find. Moreover, some authors have claimed that in the cat typical type I neurons are absent (see 239). In the myenteric plexus of the sheep and goat Dogiel's types I and II neurons are both present. The high number of small type I cells in the duodenum gives way to a high proportion of small type II cells in more distal regions of the small intestine (319).

Recent investigations, however, have proved that the enteric neurons can be divided into many more types than originally stated. Nowadays, about ten or more distinct neuronal types were distinguished on morphological, histochemical, biochemical, electrical and pharmacological grounds as well as on the basis of their modes of action (35; 246; 473; see 473). But, until now, it seems not possible to determine the functional significance of the enteric neurons from morphological studies only (674). However, the orientation of the processes, arising from the soma, might give a clue as to the extent of the receptive field of an afferent fibre or of the influence of efferent neurons. The vast majority of the enteric processes seems to run circumferentially not longitudinally and are intraganglionic. The soma morphology, the length and orientation of smooth and varicose processes are varied and complex (349). Intracellular injection of a fluorescent dye or HRP injections reveal that the neurons from the myenteric plexus of the guinea-pig have one to seven processes which usually arise from the poles of the oval soma (212; 563). These studies allow the axonal projections of individual neurons to be followed and have shown that there are at least twice as many aborally-directed processes as orally-directed processes (see 438).

Electrophysiologically, the occurrence of long, interganglionic soma processes has been reported. The fact that twice as many of these processes was found to leave the ganglia aborally than orally is in keeping with the electrophysiological observations that most long synaptic pathways in the myenteric plexus are aborally directed (see 348). Pathways from the ganglia project

up and down the intestine within the plexus to the circular muscle layer (including the deep muscular plexus) and, where it is innervated, to the longitudinal muscle. Other projections go to the submucosal plexus (246). Finally, some neurons send their axon(s) away from the intestine to enter the mesenteric nerve bundles and run towards the CNS. Some of them form synapses in the prevertebral ganglia. Hence, these neurons correspond to the intestinal afferent nerve cells (212; see 246; 673).

In conclusion, *Auerbach's plexus, a continuous nervous network running uninterruptedly from the esophagus to the internal anal sphincter, is confined to the connective tissue layer separating the longitudinal and circular muscle layer. Due to this topography myenteric ganglia most probably undergo considerable variations in size and shape during intestinal contractions. This is clearly reflected in the appearance of the myenteric ganglia and neurons. The shape of the meshwork, the dimensions of the meshes, the size of the ganglia, the neuronal density as well as the neuronal morphology show considerable variations depending on the species, the topographical localization in the gut and the age of the animal.*

Generally speaking, the plexus seems better developed in the sphincter regions. Neurons are more densely packed in the smaller species, the number of neurons/unit surface area of the intestine is inversely related to the body weight while the relative number of glial cells, the percentage volume of neuropil and the average neuronal size is directly related to the body weight. In addition, the neuronal size has been found to correlate with the thickness of the intestinal musculature.

The morphology of the perikarya and their processes is often used to classify the myenteric neurons into different types (Dogiel's type I, II and III and most likely others). Intracellular injection techniques have clearly shown that the majority of the neuronal processes in the myenteric plexus are aborally directed. It now seems clear that different neuronal types are distributed among the enteric ganglia. Thus, no ganglion can be regarded as motor, sensory or associative but as a microcosm of the ENS with the potential to contain nervous elements that are representative for the region in which the ganglion is found.

Physiological support for Dogiel's classifications have been found.

The majority of Dogiel type I neurons are neurophysiologically classified as type 1/S while Dogiel type II neurons are referred to as type 2/AH neurons. In addition, the Dogiel type I neurons showed immunoreactivity for VIP and ENK.

Plexus Muscularis

Subsidiary aggregations of nerve fibres, without ganglion cells, are found within the circular and longitudinal smooth muscle layers, as a deep plexus in the submucosa resting on the circular muscle, within the muscularis mucosae and within the loose areolar connective tissue of the lamina propria (275; see 758). The density and distribution of the nerve fibres in and between the muscle bundles varies both along the length of the gut and across the transverse plane of its wall (61; 716).

The longitudinal muscle layer is sparsely innervated (148), although cholinergic nerves seem more densely concentrated in the longitudinal layer than in the circular muscle (see 384). Fine nerve bundles run parallel to the muscle fibres. In addition, nerves, passing between the mesentery and the enteric plexuses, run through the outer muscle layer near the mesenteric attachment. Apart from these, there are few or no fibres in the longitudinal muscle layer of small mammals such as the rat or the guinea-pig. In cases where the longitudinal muscle is thicker (taeniae coli, rectum) small axon bundles run parallel to the muscle (246). In the large intestine of the cat, on the contrary, the longitudinal muscle layer seems as densely innervated as the circular layer (716; see 716). In addition, axons of the tertiary component of the myenteric plexus lie on the surface of the myenteric plexus and face towards the outer muscle layer. They may, likewise, be regarded as part of the innervation of the longitudinal muscle layer (see 239).

The circular muscle layer receives, as a rule, the densest innervation (see 239; 716; 758). However, in rat the plexus is, at the pyloric level and on the inner surface of the longitudinal muscle layer in particular, not as strongly developed as in the other gastric regions. The densest intramuscular plexus has been found in the duodenum while in the large intestine the morphology of the plexus strongly resembles that of the stomach (758). In a wide variety of the species that have been examined small bundles of 1 to 5 axons have been seen to branch off from the myenteric plexus and penetrate both muscle layers where they run parallel to the muscle cells. These bundles were normally interconnected by many fine obliquely running nerve bundles (see 239; 758; see 758). Such nerve bundles have been found throughout the full thickness of the circular muscle. Furthermore, nerve bundles, connecting the myenteric and submucosal plexuses, transverse this layer in a similar manner. Some axons, especially in the small intestine, leave these bundles and pass obliquely through the circular muscle and become part of the deep muscular plexus (plexus muscularis profundus) first discovered by Cajal (148; see 239; 246; 758). This plexus lies adjacent to the inner (mucosal) aspect of the circular muscle and contains few or no ganglia consisting mainly of numerous small anastomosing nerve bundles. These bundles run parallel to the muscle cells. In this manner a thin layer of smooth muscle cells is separated from the rest of the circular muscle layer (see 239; 246). In the rat' s intestine delicate nerve bundles have been seen linking the deep muscular plexus with the submucosal plexus (758). The function of the deep muscular plexus is not known, but as it axons have the same origin and the same neurochemical spectrum as the outer axons in the circular muscle layer, it may be postulated that the circular muscle layer is innervated in an asymmetrical way (compare the arterial innervation) (see 239).

Small (3-5), medium (20-40) and large (about 70 axons) nerve bundles have been found in the connective tissue bands of the circular muscle underlying the taenia coli. Only small nerve bundles were found towards the serosal surface, while medium and small nerves enter the muscle bundles. The large nerve bundles are more or less completely surrounded by the processes of fibroblasts, whereas the smallest nerve bundles frequently do not have any investing connective tissue cell sheath. In transverse sections the nerve bundles have been found to be enclosed partially or completely by the processes of Schwann cells. The axons are arranged within the Schwann sheath either as groups of 2-30 fibres or as individual fibres which are usually situated on the periphery of the bundle (61).

In conclusion, *nerve fibres, but no nerve cells, are found in the smooth muscle layers of the gut. In all species investigated the circular muscle layer receives as a rule the densest innervation throughout the gastrointestinal tract. The longitudinal muscle layer and the muscularis mucosae are sparsely innervated except in the large intestine of the cat where neither the circular layer nor the longitudinal layer show any differences in the density of their nervous network .*

Plexus Submucosus
(Meissner's plexus)

Embedded in the connective tissue between the circular muscle layer and the mucosa, lie ganglia and their interconnecting nerve bundles forming, in a similar way as the myenteric plexus, a nervous network called the submucosal plexus. This plexus is continuous around the circumference of the gut and along its length (see 239; 246; 319; 343; see 473; 758; see 758). Meissner's plexus differs, just like the myenteric plexus, in form and extent in the different parts of the gut and in the different species.

In the small intestine of the cat and the opossum, for example, the plexus is characterized by finer meshes and more and smaller ganglia than the myenteric plexus. In other segments of the gut ganglia are sparse, small and very irregularly distributed (126). Since the meshes of the submucosal plexus are more irregular and, as a rule, smaller than those of the myenteric plexus and since the ganglia are smaller, are distributed at various levels, hold fewer cells in total (mean 8 neurons/ganglion in the guinea-pig small intestine) and contain neurons that widely vary in size and character, the submucosal plexus seems in its entirety less regular and structurally more complex than the myenteric plexus (126; see 239; 246; 319; see 473). In addition, in rodents and rabbits the submucosal plexus is arranged in tiers with fasciculi linking ganglia throughout the thickness of the submucosa (674).
Several authors have subdivided the submucosal plexus, according to the species (cat, pig, monkey) they studied, into two or three subplexuses, i.e. the plexus of Henle (plexus entericus internus), the intermediate plexus and the plexus of Meissner (246; see 246; 319; 674; see 674). In the pig it is possible to pull apart two fibrous sheets that lie closely apposed in the submucosa. Each of them contains a nervous plexus quite different in character from the other. The sheet lying close against the inner side of the circular muscle contains Henle's plexus, the other sheet contains Meissner's plexus (319). In most parts of the small intestine Henle's plexus is poorly developed, thin, very irregular and wider-meshed than the rest of the submucosal plexus. In contrast, there is a great development of this plexus in the region of the ileo-colic sphincter and in the distal part of the rectum in the different species studied (mouse, rat, hamster, guinea-pig, rabbit, cat, pig, sheep, goat, monkey). In these regions the plexus even seems as prominent as the myenteric plexus both with regard to the fibre tracts and the number of ganglion cells. The large ganglia contain large and small neurons. This notable development of Henle's plexus in the sphincteric regions must probably mean that the plexus is very much concerned with the function of the sphincter and this would further suggest that it is concerned in the control of the circular muscle in general. In the feline colon the appearance of this plexus seems not very different from that of the small intestine. Finally, an almost complete absence of Henle's plexus has been noted in the colon of herbivores (319; see 319).

However, in the different parts of the intestine these subplexuses are often so intimately interconnected that the distinction is difficult to justify. In the cat and opossum a distinct between Henle's and Meissner plexus is even impossible (126). Moreover, in their recent and extensive study of the ENS Furness and Costa (1987) clearly proved that the confusing subdivision of the submucosal plexus into the plexus entericus internus of Henle, the plexus submucosus of Meissner, the plexus submucosus externus (Schabadasch), the plexus submucosus internus (Meissner) can be carried back to a historical misinterpretation of Henle's work by Schabadasch and to the subsequent adaptation of Schabadasch's terminology by many workers (see 239). Therefore, it is suggested that the nomenclature of all the separate components of the submucosal plexus is abandoned and that the term "submucosal plexus" or "Meissner's plexus" is used.
Only in some cases a division into outer and inner components might be justified (see 239). Furthermore, there is as yet no reason to distinguish between these subplexuses on a functional principle (246; see 246; 319; 674; see 674).

In different species that have been studied considerable variations in the number of ganglia/cm^2 were found along the gastrointestinal tract.
In the rat (see 239; 758) and cat (126; see 239) no ganglia were observed in the esophageal submucosa. In the opossum, where abundant submucosal glands were found in the esophagus, 4.5 to 9.7 ganglia/cm^2 were counted (126). In other species i.e. cat, dog and man esophageal ganglia are extremely rare (see 239; 715).

In the rat duodenum the submucosa is characterized by an increasing number of ganglia, while in the caecum and large intestine numerous polygonal ganglia have been observed (758).

In the feline stomach the submucosa has few ganglia (2.2 to 7.1/cm^2). This number increases drastically in the small intestine (90.4 to 188.7) where the submucosa has more (and smaller) ganglia than the myenteric plexus. In the large intestine, finally, the number decreases gradually (17 to 41.4) to a low number (10.5 ganglia/cm^2) in the rectum. In opossum's gut a comparable arrangement was found (126).

In all species examined so far the submucosal ganglia have, compared with the corresponding myenteric ganglia, a smaller percentage volume of neuropil, a much smaller number of glial cells and (except in the mouse ileum) smaller neurons. In addition, a positive correlation between the size of neurons and the size of the glial cells has been demonstrated. Consequently, (except in mice) glial cells in the myenteric ganglia are larger compared with those of the submucosal plexus (261).

Henle's and Meissner's plexus are quite different as regards the weave-pattern of their fibres, the appearance of their ganglia and the character of the ganglion cells they contain (319). In the small intestine of the pig Henle's plexus shows a typical architecture i. e. thin fibre tracts and small, irregular and sparsely distributed ganglia. The ganglia are further characterized by their typical form and size and contain a remarkable small number of neurons that are, in addition, loosely arranged. Based upon these characteristics Henle's plexus can be defined as the most irregular plexus of the swine intestine (319; see 319; 714).

In Meissner's plexus the meshes are much smaller and of a more regular pattern than in Henle's plexus. The ganglia are compact and numerous (319).

The intermediate plexus is usually found on the inner fibrous sheet of the submucosa. Coiled fibre bundles join both the intermediate plexus and Henle's plexus. The ganglia, being compact and enclosed in a very prominent capsule, are present in certain regions of the gut only. They have a characteristic appearance and are associated with compact and convoluted fibre tracts. In the pig, for example, they are very prominent in the region proximal to the ileo-colic sphincter (319).

In rodents (mice, rat, guinea-pig) several of the medium-sized submucosal nerve fibres terminate in the mucosal membrane. Some fibres branch repeatedly in the submucosal plexus and again shortly after piercing the muscularis mucosae. Others form coils of varying complexity and still others follow, for considerable distances, a course parallel to the muscularis mucosae before branching. The mucosal branches of the fibres were often traced for considerable distances in the subglandular and interglandular connective tissue. In cats and primates most of the submucosal ganglia were found adjacent to the circular muscle coat. Many of the fasciculi in both groups of animals contain nerve fibres which traverse the submucosal plexus obliquely and branch as they passed towards the mucous membrane (674). In the rat intestine two categories of submucosal nerve bundles have been established :
Firstly, there are bundles (5-10 axons) that run parallel to the longitudinal axis of the alimentary canal. They form a wide-meshed nervous network extending throughout the whole submucosa. At the border with the tunica muscularis fine nerve bundles split off and form a narrow-meshed network that furnishes small nerve bundles to the circular muscle layer. An identical delicate nervous network is formed at the boundary of the tunica mucosa. From this network nerve fibres spread out into the lamina muscularis mucosae.
Secondly, bundles (1-3 axons) in the adventitia of the blood vessels form a perivascular plexus. Numerous nerve fibres interconnect both the wide-meshed and the perivascular plexus (758).

In the guinea-pig small intestine nerve bundles, linking the submucosal ganglia together, are much finer than the myenteric interganglionic bundles (246). As in the myenteric plexus, submucosal ganglia and their internodal strands contain processes of glial cells and neurons (intrinsic and extrinsic) (473). Connections can be traced from the submucosal plexus to the myenteric plexus, to the mucosal plexus and also to paravascular nerves following the submucosal arteries (246). Nerve fibres, running from the submucosal plexus to the mucosa, most likely arise from submucosal afferent neurons. Some authors further postulated that nerve fibres, linking the myenteric and submucosal plexus, are processes from submucosal neurons (674; see 674).

As studied by the histochemical method for AChE, the density of Meissner's plexus in the horse seems fairly uniform along the entire large intestine. In addition, the submucosal plexus has been found to be less dense than the myenteric plexus (786; 787).

The neuronal density of the submucosal plexus has been studied infrequently and only in a few parts of the gut and in a few species. The results of the available lit-

erature on this subject are summarized in table 3 (see Addendum/ Part I/ table 3). From these studies it may be concluded that:

- In the mouse the submucosal neuron density decreased gradually along the length of the small intestine (813).
- Contrary to the general rule, the submucosal plexus in the cat small intestine holds more than twice as many ganglion cells as the myenteric plexus. There are over five million myenteric cells and two to three times this number of submucosal neurons. With the possible exception of the proximal duodenum, the distribution of the cells is fairly uniform with no evidence of a gradient within the small intestine (665).
- In the small intestine of the sheep the neuronal spatial density of Meissner's plexus was about 90% higher as compared to Auerbach's plexus (813).
- In the rat (758), cat and opossum (126) the esophageal submucosa is devoid of nerve cell bodies.
- In the stomach of the rat, cat and opossum the neuronal density in Meissner's plexus is considerable lower than in the small intestine where the highest neuronal density of the gut is found (126; 758).

In the cat and opossum a gradual decrease in the nerve cell density has been seen along the large intestine (126).

In the submucosal ganglia neurons are frequently arranged in the form of a capsule about a core of mainly small nerve fibres that are continuous with fasciculi entering the mucosa (674; 718; 758). In the guinea-pig the submucosal neurons show little variation in average size along the length of the gut. They are in general smaller and more homogeneous in size than those of the corresponding myenteric ganglia (261; see 473). The average neuronal size in the ileum was greatest in the sheep and smallest in the mouse with intermediate values in the guinea-pig and rabbit (261). The majority of the submucosal ganglia contain pseudounipolar or bipolar neurons, a morphology characteristic of primary afferent cells (see 275; 673).

The great majority of the submucosal neurons were usually classified as type II neurons, although Furness et al. (1985) classified the neurons in Meissner's plexus of the guinea-pig small intestine as type III neurons (see 239). In the pig small intestine Dogiel's type I and II neurons were observed in Henle's plexus, where they form aggregations in the same or in adjacent ganglia. However, a high proportion of type I neurons in Henle's plexus (as is in Auerbach' s) was normally seen (319). Type I cell aggregates have unequivocally a manifold better vascularization than the type II neurons. Processes of the uni- or bipolar type II cells reach the plexus of Meissner and to some extent the plexus muscularis profundus. Axons of the type I neurons essentially ramify in the plexus itself, though some of them reach the myenteric plexus (713; see 713; 714; see 714). In the cat, pig and sheep Henle's ganglion cells are of the same type as those of Auerbach's plexus. In sheep they are small, multipolar and loosely distributed in the ganglia, while those of Meissner's plexus are unipolar and arranged compactly. In the cat the neurons of Henle's plexus are type II neurons both in the small and large intestines (319).

The ganglion cells of Meissner's plexus are quite different from those in Henle's plexus. They are all small (diameter +/-25 µm), uni- or bipolar, globular or pear-shaped with an eccentrically-placed nucleus. In many unipolar cells the axon divides not far from the cell body into two branches suggesting that they are probably pseudounipolar cells. These cells are different from the unipolar cells that are normally classified as Dogiel's type II neurons. In fact, they closely resemble the neurons of the dorsal root ganglia (319).
In the small ganglia of the intermediate plexus all the neurons are most probably unipolar. In appearance the cells are very different from Auerbach's and Henle's neurons i.e. they are quite unlike Dogiel's types I, II and III, but they resemble the neurons of Meissner's plexus. Consequently, it has been argued that the intermediate plexus may be a modified form of Meissner's plexus (319).

Arteries and arterioles in the gut wall have two sets of accompanying nerves i.e. a perivascular plexus (supplying the arterial innervation) and paravascular nerves which follow the arteries and arterioles within the gut wall and in the mesentery. There is a particularly profuse network of arterioles in the submucosa. Thus in sections through this layer the vessels are associated with many nerves. In the sphincter regions the capillaries are very numerous and are characterized by the densest innervation of all gastrointestinal vessels (see 716). There is much evidence from the literature that the vascular smooth muscle is innervated by NA-ergic nerve fibres (255; 262; 466; see 716) and NANC nerves (see neurochemistry; see functional significance). Very few nerves seem to supply veins or lymphatics within the gut wall (246), though such nerve fibres were clearly seen in the duodenal mucosa of rat, cat and dog (716; 717).

In conclusion, *the plexus submucosus is confined to the space between the mucosa and the circular muscle layer. As compared to the myenteric plexus the meshes are irregular and larger, the ganglia smaller, the neuronal density lower (cat excepted). In addition the plexus differs in form and extent in the different parts of the gut and between the different species. In some species, two subplexuses (Henle's and Meissner's), which can be physically separated from each other, have been recognized. The outer plexus (Henle's) appears to be a motor plexus as it contains neurons of the same type as those found in the myenteric plexus. The inner plexus (Meissner's plexus) is (probably exclusively) composed of small unipolar (pseudounipolar) or bipolar neurons. They appear to be sensory as they closely resemble the small neurons found in cerebro-spinal ganglia. An "intermediate" plexus seems to be present only in certain regions (sphincters) of the gut. As the ganglion cells resemble the nerve cells of Meissner's plexus the intermediate plexus may be considered as a modified Meissner's plexus . New insights, however, indicate that this distinction in submucosal subplexuses is morphologically as well as functionally difficult to justify .*

Plexus Mucosus

The mucosal plexus consists of fine nerve bundles and axons. Small groups of enteric mucosal neurons were reported by some investigators in the stomach and in the small and large intestines. Earlier authors, however, claimed that these ganglia are ectopic submucosal ganglia (see 239). A subglandular plexus (lying in the lamina propria adjacent to the muscularis mucosae), a periglandular (interglandular) plexus, an intravillous plexus and a subepithelial plexus represent the morphological components of the mucosal plexus in the small intestine (see 239; 246; 695) As nerve bundles pass through the submucosa up to the mucosa, few fine nerves run in the muscularis mucosae parallel to the long axes of the smooth muscle cells (see 239; 246). In the guinea-pig nerve fibres penetrate this muscle layer to innervate blood vessels and secretory glands of the mucosa. Nerves, innervating the blood vessels, form a rich mesh surrounding the vascular wall (the perivascular plexus) (148).Most mucosal fibres arise from neurons within the gut wall, mainly in the submucosal plexus, though some stem from neurons situated outside the gut including those in the vagi and prevertebral and spinal ganglia.(see 239; 246; 695).
In the esophagus of the rat no nerve fibres have been observed in the tunica mucosa (758). In rodents the esophageal submucosa is devoid of ganglia. The submucosal plexus consists mainly of medium-sized nerve bundles from which finer nerve bundles branch off and independently passed towards the mucosa (674). In different species (rat, hamster, guinea-pig, rabbit, cat, dog and pig) relatively dense nerve networks are found in the tunica mucosa. They are more pronounced in the stomach and small intestine than in the large intestine (630; 716; see 716; 717).

In the non-glandular part of the rodent stomach both the submucosal and mucosal plexuses are similar in appearance to those seen in the esophagus. In the glandular part of the stomach and in the stomach of the other animals examined the larger and smaller submucosal fasciculi were joined by processes originating from neurons in the submucosal ganglia. Individual medium-sized nerve fibres form coils of varying complexity in the submucosa and enter, after piercing the muscularis mucosae, the mucous membrane (674). As a rule the densest plexus has been found in duodenal villi (630; 617; see 617; 717). In all the animals studied so far, nerve fibres branch off from Meissner's plexus, from the perivascular plexuses and from the plexus

within the lamina muscularis mucosae. They penetrate the mucosa and build up a very dense network around the glands and within the intestinal villi. Thus, each tubular gland becomes, mainly in its lower and upper third part, surrounded by a nervous network. In general the crypts of Lieberkühn are surrounded by a denser plexus than the Brunner's glands. It was found that most of the axons in the mucosa were cholinergic, although adrenergic and NANC axons also take part in the innervation of the glands. All these findings provide a morphological basis on which to speculate on the influence of this nervous network and on the different functions of the intestinal mucosa (718; 758; see neurochemistry and functional significance of the ENS).
The colonic mucous membrane of the mouse receives an extensive autonomic innervation. Nerve fibres enter the mucous membrane through the muscularis mucosae and ramify in the lamina propria where they were often seen in close proximity to smooth muscle cells. A close membrane to membrane relationship is seen between some nerve fibres and smooth muscle cells. The nerve fibres entering into this type of relationship may perform a motor function. Other fibres may be sensory, while still others accompany blood vessels or passing along the basal aspect of the epithelium subjacent to the basement membrane (695). In the proximal two-thirds of the rodent large intestine the arrangement of the submucosal and mucosal plexus is nearly similar to that of the small intestine except for the facts that medium-sized fibres form coils, that there are appreciably fewer neurons in the submucosal plexus and that large nerve fibres have also been seen in the submucosal layer (674).

Until now, no nerve fibres have been demonstrated in the gut epithelium of any species studied. However, the distance between the epithelial cells and free nerve endings has sometimes found to be so small that the existence of a neuro-cellular complex may be hypothesized. Most probably these complexes represent the morphological substrata for the innervation of the intestinal epithelium (603; 630; 674; 716; see 716; 717; 718; 719). Furthermore, in the colon of the mouse a single basal process of an argentaffin cell has been seen to come into intimate relationship with a bundle of unmyelinated nerve fibres (673; 695), while ultrastructural studies have demonstrated the presence of bundles of non-myelinated nerve fibres close to the basal lamina of enterochromaffin cells in the guinea-pig duodenum (490). Finally, in a human fetus (5 months) direct contacts between non-myelinated nerves and basal-granulated cells have been likewise observed. This type of neuroendocrine complex is apparently quite similar to that seen in the chemoreceptor complex of the carotid body and in the neuro-insular complex in the pancreas (577; see 577).

In conclusion, *the plexus mucosus is a neuron-free nervous network built up by delicate nerve fibres the majority of which arise from the submucosal plexus. Subglandular, periglandular and intravillous parts can be recognized. The appearance of the plexus varies along the gastrointestinal tract but as a rule the densest network is found in the intestinal villi.*
Though no nerve fibres have been ever seen in the epithelium, "direct" contacts between free nerve endings and epithelial cells, the basal granulated enterochromaffin cells in particular, have been observed in different species. In this way intramural nerve fibres can influence the release from the entero-endocrine cells.

Ultrastructure

The ultrastructural characteristics of the enteric ganglia are unique and differ in many respects considerably from that of the other autonomic ganglia. Thus, enteric ganglia more closely resemble the neural tissue of the CNS and they share, both in vertebrates and invertebrates, with the CNS a morphological organization that is common to systems with intrinsic integrative capability. This is not shared with other autonomic ganglia (135; 136; see 239; 284; 287; 516; 798; 799).
The following ultrastructural peculiarities have been observed in the enteric ganglia:

- Numerous neurons and a remarkable diversity of neuronal types.
- Numerous glial elements.
- A dense synaptic neuropil with different terminals and extensive synaptic connections.
- Densely packed neural and glial elements leading to the virtual absence of an intraganglionic extracellular space.
- The absence of endoneural and perineural sheaths.
- The absence of connective tissue.
- The absence of blood vessels (at least in some species) and isolation (basal lamina) from the surrounding tissue, creating a physical barrier (56; 132; see 239; 273; see 273; 275; see 275; 339; 345; 346; 347; 395; 473; see 473; 573; see 573; 691; see 796; 798; see 798; 799)

numerous neurons and a remarkable diversity of neuronal types

Perhaps the most striking ultrastructural feature of the myenteric plexus, with reference to the CNS, is the large quantity of neurons and the diversity of the neuronal types and axon terminals (957).
Neuron density in the myenteric plexus can be as great as 50.000 cells/cm^2 of gut surface (339). The majority of the perikarya are distinctly irregular in shape with deep invaginations and many cell processes though some neurons are smooth-surfaced (256). All nerve cell bodies are characterized by large eccentrically placed nuclei with finely granular nucleoplasm, prominent nucleoli and sparse condensations of chromatin. In size, shape, and other aspects of the fine structure the myenteric neurons vary widely. Myenteric neurons have been classified into eight or nine morphologically different types by means of their size, the distribution of organelles, their location of the neurons and their relationship to satellite cells (for detailed description see 239). Whether these different morphological categories have any functional significance is not clear (106; 108; 135; see 239; 256; 259; 275; 473; 573; see 573; see 796). The different neuronal types in Auerbach's ganglia appear at first somewhat randomly distributed. But, more detailed analysis of their distribution and projections reveal that they are arranged in an orderly fashion, that they make organized connections within the enteric plexuses and that each type projects in defined directions for defined distances in particular layers of the gut wall (473). In spite of differences in their projections and functional specializations, it has proven difficult to classify the neuronal cell types using ultrastructural criteria alone (256). Indeed, no correlation has been made between the types of neurons identified ultrastructurally and the types identified histochemically or physiologically. Moreover, it is still not possible to identify the exact neurotransmitter of most axons from the morphology of their axon terminals. Although, the multiplicity of the neuronal types and varicosities does highlight the fact that there are not only many neurons in the enteric nervous system but many types of neurons as well (275).
Submucosal neurons in the guinea-pig small intestine, in contrast, could not be divided into different categories on the basis of either their shape, size, organelle content or types of process (473).
Submucosal neurons of the guinea-pig small intestine do not vary very much in their ultrastructural features and they appear to belong to one class (see 239). Indeed, most of these submucosal neurons have an irregularly oval shape with a large, smooth, oval or round nucleus that is often eccentrically situated (473).

numerous glial elements

In the older literature, nucleated, satellite cells were described around enteric neurons and in the nerve strands. They were referred as Schwann cells. However, electron microscopy has shown that these satellite cells resemble more the glial cells of the CNS than the Schwann cells of the peripheral ganglia. Hence their name, enteric glia (see 239). Neurons and glial cells represent the two major cell types within all enteric ganglia. In the rat, guinea-pig, rabbit, cat and sheep glial cells always outnumber neurons (in mice 1.1 times; in sheep 4.5 times). In the myenteric plexus of the guinea-pig ileum, for example, the glial cells are twice as numerous as neurons and in the interconnect-

ing nerve strands even many more glial cells are found. In the submucosal ganglia (the ratio here is about 1:1) of the same region and in the plexuses of the other gut regions in the guinea-pig and other species they appear, on the contrary, relatively less numerous (see 239; 256; 473).The functional significance of the glial cells lies in the fact that enteric ganglia, the myenteric ganglia in particular, are most certainly exposed to very intense mechanical stresses during the mechanical activity of the muscularis externa. Thus there are changes in the width and thickness of the ganglia, in the shape of the individual neurons, and there is a sliding of structures past each other (257). Glial cells have processes rich in gliofilaments (these filaments were found to be immunologically identical to the filaments in the astrocytes of the CNS in that both cell types stain with antisera against glial fibrillary acidic protein) with which they are firmly anchored to the surface of the ganglion. Glial processes, reaching the ganglion surface and abutting on the basal lamina, display conspicuous incrustations of electron-dense material. In this way the gliofilaments are anchored to dense bodies beneath the cell membrane at the surface of ganglia. Mechanically some glial cells form, by means of their radial processes, robust cross bridgings spanning the full thickness of a ganglion from one surface to the other (136; see 239; 256; see 256; 257; 260; 275). Consequently, it has been suggested that the enteric glial cells, because of their shape and their richness in gliofilaments, may be important in conferring structural stability to the ganglia, in holding the ganglion together and at the same time in allowing structural rearrangement of the ganglia during muscular contractions (256; see 256). Moreover, numerous specialized contacts are found between vesicle-containing nerve endings and glial cell bodies or processes (256). In this way glial cells may , as in the CNS, play a role in the release and/or uptake of transmitter substance(s) in enteric ganglia (573; see 573).

Other non-neuronal elements closely associated with Auerbach's plexus are satellite cells, fibroblasts and occasionally macrophages (136).

Cajal (in 1911) introduced the term autonomic "interstitial cell" to describe a type of cell closely associated with Auerbach's plexus. The shape of these cells and their staining properties led Cajal and many other investigators to consider these cells as neurons or neuron-like. Indeed, the cells are stained with a number of methods (methylene blue, silver impregnation, osmic acid) used to stain nerve cells and their processes. In addition, the interstitial cells of Cajal stain for cholinesterases, are weak for monoaminooxydase and show some APUD characteristics (see 239). Furthermore, the long, sometimes varicose, processes of the interstitial cells of Cajal occasionally enveloped macrophage-like cells containing lysosomes and coated vesicles (655). But, since there is no increase in the frequency of these cells nor any indication of phagocytic activity in the tissue which showed considerable axonal degeneration they are not considered to be macrophages (136; see 136).

Electron microscopy, however, has shown that Cajal's interstitial cells are structurally not similar to neurons but do resemble fibroblasts or smooth muscle cells. Due to the existence of their nexus-like contacts with smooth muscle cells, they seem to be specialized in that they are closely associated with axon bundles and form close contacts with smooth muscle cells (136; see 136; see 239; 259; 635; 637). Nevertheless, despite the numerous studies devoted to Cajal's interstitial cells the nature and role(s) of these cells in the gastrointestinal tract remains mysterious and, as a consequence, different theories remain open for discussion (see 239).

dense synaptic neuropil with different terminals and extensive synaptic connections

Substantial portions of the myenteric ganglia, especially in the large intestine of the guinea-pig, rhesus monkey and man are occupied by a complex neuropil, which is a term including all the neuronal (intrinsic and extrinsic) processes, and the glial cells and their processes. In the submucosal ganglia the neuropil also takes up a considerable proportion of the space, often occupying the center of the ganglion with nerve cell bodies arranged on either side. The submucosal neuropil contains similar types of processes to those seen in the myenteric neuropil and may also be the location for nerve-nerve interactions (473).

In any part of a ganglion vesicle-containing axons are extremely numerous. The great majority of them are varicosities or ovoid expansions distributed along the length of thin axons. Frequently, the distinction between axons and dendrites is dubious and the postsynaptic process cannot always be identified with certainty. Moreover, axons are usually packed into bundles surrounded, but not penetrated, by glial cell processes. Occasionally, nerve endings of different morphology are directly apposed to each other (256).

Morphological and functional studies have indicated that extrinsic neurons likewise contribute to the formation of the enteric neuropil. However, the large majority of the nerve processes in the neuropil most probably arises from the enteric neurons themselves. Evidence for such intrinsic connections first came from the physiological work of Bayliss and Starling (in 1900) and Langley and Magnus (in 1906), when they showed that reflexes could be elicited in isolated segments of the intestine. Earlier morphological studies also indicated the presence of connections between the ganglia within one plexus and between the ganglia in different plexuses. Intracellular recording techniques later confirmed that intrinsic neurons receive inputs from other intrinsic neurons (see 139; see 239; 345; 346; 347; see 473; see 673).

Vesicles are regarded as the storage sites of transmitter substances and so, most probably, the clustering of vesicles indicates the places were neurotransmission occurs. Axon terminals have been classified into eight (135; 395; see 796), ten (246), fifteen or even more morphologically distinct types on the basis of the size, the shape and the content of the synaptic vesicles (56; 106; see 106; 256; 275; 303; 473; 573; see 573). This classification is not meant to be exhaustive and attempts to relate the different profile types to the various putative neurotransmitters have been largely unsuccessful. Moreover, interpretation is further complicated by the growing evidence that different transmitters, often with a different chemical nature, may coexist within the same nerve terminal (56; see 106; see 239).

According to the size, appearance and proportion of vesicles in the axonal varicosities at least three different types of neuronal processes can be distinguished in the myenteric neuropil i. e.

- Processes that have an organelle content and electron density similar to the nerve cell bodies.
- Varicose processes having round or fusiform dilatations containing variable numbers and types of vesicles and
- Relatively straight processes with microtubules, microfilaments and mitochondria. These probably represent the intervaricose and non-varicose segments of axons (56).

Neurosecretory vesicles are pooled in the terminal area of nerve fibres where preterminal axons widen into varicosities filled with vesicles. Four different kinds of synaptic vesicles i. e. cholinergic, NA-ergic, serotoninergic, and purinergic or peptidergic occur both in perikarya and in the neuropil (56; see 239; 638; see 638; 796; see 796). Hence, as determined by the features of the synaptic vesicles in the presynaptic endings, a given enteric ganglion cell may receive extensive synaptic input from different kinds of neurons (259). Other investigators, on the contrary, defined six major groups, as well as some subgroups (see 239). However, different authors, even when they have studied the same areas of the gut, proposed different classifications of the nerve profiles. Indeed, the vesicles, their content in particular, vary so widely that classification of the terminals on this basis are extremely difficult to make and are still uncertain. Thus, it seems very difficult to classify the different profiles in the enteric ganglia by their sizes and their populations of vesicles (see 106; see 239; 256).

Some synaptic endings contain almost exclusively small agranular vesicles. Due to their structural similarity with cholinergic nerve endings at the motor end plates and the vast amounts of ACh, which is known to be stored and released by the myenteric plexus, these endings are regarded as cholinergic. However, such endings are not numerous and therefore they cannot account for all the cholinergic endings one expects to find in a ganglion. Hence, many other endings that contain, beside a majority of small agranular vesicles, large granular vesicles as well are probably also cholinergic (106; 256; see 573).

Endings with small and large granular vesicles, with or without a dense core, are numerous in the wall of the intestine. They are found in the enteric ganglia, in intramuscular nerve bundles and around the blood vessels. They are identified as adrenergic on the basis of their structural similarity to adrenergic endings in other organs, as well as on the fact that their granularity is enhanced by the use of an adrenergic "false transmitter". Furthermore, they disappear after extrinsic denervation of the gut, some react to 5- and 6-OH-dopamine and using autoradiography vesicles have been shown to contain NA (237; see 237; see 239; see 256; 260; 300; see 638; see 758). However, not all of these vesicles degenerate after extrinsic denervation and not all of them accumulate 5- or 6-hydroxydopamine. Moreover, ultrastructural examination of the myenteric plexus has revealed that NA-ergic terminals are a minor component (far less than 1 % of all varicosities in the plexus) (see 239; 275; see 275; 499). Studies on the sites of uptake of exogenous tritiated NA and of an adrenergic "false transmitter" suggest that the overwhelming majority of the NA-ergic endings in the myenteric

plexus are located near the surface of the ganglia i. e. at the interface with the connective tissue space. Here they form, without an intervening supporting cell process, complexes with other varicosities that are non-adrenergic (111; 275; 400; 636). Most probably, they represent the morphological substrata of the reciprocal adrenergic-cholinergic axo-axonal synapse of the gastrointestinal tract (275; see 275; see 638). Within ganglia fibres are located in close proximity to ganglion cells, though no membrane specializations have been detected at the sites of close contact of the adrenergic boutons with the dendrites and cell bodies of the intrinsic nerve cells (see 256; 275; 278; 499; see 638).
Chemical labelling techniques (immunohistochemistry, chromaffin reaction and the uptake of false transmitter) applied to the guinea-pig small intestine have demonstrated in the myenteric ganglia numerous axons profiles with elongated vesicles (80 nm long and 30 nm wide). These axons profiles occur, as a rule, near the surface of the ganglia. In man many of these axons are directly exposed to the extraganglionic space and do not have a glial covering. Both in the guinea-pig and man these axons profiles are also found immediately adjacent to nerve cell bodies and their processes, although synaptic specializations have not been demonstrated. These profiles, finally, degenerate after extrinsic denervation (239; see 239).
Electron microscopic studies on the axon profiles in preparations known to be innervated by NANC nerves have led to the discovery of opaque, large, granular vesicles in both adrenergic and cholinergic nerves. These vesicles have a less prominent halo between the granular core and the membrane and are, in addition, unaffected by 6-hydroxydopamine, a substance which destroy the adrenergic nerve terminals. Furthermore, they contain a number of different substances (e. g. they are serotoninergic, and purinergic or peptidergic) which probably act as neurotransmitters (108; see 239; 300; 638; see 638; 716; see 716). Fibres containing this type of vesicles have been seen in the enteric ganglia and, most probably, belong to intrinsic neurons. Because of the similarity of their vesicles with those of polypeptide-storing neurosecretory nerves, they have been designated "p-type fibres" (polypeptide fibres) (56; see 56; 723). Numerous intrinsic p-type fibres have been found in the myenteric plexus and they have true synaptic contacts with the processes of nerve cell bodies, indicating a distinct functional role of this widely distributed system within the ganglion (and probably also in the smooth musculature of the gut) (56). Moreover, the granular core of the vesicles in the p-type fibres and the accumulation of an amine precursor analogue in combination only with a possible storage of a polypeptide substance (or an ATP-like substance) resembles the situation in several diffusely distributed endocrine cell systems.
There are relatively few structurally identifiable synapses on the myenteric perikarya but there is a close apposition between the nerve processes in the neuropil. Thus, the enteric neuropil constitutes the morphological basis for an integrative circuitry and it may be postulated that many neuro-neuronal interactions in the enteric ganglia occur in the neuropil distant from the nerve cell somata (56; see 239; 256; 473; 798; see 798). Indeed, the ENS receives sensory information from chemo- and mechanoreceptors, processes this information and generates an output that is appropriate for the control of the effector system. These are properties that are usually thought of as being associated only with the CNS (339). So, unlike other autonomic ganglia, Auerbach's plexus possesses considerable intrinsic integrative capacity (395).

virtual absence of an intraganglionic extracellular space

Perikarya and glial cells, as well as their processes, are as in the CNS tightly packed together in a way similar to the CNS. Thus, there is a virtual absence of extracellular space. This texture provides, of course, some limitation to the free diffusion of certain substances through the ganglia. Other autonomic ganglia, in contrast, are more loosely organized and contain collagen, fibroblasts, macrophages, mast cells and chromaffin cells in addition to neurons and supporting cells (see 239; 256; 273; 275; 473).

absence of endoneural and perineural sheath

The ENS differs from other regions of the peripheral nervous system (PNS) in that it lacks endoneural supporting sheaths. In all other autonomic ganglia the perikarya are fully sheathed by satellite cells (except small areas of certain dendrites in sympathetic ganglia) (see 239; 256; 257; 273; 275; 287; 473). Myenteric neurons are rarely completely enclosed in glial cell processes and submucosal neurons are never. The glial cells separate only groups of neurites and rarely form,

as is the case in monkey and man, a sheath around an individual axon. Therefore, endoneuronal supporting sheaths certainly do not provide a barrier between neurons and connective tissue or interstitium. Thus, large areas of the perikaryal surface and their large processes lie directly beneath the basal lamina and the connective tissue surrounding the ganglion. Consequently, parts of the surface of many enteric neurons are directly covered by and in contact with these tissue layers. Moreover, some neurons at the edges of ganglia have both their serosal and luminal surfaces exposed to the extraganglionic space .Where the neuronal plasma membrane is in contact with the basal lamina the cytoplasm directly beneath often contains a band of microfilamentous material. The sites, where axonal varicosities are bare and uncovered by supporting cell processes, are probably the regions where axons can release their transmitter substance (see 239; 256; 257; 273; 275; 287; 473). Further, most nerve processes are in direct membrane-to-membrane contact with each other. As a consequence, blood-borne substances, diffusing through the capillary endothelium, can directly reach the surface of neurons and neuronal processes.

absence of connective tissue

Connective tissue does not penetrate into the enteric ganglia but lie outside them. In the guinea-pig this exclusion from the myenteric plexus gradually takes place during development and is complete soon after birth. In late embryonic stages "islands" of collagen fibres are still found inside some ganglia and they only disappear after birth. However, in larger species i.e. the cat, sheep and man some of the largest ganglia may contain minute septa of connective tissue (see 239; 256; 473; 758; see 758).

absence of blood vessels

As a rule autonomic ganglia have their own capillary networks though the interior of the myenteric plexus is totally avascular (256; 259; see 273; 473). Myenteric capillaries are, moreover, quite different in their fine structure from these found elsewhere in the gut. Ordinary enteric capillaries are fenestrated and thus exceedingly permeable. Capillaries, lying just outside the myenteric plexus, are much thicker walled and nonfenestrated (287). In addition, cerebral and myenteric capillaries are similar in that both have impermeable junctions that prevent the passage of intravascular tracer molecules (labelled albumin or horseradish peroxidase) between endothelial cells (287). However, there is a slow leakage of macromolecular tracers out of the myenteric capillaries but a backup system of phagocytotic cells removes this material and prevents the tracers, leaking out of vessels, to reach detectable concentrations in the extravascular space or within the myenteric plexus (275; see 275; 473; see 796).

isolation (basal lamina) from the surrounding tissue, creating a physical barrier

Enteric ganglia are not encapsulated, but lie in the connective tissue between the muscle layers or in the submucosa (see 239). However, in the embryo there are numerous direct appositions of muscle cells and elements of the ganglia. These appositions disappear as the amount of connective tissue surrounding the ganglia increases (256). In this way ganglia become clearly separated from the muscle cells and connective tissue, the capillaries, fibroblasts, interstitial cells and other cell types lying outside the ganglia (256). Finally, they become surrounded by a continuous basal lamina, a thin disorganized layer of collagen (see review 258). In the guinea-pig small intestine submucosal ganglia are surrounded by a basal lamina and an incomplete irregular sheath composed of the fine processes of fibroblasts (473). This structural arrangement may have important functional implications because it has been found that the basal lamina may present a physical barrier to the entrance of substances from the extraganglionic space (798). In long-term organ bath experiments, for example, bacteria infiltrate the muscle and appear very numerous around the ganglia of the myenteric plexus. They are, however, not found inside the ganglia themselves. Hence, a blood-myenteric plexus barrier to macromolecules may be hypothesized. This barrier resembles the blood-thymic barrier and may be functionally analogous to the blood-brain barrier (256; 257; see 273; 473; 691; see 796). Indeed, it has been claimed that the enteric ganglia receive their nutrients by diffusion from the outlying blood vessels through the connective tissue and the basal lamina that surround them (239).

I. 3. 4. NEUROCHEMISTRY OF THE ENS

The classical picture of the innervation of the gut is one of intramural cholinergic excitatory neurons controlled by preganglionic parasympathetic (cholinergic) vagal and sacral fibres opposed by postganglionic sympathetic inhibitory (adrenergic) fibres modulating in consequence the final common pathway to the intestinal smooth muscle cells (see 239; 514; 552). However, new insights, mainly based upon the results of histochemical, immunohisttochemical and radioimmunological techniques, reveal an abundance of putative and established neurotransmitter substances. It is now realized that no other region of the ANS has such a large spectrum of neuroactive substances as the ENS. Moreover many of these substances, observed in the enteric nerve cell bodies and in their processes, have been shown to be present and active in the brain as well (brain-gut axis) (see 239; see 273).
In the following paragraphs the classical transmitter substances as well as some of the newly discovered transmitter substances of the ENS will be briefly reviewed.

Acetylcholine

In all the mammals (mouse, hamster, rat, guinea-pig, cat, pig, horse etc.) that have been studied, both the myenteric and submucosal plexus demonstrate AChE activity in a high proportion of their neurons and nerve fibres. The intensity of the AChE reaction shows considerable variations along the gastrointestinal tract (see 239; 246; 319; 387; 388; see 388; 462; 473; 666; 695; see 695; 716; 758; see 758; 785; 787). Moreover, choline acetyltransferase (ChAT, the enzyme involved in the formation of acetylcholine) has been demonstrated in the myenteric perikarya (Dogiel type I cells) and submucosal perikarya, while the myenteric plexus was found to be the major site of ACh synthesis (see 239). Intense AChE activity is considered to be a tentative suggestion of the cholinergic nature of a neuron (involved in the excitatory component of the peristaltic reflex) (246; see 448). Sensory neurons usually exhibit a low level of AChE activity so the unstained or lightly staining neurons are most probably sensory (695; see 695).
Myenteric ganglion cells in the canine esophagus stain intensely for AChE (388).
After in toto staining of the stomach and intestine of the rat large AChE-positive nerve bundles have been found to form a distinct rectangular AChE-positive nervous network between the longitudinal and circular muscle layers. Cholinergic nerve bundles split off from the myenteric plexus and penetrate the musculature. Principally, these cholinergic fibres run parallel to the longitudinal axis of the smooth muscle cells but interconnecting nerve fibres have always been observed. Furthermore, smaller nerve bundles branch off from the larger ones and constitute within the former network a secondary and tertiary AChE-positive plexus (758).
Basically, the same picture was observed in whole mount preparations of the equine large intestine. However, the density of the AChE + nervous network increased in a cranio-caudal direction, the densest network being always found in the transitional segments i.e. the ostium caeco-colicum, the flexura pelvina and the colon transversum (785; 787).
In the intestine of the rat an irregular AChE + nervous network, consisting of ganglia and rather fine interconnecting nerve bundles, has been observed in the submucosa (758). In the large intestine of the horse ganglia of varying size and shape, as well as their interconnecting nerve bundles, show AChE reactivity throughout the submucosa. Contrary to the myenteric plexus no specific distribution pattern in the AChE + nerve structures has been noticed (785; 787).
In histological sections of the intestine of the rat large and small neurons, showing a varying AChE activity, have been observed in the myenteric and submucosal ganglia respectively. Spots with a very high AChE activity were regularly seen at the outer surface of the perikaryal membrane (758). Interestingly, double staining with antibodies raised against ChAT and CGRP, CCK, NPY, SOM and Sub. P demonstrated that some neurons of the submucosal plexus react for ChAT alone, for ChAT/Sub. P and, finally for ChAT/CGRP/CCK/NPY/SOM (239; see 239). AChE-positive nerve fibres have been observed running between the ganglion cells and within the interganglionic nerve strands (343). In the muscularis mucosae a delicate AChE + network was found, except in the esophagus, where this network is replaced by a few very fine cholinergic nerve fibres. In the stomach this network holds very small ganglia (1-2 neurons) from which nerve bundles branch off towards the mucosa. In the small intestine the network send several fibres to the circular muscle

layer where they come into contact with the intramuscular plexus. An AChE-positive perivascular plexus has not always been seen (758).
In contrast no AChE-positive neurons, nerves or perivascular fibres have been observed in the mucosa of the gastrointestinal tract of the rat, though a well established delicate AChE-positive nervous network around and between the glands of Lieberkühn was, normally, encountered (758). In the cat delicate, small-meshed AChE-positive nerve nets have been found to enclose the Brunner glands in a "basket-like" fashion. This arrangement is suggestive of a functional innervation (448).
Ultrastructural studies have revealed agranular and large granular vesicles in the majority of axons observed in the mucous membrane of the mouse colon. The fact that they are not depleted by reserpine and that they can be identified in axons with a positive cholinesterase reaction tends to indicate that these vesicles are closely concerned with a cholinergic innervation (695).

Noradrenaline

Mesenteric nerves contain 12-15 times more NA than adrenaline and NA is released within the gut wall at the endings of the mesenteric nerves (237; see 239). Preterminal portions of adrenergic axons normally enter the wall of the gut along arteries (65; see 656) and anastomoses with one or two ganglia as soon as the intestinal wall is reached (382). After penetrating the serosa, paravascular nerves give rise to small, smooth filaments that supply NA terminals to various intramural structures. NA- ergic fibres appear smooth in their nonterminal parts (237). It is believed that NA is released from the varicosities along the entire length of the terminal portion (255; 498). The length of intestine supplied by NA nerves following any one artery is approximately proportional to the area of supply of the artery and its branches. The adjacent field is overlapped by a maximum of +/- 20 % (237; 239; see 239; see 573).
Most NA-ergic cell bodies supplying the gut lie in the prevertebral ganglia (celiac, superior and inferior mesenteric, pelvic ganglia) (see 237; see 239; 473; see 758). Hence, the perikarya of the mesenteric NA (inhibitory) nerves to the stomach, the small intestine and the ileo-colic sphincter are in the celiac and the superior mesenteric ganglia respectively, while at least in rabbits and cats, the sympathetic fibres to the colon and rectum, originate from the inferior mesenteric ganglia (see 239). This morphological finding was further supported by the fact that celiac ganglionectomy in the cat causes a disappearance of catecholaminergic nerves innervating the intestine (see 239; 387).
Moreover, in the celiac ganglion 50 % of the neurons are NA/NPY, 21 % NA/SOM and 25 % NA/- (239; see 239). NPY-IR was found to be closely related to the distribution of markers for NA neurons such as the catecholamine-synthesizing enzymes dopamine-β-hydroxylase (DBH) or tyrosine hydroxylase both in sympathetic ganglion cells and in terminals. Coexistence of these enzymes with the NPY can directly be demonstrated in the same neuronal cell bodies of the sympathetic ganglia. Additional evidence for coexistence of NPY and NA in the nerve endings has been provided by pharmacological studies (484). Only a proportion of sympathetic NA nerves seem to contain NPY and these neurons innervate target organs such as blood vessels (the major target), the muscle of the heart, the spleen and the vas deferens suggesting a chemical heterogeneity and a morphological basis for a functional differentiation of the sympathetic nerves. In the sympathetic system, both NA and NPY cause vasoconstriction, whereby NPY exhibits a slowly developing, long-lasting effect. Furthermore, there are examples of considerably fewer NPY-IR nerves than NA nerves on the venous side of the vascular tree. This may indicate a heterogeneity of sympathetic vascular nerves and possibly a separate control of blood flow (arterial nerves) and blood volume (venous nerves) (350; see 350; 484; see 484).
Intramural NA neurons are a rare occurrence in the gut (108; see 239). No fluorescent catecholaminergic nerve cell bodies have been identified in the large intestine of the eel, trout, toad and rat. Such perikarya were, in contrast, observed in Auerbach's plexus of the lizard large intestine (632) and the avian stomach (108; 153; see 153; 473; 758; see 758). In mammals a few NA-ergic neurons have been seen in the esophagus and colon of the rat and in the stomach of the guinea-pig (678). Only in the proximal colon of the guinea-pig does the myenteric plexus contain a substantial number (+/-10.000) of NA cell bodies (150; see 153; 237; see 239; 253; 254; 473; 710).

The histochemical method of Falck and Hillarp (Formaldehyde Induced Fluorescence, FIF) (207) has revealed a well-developed, narrow-meshed plexus of varicose, intensely green fluorescent catecholaminergic nerves in the enteric ganglia, NA axons in the interconnecting strands (for up to several mm) and a comparatively sparse NA supply to the non-sphincter

smooth muscle throughout the alimentary tract of non-mammalian and mammalian species including man. In the rat most NA fibres have been seen in the peripheral parts of the ganglia. It was further observed that most neurons, and those of the myenteric plexus in particular, have varicose NA- ergic axons close to them though true pericellular endings, possibly reflecting a different function for these neurons, have rarely been seen (56; 61; 108; 114; 150; 207; 237; see 237; see 239; 246; 256; see review 322; 343; 387; 473; 546; 565; see 566; see 573; see 632; see 642; 688; see 688; 758; see 758; see 796; see 799).

Along the gut the distribution of NA fibres within the myenteric ganglia appears fairly uniform in spite of distinct differences in the size and shape of the ganglia (150; see 239). Nevertheless, some investigators have argued that in the guinea-pig and man myenteric adrenergic fibres increase in number in a caudal direction (see 758). Moreover, in the small intestine of the pig an unequivocal relation between the adrenergic axons and Dogiel's type I, II and III neurons has been noticed. Type II neurons were obviously privileged. In principle, the relation between adrenergic varicose axons and neuron types is identical in Auerbach's and Meissner's plexus (668).

Beaded fluorescent NA fibres have been observed in all internodal strands (237; 473; see 473). In the rat the largest nerve bundles contain on the average 10 fluorescent catecholaminergic nerve fibres, the smallest bundles 1-3 (758).

The intestinal smooth musculature is characterized by a sparse NA-ergic supply, although in the sphincteric circular muscle a dense NA-ergic innervation is normally observed (237; see 239; see 758). These anatomical observations have been largely confirmed by modern techniques e.g. immunohistochemistry (236; see 239). Moreover, in the rat the distribution pattern of the intramuscular NA-ergic nerve fibres strongly resembles the results seen in AChE preparations (758). In small mammals there are almost no NA fibres within the longitudinal muscle layer of the small and large intestines, the taeniae and the longitudinal muscle of the rectum in the guinea-pig, cat and dog being exceptions (see 239).

No adrenergic cell bodies have been observed in the submucosal plexuses of any of the mammalian species studied (237; see 239; 758; see 758). However, pericellular NA networks, similar to those of Auerbach's plexus, were formed around most submucosal neurons in mammals including man (61; 114; see 239; 387; 565; 566; see 632; 758; see 758). These NA fibres are of extrinsic origin even in the proximal colon of the guinea-pig where NA myenteric neurons have been demonstrated. In the small intestine of the guinea-pig only the VIP/DYN submucosal neurons, lying in the central part of the larger ganglia and projecting to the mucosa, receive NA nerve terminals (see 239).

In the rat's esophagus NA fibres have been seen only in the tunica adventitia of the blood vessels (758). In the rat, guinea-pig, rabbit and cat the gastric submucosal ganglia have been reported not to be supplied with adrenergic fibres (237; see 237), although this finding disagree with the observations of others investigators (544; 758; see 758).

Fluorescent axons are relatively scarce in the duodenal submucosa. In the cat Brunner's glands have a rather sparse adrenergic innervation, although it is possible to demonstrate a specific functional adrenergic innervation of these glands (448; 565; 631).

Few, if any, fluorescent nerves have been observed in the lamina muscularis mucosae. Some authors even claimed that there is no adrenergic innervation of this smooth muscle layer. But as this muscle layer is generally very thin, the smooth muscle cells might be directly affected by the sympathetic transmitter released from adjacent perivascular nerves (631). In the guinea-pig stomach, ileum and rectum on the other hand NA fibres, running parallel to the smooth muscle cells, have been reported. Especially in those regions where the muscularis mucosae is thicker (cardia, anal canal) a greater number of NA fibres has been found (57; 149; 150; 237; see 239; 262).

In the rat's intestine nerve bundles, usually containing 1-3 NA fibres, have been observed in the delicate nervous networks present at the border zones between the tunica submucosa and the tunica muscularis on one hand and the mucosa on the other hand (758). Around all submucosal blood vessels a clear NA-ergic perivascular plexus is seen. Frequently, delicate nerve fibres, interconnecting the perivascular and submucosal plexus, were encountered (758).

Most fluorescent mucosal nerves are, as perivascular plexuses, associated with small blood vessels and, especially in mammals, with arterioles (387; 565; 566; see 631; 758). These fibres leave the immediate vicinity of blood vessels and extended towards the mucosal epithelium. In the small intestine of the rat, guinea-pig and rabbit and in the human large intestine NA fibres form an open network around the basis of the mucosal glands, while a few fibres extend into the villi except in the rat in which no fluorescent nerves have been ob-

served in the villi (150; see 237; see 239; 247; 262; 387; 552; see 631; 758).
A dense perivascular NA network has been demonstrated around the mesenteric and intramural arteries, while some NA fibres accompany the central lacteal and the mesenteric lymphatics (239; see 239).
The different targets in the gut wall are supplied by separate groups of NA neurons. In the guinea-pig small intestine, for example, NA axons containing NPY (NA/NPY) innervate intestinal arterioles, NA/SOM axons the submucosal ganglia, and some fibres in the circular muscle layer, "pure" NA axons supply the myenteric plexus (239; see 239).
Small granular vesicles, usually associated with adrenergic nerves, have occasionally been seen in the mucous membrane of the mouse colon. Most probably, therefore the colonic mucous membrane receives a sparse adrenergic innervation in this species (695). In ultrastructural studies catecholamine containing varicose fibres have been observed near enterochromaffin cells (12; 262; 387; 490; 554; 577). In the crypts of the guinea-pig duodenum no typical synaptic arrangements have been observed between the unmyelinated nerve processes and the basement membranes of enterochromaffin cells. But, the minimal distance between these cells and nerve bundles, containing small electron-dense vesicles (probably CA-fibres), was within the functional limits of the "autonomic gap" (490). Indeed, peripheral autonomic neurons can exert their action on effector cells by the release of transmitter even without true synapses, provided that the distance of the "autonomic gap" does not exceed 100 nm (111; 552). In the rat's ileum a membrane to membrane contact between single axons (in a bundle of non-terminal axons) and the basal membrane of the epithelial cells has often been found. In a few cases a direct contact, with a true synaptic specialization, between nerve terminals and basal-granulated cells is seen. The synapsing nerve terminal, containing both large dense cored and small round empty vesicles, most probably represents an adrenergic terminal (552; see 552).
In the guinea-pig's stomach, ileum and rectum varicose adrenergic fibres have been demonstrated in intimate relationship to the secretory epithelial cells (262).
The arteries within the gut wall are surrounded by a dense plexus of NA nerves at their adventitio-medial border (238; 253). The intramural veins are very sparsely innervated, but the nerve supply increases as larger vessels are formed in the mesentery and becomes dense in the walls of the superior mesenteric and hepatic portal veins (237).

***In summary**, AChE activity is found throughout the length and width of the gastrointestinal tract of all animals so far investigated. In this way nearly every structure of the gut wall is most likely innervated by cholinergic nerve fibres. The intensity of the AChE reaction in ganglion cells and their processes, and the density and distribution of the AChE + nervous network show, however, considerable variations between and within species.*
Fluorescence microscopy and immunohistochemistry have demonstrated an extensive NA-ergic nervous network in the wall of the gut and no or very few catecholaminergic perikarya (except in the proximal colon of the guinea-pig). In contrast numerous varicose NA-ergic nerve fibres, surrounding most nerve cell bodies in a basket-like formation, have been observed in the enteric ganglia and in Auerbach's plexus in particular. Relatively few catecholamine-containing nerves have been observed in the internodal strands and in the muscles, except in the sphincters. Thus, the adrenergic control of the gastrointestinal tract primarily takes place in the myenteric plexus.
An intimate functional "contact" between NA-ergic axons and epithelial cells (enterochromaffin and secretory) has been observed. Probably, these axons control the release from the enterochromaffin cells and, as a consequence, can influence indirectly a large spectrum of intestinal functions.
Finally, distinct perivascular NA nerve plexuses have been seen throughout the gut wall.

the NANC system

The autonomic nervous system has been classically considered to be bipartite. However, a number of morphological (56; 135; 246; 698; 723), electrophysiological and pharmacological observations indicate the existence of other types of nerves (73; see 239; see 629; see 723). The first suggestion for the presence of NANC autonomic nerves in the gut dates back to Langley (in 1898), when he found that stimulation of the vagal nerve cause gastric relaxation. At that time it was thought that this effect might be ascribed to occasional sympathetic fibres running in the vagus. However, this has since been found not to be the case since the pattern of response differs from that obtained after adrenergic nerve stimulation (see 108).

Burnstock (in 1963) postulated the existence of non-adrenergic inhibitory nerves in which purine nucleotides act as neurotransmitters (108; see 108; see 350). Further research established ATP as a co-transmitter with NA in sympathetic nerves supplying the vas deferens, taenia coli and a number of blood vessels. Moreover, it is likely that various peptides, particularly NPY but also SOM, ENK, VIP and vasopressin-like peptide, coexist with ATP and NA in sympathetic nerves (105; see 105). ATP and NA act synergistically potentiating each others actions and they help each other to terminate neurotransmission by acting on prejunctional receptors inhibiting the release of transmitter (105). Co-transmission of ATP and NA seems to involve different postjunctional mechanisms. Indeed, ATP produces contractions via an electromechanical coupling mechanism involving voltage-dependent Ca^{2+} channels, while NA produces contractions via a spike-independent mechanism involving receptor-dependent Ca^{2+} channels. It is now proposed that ATP is a primitive neurotransmitter that has been retained as the principal transmitter in some nerves and that, during evolution, it has been utilized as a co-transmitter to a variable extent in other nerve types (105; see 350).

Subsequently, it has been claimed that the NANC nerves, which supply intestinal smooth muscle, are "peptidergic" rather than purinergic (108; see 108). The first morphological suggestion for the existence of enteric peptidergic nerves was given by Baumgarten in 1970, when he described nerve fibres with an ultrastructural similarity (large opaque vesicles) to peptidergic nerves in the hypothalamo-hypophyseal system. Because of this profile they were named p(eptidergic)-type nerves (56; 73; see 73; see 629). In addition, quinacrine fluorescence, known to be selective for NANC nerves, showed numerous positive perikarya in the enteric ganglia (1045/cm^2 in the rabbit's ileum; 2633/cm^2 in the rat's stomach) (153). Today, a considerable number of biologically active peptides, first extracted from the brain or gut, are known to have a very wide distribution in the ANS. These brain-gut neuropeptides include vasoactive intestinal polypeptide (VIP), Sub. P, Sub. K, somatostatin (SOM), enkephalin (ENK), bombesin, neurotensin, galanin, calcitonin gene related peptide (CGRP), gastrin/cholecystokinin (gastrin/CCK), neuropeptide Y (NPY), peptide HI (PHI) and probably other peptides. Most of them are localized both in neurons and nerve fibres (neurotensin, however, can not be demonstrated in the enteric neurons) (77; 108; see 108; 117; 153; see 153; see 239; 246; 353; 354; 359; 379; 458; 589; see 629; 678; 698; 723).

Several peptides have been identified in the extrinsic autonomic nervous supply to the gut. Immunohistochemical studies, using antibodies to Sub. P, CCK/gastrin, SOM,VIP and CGRP have stained nerve cell bodies of the nodose ganglia (see 170) and there is immunohistochemical evidence for the transport of these peptides in afferent fibres (170; see 170; 340; see 340). The mean rate of transport for CCK-8, Sub. P, VIP and SOM is closely similar in different species and varies from 0.8 to 4.0 mm/hour. Allowing for the fact that only about 30% is moving, the true rate is probably closer to 10 mm/hour which is within the range for fast axonal transport (170). A central accumulation of VIP, Sub. P, SOM, ENK and gastrin/CCK immunoreactive material has been found in the vagal, splanchnic, pelvic and sciatic nerves after ligation. In man very high numbers of Sub. P-, medium numbers of ENK-, and low numbers of VIP-IR fibres have been seen above the ligation. These results suggest that the afore-mentioned peptides are most likely transported towards the periphery (73; see 73; 88; 170; see 170; 268; 379; 479; 489; 497; see 497; see 605; see 629; see 677; see 723). However, ligation experiments of the lumbar splanchnic nerve of the guinea pig indicate that there is little or no transport of CCK, SOM or VIP towards the periphery (see 170). In addition, after ligation of the porcine vagal nerve there is a considerable accumulation of Sub. P and VIP both above and below the ligature indicating that these peptides are transported in both directions (325; see 325; 489; 497). Furthermore, in the cat and pig VIP has been shown to be released in response to vagal nerve stimulation (see 629). However, the relatively low concentrations of these peptides in vagal nerve extracts, the failure of the extrinsic denervation of the guinea-pig ileum to alter the peptide-

containing (VIP, Sub. P) and AChE + nerve fibres, the fact that vagotomy leaves the innervation pattern of the jejunum completely unaffected and, finally, the accumulation rates of peptides on the cranial side of ligatures all suggests that the vagal peptidergic nerves do not provide a quantitatively significant contribution to the peptidergic nerve supply of the gut (73; see 73; 118; 232; 325; 379; 489; see 489; 497).

The phenomenon of the coexistence of the different established and putative transmitter substances in the same neuron seems widespread both in the central and peripheral nervous systems. Thus, the classical view of the neuron as a rather rigid entity with a unique neurotransmitter has, to a larger extent, to be amended. Indeed, the neuron now appears as a flexible and adaptable system with several messengers synthesized and liberated and with diverse graded responses (122). Numerous coexistence situations have been observed in the CNS. It is important to note that different types of combinations of compounds can be recognized. For example a classical transmitter e.g. NA, together with one one or more peptides in the same neuron. In addition, neurons containing more than one peptide have also been seen. A common principle often observed is that only a subpopulation of certain systems seem to exhibit a certain combination of messengers. Initially, catecholamines and 5-HT were identified as classical transmitters, but more recently there are also multiple examples involving ACh and GABA (350). Several biologically active polypeptides are present in autonomic motor and sensory neurons (see 484) and recent research has provided many examples of the coexistence of classical/peptide, amine/peptide and peptide/peptide neurotransmitters in the same neuron (51; see 51; 170; see 170; 350; see 677).

The coexistence of neurotransmitters (classical and putative) in the same neuron in the peripheral ANS has been demonstrated by several investigators (73; see 73; see 239; see 338; 352; 379; 411; 473; see 473; 481; 482; 483; see 484; 485; 486; 487; 488; 680; see 680). In the celiac, the superior mesenteric and the inferior mesenteric ganglia of all species examined so far, dense networks of Sub. P, VIP and ENK nerve terminals; 5-HT/ENK; VIP, VIP/NPY, VIP/NA; SOM, SOM/NPY, SOM/NA; NPY and NPY/NA cell bodies are present (see 73; see 108; see 239; see 338; 354; 356; see 356; 411; 471; 478; see 677; see 678; see 680). In addition, the splenic nerves contain vesicular Sub. P (108), in the superior cervical ganglion of the rat some ganglion cells contain both ENK and NA (73; see 73; see 239; 323; see 323; 350; see 350; 354; 356; see 356; 471; 473; see 514; 678; see 678; 680). VIP has been demonstrated in some sympathetic ganglion cells and in AChE-positive nerve fibres to exocrine glands in the cat (478). Coexistence implies that, if mechanisms exist that allow selective activation of the two types of vesicles upon arrival of nerve impulses, it should be possible to obtain a differential release. Such activation could be frequency coded: at low nerve impulse flow small vesicles release the classical transmitter, at higher frequencies large vesicles release both peptide and classical transmitters (350; see 350). This seems to be the case, since autonomic transmission is characterized by a frequency-dependent, chemical coding of multiple signals (484). Indeed, changes in the frequency of stimulation of a neuron containing one signal substance will change the amount of released neurotransmitter at a given synapse. But, changes in the firing frequency of a classical neurotransmitter/peptide neurotransmitter-containing neurons may alter which of the coexisting neurotransmitters is released or in what stoichiometrical proportion they are released. Thus, it appears that there is a direct translation of the frequency code into a chemical code at the level of the individual nerve terminals of a neuron with more than one signal substance (51).

In the periphery neurons, containing different types of coexistence combinations, may project differentially. Preganglionic fibres containing VIP/DYN-IR terminate virtually exclusively in those parts of the sympathetic ganglia that contain SOM neurons. These fibres, in all probability originate from the gastrointestinal wall. This would suggest that mainly the SOM-IR cells are involved in the reflex loop between ganglia and intestine (see 350). Thus, subdivision of transmitter specific groups of neurons by a peptide may be related to the target areas of the projections (350).

Some SOM/NA, NPY/NA and NPY, NA/VIP ganglion cells have been found to project to the gastrointestinal wall (see 239; 354; 356; 471). It has been definitely demonstrated that the NA/SOM and NA/NPY-IR cell bodies occupy different territories in the ganglion. Only a small degree of overlap exists and a few cell bodies contain both peptides (350; see 350; 484; see 484). A close relation between NA/NPY neurons and the vascular system (mainly intestinal blood vessels) has been observed (see 350). In contrast, the NA/SOM neurons project to the submucosal ganglia and the mucosa. A third population of NA nerves lacking the two peptides project to the myenteric ganglia. Taken together, these findings strongly suggest a distinct chemical coding of neuron populations in the

prevertebral ganglia and gastrointestinal tract and that these subgroups of peripheral neurons may be involved in the regulation of different peripheral functional events (350). It has been possible to show that NPY inhibits the release of NA evoked by electrical stimulation. NPY seems to have at least three effects on the sympathetic neuro-effector junction. There is a prejunctional effect, expressed as suppression of stimulated NA release, and two post-junctional effects i.e. a direct response, which is not mediated via adrenoreceptors, and a potentiation of the NA-evoked response. The possible end result of these diverse actions may be an improvement in the "economy" at the sympathetic neuro-effector junction, which is reflected in a reduced NA demand and a suppression or shortening of the NA release process after nerve stimulation. NPY does not seem to evoke all three effects at every sympathetic neuro-effect junction. Moreover, the potentiating effect of NPY is not limited to NA, since the effects of e.g. histamine are also enhanced. Finally, NPY is not the only peptide that is capable of exerting the three actions at the sympathetic neuro-effector junction. Its chemical relative PYY, and to some extent PP, are also capable of inducing the same effects. The functional role of NPY in the periphery is probably not related exclusively to noradrenergic neurotransmission. NPY seems to have effects on vascular smooth muscle that are unrelated to adrenergic receptors. Furthermore, NPY has been demonstrated by immunohistochemistry in other neuronal systems apart from the sympathetic one (323; see 323; 350; see 350).

In sympathetic ganglia APP has been found in a NA cell population complementary to the NA/SOM cell bodies (350; see 350; 484; see 484).

ENK immunoreactive cells have been observed both in the gastrointestinal wall (680; 681) and in sympathetic ganglia (338; see 338; 354; 489). Sensory fibres, containing Sub. P most probably project to the gut via sympathetic ganglia (73; see 73). In the ileum of the guinea-pig vascular Sub. P-containing fibres have been found to originate from outside the intestine; vascular VIP nerves, in contrast, were of intrinsic origin (see 325). Moreover, some peptidergic neurons project outwards from the gut wall since a few VIP neurons have been shown to project from the gastrointestinal wall to the mesenteric ganglia (see 629).

Sub. P, VIP, SOM or ENK-containing nerve fibres and cell bodies are particularly numerous in the enteric plexuses (246; 678; 723). These neurons are predominantly "short" neurons with their cell bodies in the gut wall, an arrangement which provides considerable nervous autonomy to the gut (723). The vast majority of the different enteric peptidergic nerves seem to issue from intrinsic cell bodies (see 73; 497; 723). The bulk of the evidence indicates that enteric peptide-containing nerves constitute separate systems independent of each other. Hence, each of these peptide-containing nerves has its own characteristic topographic distribution within the gut (246; 497). It is most likely that they are responsible for the NANC neural events in gastrointestinal functions such as motility, secretion/absorption etc (see 629). In addition, it has b een found that some neuropeptides have a dual localization in the gut in that they occur in endocrine cells as well (246; 723; 725; vide infra). Ligation and tissue culture experiments support the hypothesis that the vast majority of the different enteric peptidergic nerves issue from intrinsic cell bodies (see 73; 497; 723). Indeed, various peptide-containing nerves have been identified in mouse small intestine grown in tissue culture (497; 681). Moreover, by separately culturing the two ganglionated plexuses from the guinea-pig gut immunostaining of the submucosal plexus has revealed only VIP fibres and, conversely, cultures of the myenteric plexus show only Sub. P immunoreactive fibres. Thus, the VIP and Sub. P fibres, found in their respective plexuses, may represent interconnections running across the muscle coat (73; see 73). Furthermore, in autotransplants of the gut AChE + and peptidergic nerve supplies are unaffected (73; see 73; 118; 325; 379; 497). However, the situation becomes more complex since a coexistence of two peptides in the same enteric neuron has been observed. For example, SOM/ gastrin-CCK neurons in the submucosal plexus of the proximal colon of the guinea-pig (478; 678), and Sub. P/ENK in some myenteric neurons in the cat ileum (171). It has been suggested therefore that the coexistence is usually between structurally related peptides, although dissimilar substances such as peptides and amines do coexist in certain species (73; see 73).

Peptidergic nerves, with other intrinsic autonomic fibres, appear to form an autonomous unit in the gut. This unit, receiving relays from outside, can continue functioning when these influences are disturbed (note that gut motility is not significantly altered following extrinsic denervation) and it is only when the intramural "minibrain" itself is damaged that the mechanism really ceases to run smoothly. This is clearly demonstrated in examples of gut diseases where peptidergic nerve abnormalities occur (73; see clinical aspects). All these findings point to a greater complexity of the autonomic nervous system than envisaged in the classical

studies (354; 356) and the concept of a bipartite (cholinergic and adrenergic) autonomic innervation of the gut has been seriously challenged (395; 593). Indeed, the classical noradrenergic and cholinergic transmitter systems in the periphery can now be subdivided into different populations of neurons on the basis of the coexistence of specific peptides with NA or ACh. Therefore, a concept of multiple synaptic messengers is emerging in which the peptides can act together with and/or interact with the classical transmitters at the pre- and/or postsynaptic levels (484). These various types of compounds differ in parameters such as time course and mechanisms of action. For example, amino acids seem to be involved in fast transmission processes, whereas neuropeptides and monoamines often exert effects with slow onset and long duration (350; see 350).

Serotonin (5-HT)

5-HT has been found in a population of enterochromaffin cells (references see addendum/Part III/review literature 5-HT). No non-neuronal cells other than EC cells (except mast cells in rats and mice) have been found to contain 5-HT (see 239; 275).

NA-, 5-HT-containing and non-5-HT amine-handling nerves can be distinguished in the enteric ganglia (see 239; 473), although it must be stressed that in the normal, untreated intestine FIF has failed to demonstrate 5-HT. It is only after injection of tryptophan or the use of a monoaminoxidase inhibitor that a 5-HT (yellow) fluorescence reaction can be observed in the myenteric plexus (see 239).

Amine-handling neurons have been found in both the myenteric and submucosal plexuses of mice, rats and guinea-pigs (236; 246; 288; 289; see 473). They contain the enzymes aromatic amino acid decarboxylase and monoaminoxidase (MAO), and they take up and retain catecholamines and indoleamines (246) although no tyrosine or dopamine-β-hydroxylase has been found. This indicates that they do not synthesize a catecholamine (246; 249). Consequently, intrinsic amine-handling neurons are probably not catecholaminergic but indoleaminergic (145; 246; 249; 250). The transmitter of these "amine-handling" neurons is most probably 5-HT (275). Both in culture and in situ enteric neurons convert ^{3}H L-tryptophan to ^{3}H 5-HT (181; 210; see 239; 275; 315; 640) and since enteric 5-HT neurons persist in culture they must be intrinsic to the gut. As a consequence, the non-mucosal 5-HT, biochemically detected in the gut, is undoubtedly present in the enteric neurons (275). Enteric non-5-HT, amine-handling neurons can take up and retain catecholamines and indoleamines and can decarboxylate amine precursors and store the decarboxylated product (141; 147; see 239; 250; see 473). However, it is unlikely that they use a catecholamine as transmitter since the decarboxylating enzyme of these neurons is immunocytochemically different from that of the sympathetic NA neurons (236; see 239; 246; see 473).Moreover, immunohistochemistry has revealed that 5-HT enteric neurons and the enteric non-5-HT amine-handling neurons are two separate neuronal populations that have different distributions (see 239). However, amine-handling properties may also be related to the synthesis and storage of peptide neurotransmitters (236; 246; 288; 289; see 473). In addition, the coexistence of 5-HT with Sub. P, GABA and NA has been found respectively in the ventral part of the spinal cord, in some pontine neurons and in the pineal gland (see 350).

5-HT immunohistochemistry has clearly established the presence of 5-HT neurons in the enteric plexuses (see 239). About 10 % of the submucosal neurons and 0.5 - 2 % of the myenteric neurons in the guinea-pig ileum are bound by antibodies to 5-HT, although some authors have not observed 5-HT immunoreactive perikarya in the submucosal ganglia (141; see 473). Myenteric 5-HT immunoreactive neurons have many short thick processes and one long fine process (cfr. Dogiel's type I) (236; see 473). Varicose 5-HT immunofluorescent axons, with fine faint intervaricose segments, ramify extensively in the enteric ganglia and contribute to the deep muscular and mucosal plexuses as well (147; see 239; 246; 250). In the guinea-pig myenteric plexus they project as far as 20-25 mm in the caudal direction. Along their course the processes gave off small varicose branches that wind around some of the myenteric neurons (242; 473; see 473). Most fluorescent fibres in the submucosal ganglia are derived from myenteric perikarya about 12 mm oral to the submucosal ganglia they innervate (242; see 473).

Substance P (Sub. P)

Sub. P belongs to the tachykinins. This is a family of peptides structurally related to Sub. P which occurs naturally both in invertebrates and vertebrates. It is so termed because of its ability to produce a rapid contraction of smooth muscle. Each membre shares the common C-terminal sequence, Phe-X-Gly-Leu-Met-NH_2.

It is evident now that at least three tachykinins occur in mammals in addition to Sub. P (52; see 52).
Sub. P is the most abundant of the vagal peptides since it occurs in concentrations roughly ten times higher than those of CCK-8, SOM and VIP in the cervical vagus of several mammals i.e. the guinea-pig, cat, rabbit, rat for Sub.P; the rat for VIP and the cat, guinea-pig and rat for SOM (170). Sub. P may have a direct action on neuronal cell bodies of the nodose ganglia (340; see 340). Nevertheless, there is little evidence to indicate a vagal Sub. P supply to the stomach, pancreas or portal vein in the rat. It seems that the upper gastrointestinal tract is an unlikely destination for vagal Sub. P fibres, and it is more likely that the various thoracic structures, including the bronchi, trachea and baro- and chemoreceptors are supplied by these fibres (170; see 170; see above).
Sub. P has a dual localization in the gut wall i.e. in some endocrine cells and in nervous elements (142; see 239; 325; see 325; 357; 560; 589; 605; 617; see 629; 723; 724; see 724). In cultures of the enteric plexuses Sub. P has been seen to originate mainly from the myenteric plexus (617; 723).
Sub. P-containing neurons have been found in all areas of the gut and represent about 3-16 % of the cell bodies. They are principally encountered in the myenteric plexus (3.5-5.1 % of the total myenteric neurons) where, as a consequence, the majority of Sub. P fibres has been observed (19; 52; see 52; 73; see 73; 139; see 139; see 239; 473; see 497; 518; 560; 589; see 680). Sub. P neurons are characterized by relatively short but profusely branching processes that have very localized projections to the muscle, the other neurons and the glands in a narrow ring of the intestinal wall. Immunohistochemistry has further revealed a multiple peptide content in some myenteric neurons of the guinea-pig small intestine. Indeed, some Dogiel type Ib neurons show immunoreactivity for Sub. P/VIP/DYN/GRP/CCK, while type II neurons react with antibodies raised against Sub. P, SOM and CCK in various combinations. Unclassified neurons have been found to be immunoreactive for Sub. P/ENK or Sub. P/ENK/DYN (239; see 239).
Sub. P-IR fibres ramify extensively around myenteric neurons in such a way that each neuron appears to be supplied by a very dense Sub. P network (139; see 139; 141, see 239; 325; 354; see 473; 560; 589; 605; 723). The majority of the myenteric Sub. P fibres most probably originate from cell bodies in the same or adjacent ganglia (139; see 139; 354; 473). However, in the cat, spinal ganglionectomy (Th_5-L_2 bilateral) greatly reduces Sub. P-IR fibres in the myenteric plexus. Similarly, in the rat, splanchnic (although not vagal) lesions have been found to reduce a population of Sub. P-IR fibres in the myenteric plexus (see 170). This topography of Sub. P is in agreement with the postulated role for Sub. P as a transmitter in the excitatory interneurons within the myenteric rather than in the submucosal plexus (73; see 73; 616). Thus, Sub. P appears to be involved in non-cholinergic transmission in the myenteric ganglia (139). Additionally, abundant Sub. P fibres are present in the circular muscle coat where they may act directly on the smooth muscle cells to increase contractions (19; 52; see 52; 139; see 139; see 239; see 497; 560; 589; 605; 723). These data, taken together, suggest that the main targets for Sub. P are most likely the intestinal smooth muscles and the enteric (mainly myenteric) neurons (73; 325; see 325; 605; 616; see 629; 723).
Submucosal Sub. P-containing neurons (2.8-11.3 % of the total number), projecting principally to the mucosa, have been found in all segments of the gut, except the stomach of the rat and guinea-pig (73; 139; see 139; 141; see 239; 325; see 325; 473; see 473; 605; 680). High concentrations of Sub. P are also present in man in the mucosa and submucosa (220; see 220).
A very dense network of Sub. P nerve terminals is observed around the cell bodies of Meissner's plexus (325; 354; see 354; 589; 605), except in the guinea-pig in which only a moderate number has been detected (473). However, no effects of Sub. P on the submucosal neurons have been described (73; see 73). Most of the Sub. P terminals originate from myenteric neurons (141). The rich periglandular and subepithelial plexuses of varicose Sub. P fibres originate partially from myenteric ganglia and partially from submucosal ganglia (see 139). Sub. P neurons of the gut receive inputs from excitatory cholinergic interneurons, which run relatively long distances up and down the intestine. It is now clear that Sub. P containing enteric neurons are arranged in a highly ordered fashion and that they are part of multineuronal pathways within the intestinal wall (139; see 139).
At least in the guinea-pig, some fibres originate from extrinsic cell bodies. Producing lesions in the extrinsic innervation of the guinea pig ileum reduces the population of Sub. P fibres projecting to the submucosa and to blood vessels. These fibres reach the gastrointestinal tract via mesenteric and perivascular nerves and via vagal and sacral autonomic nerves (52; see 52; see 139; see 170; see 239).
It has further been suggested that some Sub. P fibres in

the intestines are afferent (357; see 497; 793) and that they reach the central nervous system via the vagal nerves (268; see 497). A sensory function for Sub. P. fibres has been suggested in the following literature (73; 88; see 88; 139; see 170; 325; see 325; 340; see 340; 355; see 571; see 629; see 680). The cell bodies of the Sub. P neurons lie most likely in the dorsal root ganglia (in the rat 10-20% of the neurons show Sub. P-IR) .In the rat and guinea pig 40-60% of the spinal afferent neurons to the stomach, and the portal vein/ hepatic artery contain immunoreactive Sub. P. Capsaicin, a substance that rather selectively damages unmyelinated afferent neurons, causes the disappearance of those Sub. P-IR axons which are lost after extrinsic denervation (52; see 52; 170; see 170; 340; see 340; see 571) and a depletion of both spinal and vagal Sub. P, but there is little or no difference in the concentration of Sub. P in stomach extracts (170; see 170). Nevertheless, the contribution of extrinsic nerve fibres to the Sub. P content of the gastrointestinal tract seems to be minor, at least in the rat and guinea-pig (in contrast to the cat and dog) since neither chronic extrinsic denervation nor systemic treatment with capsaicin changes the Sub. P content of the gut (52; see 52; vide supra). Finally, Sub. P fibres are frequently seen around intramural blood vessels, where they may mediate the function of a vasodilator (73; 139; see 239; 325; see 325; 605; see 629; see 723). These perivascular Sub. P fibres are thought to have an extrinsic origin (52; see 52; 139; see 139; see 239).

Vasoactive Intestinal Polypeptide (VIP)

In the mammalian gut VIP is located in nervous elements exclusively (see 108; 118; see 118; see 121; see 188; 201;203; see 239; 248; 325; see 325; see 379; 455; 457; 458; 518; 605; see 677; see 680; see 723; 725). It is now clear that the early observations, indicating that VIP also occurred in entero-endocrine cells, was due to the fact that the primary antisera were locating substances with a homology to secretin and glucagon. These substances have similarities with the N-terminal of VIP (see 239). VIP nerves occur in all layers of the gut and they may be considered as the most abundant of all peptidergic fibres, hence constituting a quantitatively very important nerve population in the gut. In accordance with the three main actions of VIP on the gut i. e. muscle relaxation, stimulation of water and electrolyte secretion and vasodilation, VIP nerves may be expected to be found around blood vessels, in the enteric plexuses and, probably for the largest part, in the circular muscle coat and mucosa (73; see 239; 248; 325; see 325; 354; 458; 605; see 677; see 680; 723).

VIP-immunoreactive nerve cell bodies and fibres occur in both plexuses throughout the gastrointestinal tract (73; see 73; 118; see 239; 242; 246; see 246; 248; 325; see 325; 354; 379; 458; 497; see 497; 518; 605; see 605; 677; see 677; 678; see 680; see 723). In the enteric plexuses VIP-IR neurons are the most abundant, followed by Sub. P, and with only a few SOM and ENK-immunoreactive neurons (518).

VIP neurons represent about 2-8 % of the myenteric neurons (246; 497; 677; see 723). Both in the myenteric and submucosal plexuses VIP-IR neurons were seen to be surrounded by numerous Sub. P-IR nerve fibres (677). However, in the guinea-pig caecum Auerbach's plexus is devoid of VIP-containing perikarya (393; see 497), while in other species (dog) more VIP-containing neurons occur in the myenteric plexus than in the submucosal plexus (518). Furthermore, physiological studies have established that VIP excites about 45 % of the myenteric neurons in the ileum of the guinea-pig (see 246; 792) and that VIP stimulates the release of ACh from myenteric neurons. This release is susceptible to inhibition by SOM (809).

NPY coexists with VIP in certain non-adrenergic enteric neurons and in non-adrenergic neurons in pelvic ganglia (323; see 323). In the guinea-pig small intestine a very complex overlap of chemical markers in the myenteric perikarya has been found. Indeed, Dogiel type Ia neurons may show immunoreactivity for VIP/ DYN/ENK/GRP/NPY; and type Ib neurons for VIP/ DYN/ENK/GRP/CCK. Antibodies against VIP/DYN stain some type III neurons. Finally, some unclassified myenteric neurons are immunoreactive for VIP/DYN/ ENK/GRP; VIP/DYN/ENK/GRP/NPY; VIP/DYN/ GRP and, finally, VIP/DYN/GRP/NPY (239; see 239). Within the myenteric ganglia VIP-containing nerves form a fairly dense network, some fibres making ring-like arrangements around nerve cell bodies (143; see 239; 248; 325; see 325; 458; see 497). Myenteric VIP-ergic neurons project in an anal direction and supply the circular muscle coat. In this manner numerous VIP nerves are seen in the circular muscle layer (see 121; 239; 248; 325; see 325; see 379; 605; see 605; see 723). These observations support the idea that VIP could be the transmitter released by enteric inhibitory nerves that are involved in the descending inhibitory component of the peristaltic reflex (148; see 246; 248; see 248;

see 325; 347). Moreover, a particularly high number of VIP-containing nerve fibres have been reported in all gastrointestinal sphincters (18; see 239; see 325; 379; see 497; see 723), although this seems now not as general as initially assumed (379). In the longitudinal muscle layer, in contrast, VIP fibres are almost absent, which explains the recently reported lack of a direct effect of VIP on longitudinal smooth muscle preparations of the guinea-pig (325; see 325; 605; see 605).
As a rule the majority of the VIP-ergic neurons have been encountered in the submucosal plexus where 25-50 % neurons are immunoreactive for VIP (73; see 73; 239; 246; 379; 497; 605; 677; see 723). In addition, double staining for VIP and DYN has revealed in the small intestine of the guinea-pig DYN immunoreactivity in all submucosal neurons that stain for VIP (239; see 239). VIP submucosal neurons probably supply the dense VIP innervation of the submucosa and mucosa (118; 239; see 239; 248; see 325; 497; 677; see 723). In the mucosa VIP nerves form a network around the gastric and intestinal glands. In the small and large intestines the mucosa has numerous VIP fibres extending up to the epithelium. In this way single nerve terminals, running just beneath the epithelium, come into a close contact with secretory mucosal cells and blood vessels (118; see 239; 325; 354; 379; 605; see 680; 723). This morphology fits well with the peptide's role as a stimulator of secretion (73; see 73; 118; see 239). In addition, some VIP axons observed in the mucosa may correspond to pseudo-unipolar sensory neurons involved in the intestino-intestinal reflex. Some mucosal nerves form synaptic connections with prevertebral ganglion cells and, recently, a significant concentration of VIP has been found in the cell bodies of the dorsal root ganglia (see 170) and in the dorsal horn of the spinal cord (379).
VIP nerves have been found in the wall of intramural blood vessels (118; see 239; 354; 379; see 680; 723; see 723) and evidence has been given for a VIP release from enteric vasodilator nerves (190; 205; 206; see 246; see 605).
Finally, it may be interesting to note that in the cat salivary gland both the sympathetic and parasympathetic nerves contain a biologically active peptide. NPY is present in a population of the sympathetic noradrenergic fibres, mainly innervating blood vessels and VIP occurs in the parasympathetic cholinergic fibres (350; see 350). Indeed, several lines of evidence suggest that VIP-IR neurons, which project to exocrine glands in the cat, are cholinergic (see 51; see 484). The light microscopical evidence of a co-storage of VIP and ACh has been supported by ultrastructural studies in which VIP was localized to fibres containing small, agranular vesicles like those found typically in cholinergic nerves (see 73; see 239). In addition, VIP and PHI are co-synthesized in the same precursor molecule. In consequence, some autonomic neurons to the exocrine glands contain both VIP- and PHI-IR (484; see 484). Both VIP and ACh are released on parasympathetic stimulation of the submandibular gland of the cat. Low-frequency stimulation causes preferential release of a classical transmitter (ACh, mainly stored in small vesicles), whereas high-frequency activation also induces the release of peptide (VIP, from large dense-cored vesicles (484; see 484). VIP potentiates ACh-induced secretion and when ACh and VIP are infused together additive effects on blood flow are observed (350; see 350). VIP causes vasodilation but no secretion per se, its action being to enhance the secretory response to ACh. The VIP-induced potentiation of cholinergic salivary secretion may in part be due to the additional increase in blood flow and also to a direct effect of VIP on secretory elements (484; see 484). Moreover, VIP markedly affects cholinergic binding in the cat salivary gland (350; see 350). In addition, the release of ACh from all of these ACh/VIP-containing nerves is regulated via a muscarinic autoreceptor feedback system. Via these receptors ACh inhibits the release of VIP and VIP acting at VIP receptors inhibits the release of ACh (51; see 51). Furthermore, parasympathetic nerve stimulation is also accompanied by an increase in the output of PHI (see 484). In consequence, the parasympathetic control of exocrine gland secretion and blood flow seems to depend on a multi-messenger system involving the classical transmitter ACh and at least two peptides i.e. VIP and PHI (484).

Somatostatin (SOM)

In man and many animals (e.g. mouse, rat, guinea-pig) SOM is principally localized in endocrine cells and in neurons and nerve fibres of the enteric plexuses (21; 73; see 73; 146; see 146; 148; see 239; 325; see 325; see 356; 357; 358; 359; see 397; 605; see 605; see 629; 679; 681; 723; see 723).
In the guinea-pig about 17 % of the submucosal neurons and 3 % of the myenteric neurons react for SOM (246; 379). However, no SOM-IR neurons have been found in the rat stomach, while in all other areas of the intestines of rats and guinea-pigs 2-14 % of neurons contain SOM (see 239; 497). The SOM- ergic neurons

of Auerbach's plexus project in an anal direction (see 246) and end mainly within the enteric plexuses. Some fibres form a basket-like arrangement around nerve cell bodies, though other neurons appear to be only sparsely innervated (144; 246; 497). The coexistence of SOM with other transmitters in the intra- and extramural neurons has recently been demonstrated (see above).
SOM-immunoreactive nerves were first reported by Hökfelt et al. in 1975 (359). Most nerves are intrinsic (see 239; 246) although SOM stores with an extrinsic origin have also been found (148; 354; see above). Unlike other peptides (Sub. P, VIP and ENK) SOM nerve fibres are principally localized in the enteric plexuses and they are sparse in the intestinal musculature (73; see 73; see 239; 325; 356; 379; 497; see 497; 723). In the rat and guinea-pig SOM-IR fibres have been found in the myenteric ganglia of both the large and small intestines, and also in the stomach where they are relatively scarce (see 497; 678). Due to this topography SOM, most probably, has little or no direct effect on the intestinal musculature (131; 146; see 146; see 246).
SOM-ergic fibres in the submucosal plexus of the guinea-pig small intestine appear to originate from submucosal perikarya (144; see 497). However, only a few SOM nerve fibres can be observed and these form a network around the intestinal crypts or are associated with the submucosal arterioles (see 239; 325; see 497; 723).

Enkephalins (ENK)

Both leu- and met-ENK immunoreactive neurons and fibres have been observed in the gut of several species (mouse, hamster, rat, guinea-pig, rabbit, cat, dog, pig, monkey and man) (73; see 73; see 239; 325; 379; see 379; 472; 473; see 473; 518; 605; see 605; 723). In cultures of the enteric plexuses ENK is seen to originate mainly from the myenteric plexuses (617).
Counts of immunoreactive nerve cell bodies indicate that about 4-25 % of myenteric neurons contain an ENK- like substance (473; 605).
ENK immunoreactive axons are particularly numerous in the stomach, and in the small and large intestines. They provide a rich innervation to the circular smooth muscle, the deep muscular plexus and the myenteric plexus where they come close to ganglion cells (73; see 73; 379; 473; 605; see 605; 723). ENK- ergic nerve fibres have a distribution very similar to that of Sub. P. They predominate in the smooth muscle layer and in the myenteric plexus (325). Contrary to what may be postulated from this distribution, there is little or no direct effect of ENK on the intestinal musculature of the guinea-pig (473). However, its inhibitory role on the neuronal activity is in agreement with the fact that ENK is mainly found in the myenteric plexus (73; see 73).
As a rule very few ENK-containing axons and no positive nerve cell bodies have been observed in the submucosa or mucosa, though ENK-like immunoreactivity has been seen in fibres in the submucosal plexus itself (see 239; 473). Moreover, in the hamster, rat and guinea-pig nerve fibres, containing leu^5-and met^5-ENK are most numerous in Meissner's plexus (472). In the human gut scattered ENK- ergic nerve fibres are present in the muscularis mucosae (73; see 73; see 239).

Bombesin

Bombesin-IR perikarya have been observed in the dorsal root ganglia, but it is as yet not clear if this peptide occurs in visceral afferents (see 170). At least in the rat there appears to be a rich distribution of bombesin-like immunoreactive fibres in the gastric mucosa unlike in the intestinal mucosa where they are few. Nerve cell bodies in the myenteric plexus are provided with an abundant supply of bombesin fibres. On the basis of these findings it has been suggested that bombesin-like peptides are neurotransmitters in the gastrointestinal tract and could play a role in the modulation of motility and in the release of other neurochemical substances (gastrin) (see 188). In this respect it has been found that bombesin functions as a neurotransmitter of excitatory interneurons in the myenteric plexus (see 121; see 379).

These data concerning the neurochemistry of the enteric NANC system show clearly that the gastrointestinal tract is richly innervated by a complex and interconnected network of autonomic nerves including a large and complex peptidergic system (73; see 239; 379; 605). Although peptidergic nerves are distributed throughout the entire length and width of the gut, marked differences are observed between them in respect to their localization within the various layers of the gut wall. Nerves containing particular peptides often have a characteristic distribution within the gut wall and a well-defined set of actions compatible with their localization (356; 605). Peptidergic nerves form with the other intrinsic nerves what appears to be a largely autonomous unit that may be considered as a "minibrain" under the general influence of the central nervous

system but able to function, to a certain extent, by itself. Although often grouped together, peptide-containing nerve cell bodies and fibres of any particular type seem at first more or less randomly distributed throughout the enteric ganglia. This apparently random distribution differs significantly from the central nervous system where nerve cells tend to be organized into morphologically or functionally similar nuclei or groups and from other autonomic ganglia where cells with similar functions and chemical properties are grouped (473). However, more detailed studies of the distributions and projections of peptide neurons in the gut indicate that the connections which the different types of neurons make within the ganglia are highly ordered. Thus there seems to be an ordered projection of neurons with 5-HT-, ENK-, VIP- and CCK- like immunoreactivity within the myenteric plexus. For example in the guinea-pig small intestine myenteric neurons containing a SOM-like substance project caudally and have processes averaging 10 mm in length. Some SOM-immunoreactive fibres end in basket-like groups of varicosities around SOM + neurons while other fibres end around non-reactive neurons (242; 243; 244; 473; see 473).

The characteristic architecture of the enteric peptidergic nervous system supports earlier suggestions of an autonomous enteric brain and provides anatomical support for the numerous separate and well defined spectra of actions (379). Moreover, recent evidence for the coexistence (co-release) of different substances in various combinations in the same neuron, have made the picture of the enteric innervation even more complex (239; see 239). The functional significance of coexistence is, at present, difficult to evaluate but coexistence should not be looked upon as a uniform phenomenon. Evidence has been obtained that classical transmitter and peptide are co-released and interact in a cooperative way on effector cells. In addition to enhancement, there is also evidence that other types of interaction may occur. For example, the peptide may inhibit the release of the classical transmitter. Thus, multiple types of interaction may provide a mechanism for obtaining differential responses and for conveying an increased number of messages. Indeed, multiple messengers may provide the means for increasing the capacity for information transfer in the nervous system. Moreover, it may be argued that the coexistence phenomenon provides a basis for the view that peptides may well be involved in other functions since they are present in neurons which already have a transmitter at their disposal i.e. the classical transmitter. Indeed, the different types of compounds do not necessarily have to be involved in the process of transmission at synapses, but could also participate in other events, such as, trophic effects or the induction of other types of long-term events in neurons and effector cells (cfr. Sub. P exerts growth stimulatory effects on smooth muscle cells) (350; see 350).

In summary, during the last decade modern techniques, radioimmunoassay and immunohistochemistry in particular, have profoundly changed our understanding of the innervation of the alimentary canal. Many morphological arguments can be given to maintain that a complex control system, built up by extrinsic and intrinsic nervous elements, manage the gut. Within this control system a large spectrum of substances, e.g. 5-HT; non-5-HT amine handling; purines and peptides, have been found besides the classical neurotransmitters ACh and NA. These NANC substances, which most probably function as neurotransmitters/modulators in this system, occur as separate entities or in various combinations with each other (coexistence, co-release).

In the gut 5-HT has been found both in some enterochromaffin cells and in the intramural nervous elements. 5-HT immunoreactive perikarya of the myenteric plexus project caudally within this plexus and to the submucosal ganglia where they ramify extensively. They further contribute branches to the deep muscular plexus.

Peptidergic nerves have a predominantly intrinsic origin, although a number of outside connections occur. They are found along the entire length and breadth of the gastrointestinal tract and constitute a major component of the ENS. VIP, Sub. P and ENK are the most abundant peptides of the gut wall and all display a characteristic pattern of distribution.

Sub. P. has, just like 5-HT, a dual localization in the gut i.e. in the epithelial endocrine cells and in the intestinal nervous tissue. Sub. P. immunoreactivity has been found mainly in the myenteric neurons and in the tunica muscularis. Fibres ramify extensively and form a dense network around the enteric neurons (especially in the myenteric plexus). Thus, the main target organs of Sub. P. are the enteric neurons and the intestinal musculature and therefore the peptide may have a role as a transmitter in excitatory interneurons. In

different neuronal type (Dogiel types I and II and unclassified neurons) Sub. P most probably coexists with VIP, ENK, SOM, DYN, GRP and CCK in various combinations. Sub. P. has further been seen around intestinal blood vessels, in the mucosa and it has recently been suggested that it may have a sensory function as well.
In the mammalian gut VIP, representing the most abundant peptide, is found in all layers. Most VIP-containing perikarya occur in Meissner's plexus (species differences) from where they probably supply the dense VIP innervation of the mucosa and the gastric and intestinal glands. In the intestinal villi some fibres extend just beneath the epithelium. Within the ganglia VIP fibres form a dense ring-like network around some neurons. VIP nerves are numerous in the circular muscle layer and in the sphincter regions in particular. This localization is compatible with the main actions of VIP on the gut (muscle relaxation, stimulation of the secretion and vasodilation). A complex overlap of VIP with other chemical markers such as ENK, DYN, GRP, NPY, CCK has been discovered in different types of myenteric neurons.
SOM is principally found in endocrine cells as well as in neurons and nerve fibres in both enteric plexuses. Few SOM- ergic neurons have been observed in the myenteric plexus. They project in a caudal direction and end within the myenteric or submucosal ganglia. Few fibres occur in the intestinal musculature and mucosa. The coexistence of SOM with other peptides in the enteric neurons has been confirmed by different investigators.
ENK occur in neurons and nerve fibres in the gut in different species. Immunoreactive perikarya have only been encountered in the myenteric plexus, while ENK-ergic nerve fibres provide a rich innervation of the circular muscle layer, the deep muscular plexus and the myenteric plexus and in some species (guinea-pig) also in Meissner's plexus. Very few ENK-containing axons have been observed in the submucosa and mucosa and none of the perikarya are positive.
Finally, the distinction between neurons and endocrine cells is becoming increasingly vague since several peptides have a dual localization i.e. in entero-endocrine cells and in the intestinal nervous tissue.

I. 3. 5.
FUNCTIONAL SIGNIFICANCE OF THE ENS

Langley distinguished the enteric ganglia from other autonomic ganglia on a morphological basis and on clear indications that they contain interneurons that do not receive any direct synaptic input from parasympathetic preganglionic fibres (797; see 797). Indeed, there are considerably more enteric neurons than CNS fibres reaching the gut (see 638). The human vagal nerves, for example, hold fewer than 20.000 fibres (afferent and efferent) as they pass through the diaphragm while the number of enteric neurons is so large (approximately 10^7 to 10^8; small intestine of the guinea-pig 6 x 10^6 neurons) that it rivals the number in the spinal cord (246). CNS inputs impinge on the enteric ganglia and, in consequence, the extrinsic effects on the digestive tube are realized through the ENS. As the great numerical discrepancy suggests most neurons of the ENS are not connected to the CNS at all and receive probably only an input from the postganglionic autonomic nerves. Based upon this morphology the ENS must, in consequence, be able to perform many functions independently of the CNS. This morphological phenomenon and the physiological independence of the ENS support the hypothesis that the direct influence of the CNS on enteric neurons, and hence on the normal functioning of the ENS, might be minor. Therefore, motility is not irreparably harmed by severing the connections between the ENS and CNS (35; see 239; 246; 273; 275; 399; 629; see 638; 741; 745; 758; see 758). Intrinsic ganglia, therefore, most probably contain the basic circuitry both for processing the information supplied by extrinsic command signals on the one hand and by the various kinds of sensory receptors, present along the gut on the other hand and for generating precise patterns of neural outflow (723; 796). Thus, the intrinsic innervation must be involved in the regulation of the different motility patterns observed in the alimentary canal (573). The functional impact of the intrinsic innervation on the normal motor activity of the gut is clearly illustrated by the intestinal obstruction brought about when a segment is congenitally deprived of its ganglion cells (cfr. Hirschsprung's disease in man, the piebald and lethal spotted strains of mutant mice and the lethal white foal syndrome in the horse) or when the loss is acquired (cfr. Chagas' disease in man, Grass Sickness in horse) (see clinical aspects). Although the

aganglionic segments receive an adrenergic and a cholinergic innervation, the absence of enteric ganglion cells eliminates the local coordination and integrative activity associated with the intrinsic ENS elements. This results in defective motility and a permanent obstruction of the intestinal segments involved.(see 239; 27; 273; 275; see 275; 377; 741; 745; 752; 799; see clinical aspects).

This concept of the unique nature of the ENS was strongly supported by the results of subsequent neurophysiological investigations (797; see 797). Nevertheless a complex interplay of hormonal and neuronal signals generated by extrinsic and intrinsic neurons, was shown to regulate the different intestinal functions and local blood flow. Hence, it is now believed that the ultimate function of the ENS is most likely the control and coordination of the various effector systems (muscles, mucosa, blood vessels) (see 239; 573; 723; 796).

Portions of the intestine stimulated in vitro respond by contraction proximal to the point of stimulation and by relaxation distal to the point. It was believed that this stereotyped reaction (reflex), described as the "law of the intestine" by Bayliss and Starling in 1899, was due to a local nervous reflex (275; see 275; 674; see 812). The term "reflex" suggests the existence of classical reflex arches within the ENS (116; see 239; 246; 273; 275; 399; 629; 758; see 758; 797). Although it was argued that intestinal reflexes are rather primitive in their organization, they probably subserve multiple functions and are appropriately designed for the regulation of the intestinal musculature (161). Furthermore, some enteric nerves may not be directly involved in reflex pathways but rather in their modulation (473). In addition, the gut is capable of far more complex patterns of motor activity than simply the peristaltic reflex since not all intestinal motility is propulsive. Indeed pendular non-propulsive movements which may have a mixing function have to be appropriately integrated and coordinated with the propulsive activity for the normal digestive processes (see 275).

Although, intestinal smooth muscle has an inherent tone and exhibits spontaneous rhythmic activity it normally functions under a nervous control which is primarily from two different levels. First by stimuli from the CNS (brain and spinal cord) and the prevertebral ganglia (celiac, superior and inferior mesenteric ganglia). Second, and the more important, is the programming and integration that is performed by intrinsic machinery within the ENS itself (58; see 164; see 239; 339; 674; see 674; 761). The degree of CNS control over the intestine is not uniform along the gastrointestinal tract but varies from region to region. The small intestine, for example, appears to function quite normally in the absence of CNS input whereas the oesophagus, stomach and colon are more dependent on CNS input for their normal functioning (58; see 164; 674; 741; 745). An autotransplanted small bowel segment generates migrating motor complexes. Thus, the ENS is self-sufficient and must possess all the basic elements of a reflex arch i. e. receptors (sensors), interneurons and neurons making either inhibitory or excitatory connections with their principal slowly-responding target organ: the intestinal musculature. It has been found that integration between the excitatory and inhibitory influences is realized by a temporal separation of the activity in the two types of nerve fibres (343; see 761; 796). Since the ENS is considered to be an integrative system three functional subdivisions may be recognized.

Sensory receptors constitute the first subdivision. They transduce changes in chemical and mechanical energy into a steady flow of neural information that is transmitted to processing centers within the enteric ganglia and the CNS.

The second subdivision consists of an internuncial circuitry (the central processing unit) that processes the sensory information and generates appropriate signals for controlling the outflow of commands in motor neurons to the effector systems. Internuncial circuitry also contains neural networks that are preprogrammed to generate cyclical stereotyped patterns of motor behavior. The role of sensory feedback to these networks is one of adjustment and modulation.

Motor neurons, finally, represent the third (functional) subdivision. Discharges of the motor neurons represents the final decision of the integrative circuitry and the final common pathway to the effector. Coordination of secretory activity, mucosal transport, blood flow and motility is required for efficient progression of the digestive processes and there are many indications that the ENS has a major role in unifying these functions (see 239; 796; see 796).

As it is clear from the discussion above that all intestinal functioning is based upon intestinal reflex activity. In consequence, all the classical components of a reflex arch i. e. receptors, afferents, central processing circuitry, efferents and effectors, must be present in the gastrointestinal wall in order to build up the intestinal reflex arch. Moreover, each link must function smoothly in order to execute the different intestinal functions in a controlled and coordinated manner (see 239; 796; see 796).

First link: the intestinal RECEPTORS (Input data)

A variety of receptors has been found within the intestinal musculature, the submucosa and the mucosa itself. However, the exact type of information conveyed by the different intestinal receptors is not always fully understood, since their functions have been difficult to judge from their morphological appearances (35; see 35). Nevertheless, the variable activation of the intestinal receptors most probably relates lumen contents to an activation of intrinsic neurons through the integrating circuitry within the enteric ganglia. Indeed, G.I. hormones, released after a meal, act in many cases to enhance or depress the release of ACh from intrinsic neurons and may tune their sensitivity to stimuli from luminal contents (see 157; see 796; 797).

Histologically, no real sensory structures nor sensory neurons have been described within the ENS. However, Meissner's plexus contains, probably exclusively, a special type of ganglion cell (bipolar, unipolar or pseudo-unipolar) that bears a distinct resemblance to the dorsal root perikarya (see 796). Moreover, it is possible that there are no intrinsic sensory neurons per se. Rather the multipolar ganglion cells might be multifunctional in that some processes may function as sensory detectors, while others may function as dendrites receiving synaptic input(s) (796). Additionally, the behavior of enteric mechanoreceptors is similar to that of mechanoreceptors discharging in intestinal afferents. So the question rises of whether the same mechanoreceptors provide sensory information to both the CNS and the local integrative network of the enteric ganglia (see 798).

Physiologically (electrical recording techniques) the existence of chemo-, mechano-, thermo- and noci-receptors have been demonstrated within the gastrointestinal wall as well as afferent nerve fibres projecting from there via vagal, pelvic, mesenteric and splanchnic nerves to the CNS. They can be divided into separate populations with different functional properties (see 246; 390; see 390; 796; see 796). Thus, undoubtedly enteric sensory receptors provide both the ENS and CNS with a steady flow of information on the prevailing conditions within the intestinal lumen and wall (573; 797).

Chemoreceptors

Chemical stimulation of the mucosa elicits enteric reflexes. Enhanced contractile activity is seen proximal to the chemical stimulus, while inhibition occurs distal to it (see 239). In addition, chemoreceptors have been described in the spinal afferent pathway from viscera (390; see 390). These receptors react to osmotic pressure, certain fats and organic acids (see 239; 573).

In the gastric mucosa acid and alkaline chemoreceptors specifically sensitive to the presence of acidic (pH: 3) or basic (pH: 8) solutions respectively, have been discovered. They discharge continuously for as long as the stimulus is present (see 28; 158; see 239; 573; see 796). However, gastric mucosal chemoreceptors can no longer be regarded as pH receptors (see 582) since the response to acids is not related to their pH value per se but rather to their titratable acidity (cfr. titratable alkalinity for duodenal receptors) and their molecular weight (see 28). Furthermore, it seems that since the chemoreceptors signal the passage or presence of gastric contents, they could also be involved in some way with the sensations of immediate satiation following distension of the stomach with food (582; see 582).

There are mucosal receptors which respond to only one group of substances (carbohydrates or amino acids) and not to mechanical, osmotic or thermal stimuli. The two best characterized types in the cat are glucoreceptors and amino acid receptors.

(1) Glucoreceptors: although glucose is the most potent stimulus, some of them respond to carbohydrates other than glucose (e.g. D-levulose, D-galactose, lactose, maltose). Their discharge is prolonged and very slowly adapting. The receptors appear to be more concentrated in the duodenum and proximal jejunum than in more distal regions of the gut.

(2) Amino acid receptors respond only to amino acids in the lumen of the small intestine. These afferents are not spontaneously active. The discharge is prolonged and slowly adapting (cfr. glucoreceptors). A single unit can respond to amino acids from each of the three groups (neutral, acidic, basic) of amino acids (28; see 28).

Installation of fatty acids into the duodenum causes initial excitation followed by sustained inhibition (see 239).Slowly-adapting chemoreceptors responding specifically to glucose, hypertonic NaCl, potassium oleate, sodium dioxycholate, tryptophan or organic acids, have been recorded in vagal afferents originating from the small intestine (see 28; see 239; see 573; see 796; 797). In addition, in the rat and sheep there are fast-

adapting receptors, which respond to stroking of the gastric mucosa or epithelium. The vast majority of them are most probably polymodal receptors since they have a degree of chemosensitivity (28; see 28).

Mechanoreceptors

Localized distention (liquid distention seems the most appropriate stimulus) elicits single or multiple transient, graded contractions of the circular muscle, the longitudinal muscle contracting also on the proximal side (up to 7 cm) with graded relaxations (up to 10 cm) on the distal side. This indicates the existence of an intrinsic ascending excitatory pathway and a descending inhibitory pathway. Moreover, it has been demonstrated that the relaxation on the distal side of the stimulus depends on the activation of enteric inhibitory neurons supplying the muscle. However, distention of the colon elicits an inhibitory reflex that acts on the distended area to allow further distension. This inhibitory reflex apparently seems to be involved in the local accommodation of the colon. Thus, it seems that in the storage regions of the gut (the stomach and large intestine) there is a reflex of accommodation, while in the small intestine a local excitatory reflex predominates (see 239).

Mechanoreceptors have been discovered within and outside the gut wall. Within the gastrointestinal wall they occur in the mucosa, submucosa, muscle and serosa. In the cat and sheep three types of mechanoreceptors have been identified in the region of the LES i.e. mucosal, muscle and serosal mechanoreceptors. All three types of mechanoreceptors are likely to be transiently stimulated by gastric contents entering the esophagus during vomiting and hence may play a role in its coordination (28; see 28).

Direct electrical recording from intestinal afferents has established the existence of mechanosensitive units. Generally, the pattern of distribution of the majority of the mechanosensitive sites is related to the path of the neurovascular radicles. As a result, one axon does not normally innervate more than one segment of the small intestine although there is a restricted overlap between the sensory fields of adjacent neurovascular bundles. Some units have receptive fields resolved into 1-5 sensitive points, which are separated by up to 1 cm, and are located at sites of arterial division in the mesentery or where the intestinal arteries enter the musculature. These sensitive spots responded to light mechanical stimuli and distortion (390; 540). Intestinal distension increases the tension in the bowel wall and the length of the distended segment. This invariably causes distortion of the adjacent mesentery and hence of the mesenterial mechanosensitive units. In this way many mechanosensitive units near hollow viscera (bowel or gallbladder) behave as "in series" tension receptors (540). Vagal units have receptive fields which are entirely confined to the bowel wall. Their endings, responding to changes in wall tension, are in the muscle layers. The majority of the vagal units consist of a single sensitive area and normally do not innervate the mesentery (540; see 540).

The majority of mechanoreceptors in the spinal afferent pathways from viscera have receptive fields in a mesentery or peritoneal ligament and/or the adjacent viscus. They consist of between one and nine mechanosensitive spots distributed along the course of the nerves (periarterial nerves) innervating the viscera (390).

Splanchnic units often include endings in the peritoneum, while their intramural endings are mainly serosal. Lumbar splanchnic afferents course along the branches of the caudal mesenteric artery and typically have punctated sites of mechanosensitivity (540; see 540). About 120-350 Pacinian corpuscles are present in the mesenteries and pancreas of the cat. These sense organs have large myelinated fibres and are extremely sensitive to vibrations transmitted from outside the animal (390; see 390).

Pressure receptors, responsible for activating the peristaltic reflex, seem to be located in the intestinal mucosa (see 275; see 573; 797). Pinching of the gut stimulates the mechanoreceptors to fire at a higher frequency. These receptors appear to signal movement of the mucosa resulting from contraction of the muscularis mucosae as well as the presence and movement of luminal contents (see 573; see 796). Stroking the mucosa of the small intestine of the dog elicits an excitation on the proximal side and an inhibition on the distal side of the stimulus (the "mucosal" reflex). In the rabbit the magnitude of the response is related to the strength of the stimulus, and strong stimuli elicit a series of contractions (see 239). Esophageal mucosal mechanoreceptors in the cat and sheep rarely have a spontaneous discharge and do not directly respond to moderate distension or contraction of the LES. They are activated by strong and rapid distension, strong digital compression, stroking of the mucosa and strong and rapid saline injections. They can monitor the consistency of food passing through the sphincter (28).

Receptors which respond only to stroking of the intes-

tinal mucosa appear to be relatively rare (cfr. stomach). In the sheep duodenum only 20% of the mechanoreceptor units do not respond to some chemical stimulus. Two populations of mucosal mechanoreceptors i.e. "low threshold" and spontaneously silent and "high threshold" (receptive field area 1 mm^2) and spontaneously active have been discovered (28). Small "nerve" cells with elongated processes occur in the gastric subglandular layer of the pig and cow and beneath the crypt epithelium in the small intestine of the rat. These neuron-like cells may possibly belong to the sensory apparatus and innervation of the gut (68; 553; see 553; see 758). Peristaltic movements cause mechanical distortion of the ganglia and alter the pattern of discharge of the nerve cells (mechanosensitive units) (see 275; 573).

Light touch sensation is, however, absent from the esophageal and gastric mucosa. Firm probing or squeezing of the mucosa produces a sensation of pressure, but such stimuli could evoke activity in mechanosensitive vagal afferents in all three gut layers (28; see 28).

Sensory receptors (neurons) in Meissner's plexus respond to intraluminal distension. The mucosal reflex, induced by these receptors, is unimpaired by extrinsic denervation but is abolished by asphyxia, stripping of the mucosa or by application of cocaine which suggests that the sensory nerve endings are in the mucosa (see 239; see 573; 744). Removal of the submucosal plexus further prevents the distension-evoked descending excitation but does not prevent descending inhibition. However, it has been argued that neither the inhibitory nor the excitatory phase of peristalsis requires the presence of the mucosa and submucosal plexus (see 239; see 796). Consequently, the sensory receptors of the reflex initiated by distention are also in the myenteric plexus and the external muscles layers (see 239). In the small intestine of the cat induction of peristaltic waves to mucosal stimulation is completely prevented by circumferential cuts through the myenteric plexus. Hence, impulses, originating in the mucosa, pass to Auerbach's plexus, travel along the intestine in this plexus and, finally, end in the muscle (see 239).

Muscle mechanoreceptors appear to be concentrated at the gastric and oral extremities of the thoracic esophagus (28). Esophageal afferents with receptors in the muscle layers can signal the passage of a bolus by the distension it might produce or by the sequential contractions of the esophagus which are used to propel it towards the stomach (28). In the conscious sheep the afferents only discharge in response to a distension if the level of distension is sufficient to evoke a local contraction of the muscle (see 28).

The gastric stretch receptors seem to be suited for signalling the degree of distension of the stomach and the slow arrival of food or fluid into the stomach. Such receptors have also been described in the cat, goat and sheep (582; see 582). With the gradual entry of food or fluids there would be a gradually increasing activity in the gastric stretch receptors, which would continue to increase in proportion to the amount of food or fluid ingested (582).

Some muscle cells within the innermost portion of the circular muscle have been found compared to other muscle cells to be much more elongated, to have a smaller diameter and thinner processes (surrounding nerve bundles), to be better innervated and to have closer contacts with nerve boutons. These structural peculiarities suggest that these muscle fibres could function as length detectors analogous to intrafusal skeletal muscle fibres (796; see 796). Studies on the dog stomach suggest that proximal muscle contraction as well as proximal distention can elicit distal inhibition. Thus, these mechanoreceptors are probably in series with elements of the circular muscle and are most likely associated with them (157). It has been hypothesized that ENK- ergic fibres in the smooth muscle layer of the gut act as stretch receptors (see 723).

Distention of the rabbit's small intestine causes an excitatory effect on specific mechanosensitive neurons in Auerbach's plexus, which regulate the contractions of the intestinal muscle and produce the peristaltic movements (812). But, mechanosensitivity is not a general property of all myenteric ganglion cells. So, at least in rabbit's small intestine, Auerbach's neurons may be classified in two main categories. The first category consists of mechanosensitive neurons, the second consists of neurons whose activities are not changed by gut distension (812). The receptive fields of the mechanosensitive neurons are limited to the region of the ganglion. The discharge patterns of these neurons are similar to those recorded in gastrointestinal afferent fibres within the vagal and splanchnic nerves (see 796; 812). Direct electrical recording from single units within Auerbach's plexus and from afferents within the vagal and splanchnic nerves reveals three subsets of mechanosensitive units. These subsets are differentiated from one another by their pattern of adaptation to mechanical distortion (573; see 796; 798; 812).

One kind of unit behaves like a typical slowly adapting mechanoreceptor, the second like a fast adapting mechanoreceptor and the third like a tonic-type recep-

tor which discharges a stereotyped train of spikes independent of the original stimulus (275; see 275; 573; see 573; 796; 797; 798; 812; see 812). Some units are entirely peritoneal, others only intramural and still others have been established in both (540).

Slowly adapting mechanoreceptors (SAM) show sustained discharge without adaptation during a constant stimulus. The frequency of their discharge is in direct proportion to muscular contraction, indicating that they are connected "in series" with intestinal muscle. Distension of the intestine within the physiological range as well as contraction and compression of the muscle have been considered to be the adequate stimulus. Stretch receptors have been reported in the esophagus, stomach and small intestine of the rat and cat and, most probably, in the forestomach of the sheep. In the sheep they appear to be located in the longitudinal muscle layer of the duodenum. Esophageal muscle mechanoreceptors in the cat and sheep have a relatively high level of spontaneous discharge which increases during distension. These receptors are also stimulated by contraction of the sphincter evoked by vagal stimulation. All the receptors are slowly adapting. The muscular mechanoreceptors serve to monitor the level of tone in the LOS and in this way play a role in its reflex control. In the anal and rectal mucosa SAM with punctuate receptive fields have also been described. It has been postulated that the SAM provide the integrative circuitry with quantitative information about the volume changes of the viscus and accurate information on the stimulus energy (28; see 28; 540; see 573; see 796; 812; see 812). SAM can be reactivated repeatedly (see 798). The vagal SAM are confined to the walls of the viscera and respond to tensions in them. They appear to be involved in the reflex regulation of gastric volume. Splanchnic SAM have a wider distribution as they are present within the viscera and external to them. Hence, they are also excited by changes in tensions on the mesenteries. Splanchnic afferents, which show no consistent relation to volume, do not have a major effect on the size of the gastric reservoir and are probably implicated in the reflex inhibition of spontaneous gastric movements (28; 540; see 540; see 573). In addition, the range of conduction velocity is another difference between splanchnic and vagal SAM. The vagal afferents are almost entirely C fibres with a few delta units (540; see 540).

Fast-adapting mechanoreceptors (FAM) are represented by epithelial and mucosal receptors identified both in the stomach and small intestine of the rabbit, cat and sheep (see 573) and in Auerbach's plexus of the rabbit small intestine (812). They give an intensity dependent discharge at the onset of the stimulus but quickly adapt (see 798). It has been postulated that they provide information on the mechanical properties of the food, on changes in the passage of intestinal contents and on the rate of change of stimulus energy (573; 796).

Tonic-type mechanoreceptors (tonic type enteric neurons) (TTEN) are insensitive to deformation itself. They signal, with a stereotyped all or nothing response, only the mechanical distortion of the ganglion. Once activated they generate the characteristic train of action potentials independent of the original stimulus and their discharge continues for many seconds after termination of the original stimulus. When the discharge stops the units are refractory to reactivation for a prolonged time (up to 2 min) (After Hyperpolarization = AH neuron) (246; 275; 395; see 573; see 796; 797; see 798; 812). Interestingly, the activities of these neurons were inhibited by stretching the longitudinal muscle in a longitudinal direction (812). TTEN are multipolar neurons, in which 5-HT is the chemical transmitter. Their somal membranes have been found to behave differently from the higher excitable membranes of their processes. Thus, they can operate as a multifunctional integrative unit with distinct kinds of integrative functions occurring in different topographic regions of the same neuron (796; 797). Independent spike initiation in each of the cell processes might be advantageous in terms of neural economy because one neuron can in effect acting as several separate neurons. In addition some enteric neurons may have a multifunctional involvement in the simultaneous control of more than one kind of effector (796).
Earlier workers suggested that the TTEN are sensory neurons since no synaptic input could be demonstrated (797). But the discharge of SAM always precedes, with a constant time interval, the discharge of the TTEN suggesting that the latter may be triggered by inputs derived from the SAM. Hence, TTEN are most probably higher order neurons activated by input derived from other "mechanosensitive" units. Consequently, TTEN may only be involved in the intrinsic integrative mechanisms for the neural control of gastrointestinal motility (see 275; see 573; 796; 797; 798). Depending on the nature of the connections, the activity of the TTEN would produce either excitation or inhibition of an inhibitory neuron (798).

Mechanosensitive units recorded in the myenteric ganglia appear to correspond to the "deep" tension receptors discharging in vagal afferents. Light microscopy has shown receptor-like structures within the connective tissue capsules of Auerbach's ganglia. Electron microscopy, in addition, has demonstrated peculiar contacts between neuronal terminals and the intraganglionic surface of the periganglionic sheath. These contacts might represent sensory structures (see 796). Thus, the generator region of the "deep tension receptors" is most probably within the periganglionic connective tissue (see 796; 797).
Two other types of mechanoreceptors have been identified. One group, located in the serosa, responds to serosal probing, stretching and distension of the duodenum with a balloon. Activity is only weakly stimulated by evoked duodenal contraction. The second group is located in the lesser omentum. These two groups of receptors have similar characteristics to splanchnic receptors (see 28). Serosal mechanoreceptors in the esophagus of the cat and sheep are activated by stretching or stroking the serous membrane and are insensitive to contraction of the LES or superfusion of the mucosa. Their discharge is rapidly adapting (28).

Pain receptors

Pain from the abdominal and pelvic viscera seems to be mediated almost entirely through sympathetic afferent fibres. Visceral pain could originate from specific nociceptors (see 582).
The functional properties of some splanchnic units strongly suggest that they may be involved in the mediation of visceral pain. Tension on mesenteries or blood vessels, excessive distension, powerful contractions of the bowel and chemical stimulation of splanchnic afferents all excite the splanchnic receptors and are accompanied by pain (540; see 540; see 582). Splanchnic afferents have been found to react vigorously to traction on the mesentery, a stimulus that induces the strongest visceral pain sensations both in humans and animals (83). Visceral afferent fibres in the lumbal splanchnic nerves are activated by colonic distension and may be involved in visceral nociception from the colon. However, it remains doubtful whether the afferent distension-sensitive units subserve only nociceptive functions. Most probably there is a set of spinal afferents which are relatively "unspecific" to the stimuli, as they react to mechanical stimuli eliciting pain (83; see 334). Moreover, it is almost certain that pain can be produced through non-nociceptive inputs provided that the intensity of the sensory input is adequate (intense activity not being an essential requirement) and that the normal pain-blocking inputs are absent (582). In addition, the functional homogeneity of the spinal visceral afferent neurons suggests that the same population of afferents encodes various events that give rise to non-nocuous and noxious sensations, a number of reflexes, and to the regulation of viscera. Thus, no convincing experimental data exist which justify a subclassification into visceral "nociceptive" and other functional subgroups. Noxious and innocuous events in the visceral domain are encoded in the intensity of the discharge of the same population of visceral afferent neurons (390).

Themoreceptors

Themoreceptors have also been described in the spinal afferent pathway from the viscera. In the cat slowly adapting receptors responding to the temperature have been demonstrated in the esophagus and stomach (see 28). The lower esophagus is much more temperature sensitive than the stomach. Activation of the thermoreceptors can modify esophageal and gastric motility. The gastric mucosa is temperature sensitive, and stimuli > 40°C and < 18°C are appreciated as hot or cold, respectively (28).
In the cat duodenum vagal afferents which are activated by luminal perfusion with warm (38°-51°C) or cold (36°-10°C) solutions, have been identified. These receptors are insensitive to mechanical or chemical stimuli (see 28). Heating (> 46° C) the wall of the small intestine of the cat elicits an intestino-intestinal inhibitory reflex. Intra-abdominal heating (42-44° C) causes thermoregulatory changes in sheep and these can be abolished by splanchnic nerve section. Finally, mechanosensitive units in the retroperitoneal space also respond to heat stimuli. Consequently, responses to intra-abdominal heating need not depend on a specific group of afferent units excited by heat (390; see 390).

Polymodal receptors

Such receptors respond to mechanical stimulation of the mucosa and to one or more chemical stimuli. This type of receptor appears to be the most common (28). They have been found in cat and rat in which some duodenal units have a rapidly-adapting response to mu-

cosal stroking and slowly adapting response to acid. In the sheep 80% of the mechanosensitive units of the duodenal mucosa also responded to a variety of chemicals including sodium hydroxide (28; see 28).

Second link: the AFFERENTS
(fibres conveying information to the intramural and extramural reflex centers)

Taken as a whole, enteric reflex centers receive information from two different sources i. e. from the intramural receptor system via "intrinsic" afferents, connecting the various intestinal receptors with the intramural and extramural nervous centers and from centers located outside the gut via extrinsic efferents conveying extramural input to the intestine.
There is ample morphological and neurophysiological evidence that the gut is innervated by intrinsic primary afferent fibres (380; see 674) and for a sensory nervous input to the intestine (68; 553). Sensory information from the gut itself is brought into the different reflex centers via intrinsic afferent nerve fibres. The first, and probably the more important, is the afferent input to the intramural plexuses. This information is next conveyed to the prevertebral ganglia and finally to the CNS (spinal cord, brain stem) where it is integrated with inputs from other receptors (eyes, pain receptors etc.) (see 573). +/- 80% of the vagal fibres are claimed to be afferent, while many splanchnic afferents have been found to stem from the gut (28; 157; see 204). There is little relationship between the mass of abdominal visceral tissue innervated and the number of afferent (and efferent) fibres. This may mean that afferent vagal fibres branch profusely to give receptive fields of adequate size to cover the whole organ (28).
Studies have been made of the afferent fibre number of the hypogastric, lumbar splanchnic, lumbar colonic and pelvic. The lumbar innervation uses about 4600 afferents and the sacral innervation about 7350 afferents.In the cat, the estimated number of spinal visceral afferents is about 1.5-2.5 % of the total number of spinal afferent neurons supplying the periphery, which may amount to about 1-1.5 million (see 390). In total, about 22.000-25.000 primary afferent neurons appear to be responsible for signalling afferent information from the abdominal and pelvic viscera in the cat (390; see 390). The numbers of fibres in the pelvic, lumbar splanchnic and renal nerves of the rat appear to be about 40, 20 and 33 %, respectively, of those in the cat (390).
Afferents from different intra-abdominal nerve trunks enter different segments of the cord. There is a degree of overlap between these groups of afferents in the dorsal roots, but each nerve shows a peak of afferent innervation in one or two adjacent segments (390).
Immunohistochemical studies suggest that neuropeptides (e.g. Sub. P and VIP) may, likewise, play an important role in visceral sensory mechanisms. In addition, in the spinal cord some peptidergic afferent fibres have a distribution very similar to that of certain components of the visceral afferent pathways.
Moreover, the coexistence of peptides in visceral afferent neurons raises the possibility that individual neurons may release multiple peptide transmitters at their central terminanls as well as at their peripheral terminals. Furthermore, the coexistence in afferent neurons of inhibitory peptides (ENK) and excitatory peptides (VIP and Sub. P) raises the possibility of complex transmitter interactions at visceral afferent synapses (see 28; 160).
Approximately 30% of the mesenteric axons arose from cells in the gut wall. They course centripetally and are thought to synapse in the prevertebral ganglia. In this way it is likely that they are involved in the peripheral inhibitory intestino-intestinal reflex (237; see 540; 644; 674; see 674). Many unmyelinated afferents have likewise been demonstrated in the pelvic nerves. In the cat approximately 30% of the sacral ventral root fibres are unmyelinated and arise from pelvic viscera including the colon and anal canal. The majority of these fibres are afferents (see 540; see 674).
Extrinsic information reach the intramural reflex centers via extrinsic efferent fibres (vagal, splanchnic, pelvic).
Branches of the vagal and pelvic nerves run within the wall of the stomach and the colon and rectum respectively. These "intramural" parasympathetic nerves course between the external muscle coats. The pelvic nerves can be, depending on the species, as long as 10 to 80 % of the length of the colon. These "intramural" nerves constitute a special component of the intramural neuronal circuitry (see 239).
Parasympathetic fibres synapse in the intramural ganglia and innervate both the excitatory and inhibitory enteric ganglion cells. ACh is the transmitter of these preganglionic fibres (58; see 638). However, at the level of the diaphragm the vagal nerve contains only +/ -20% efferent fibres (see above). Thus, the preganglionic parasympathetic fibres have most probably

only a modulatory action on the intramural reflex centers (28; 157; see 204; 573; see 796; 797). Furthermore, in the cat colon parasympathetic efferent firing increases during segmental contractions and is correlated with rhythmic contractions of the circular muscle. This phenomenon is abolished by atropine or ganglionic blocking agents indicating that the firing is produced by neurons in the intrinsic plexus (161). In addition the ENS contains subsets of neural circuits preprogrammed for control of distinct motility patterns. These pre-existing programmes receive command inputs from the CNS via vagal fibres. This situation is analogous to other neural control systems in which activation of single "command neurons" releases extensive coordinated motor responses (see 796). Only a small portion of vagal fibres pass to the prevertebral ganglia and from there to the small and large intestine. Thus, it is usually presumed that the vagal control over motor function of the gut plays a greater role on the proximal portions of the gut (esophagus, stomach and small intestine) of the gut. This hypothesis is supported by the finding that below the small intestine vagotomy results in little obvious impairment of normal motility and other digestive functions (157; see 157).

Sympathetic, postganglionic fibres innervate mucosal mucus-secreting cells, vascular and intestinal smooth muscle, and intramural nerve cell bodies (58, 159). The vast majority of these sympathetic fibres synapse directly with ganglion cells of Auerbach's and Meissner's plexus. Only a few reach the gut muscle per se being virtually absent from the longitudinal layer of the smooth muscle. Catecholamines inhibit the ACh release from cholinergic enteric neurons by acting on α-receptors (157; see 157; see 237; see 275; see 573; 797). Brain stem or spinal cord stimulation can increase intestinal motility by reducing the inhibitory action of adrenergic nerves (237; 797). In addition, NA has been found to augment the excitability of TTEN in guinea-pig small intestine (157; see 157; see 237; see 275; 797) Recently the presence of NANC transmitter substances in the classical efferents to the gut has been demonstrated (see neurochemistry).

Third link: the INTRAMURAL GANGLIA (Central Processing Unit)

Functionally the myenteric plexus seems primarily concerned with peristalsis, Henle's plexus being motor to smooth muscle and the submucosal plexus controlling absorptive and secretory functions (see 319; 325; see 573; 714). Within these plexuses neurons send some of their processes in the fibre tracts to the adjacent ganglia and, in turn, receive synaptic input from axons projecting into the same fibre tract from adjacent ganglia (796; see 796). Circumferential myotomies performed on the rat small intestine have showed degenerating fibres in the myenteric plexus up to +/- 10 cm distally and +/- 7.5 cm proximally from the cut. In the submucosal plexus some degenerating nerve fibres occurred as far as 5 cm distally from the lesion. Degenerating intramuscular nerve fibres were only found close to the cut. Comparable results have been obtained in the small intestine of the dog (see 239). The retrograde fluorescent labelling technique showed that in the guinea-pig small intestine +/- 75 % of the myenteric neurons project distally, +/- 18 % proximally and +/- 6 % circumferentially (see 239). The projections of the myenteric neurons (VIP; GRP; ENK; CCK; DYN) to the sympathetic prevertebral ganglia are probably parts of the afferent link in the intestino-intestinal inhibitory reflexes (239; see 239).

Recent immunohistochemical observations have revealed the existence of numerous neuronal connections between myenteric ganglia. In the guinea-pig, short projections provide terminals (Sub. P; SOM; NPY) in the same or adjacent ganglia, while long distally directed projections (up to 50 mm), containing 5-HT; VIP; SOM; GRP; CCK; DYN, have also been encountered (239; see 239).

This connectivity would enable a neuron in one ganglion to activate the soma of the second neurons in another ganglion, thus switching ongoing electrical activity in the dendrites of the second neurons or to influence the axon that projects in the interconnecting fibre tract to the adjacent ganglion. These specialized properties of the neurons and their synaptic input provide a mechanism whereby the integrative circuitry of one ganglion can control the information input it receives from an adjacent ganglion (796; see 796). Moreover, the projections from the myenteric plexus to the submucosal plexus imply that there is a direction and/or coordination of the secretomotor reflexes occurring in the submucosal plexus through the myenteric plexus (see 239).

Ultrastructurally, the myenteric ganglia show a striking resemblance to the CNS. Thus, there is a morphological basis to postulate that the neuronal integrative circuitry in the myenteric ganglia resembles that of the CNS (see above). Moreover, electrophysiological synaptic

mechanisms similar to those of the CNS have been found in enteric ganglia. Indeed, both systems utilize many of the same neurotransmitter substances. Enteric ganglia process, probably via inhibitory and excitatory interneurons, the incoming sensory information and generate appropriate signals for controlling the outflow of commands in motor neurons to the effector systems. Finally, the ganglia contain neural networks that are programmed to generate cyclical stereotyped patterns of motor behavior not built on a series of reflexes (796; 797). Thus, taken as a whole, there must be a complex wiring of various morphological and functional distinct neurons within the enteric plexuses. These neurons constitute an independent integrative network that is capable of carrying out its integrated functions in the complete absence of extrinsic nerves, reflecting in this manner, the high degree of autonomy of the gut (see 157; 497). In contrast, the integrity of this network is essential for the normal functioning of the intestine (35; 573). The nature of most neurons within the intramural chains is probably cholinergic, though the inhibition of circular muscle at the end of the chain is clearly by means of non-adrenergic inhibitory neurons. The details of how the various neuronal elements are morphologically and functionally integrated are still lacking but they seem to form a continuous communication system that encompasses the entire digestive tract enabling impulse propagation from its most proximal to its most distal parts (see 157; see 239; 325; see 573; 797).

Extracellular recordings have classified the enteric neurons from within the small intestine of the guinea-pig, cat and dog into sensory and motor neurons. The electrophysiological properties of the sensory neurons have already been discussed (see first link). The enteric motor neurons may be further subdivided into single-spike and burst-type neurons (246; 275; 395; see 573; see 796; 797; 798; 799).

Single-spike neurons are unaffected by mechanical stimulation and discharge, are independent of any synaptic input, which is random at low frequencies. In many animals (e.g. guinea-pig, cat) these neurons are sensitive to ACh, other nicotinic-cholinergic agonists, 5-HT and to caerulin. Their discharge rate is, on the contrary, reduced by NA (acting at α-receptors) and by morphine (see 239; 246; 275; 395; 573; 796; see 796; 797; 798; see 798; 799).

Burst-type neurons have brief periods of high frequency discharge followed by intervening quiescent periods (see 239; 246; 275; 395; see 573; see 796; 797; 798; 799). On the basis of the regularity, or lack thereof, in the interburst intervals they have been subdivided into erratic and steady bursters. It has been shown that the intervals between bursts of spikes are dependent on the ambient temperature. This thermosensitivity has led to the suggestion that burst-type neurons could function as deep body thermosensors (275; 796; see 796; 797; 798).

Erratic bursters are characterized by irregular interburst intervals and by periods of continuous discharge. This erratic pattern of activity is probably generated by a synaptic input.

The discharge of steady burst-type neurons occurs in the absence of any overt stimulation with relatively low variance of the interburst interval. In consequence, the timing of the bursts is most probably determined by an intrinsic endogenous oscillatory pacemaker, the generation of spikes failing during some cycles of the oscillator (see 239; 275; see 275; 395; see 573; 797; 798; see 798). The firing patterns of steady bursters are unaltered by ACh and putative neurotransmitters. This is consistent with the view that they may be endogenous oscillators that do not receive synaptic input from other neurons and that erratic burst-type neurons may be driven by input from the steady-burst neurons to release their transmitter at the circular muscle (see 275; 797; see 798).

It is suggested that the activity of the burst-type neurons reflects the spontaneous activity of enteric neurons. The functional significance of the continuous discharge of the burst-type units might be the tonic release of an inhibitory transmitter substance and the realization of a continuous inhibition of the spontaneous myogenic activity of the circular muscle layer (797; 798; 799; see below). Thus, disinhibition of the circular muscle occurs either when the excitatory drive on the inhibitory neurons is removed by presynaptic inhibition of ACh release from the steady-burst type neurons or by presynaptic inhibition of release of the inhibitory transmitter from the erratic bursters (see 573; 797).

On the basis of *intracellular* recordings two distinct types of enteric ganglion cells are distinguished.

The *first type* was referred by Hirst et al. (347) as AH neurons and by Nishi et al. (563) as type 2 neurons. AH/2 neurons are bipolar and send their processes in opposite directions within the ganglia. They are characterized by a high resting membrane potential, by a low input resistance, by long-duration After Hyperpolarization and, finally, by tetrodotoxin-resistant action

potentials. AH neurons do not receive a synaptic input. This was confirmed by electron microscopy their somata being shown to be devoid of synapses (see 157; see 239; see 343; see 812). However, recently, evidence has been given that AH neurons receive a "weak" cholinergic input from a local source (see 239) and it was postulated that neurons without synaptic input might be mechanoreceptors (see 798) or sensory neurons (343; see 348). Thus, it may be that AH neurons are the intracellularly recorded equivalent of the extracellularly recorded tonic-type enteric neurons (TTEN) (275; see 275). It has therefore been argued that AH cells are, most probably, sensory neurons in a descending inhibitory pathway located entirely outside the submucosal plexus. Their activation may be important in the delay before the onset of descending excitation (see 157; see 345; see 348). Finally, some authors have claimed that AH neurons have Dogiel's type II morphology and represent about one-third of the myenteric neurons (see 239).

The *second type* of ganglion cell was named the S (synaptic) cell by Hirst et al. (347) and the type 1 neuron by Nishi et al. (563). S/1 neurons are slowly adapting and respond to depolarization with a continuous discharge of spikes. The frequency of this discharge is a direct function of the intensity of the applied current (see 239; 275). In S/1 neurons each action potential is followed by a brief after-hyperpolarization typical for autonomic neurons (see 157; see 239; see 246; see 343; see 348; see 573; see 796; see 798; see 812). Probably some S cells act as interneurons, while others make excitatory/inhibitory connections with the circular smooth muscle (343). Indeed, there is morphological, physiological and pharmacological evidence that myenteric neurons, supplying the circular and longitudinal muscle, belong to the class of S-neurons. A correlation of neuronal shape with electrophysiological properties shows that Dogiel's type I neurons (or neurons with a similar morphology) are S neurons (239; see 239). These neurons send projections directly to the underlying musculature 1-3 mm along the intestine (239, see 239). Radial stretch of the intestine evoked excitatory synaptic potentials in most S cells distal to the stretched area (343).

Neuronal connections in the myenteric plexus run predominantly in distal direction. Two descending nerve pathways in the myenteric plexus have been found. One may mediate descending inhibition of the circular muscle layer while the other may mediate descending excitation of both muscle layers. Axons of the descending pathways are less than 2.5 cm long. Therefore, long (5 to 7 cm) descending pathways appear to be polysynaptic with each neuron in the pathway receiving several excitatory inputs in a cascade arrangement (see 573; see 796). Virtually all S cells receive projections from one of these descending pathways (343; see 345). AH and S neurons are both present in the myenteric plexus, about two-thirds being S type neurons (see 246; see 343; see 798). Finally, some myenteric neurons have properties intermediate between the two major types. They are usually found to be AH/2 neurons that are partially activated by ongoing release of 5-HT within the ganglion (see 239; 796).

There is no clear electrophysiological evidence for the existence of pathways in the myenteric plexus. In the rabbit, for example, evoked potentials could be detected 15 mm distal to stimuli. Hexamethonium causes some reduction in the amplitude of the evoked potentials, while they are abolished by tetrodotoxin. Evoked potentials have also been recorded from circumferential pathways over a distance of 3 mm (see 239). In addition, intracellular recordings have recorded antidromic action potentials from S neurons at a longitudinal distance as far as 25 mm. But, as these antidromic responses are rare at 25 mm, they are probably due to the stimulation of a chain of interconnected neurons. For AH neurons, on the contrary, this distance is not greater than 1 mm. More recently, it has been demonstrated that most axons in the circular muscle layer run for short distances i.e. less than 2 mm in distal direction, while a small number of neurons send their processes 25-30 mm distally (see 239).

In the submucosal plexus nearly all neurons seem to be of the S type (see 239, see 246). In the guinea-pig small intestine each S type neuron receives an excitatory synaptic input from a number of presynaptic fibres (ACh). In contrast to the myenteric S cells, about one third of the submucosal S neurons receive, in addition to their excitatory input, an inhibitory synaptic input. Inhibitory potentials can be mimicked by the local application of catecholamines and dopamine (see 343). Both synaptic inputs survive extrinsic denervation (see 343; 345; see 798). In addition, a small proportion of the submucosal neurons do not receive any synaptic input. Like AH cells of the myenteric plexus they may be sensory in nature though their properties are different (see 343).

No preferred pathway directions have been demonstrated in the submucosal plexus of the rabbit small intestine. Evoked potentials run about 4 mm proximally, distally, circumferentially and obliquely. Intracellular recordings in the guinea pig small intestine have been

found to evoke potentials at a distance of 6 mm (see 239).

From the foregoing it is apparent that the earlier concept of the enteric ganglia, functioning only as simple relay-distribution centers for information, is no longer tenable. Ganglia do indeed receive command signals transmitted from the CNS along parasympathetic and sympathetic pathways, but this constitutes only one kind of input to a system that also contains pattern-generating and integrative circuitry for processing the complex sensory information derived from receptors along the gut (797). Consequently, there are morphological and functional arguments to consider the enteric ganglia as "little brains scattered along the alimentary canal" (797). Information within this intrinsic integrative system is processed by means of neurochemical substances, which function to transmit (neurotransmitters) or to modulate (neuromodulators) the incoming information. Neuromodulation, which is defined as the regulation of transmitter release from nerves by the action of neurohumoral agents on prejunctional receptors, is of physiological importance. For example, ACh inhibits the release of NA from stimulated enteric sympathetic nerves, while the prejunctional action of catecholamines modulates cholinergic nerve activity. It therefore seems likely that cholinergic nerves inhibit the release of NA (and ACh) as adrenergic nerves do for the release of ACh (108; 275; see 275; 629). Moreover, modern electrophysiological and immunohistochemical studies have made it possible to demonstrate highly ordered projections within the digestive tract. At least in the guinea-pig motor neurons to the circular muscle layer are located entirely in the myenteric ganglia, whereas those for the mucosa are found in Meissner's plexus. Intrinsic proximally and distally orientated projections between the myenteric ganglia have been established, but they are short (15-30 mm) as compared to the length of the intestine. Circumferential projections are even shorter. Projections between Auerbach's and Meissner's plexus have likewise been found. As yet there is no evidence for projections from the submucosal to the myenteric plexus (see 239).

Sympathetic fibres to the gut originate from the spinal cord and the prevertebral ganglia. Electrophysiological studies have shown that prevertebral ganglia possess a nervous circuitry that is able to integrate inputs from both the gut and the CNS and to mediate inhibitory inputs to the gut and the extramural intestinal reflexes (161; see 573; 642). The spinal cord is probably the source of the neural inhibition of the gut since spinal cord lesions, ventral root section or removal of the spinal cord induce intestinal hypermotility (see 642). Because of the relatively small number of adrenergic fibres in the muscularis externa and the dense accumulation of these fibres around ganglion cells, it has been suggested that the adrenergic inhibitory mechanism primarily takes place at the myenteric ganglia where NA may act directly on perikarya, or on terminals or on both (see 237; see 239; 387; 796).

Morphological and functional investigations have revealed a considerable variety of neurons, with characteristic morphological and electrical features in the enteric ganglia. Up to nine morphologically distinguishable neurons have been found in the enteric plexuses and some nerve profiles have been found to hold a complex mixture of vesicles suggesting that they may contain multiple transmitters/modulators (see 108; 339; see above). Some of these neurons are responsible for the powerful NANC inhibitory or excitatory responses of the smooth muscle, whilst others are sensory or may represent various types of interneurons. Still others may supply blood vessels and mucosal epithelial cells. Hence, it seems unlikely that the NANC nerves of the gut represent a single population with only one transmitter substance (108). Histochemical, immunological (radioimmunoassay, immunohistochemistry) and physiological studies have identified numerous substances both in the ENS and CNS (see 35; 108; see 188; see 239; 339). Neurotransmitters functioning in the intrinsic central processing units and responsible for gut motility are classified as cholinergic, adrenergic and NANC (629). The NANC system has been shown to hold different putative neurotransmitters such as adenosine-5'-triphosphate (ATP), 5-HT and the neuropeptides Sub. P, VIP, SOM, ENK, gastrin/CCK, neurotensin and bombesin (108; see 130; see 164; see 239). Sub. P, VIP, ENK, SOM are widely distributed throughout the alimentary tract, whereas gastrin/CCK and neurotensin have a more restricted distribution. Moreover, a co-storage of classical/NANC and NANC/NANC neurotransmitter in the same neuron has been observed (see above). This morphological and neurochemical complexity reinforces once more the hypothesis of the considerable integrative capacity of the ENS (108; for a review see 239; 339). Many studies have demonstrated that ACh is a transmitter released by enteric excitatory neurons, by fibres in the vagal and pelvic nerve which form excitatory connections with enteric neurons, and by excitatory enteric interneurons (see 239). In fact, all reflexes subserving intestinal peristalsis seem to involve cholinergic transmission via

nicotinic receptors (52; see 52).
Cholinergic neurons have several important roles in the ENS. Indeed,
- there are cholinergic excitatory enteric neurons supplying the intestinal musculature,
- there are cholinergic secretomotor neurons that augment the water and electrolyte secretion,
- there are cholinergic excitatory neurons which supply gastric acid secreting cells and
- there are cholinergic interneurons (see 239).

The mammalian gut contains, in addition, powerful intrinsic inhibitory neurons the actions of which are not prevented by antagonists of the NA- ergic transmission, are unaffected by sympathectomy, and even persist when the NA- ergic axons are destroyed (see 56; see 239; see 246; see 796). These neurons represent the NANC innervation of the gut. The perikarya of the enteric inhibitory neurons are in the myenteric plexus. Their axons run circumferentially in the myenteric plexus for a few millimeters and then continue for several millimeters in the circular muscle layer. Some of these inhibitory neurons innervate the circular muscle layer close to or just distal to their cell body, while other neurons project for up to 30 mm in a distal direction giving off several collaterals. Few fibres affect the muscle proximally (0,5 - 1 mm) (239; see 239).
Purines (primarily ATP, ADP, AMP and adenosine) were at first suggested to be the transmitters in the NANC inhibitory nerves supplying the intestinal smooth muscle and sphincters (see 56; 108; see 246; 629; 799). It has been shown that in the guinea-pig ileum the inhibitory effect of ATP on cholinergic neurotransmission is due to its rapid breakdown to AMP or adenosine which in turn act on prejunctional purinoreceptors (538). These inhibitory nerves are probably involved in a cascade of descending inhibitory reflexes. The reflexes run during peristalsis ahead of the material and facilitate the passage of food by opening sphincters, by increasing the stomach size and by expanding the intestine in front of the bolus travelling through the digestive system. The nerves also participate in a vago-vagal reflex that permits the stomach to accommodate to distension. An analogous accommodation involving enteric inhibitory nerves is observed in the intestine (108; see 246).

However, there is considerable evidence for the existence of other NANC transmitters in the ENS. Immunohistochemistry especially has revealed autonomic nerves containing biologically active polypeptides (see above). In addition, there is growing evidence, based largely on autoradiographic and electrophysiological studies, that 5-HT or a related indoleamine and GABA may likewise act as transmitters in some autonomic nerves and neurons in the gastrointestinal tract (see 35; see 106; 108; see 188; 339; 514).
The gastrointestinal tract contains numerous populations of neurons each of which contains its own peptide, a mixture of peptides, or even a peptide together with one of the classical autonomic neurotransmitters (188). It is now generally believed that such neurons modulate the response to extrinsic nerve impulses and/or are involved in local reflex mechanisms as sensory neurons, interneurons or efferent neurons (108; 325; 723). Experimental evidence favours the view that enteric peptides act as neurotransmitters since they are stored in vesicles in nerve terminals, are released by nerve stimulation into the venous effluent, and mimic, in many instances, the effects of nerve stimulation. Furthermore, both immunoreactive VIP and Sub. P are present in the superfusion fluid of the intestine during electrical nerve stimulation, while exogenously applied Sub. P and VIP induces respectively strong contractions and relaxations of the intestinal muscle, respectively (29). Moreover, peptide-containing neurons are most probably involved in the communication between the enteric plexuses since a VIP-containing pathway runs from the submucosal neurons to the myenteric plexus to innervate the myenteric neurons, while a Sub. P-containing pathway runs in the reverse direction. Thus, the system of peptide-containing neurons provide the morphological basis for the functional coordination and autonomy of the gut (188; 325; see 325; 497; 723).
Neurons showing Sub. P-like and VIP-like immunoreactivity have been identified in the gut and their morphological distribution suggests that they are mainly involved in the regulation of smooth muscle activity (325; 560; 589; 723). This will be further discussed in part III.
ENK-ergic nerve fibres are numerous in the muscular coat and myenteric plexus indicating that both the smooth muscle cells and Auerbach's neurons are the potential targets (325). Opiate receptors have likewise been established as being present on the cholinergic nerve endings of the inferior mesenteric ganglion of the guinea pig. Activation of these receptors decreases the ACh release and in this manner ENK may play a part in intestinal motility. This concept is further supported by the reported interference of ENK with the peristaltic reflex (see 605). ENK nerves inhibit the firing rate of the motor neuron and are thus most probably involved

in the regulation of smooth muscle activity (325; 489). Furthermore endogenous opiates have been shown to cause membrane hyperpolarization, to reduce the output of ACh from activated cholinergic nerves and to inhibit the release of catecholamines and other transmitters (see 108; see 325; see 343; see 605; 616; see 723). These inhibitory effects could be presynaptic, postsynaptic or could result from a modulatory effect on the nerve terminal in which both ENK and an excitatory transmitter are present (437). Stimulation of enteric nerves induces a release of ENK, after which they act on the cell processes rather than on the perikarya (see 605; 616). In this manner ENK inhibits, probably presynaptically, the neuronal firing in Auerbach's plexus. Therefore, it seems likely that ENK is released from interneurons involved in the modulation of nerve-mediated excitation of the intestine (see 108; see 343; see 605; see 723) and the inhibition of the electrically evoked contractions of the smooth muscle (see 605; 616). However, it has been claimed that ENK nerves either accompany the axons of motor neurons, modulating their functional activity presynaptically by axo-axonal mechanisms, or that they are dendrites from sensory neurons inhibiting the firing rate of motor neurons (325).

SOM- like immunoreactivity has been demonstrated in the mucosa and in the myenteric plexus but not in the muscular coat (see 108; 325). This is also shown by physiological studies which indicate that SOM most likely acts on enteric neurons to diminish the output of ACh and to stimulate the enteric inhibitory neurons (131; see 239; see 246). SOM enhances absorptive fluxes in the small and large intestines and, moreover, reduces secretion induced by 5-HT or VIP (see 239). SOM inhibits the firing of myenteric neurons and, in consequence, myenteric SOM-containing neurons may be interneurons (108; 325). This is further supported by the finding that, in the small intestine, SOM inhibits the release of ACh from the myenteric nerves (see 108; see 239). Thus, by inhibiting the cholinergic excitatory neurons (inhibition releases ACh from myenteric neurons) and, most likely, by activating the intrinsic non-adrenergic inhibitory neurons SOM indirectly suppresses the gut's motility (616; 629; see 796). Finally, SOM has been shown to inhibit the release of NA from adrenergic neurons. Furthermore, SOM is well known to inhibit the release of different neurotransmitters in the brain (629) and is the "inhibitory" hormone of the gut. Thus, it blocks the release of most gastrointestinal hormones and neurotransmitters and likewise inhibits many gut functions (see 605).

Final link: the TARGET ORGANS (the intestinal musculature, epithelium and vasculature)

There are two well established final common pathways from the ENS to the target organs. These are a cholinergic excitatory pathway to the longitudinal muscle and, since ACh is only weakly excitatory on this muscle layer, a NANC inhibitory pathway to the circular muscle layer (796; 797). The density of autonomic efferent nerve endings varies in different regions of the gastrointestinal tract and this appears to correlate with the functions of the various portions of the gut. More fibres seem to lead to the stomach than to the intestine (35; 164; 438; 642).

Intestinal Musculature

One of the principal target organs of the ENS is the intestinal musculature which has some unusual properties.

Firstly, intestinal smooth muscle cells are arranged in bundles of several hundred muscle cells partially separated by connective tissue bands. Groups of smooth muscle cells form connecting bridges between these bundles. In this way a neuromuscular unit in the circular muscle layer of the small intestine consists of a myenteric motor neuron and a varicose axon. This enters the circular muscle layer and influences a band of smooth muscle (2-5 mm wide) that runs, at least partially, around the circumference of the gut. Since each smooth muscle bundle appears to have a direct input from several axons (10-50 in guinea-pig small intestine) a multiple innervation of the muscle bundles may be hypothesized (see 239). Further, the intestinal musculature behaves as an electrical syncytium (i. e. a three-dimensional core conductor). Regions of fusion (nexuses) between the plasma membranes of contiguous muscle fibres function as intercellular pathways of low electrical resistance and provide for the propagation of excitation between adjacent cells. The longitudinal muscle coat is characterized by a scarcity of nexuses, while in the circular muscle coat, in contrast, many of these structures have been observed (239; see 239; 797). In this way smooth muscle fibres are electrically coupled and, consequently, a transmitter-induced electrical current of an innervated cell can spread into and affect those muscle fibres that receive no direct

nervous influence. Thus, the autonomic nerves in the gut have a diffuse (modulatory) action rather than providing a precise point -to -point excitation as occurs in the nervous control of skeletal muscles (see 239; 796; see 796).

Secondly, the excitability of intestinal smooth muscle cells undergoes cyclic oscillations. Hence, intestinal smooth muscle contracts spontaneously and rhythmically. These contractions are inherent to the smooth muscle (myogenic) and are, in consequence, independent of any innervation (see 35; 58; 139; 159; see 239; see 246; see 674; 761). Thus, intestinal contractions may occur without the release of any (excitatory) substance (see 246). The organized excitation of the network of smooth muscle cells is, therefore, initiated by myogenic pacemaker mechanisms (electrical slow waves or slow potentials or basic electrical rhythm or pacesetter potentials) which are omnipresent and appear to be generated in the longitudinal muscle layer (see 239; 761; 797). Thus, it is now clear that the intestinal smooth muscle possesses a mechanism for the self-generation of rhythmic changes in excitability and that the movements of the intestine depend on a superimposition of the action nerves and/or hormones on this mechanism (239; see 239). However, at the moment there is still controversy as to whether the slow waves are due to oscillations in ionic permeability or to oscillations in a metabolic driven Na^+ pump (see 239). Furthermore, structural and functional characteristics of the intestinal smooth muscle innervation suggests no specialized neuromuscular junction and a non-localized release of neurotransmitter substance(s) along the axons. After release the transmitter substance persists and exerts its effect at the muscle receptors for relatively prolonged periods of time (796; see 796).

These remarkable properties of the intestinal smooth musculature (electrically coupled, spontaneous rhythmic myogenic contractions independent of any innervation) preclude a direct neuronal excitation for the coordination of the contractile patterns of the musculature. Thus, the basis for the control of the inherently excitable myogenic system appears to be **neuronal inhibition** (see 239; see 246; 796; 797). The first indication for such a mechanism was that some Auerbach neurons in the guinea-pig, rabbit, cat and dog small intestine show a continuous discharge of action potentials (797; see 797). Afterwards, many different situations have substantiated this hypothesis. Indeed, application of nerve-blocking drugs (tetrodotoxin) or local anesthetics, long periods of cold storage, surgical ablation, hypoxic vascular perfusion and congenital absence of enteric ganglion cells, all of which involve functional ablation of enteric neurons, are associated with the conversion of a hypo-irritable condition to a hyper-irritable state of the circular muscle. Thus blockade of the enteric neuron activity releases the circular muscle from an inhibitory influence and permits excitation and conduction mediated by myogenic mechanisms (see 239; 618; 797; 798; see 798; see 799). Hence, the autonomic nerve supply to the gut serves mainly to modify the excitability of the intestinal muscle and to regulate the spontaneous motor activity (see 35; 58; 159; see 239; see 246; see 674). This intrinsic inhibitory neuronal control mechanism seems similar everywhere, although various regions of the alimentary tract, and even some smooth muscle layers, respond differentially to the stimulation of their nerve supply. This appears to be related to the functional activity of each part of the gut (the presence or absence of active tone) and this in turn depends on its innervation (see 35; 157). For example, the circular and longitudinal muscle layers of the human esophagus are innervated by excitatory and inhibitory nerves. Cholinergic excitation predominates in the longitudinal layer, whilst the circular muscle responds both with contraction and relaxation to an electrical stimulation. It appears that both adrenergic and non-adrenergic pathways are involved in this inhibition whereas only a non-adrenergic relaxation has been found in the longitudinal muscle (35). Furthermore, there must be a major difference between the inhibitory outflow to circular sphincteric and non-sphincteric muscle. Indeed, the inhibitory innervation of the intestine is tonically active whereas the inhibitory ganglion cells to the sphincters are normally silent. Myogenic contractions of the former occur when the ongoing discharge of the inhibitory neurons is suppressed by an inhibitory synaptic input from the internuncial circuitry (enteric ganglia). In sphincters, on the contrary, myogenic mechanisms maintain a contractile tone (sphincteric muscle is tonically contracted in vitro i. e. in the absence of extrinsic nervous input and circulating hormones) while the sphincter is relaxed when the normally silent inhibitory neurons are activated by excitatory synaptic input either from internuncial enteric neurons or from preganglionic vagal fibres. In consequence, ablation or malfunction of the inhibitory ganglion cells or of the internuncial circuitry must lead to spasm of the non-sphincteric circular muscle and simultaneously to achalasia of the sphincteric musculature (796).

Intrinsic (non-adrenergic) inhibitory neurons appear to be ubiquitous at all levels of the gut in all the vertebrate

species that have been studied so far (796; 797). But, if tonically active inhibitory neurons continuously suppress myogenic activity of the circular muscle, then this implies that some motility patterns are dependent upon integrated disinhibition of the muscle. Therefore, the neuronal circuitry, within the enteric plexuses is most likely connected in such a way that sensory information derived from intestinal receptors ultimately leads to inhibitory synaptic effects on the discharge of the inhibitory neurons and, consequently, to a release of the muscle from inhibition. Transmural electrical stimulation or radial distension of the small intestine induces a descending inhibition (up to 5 cm) of the smooth muscle, followed by an excitation. Furthermore distention of the small and large intestines has been shown to evoke the intestino-intestinal reflex. These reflexes are most likely completely processed within the intramural nervous system. The motility patterns are due to a phase of active inhibition of the intestinal musculature. They probably involve neuronal transformation of sensory information into increased excitatory input to the intrinsic inhibitory neurons (90; see 239; see 573; 796; 798; see 798; 799). The transmitter substance released from the tonically active inhibitory neurons is probably not a catecholamine but the same substance that is released from intramural ganglion cells during transmural electrical stimulation which produces intense hyperpolarization and inhibition of the intestinal muscle (see 799). Thus, intrinsic non-adrenergic inhibitory neurons are undoubtedly of important functional significance in the control of intestinal motility, as they are continuously active in non-sphincteric regions of the bowel (796; 797). They have also additional specific functions in the adaptation of the intestine to distention (vagally induced relaxation of the lower esophageal and other sphincters, and the stomach) (796).

Thus, taken as a whole, the enteric neural control of gastrointestinal motility is mediated through enteric excitatory and enteric inhibitory neurons. These neurons act on the circular muscle coat, while excitatory neurons supply the longitudinal muscle layer (see 239). Excitatory nerves cannot control the spread of excitation within the intestinal muscular syncytium and this is largely supported by the observations that the excitability of the muscle is greatly increased when neuronal activity is experimentally or pathologically abolished (796). In addition, integration of the activity of the intrinsic neurogenic inhibitory system most probably determines whether a particular cycle of electrical slow waves triggers a response in the circular muscle (cfr. only one-third of the slow waves trigger spikes in the circular muscle of the dog in vivo), the number of muscle fibres activated and hence the force of the contractile response triggered by a particular slow wave, the distance over which excitation spreads within the electrical syncytium and, finally, the direction of spread of excitation within the syncytium (796; 797). In consequence, it may be said that the predominant function of the neuronal activity of the ENS appears to be to control (suppress) the inherently excitable electrical syncytium (intestinal muscle) and, by so doing, the continuous inhibition of the spontaneous myogenic activity of the circular musculature (see 275; 796; 797).

In their ingenious studies (based upon the technique of myotomy, myectomy and homotopic autotransplantation) concerning the origins and projections of the enteric neurons, Furness and Costa and their collaborators proved that in the circular musculature nerve fibres, showing immunoreactivity for 5-HT, Sub.P, VIP, ENK, NPY and CGRP, originate from Auerbach's neurons and/or pass through the myenteric plexus. Subsequent studies indicate that very nearly all neurites contributing to nerve bundles that innervate the circular muscle stem from myenteric nerve cells (239; see 239). Thus, powerful NANC inhibitory nerves supply the gut musculature and are most probably concerned with the propulsion of food through the alimentary canal, the reflex opening of sphincters, the "receptive relaxation" of the stomach and the "descending inhibition" during peristalsis (108; 246).

The functional significance of both the classical (ACh; NA) and NANC neurotransmitters (5-HT; Sub. P; VIP) for the intestinal musculature is further discussed in Part III of this study.

As mentioned earlier, peptidergic nerves occur in both the myenteric plexus and in the smooth muscle implying that they take part, directly or indirectly, in the regulation of smooth muscle activity. In addition, some peptides are found in the submucosal plexus and the mucosal layer and are probably involved in the regulation of epithelial functions. Conceivably, the intramural peptidergic neurons mediate and modulate the response to an extrinsic nervous impulse flow according to the local needs (723).

The distribution of ENK nerves resembles strongly that of the Sub. P nerves, indicating that ENK are primarily involved in the regulation of gut peristalsis (325; 723). ENK indirectly reduce gut motility by inhibiting the release of ACh and by reducing the firing of myenteric neurons (73; see 73; see 239). In contrast to the topog-

raphy of ENK-IR in the gastrointestinal wall (see above) ENK have little or no direct effect on the intestinal smooth muscle cells (325; 473), although it has been argued that certain endogenous opioids may exert their effect directly on the cells (629). Their major action is to reduce the output of ACh from activated cholinergic nerves, at least in part, by hyperpolarizing (lowering excitability) the neurons (see 473; 569). Met- and leu-enkephalin could be involved in the excitatory transmission to the pyloric sphincter of the cat. Moreover, ENK-ergic neurons are activated by duodenal acidification or intra-duodenal acids. They cause pyloric constriction that is blocked by naloxone or tetrodotoxin (see 239). In addition, ENK exert other actions on the gut including, the reduction of gall bladder tone and contraction of the sphincter of Oddi (see 605).
Delicate, varicose SOM-containing nerve fibres have been seen closely to nerve cell bodies within the plexuses suggesting a neuromodulatory role (73; see 73; see 239; see 325; see 356; see 723).
SOM has two actions on the intestinal musculature i.e. inhibition of the output of ACh and stimulation of enteric inhibitory neurons (see 239; see 397). *In vitro* high concentrations of SOM inhibit both the ascending excitatory and the descending inhibitory components of the peristaltic reflex. *In vivo* SOM inhibits the migrating myoelectric complex, delays gastric emptying and antagonizes intestinal propulsion (616; 629; see 761; see 796). However, in some preparations it causes contraction of gut muscle and it is not known whether SOM does so by suppressing the release of inhibitory neurotransmitter(s) from NANC neurons (629; see 761).
SOM has no direct myogenic effect. But, part of the biological actions of SOM result, most probably, from the local release of the peptide from endocrine cells (paracrine mode of action) and seem to involve the inhibition of various release processes including many gastrointestinal hormones and perhaps neurotransmitters (73; see 73; see 239; see 325; see 356; 397; see 397; 398; see 398; see 723).
Bombesin appears to be the "releasing" hormone of the gut, inducing a significant release of most gastrointestinal hormones (see 605). It may be anticipated that these features support, and broadly agree with, the idea that SOM and bombesin control and modulate gut motility, secretion/absorption and the blood flow in a synergistic or antagonistic (bombesin-somatostatin) manner (see 73).

Intestinal Epithelium

Water and electrolyte secretion

The mucosa of the stomach and intestine is richly supplied with nerve endings that are involved in the control of gastric acid and pepsin secretion, in the transport of water and electrolytes in the small and large intestines as well as in the release of gastrointestinal hormones (mainly gastrin, motilin, SOM). In the guinea-pig small intestine the mucosal plexus contains numerous VIP, Sub. P, SOM and NPY immunoreactive fibres. The majority of them seem to originate from submucosal perikarya, although a small number arise from the myenteric neurons. Thus, the majority of the secretomotor neurons to the mucosa reside in the submucosal ganglia (239; see 239).
In the stomach the vagal nerve has a dominant role in controlling gastrin and acid secretion via the enteric plexuses. Excitatory cholinergic motor neurons have been shown to control the parietal cells, while non-cholinergic excitatory motor neurons controll the gastrin secreting cells (239; see 239). Mechanical, chemical and electrical stimulation of the intestine are known to produce secretion. As this secretory process can be blocked by atropine and hexamethonium it is most likely based on intrinsic reflexes.
In the rabbit small intestine electrical stimulation of the mucosal nerves generate on ionic current which is due to the active secretion of Cl^- accompanied by Na^+ and H_2O secretion across the mucosa. The majority of the mucosal nerves appear to originate from submucosal ganglia. The secretomotor neurons involved are both cholinergic (55%) and non-cholinergic (45%). Some cholinergic neurons contain peptides (CCK; CGRP; NPY; SOM), the non-cholinergic VIP and DYN, while in the guinea-pig Sub. P seems likewise to participate in the non-cholinergic response. Both types of secretomotor neurons project to the intestinal mucosa. The relative contribution of the different secretomotor neurons varies between the species. It is now clear that the activity of the submucosal secretomotor neurons can be driven or modified by other enteric neurons that form links in enteric reflexes (239; see 239).
Pharmacological responses, histochemical studies and electrophysiological investigations of transmission in submucosal ganglia in animals and man all point to NA neurons inhibiting water and electrolyte secretion in the small intestine and probably also in the large intestine. The effects appear to be primarily mediated

through α-receptors, although a variable contribution of β-receptors has also been found. In addition, NA diminished intestinal secretion even in the presence of tetrodotoxin. This indicates that there are NA receptors on the epithelial cells. Moreover, there are also inhibitory α-adrenoreceptors on the submucosal secretomotor neurons, while NA terminals form networks around submucosal perikarya and likewise provide a sparse innervation of the mucosa. In consequence, there are actions at both sites which would diminish secretion and enhance absorption (239; see 239).

Furthermore, stimulation of the splanchnic nerves produces a drastic mucosal blanching in the proximal third of the colon as a consequence of α-adrenergic activation (see 25). Thus, adrenaline and NA both reduce mucosal blood flow and, in consequence, inhibit mucosal secretion (see 552). However, there is now clear evidence that the effects of sympathetic nerve stimulation on secretion are not simply the consequence of vascular changes (see 239).

Based upon the numerous projections of the enteric Sub. P neurons to the submucosa and mucosa it may be predicted that they are likely to be involved in secretory and absorptive functions of the gut (139). Moreover, Sub. P posses a sialogenic action and promotes secretion from other exocrine glands such as the pancreas (see 723).

Most VIP-IR neurons have been found in Meissner's plexus and numerous VIP-IR fibres have been observed in the intestinal mucosa of all mammalian species examined so far (see 239). VIP-ergic nerve fibres have been observed near the epithelial cells in the crypts of Lieberkühn and in the villi. In addition, numerous VIP receptors have recently been reported along most of the length of the intestinal mucosa. On this morphological basis it seems possible that VIP neurons participate in the control of the intestinal absorption/secretion process and that the peptide is a stimulator of water and electrolyte secretion by the gut mucosa. Pharmacological evidence has been given for the fact that the VIP secretomotor neurons are most likely involved in the final link of the ENS control over the intestinal secretion. Furthermore, VIP is one of the most powerful stimulators of exocrine secretion from the gut, pancreas and salivary glands and this secretory effect may be due to an activation of the adenylate cyclase (108; see 108; 118; see 188; 203; see 239; 325; see 325; 605; see 605; 616; 629; 723; see 723). VIP oma's produce relative large amounts of VIP to give high VIP levels in the circulation. This pathology, known as the Verner-Morrison (WDHA) syndrome, is characterized by watery diarrhoea, hypokalemia and achloremia. Removal of the tumor restores normal circulating VIP concentrations and the diarrhoea ceases. Administration of VIP, in similar quantities as those which occur in the WDHA syndrome, to experimental animals and man reproduces the classical WDHA features possibly by activating adenylate cyclase which in turn leads to an increase of the intestinal c-AMP concentrations (see 73). In addition, intraluminal installation of cholera toxin induces a substantial secretion of water and electrolytes. This response, in which pathway a cholinergic synapse may be involved, is markedly reduced by local anaesthetics and tetrodotoxin. Further investigations have demonstrated that cholera toxin induces an indirect VIP release from the intramural nervous elements. Indeed, the first step in the initiation of this reflex is the release of 5-HT from the EEC, where upon 5-HT can stimulate the sensory nerve endings in the lamina propria (see 239).

ENK have been found to suppress intestinal as well as pancreatic bicarbonate or enzyme secretion (see 605).

The presence of SOM-containing fibres in the mucosa, where they surround the basal portions of the crypts, suggests an influence of this peptide on mucosal functions (see 325). Given intravenously and intragastrically in man, SOM is a potent inhibitor of the gastric acid secretion by depressing both volumes and acidity of the secreted gastric juice (397). SOM has been found to enhance absorptive fluxes in the intestine and to reduce intestinal secretion induced by 5-HT, VIP, etc. (see 239; see 397). Indeed, SOM is known to inhibit various secretory processes in the gut (see 723). It blocks secretagogue induced secretion or decreased absorption in the rat colon, it blocks secretion in the rat jejunum, it stimulates Na^+ and Cl^- absorption in the rabbit ileum and it inhibits diarrhoea in patients with the carcinoid syndrome (see 167; see 723).

Mucus secretion

Local chemical or mechanical stimulation of the duodenum causes secretion from Brunner's glands. In the rat and man numerous VIP-IR fibres have been found near these glands, while VIP infusion increases the secretory flow as well as the bicarbonate and protein output from the Brunner's glands (see 239). Thus, it has been suggested the VIP neurons could be involved in such responses. In addition, the basal mucus secretion of the small intestine of the rat is enhanced by electrical

stimulation. Since atropine and tetrodotoxin block both the basal and stimulated release, it may be assumed that intrinsic cholinergic neurons evoke the mucus secretion from the goblet cells (see 239).

Release by endocrine cells

Few neuropeptides have been reported as acting on the endocrine cells of the gut. Nevertheless, numerous nerve fibres come close to the mucosal endocrine cells. Evidence has been presented that the release of gastrin and SOM from gastric mucosal cells is under nervous control. Indeed, SOM release is inhibited by cholinergic neurons, while non-cholinergic neurons utilizing GRP as transmitter stimulate gastrin release. Furthermore, NA fibres from the cervical sympathetic chain that join the vagal nerve are responsible for the degranulation of the 5-HT containing endocrine cells in the small intestine after vagal nerve stimulation. This stimulation induces, in addition, a release of motilin (239; see 239). Bombesin and SOM, however, modulate the release of a number of gastrointestinal hormones. Bombesin is known as the universal releaser, whilst conversely SOM is known as the universal inhibitor. Consequently both peptides may represent an example of a delicately balanced "pull-push" system controlling both the gastrointestinal hormone release and the exocrine secretion of water and electrolytes (see 73).

Finally, the autonomic innervation seems also involved in the trophic maintenance of the gut. Neural stimulation increases mitotic activity whilst denervation slows cell turnover. There is also evidence that certain peptides act on cell turnover in the gut. Bombesin, for example, causes hyperplasia of the pancreas and may have similar effects on the gut. In addition, the capacity of bombesin to simulate the release of other peptides may give it an indirect action: For example, by releasing enteroglucagon, which is widely considered to be a major factor in the regulation of intestinal mucosal growth. In a similar way SOM may inhibit the release of enteroglucagon and consequently have an anti-trophic effect. Hence, this peptide has been reported to decrease cell proliferation in the gut of the rat (see 73).

Intestinal Vasculature

Arteries and arterioles of the gastrointestinal tract are associated with two types of nerves. Paravascular nerves, using the arteries as a conduit, contain axons that supply different targets i.e. blood vessels, ganglia, intestinal smooth muscle and the mucosa. In addition, arteries and arterioles are surrounded by a continuous network of perivascular nerves (motor and sensory) that form a perivascular plexus. Very few nerves have a perivascular relationship with veins and lymphatic vessels in the gastrointestinal wall (see 239).

The control of the vasculature in the gut (and other organs) is thought to depend on both extrinsic and local influences. Extrinsic factors include noradrenergic nerves and circulating hormones, while local factors modify the blood flow in response to changes in the functional state of the organs (see 239).

Mechanical stimulation of the mucosa of the cat small intestine increases, even in the extrinsically denervated intestine, the blood flow by 30-40%, while intramural nerve stimulation increased the blood flow by 60-150% (see 239).

The splanchnic vascular bed holds about 20% of the total blood volume and some 35-40% of this blood can be displaced from there by moderate stimuli. During digestion blood flow to the small intestine increases by 31% in the dog. The flow to the submucosa increases by approximately 25% and that to the muscle by 25% and 60% in the jejunum and ileum respectively. In contrast there was little change in the mucosal flow. However, any change in mucosal blood flow tends to change secretion in the same direction and any change in secretion tends to involve a similar flow change (see 237; see 239).

There is very good evidence that the NA nerves make a contribution to the maintenance of tone in splanchnic vessels (237). NA nerves to the gastrointestinal vasculature are tonically active and they cause vasoconstriction through α-receptors (237; 246). The primary action of NA nerves on gastrointestinal blood vessels is to decrease the arterial calibre and to constrict the larger veins. Their activity is increased in order to decrease the proportion of the cardiac output feeding into the splanchnic vessels and to decrease the blood volume held in this region (237; see 239). Hence they participate in cardiovascular homeostasis by adjusting the resistance and capacity of the splanchnic vascular bed. The reduction in the mucosal blood flow (except the lamina propria) and the constriction of the larger veins persists as long as adrenergic nerve activity continues.

It results in the suppression of secretion and, in extreme cases, as reduction in absorption (237; 773). However, the decrease in intestinal blood flow in response to NA nerve stimulation declines after a few minutes despite maintained stimulation. This phenomenon is known as autoregulatory escape. There is still some controversy as to the mechanism of this phenomenon, but evidence indicates that there is a redistribution of the blood (though this is not the sole cause). Indeed, during maintained NA stimulation the blood flow recovers partially in the mucosa, substantially in the submucosa, while the flow in the intestinal musculature and the serosa remains depressed or even further reduced. Further investigations have led to the conclusion that escape from vasoconstriction principally arises from a relaxation of the innervated arteries and arterioles of the mesentery and submucosa (239; see 239).

There is also convincing evidence for vasodilatory nerves in the intestine which release neither ACh nor NA. Mechanical stimulation or irritation of the mucosa in the proximal colon elicits a local hyperemic response mediated via an intrinsic nervous reflex in the course of which 5-HT is possibly released from interneurons and VIP from the final neuron (25; see 246).

5-HT may be involved in this vasodilator response since mucosal stimulation increases 5-HT in the venous outflow and the dilatation is antagonized after 5-HT desensitization or administration of 5-HT antagonists. However, as the effect of 5-HT is blocked by tetrodotoxin it could be argued that 5-HT does not directly cause vasodilation. Most probably it is involved in the initiation of a reflex or it may be that 5-HT myenteric neurons function as interneurons in this reflex (see 239).

The blood flow in the gut is likely to be controlled to a significant extent by peptidergic nerves. VIP and Sub. P found in the largest concentrations in the gut have the same effect on the circulatory system and cause potent vasodilation. Among the various beds sensitive to both peptides is the splanchnic area (108; see 108; see 188; see 239; 325; see 325; 605; 616; 629; 723; see 723).

Pelvic nerve stimulation induces a substantial dilatation of the colonic blood vessels. The final neurons in this reflex are neither cholinergic nor noradrenergic, although a cholinergic ganglionic synapse is most probably involved in this pathway. Perivascular nerve fibres containing Sub. P and CGRP have been observed around intestinal arterioles. But, as these fibres are of extrinsic origin it is believed that they are the peripheral endings of sensory neurons (see 239). The potent vasodilatory effect of Sub. P and its localization in fine diameter fibres, which are probably sensory, around blood vessels has led to the proposal that Sub. P is involved in antidromic vasodilation (88).

Mechanical stimulation of the small intestinal mucosa causes vasodilation accompanied by increased VIP concentrations in the venous outflow of the gut. Thus, there is strong evidence for the presence of enteric vasodilator neurons which act, at least partially, through the release of VIP (203; 239; see 239).

In the distal colon and the rectum similar stimulation evokes a more widespread hyperemia which is reported to be the result of a pelvo-pelvic reflex with VIP as a possible mediator (25;203 see 246).

VIP-ergic nerve fibres have been found around intramural blood vessels. Both mechanical and chemical stimulation of the mucosa of the small and large intestines as well as electrical stimulation of the pelvic nerves evokes a hyperemia and releases VIP from the tissues into the venous effluent plasma. Administration of VIP causes an increase in blood flow matching that which occurs after mechanical or chemical stimulation. Thus, VIP is most probably the neurotransmitter in reflex hyperemia of the gut. Recently, it has been postulated that this release of VIP is mediated by 5-HT produced by enterochromaffin cells. The effect of 5-HT on VIP nerves may be paracrine, but the possibility that the nerves may be in direct contact with the 5-HT containing entero-endocrine cells has been suggested (25; see 73; 118; see 121; see 188; 239; see 239; see 379).

I. 2. 6. CLINICAL SIGNIFICANCE OF THE ENS

The ENS controls and modifies gastric acid secretion, intestinal water and electrolyte transport, motility, mucosal blood flow and several other functions. Thus, as stated earlier an intact intramural system is a necessary prerequisite for the regular coordination of normal peristalsis and other gastrointestinal functions. Therefore, it may be argued that a number of gastrointestinal disorders have their origin in neuropathological changes of enteric neurons. By analogy with the CNS, where neurological disorders may cause either gross disturbances of motor function or more subtle changes in integrative activities, it is expected that the ENS will be found to be involved in a range of gastrointestinal disorders of differing severity (239). However, several

studies indicate that the ENS reacts in a uniform manner to damage irrespective of the nature of the primary damaging agent. This reaction consists of the swelling and ballooning of axons and the reduction of specific nervous elements including total degeneration of whole nerves. The loss of substances and hence the loss of function explains the clinical symptoms, which are fairly similar in each clinical entity, and manifest themselves mainly as motility disorders of varying severity (638). As a general rule it may be said that any treatment or condition that ablates the intrinsic neurons of the gut results in tonic contracture and achalasia of the intestinal circular muscle (796). In addition, a denervated structure has an altered non-specific responsiveness to stimulation, giving an increased activity to agents that are not the usual transmitter substances (132; see 132). Moreover, autonomic nerve dysfunction may also lead to altered hormonal responses in vivo. For example, the lower esophageal sphincter in achalasia and the esophageal body in diffuse esophageal spasm show an altered smooth muscle responsiveness to several hormones (e.g. gastrin). Thus, an intestinal neural disorder may be suggested when there is an abnormality in gut response to indirectly acting stimuli (distension, feeding, drugs acting upon nerves or ganglia) despite an intact response to agents (drugs or hormones) stimulating muscle directly (see 132).

Myenteric plexus damage has been classified into three types: congenital, inflammatory, and those associated with an autonomic neuropathy (701). Achalasia of the cardia, symptomatic diffuse esophageal spasm, infantile hypertrophic pyloric stenosis, chronic idiopathic intestinal pseudo obstruction (Hirschsprung's disease), non-Hirschsprung megacolon, diabetes mellitus, amyloidosis and, finally, scleroderma are all well known examples of damage to the intestinal nerve supply (132; 701). Bacterial toxins of cholera, E. coli and Shigella, as well as several strains of Salmonella, have been shown to induce a unique pattern of action potential complexes mediated through cholinergic pathways. These disorders are thus due to an exogenous toxin, but require neural pathways for their expression (see 132). Furthermore, myenteric neuropathy is frequently found associated with malignant diseases. Indeed, infiltration with inflammatory cells (mainly lymphocytes) and destruction of the nerve cells has often been observed. Thus, a clinical presentation of the bronchus carcinoma may be megacolon (701).

There are two diseases that primarily affect the intrinsic nervous system i.e. Hirschsprung's and Chagas' disease.

Hirschsprung's disease in humans, aganglionic megacolon in piebald mice and in overo-spotted horses are congenital diseases (genetic basis) in which the rectum is usually empty and contracted, whereas the rest of the colon proximal to the constricted segment is grossly enlarged and hypertrophied (35; see 35; 73; 86; 377; 450; see 450; 672; see 672; 752; see 752; see 796). Auerbach's plexus has been found to be absent in the narrowed part of the intestine in all cases of congenital megacolon. In 80 % it was also absent in the "transitional region" and in 20 % the absence extended from the rectum into the upper part of the descending colon. (see 35; 86; 790). Since all signs of the disease disappear after colostomy or resection of the narrowed segment, megacolon is most probably attributed entirely to failure of fecal passage through the terminal constricted segment. Hence, it is thought that the aganglionic segment has no coordinated peristalsis and remains contracted (35; see 35; 73; 86; 377; see 796). In dogs deprivation of the oxygen supply to a portion of the intestine for four hours leads to death of intramural ganglion cells. Thereafter the aganglionic segment becomes tonically contracted and does not relax when coordinated propulsive waves occur proximal to it (see 35). Other, secondary, effects are abnormal discharge of myogenic action potentials and an absence of propulsive motility in the aganglionic terminal segment (see 86; 672; see 672; 752; see 752). Furthermore, in Hirschsprung's disease hyperplasia of cholinergic and adrenergic nerves has been observed in the aganglionic segment. It is thought to be due to extrinsic fibres which, during development, undergo extensive ramifications as they "search" for intrinsic neuronal cell bodies with which they normally synapse (see 73; see 233; 629). As a result of its highly integrated organization any lesion of the intrinsic nervous system of the gut would be expected to affect the peptidergic innervation as well. Indeed, the thick nerves of the aganglionic intestine show virtually no fluorescence after quinacrine incubation. In the intermediate zone there are both positive cells and large, pale abnormal fibres (266). Thus in congenital aganglionosis a reduction in peptidergic (VIP) content and innervation has been found and this reduction appears to be in proportion to the length of the aganglionic segment (see 73; 629). The finding of a lack of peptidergic nerves in the affected human bowel supports at the same time the theory that the majority of peptidergic nerves are intrinsic (see 73; see 233; 629). Moreover, the pattern of response to transmural stimulation is similar in the spastic and dilated bowel, although after cholinergic and

adrenergic blockade transmural stimulation fails to induce relaxation in the aganglionic specimens as it does in the normal colon. Thus, an alteration of the non-adrenergic inhibitory neurons is possibly involved in the motor disturbances of the aganglionic segment (233). As the larger part of the peptidergic innervation probably forms most of the inhibitory nervous supply its absence may leave the inherent excitation of the circular muscle coat uncontrolled leading to constriction. The resulting chronic constipation is further promoted by the lack of the normal VIP induced secretion from the bowel (73). In consequence, spasm of the aganglionic terminal segment of the large intestine reflects the absence of the intrinsic inhibitory mechanisms and the aganglionic segment behaves like a sphincter without inhibition and with no reflex relaxation (233; see 796).

Finally, the thickness of both the circular and longitudinal muscle layers in the terminal large intestine of piebald mice tends to be greater than in corresponding regions of normal bowel (86).

The second disorder associated with a disappearance of enteric ganglion cells is **Chagas' disease** caused by infection with Trypanosoma cruzi. This parasite has a specific affinity for the heart and the myenteric plexus. The trypanosomes live in the walls of viscera and presumably destroy the ganglion cells by the release of a toxin. Hence, there is a marked reduction in ganglion cells in the affected organs. Depletion of peptidergic nerves also occurs when there is a reduction of the intrinsic innervation. In addition, in Chagasic patients low levels of peptidergic innervation and peptide content have been found indicating the diffuse nature of the gut lesion. Furthermore, there is a varied pattern of peptidergic nerve loss reflecting the different degrees of neuronal degeneration along the length of the bowel (see 73). The lesions of intramural neurons lead to achalasia, to a failure of receptive gastric relaxation and accommodation in the proximal stomach, and to an aperistalsis in the distal stomach. Although the destruction of the myenteric plexus affects the entire bowel the usual complaint is dysphagia and constipation. In the chronic stage of the disease cardiomegaly, megaesophagus and megacolon occur. After experimental induction of the disease in rats the gastrointestinal transit time is prolonged and this prolongation is roughly proportional to the decrease in the number of ganglion cells in Auerbach's plexus. Thus, the clinical picture is similar to Hirschsprung's disease except that Chagas' disease develops in adults (see 35; see 73; 701).

In **achalasia** muscle morphology is normal. Physiological evidence from intact muscles show intact responses to direct acting muscle agonists. In contrast, lesions have been observed in the vagi, the dorsal vagal nucleus, and the brain stem and, in advanced cases, there is even an absence or degeneration of ganglion cells in Auerbach's plexus in the lower esophagus. It is now believed that this is a secondary change and not the primary cause of achalasia (see 35).

Focal destruction of the autonomic axons, a rise in the number of ganglion cells and a general nerve proliferation (neuromatous hyperplasia) have been found in **Crohn's disease** (29; 35; 77). Immunologic factors, the invasion of viruses or toxins by breakdown of intestinal barriers have been suggested as etiological agents. The morphological changes in the affected intestinal segment bear similarities to the spongy degeneration of the nervous system, the ultrastructural correlate of "slow virus" syndromes affecting the central nervous system (35). All ganglion cells show marked signs of degeneration i.e. dilatation of their endoplasmatic reticulum, destruction of the mitochondria and deposits of lipofuschin granules, in addition to a distinct neuromatous hyperplasia. These signs of degeneration are apparent only in regions with inflammatory changes. In areas free of inflammation, especially proximal to stenosis, only hyperplasia of nervous elements can be seen (638). There are also, significant changes in the peptide-containing nerve terminals and, therefore, peptidergic nerves have been implicated in the pathophysiology of Crohn's disease (see 29; see 638). VIP nerves are enlarged, thickened and distorted. There is generally an increase in the number, size and intensity of the immunostain in the mucosa and submucosa although in most cases the changes are transmural. The severe transmural inflammatory process may act as an irritant stimulus for the proliferation of intrinsic VIP containing ganglion cells. These changes in VIP innervation have also been detected in histologically normal bowel adjacent to the areas of maximal pathology. As compared to normal bowel and to samples from patients affected with ulcerative colitis a dramatic increase (+/-200%) in the content of VIP and of VIP-immunostained nerves in the gut wall has been found. VIP has a powerful effect on the gut and causes diarrhoea and disturbs motility and the blood flow. There is thus firm morphological evidence that the increase in VIP innervation could have a significant effect on gut function in Crohn's disease. However, if the aberrant VIP nerves are fully functional and release their peptide

content to the mucosal epithelium, watery diarrhoea would be expected to develop. However diarrhoea is not a typical clinical feature (see 29; 77; 629; see 638).

A significant increase in the number of ganglion cells has also been observed in **ulcerative colitis.** Nerve damage occurs especially in the inflamed areas of the gut. However, it has been reported that the intramural nervous system is involved in this inflammatory process regardless of whether the tissue samples were removed from the normal or the inflamed parts of the gut (see 638). Consequently, a decrease in neural VIP has been reported in ulcerative colitis (see 29; 77; 629; see 638).

The **irritable bowel syndrome** is characterized by an abnormal colonic motor activity in response to hormonal and cholinergic stimuli. The disease is manifested by abdominal distention, abdominal pain, constipation or diarrhoea (651). The etiology of this syndrome may be a response of aberrant smooth muscles to physiological stimuli, an abnormal release of one or more gastrointestinal hormones in response to physiological stimuli or a disordered response of the central/autonomic nervous system or gastro-entero-colonic neural reflexes to physiological stimuli (651; see 651).
Diabetes mellitus is commonly associated with clinical evidence of peripheral autonomic neuropathy. The most obvious neurological changes are in the extrinsic nerves, particularly the vagus, rather than in the myenteric plexus. Thus, the genesis of the disorder is in its essence a disturbed autonomic innervation of the gastrointestinal tract and the functional abnormalities of the esophagus are mainly due to a preganglionic involvement of the vagal fibres. Consequently, disturbances of gastrointestinal motility (decreased amplitude of contractions, abnormal peristalsis etc.) are frequent and include nausea, vomiting, constipation and diarrhoea. Hence, diabetic diarrhoea is probably due to a vagal lesion and may be the same as the post-vagotomy diarrhoea (35; 701).

Idiopathic intestinal pseudo-obstruction is a chronic disorder which manifests itself by episodes of intestinal dilatation and recurring attacks of abdominal pain with severe intestinal malfunction resembling acute obstruction. The esophagus shows features of achalasia, and except for the colon the intestine is dilated. If pseudo-obstruction occurs in the colon the clinical effect is constipation. If it occurs in the small intestine the main clinical effect may be a blind loop syndrome because of bacterial infestation of the stagnant bowel contents (35). The disorder exists in at least two forms: myopathic and neural. The myopathic form is known as primary hollow visceral myopathy and is characterized by vacuolar degeneration of the intestinal smooth muscle. In the neural form characteristic neuronal lesions are seen in the myenteric neurons (filamentous inclusion bodies) and it has been proposed that the condition results from an impairment of the neural mechanism controlling gastrointestinal functions. These neuronal changes are not seen in patients with primary visceral myopathy (35; see 35; 629). Hence, intestinal pseudo-obstruction may be due to the loss of a normal neurological control over peristalsis. In addition, there is considerable thickening of the bowel wall which may have two causes. One is that the bolus does not move adequately so that the bowel becomes overstretched activating excitatory cholinergic nerves through intrinsic reflex pathways. The greater the build-up of matter in the lumen, the greater is the stimulus for the intestine to contract around it. This may aggravate the obstruction and hence the damage it causes (237; see 701). Thus, stretch may be a stimulus for the smooth muscle to hypertrophy (see 701). The other is that, in contrast to skeletal muscle, the overall effect of the intestinal smooth muscle innervation is inhibition and, hence, to suppress contraction. In consequence, denervated intestinal smooth muscle overworks and become hypertrophied (see 701).

In visceral **amyloidosis** an amyloidotic neuropathy in both the extrinsic and intrinsic gut nervous systems has been demonstrated and the degenerative process involves all parts of the nervous system. Intramural nerve plexuses are surrounded by a massive amyloidotic wall, while the axons are swollen and electron lucent with a reduction of specific structural elements such as neurotubules, neurofilaments, synaptic vesicles and neurotransmitters. A significant reduction in the neurosecretory granules, which play a decisive role in the storage of neurotransmitters has been found and, by means of fluorescence histochemistry. Here is a depletion of catecholamines in the intramural nervous system. Furthermore, the microangiopathy, found in the intramural capillaries, indicates a trophic disturbance which may possibly play an important role in the pathogenesis of the nerve lesions (638; see 638).

Progressive systemic sclerosis (PSS) or scleroderma is a systemic disorder that may affect all portions of the luminal gastrointestinal tract. Considerable evidence

has been presented to indicate that some of the early gastrointestinal motility changes are related to neural dysfunction rather than muscle destruction. Thus, a functional neural disorder has been hypothesized in which sympathetic inhibition of ganglionic ACh release causes widespread gut dysfunction (see 35).

Cholera toxin triggers the release of 5-HT from the enterochromaffin cells which in turn may activate enteric nerves and neurons including VIP-ergic nervous elements. VIP has been shown to be released in increasing amounts from the small intestine of the cat when exposed to cholera toxin. As VIP is known to be localized almost exclusively in the intestinal nerves, these findings strongly suggest that cholera toxin activates the ENS. Consequently, choleraic secretion is, at least in part, secondary to the activation of intramural intestinal nervous reflexes and it has been claimed that such nervous mechanism(s) may account for about 60 % of the effect of cholera toxin on intestinal fluid transport (118).

Ileus is a state of no motor activity within the gastrointestinal musculature. It is argued that continuous activity of the intrinsic inhibitory neurons accounts for the low responsiveness of the circular muscle to the myogenic pacemaker (796; see 796). In different mammals including man common causes of ileus are exposure and handling of the intestines, total intestinal occlusion, laparotomy, peritonitis (peritoneal irritation), and stimulation of the splanchnic nerves (95; 234; 237; see 573; 651). Such abnormal stimulations initiate reflex activity of the adrenergic nerves and this might inactivate the peristaltic mechanisms for extended periods leading to adynamic ileus (35; see 35; see 237; 239; see 239). Hence, the mechanism for ileus is most probably an overactivity of the sympathetic nervous system (see 35; 58; see 237; 651). It is likely that spinal pathways are mainly involved in mild ileus as the condition can usually be relieved by spinal or splanchnic anaesthesia or by splanchnectomy (237; see 239; see 573; see 796). These results show that myoelectric activity of the small intestine is specifically inhibited and/or disorganized through peripheral reflex pathways (95). Nevertheless, strong and prolonged disorganization of the myoelectrical patterns of the intestine is a striking feature of any disturbance of the gut e.g. in diarrhoea irregular activity becomes continuous, intense irregular activity over the whole or a part of the small bowel results from experimental small bowel obstruction etc. (234). Adrenergic nerve activity has been found to replace the normal myoelectrical activity of the gut by a continuous spiking activity which in many cases is followed by total quiescence (796). After vagotomy, the duration of irregular spiking activity is decreased, suggesting that the ratio of irregular to regular activity is dependent on the influence of extrinsic nerves (651). It has further been demonstrated that the most prominent neural activity during ileus is the ongoing discharge of burst-type neurons (796). Adrenergic nerve activity inhibits, in addition, the action of enteric neurons, increases the tone of the intestinal sphincters, decreases motility and, therefore, the nutritional demands of the gut and, since adrenergic fibres to the gastrointestinal blood vessels are tonically active, reduces the blood supply to the splanchnic bed. The reactivity of the muscle itself is, in contrast, not impaired (see 35; 58; see 237; 651). Hence, adynamic ileus develops when the activation of adrenergic nerves leads to a long-lasting suppression of peristalsis, usually throughout the intestinal tract (239; see 239).
From the foregoing it follows that a rational treatment of adynamic ileus, especially when the basic irritation leading to increased adrenergic discharge cannot be dealt with immediately, is to use drugs which block the effect of adrenergic nerves on the enteric ganglia (phentolamine) (237; see 239). Post-operative ileus may be treated successfully by drugs blocking the release of NA (e.g. guanethidine) or by drugs blocking the sympathetic fibres projecting to autonomic ganglia (α-blockers) or by a combination of both. Alpha-blocking may be beneficial since it has been shown that stimulation of α-receptors in isolated gastric smooth muscle generates spiking activity. Alpha blocking in combination with the anticholinesterase neostigmine is not more effective (see 164; 237; see 239; see 573).

Abuse of laxatives, containing anthraquinones, over a prolonged period results, histologically, in remarkable pathological changes in the colon. Distension or ballooning of axons, reduction of nerve specific cell structures, and an increase in lysosomes up to a total degeneration of whole nerve fibres are the main features. These changes take the form of gross loss of intrinsic innervation, an atrophy of the smooth muscle and melanosis coli. This results in a serious impairment of coordinated peristalsis and probably also in a sensory dysfunction of the colon. Furthermore, the laxatives irritating the mucosa stimulate the intramural ganglia directly. The ensuing toxic degenerative damage to the submucosal plexus, in addition, proceeds in a centripetal direction. Thus, in advanced cases, also designated

as "cathartic colon", the colon is an inert tube without any active function (638).

Autonomic dysfunction leads to a variety of clinical disorders and as a rule all parts of the gut are involved (132; see 132). Degenerative changes in autonomic neurons have come to be called the **Shy-Drager syndrome** in man and Grass sickness in horse. In the Shy-Drager syndrome probably most nerve cells in peripheral ganglia, both sympathetic and parasympathetic, are affected. However, the clinical effects of sympathetic loss, mainly postural hypotension, predominate. Constipation and megacolon are actually present. Histologically, the nerve cells show a vacuolar change similar to that described in nerve cells in the sympathetic chain by Shy and Drager (see 701).

Grass sickness in the horse is characterized by difficulty in swallowing, paralytic ileus, subacute impaction of the large intestine, emaciation and severe mental depression. Extensive degenerative lesions have been found in the sympathetic ganglion cells and in the CNS (Nn III, VII, X, XII), while the primary axonal lesion (probably caused by a neurotoxin) results in the accumulation of NA in the nerve fibres (80).

Marked abnormalities of the regulatory peptide system of the gut have been observed in horses with grass sickness. A reduction in the numbers of nerve fibres immunostained for VIP, Sub. P and Enk was seen in each layer of the gut wall, and in certain grossly affected areas no immunoreactive fibres could be found. In addition, fewer enteroglucagon and somatostatin cells were present in the mucosa. Electron microscopy showed extensive degeneration of nerve fibres in the gut. P-type fibres, containing secretory granules with either VIP or Sub. P immunoreactivity, have undergone extensive degranulation with the formation of multiple vacuoles (75; 822).

PART II
THE INTRAMURAL NEURO-ENDOCRINE SYSTEM OF THE RUMINANT STOMACH:

an immunohistochemical study
using antibodies for Neuron Specific Enolase (NSE)

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Chapter 1
NEURON SPECIFIC ENOLASE AND ITS SIGNIFICANCE FOR THE STUDY OF THE ENS

II.1.1. INTRODUCTION

The gut hormone system spans most of the body but areas with the richest number of endocrine cells are the gastrointestinal tract, the pancreas an d the brain. In these regions the endocrine cells are diffusely scattered throughout the organs and not clustered discernibly as in the classic endocrine organs. So, the existence of a "diffuse endocrine system" was suggested by Feyrter in 1938. In 1966 Pearse described all these diffusely spread "clear" cells as APUD (Amine Precursor Uptake and Decarboxylation) cells (608; 724). The acronym for the common properties of these cells is APUD i.e.

Amine production and/or
Amine precursor uptake
Amino acid decarboxylase
High esterase/cholinesterase levels
High alpha-glycerophosphate dehydrogenase
Ultrastructurally identifiable "endocrine" granules
Specific peptide immunohistochemistry

The last "characteristic" at the time was almost wholly putative, since few APUD cells could be matched with a known peptide, or even with a known amine. This was due to the small number of biologically active peptides recorded, and to the lack a of sensitive cytochemical method for identifying amines (584; see 584). Thus, Pearse introduced this concept to define a system of cells, on the basis of certain common cytochemical and ultrastructural characteristics, that most probably originates from a common neural crest ancestral cell. It was further noted that the APUD cells contained neurosecretory granules. In this way the close morphological similarities between the neural system and the diffuse endocrine system were recognized (608). The subsequent discovery of identical or closely related peptides in both systems led Polak and her co-workers (in 1979) to the expansion of the original term of Feyrter into **"the diffuse neuro-endocrine system"** (DNES) (see 608). Ultrastructural studies further demonstrated that the gastrointestinal tract and pancreas harbour many peptides. Recently, it has become clear that the DNES is not confined to the gut and pancreas, but extends to nearly every tissue of the body (608; 609).

Due to the heterogeneity and complexity of the DNES, its visualization has remained, until recently, virtually impossible (608). Since 1965 several soluble acidic proteins have been isolated from the brain. Two proteins, 14-3-2 (this designation is based on chromatographic and electrophoretic properties) and S-100, both isolated from bovine brain, are of interest since it has been suggested that the former is of neuronal origin, while the latter is supposed to be present only in glial cells (see 72; 169; see 169; 197; see review 501; see 506; 513; see 580; see 584; see 608). A soluble acidic protein has likewise been isolated from rat brain (neuron specific rat protein) and was found to be antigenically similar to the 14-3-2 protein isolated from the bovine brain (513; see 580). Rigorous analysis of the brain tissue has further revealed that 14-3-2 is present in all classes of nerve cells (501; see review 501; 506). S-100, in contrast, is present in both glial cells and melanocytes, Langerhans cells of the skin, reticulum cells, chondrocytes, pituicytes, chromophobe stellate cells of the adenohypophysis and in the parathyroid of rat rabbit and man (169; see 169).

Using specific antibodies to 14-3-2, Pickel et al. showed in 1976 that the protein was localized exclusively in the neuronal cytoplasm, but not in the nucleus. Hence the term Neuron Specific Protein (NSP) was introduced (see 72; 197; see 396; see review 501; 505; see 506; see 509; 604; see 608). Further research

indicated that NSP was in fact a neuron specific isoenzyme of the ubiquitous enolase (EC 4. 2. 1. 11), a glycolytic enzyme necessary for the anaerobic conversion of glucose (129; 508; 512; see 608; 746). Intensive investigations have further showed the existence of three forms of enolase in the CNS of rat and man.

The first form is specific to neurons (cytoplasm and processes) (cfr. protein 14-3-2). Hence its name Neuron Specific Enolase (NSE) (see 129; see review 501; 505; see 506; 507; see 608; 667; see 667; 669). NSE has been first purified from rat, cat and human brain (see 129; 197; see 581; 604; see 604) but it has not been found in lower animals (129). Rat brain NSE is structurally distinct from cat and human brain protein (see 506). Later, NSE was demonstrated in gut endocrine cells (197; 613; see 667). NSE is highly acidic and displays a greater stability than the second form (NNE), described below (503; see 608). It has a molecular weight of 78.000 (see 129; see review 501; see 506; 507; 509; 510; 740).

The second form was originally localized in glial cells (cfr. S-100). Hence, it was named Non Neuronal Enolase (NNE) (see review 501; 505; see 506; see 508; see 509; see 667; 669; see 671). It is of low acidity (507).

The third form (the hybrid α–γ) represents an isoenzyme of intermediate acidity (129; see 506; 510; 512; see 608).

Subsequently, it was discovered that enolase is in fact a glycolytic dimeric cytoplasmic enzyme consisting of three immunologically distinct subunits: α, β and γ. These subunits are arranged into five possible dimeric isoenzymes i.e. three homodimers (α–α, β–β, γ–γ) and two hybrids (α–β, α–γ). Moreover, each isoenzyme was found to display a characteristic distribution. A–α occurs in cardiac muscle, liver and astrocytes; β–β in skeletal and smooth muscles; and γ–γ in neurons (see 420; see review 501; 502; see 506; see 584; see 696). The distribution of the two hybrid forms (α–β; α–γ) has not been completely elucidated (502; see 696). However, entero-endocrine cells express the neural isomer in small amounts only. Consequently it is possible that the hybrid α–γ dimer is present in these cells (218; 584).

From the above description it is clear that NSE and NNE are dimeric proteins each having identical subunits (i. e. γ–γ and α–α respectively) (see 129;see review 501; 510). In the rat, monkey and man both enolases are the major proteins of brain tissue, each representing about 1.5 % of the total brain soluble protein (see 501; 506; 508; 511). NSE levels are highest in those brain areas that have a high proportion of grey matter (e.g. the cerebral cortex). NNE is higher in white matter areas (e.g. the corpus callosum) (506; 508). About 3 % of the total soluble protein in brain neurons may be represented by NSE (see review 501; 506; 608). The peripheral nervous tissue contains less NSE compared with the CNS. In the rat, monkey and human, for example, NSE levels are lowest in the peripheral nerves and ganglia, higher in the spinal cord and highest in the brain (see review 501; 506). Furthermore, several findings support the hypothesis that NNE is present in immature neurons and is replaced by NSE during the neuronal differentiation process (505; 511). Indeed,

- NSE appears late in development (i.e. at the cessation of cell division and the initiation of synaptic contact) (506; see 506; 511; 584; see 584; 670). In rat brain, for example, NSE levels are low prior to birth and rise rapidly 5 days postnatally, while in lower brain areas (e.g. the brain stem) NSE levels reach the adult levels faster than in the cortical brain regions (505; 506; 511). In APUD cells, in contrast, the y isomer is present at a very early stage of the development (in rat from day E 12) (see 584).
-Neurons undergo a change from NNE to NSE during their migration and differentiation in vivo (505; 506; see 506; 511; 667; 670).
- The amount and enzymatic activity of NSE increases during neuronal differentiation and maturation (see 667).
- NNE is the preponderant form of enolase in cultured neuroblastoma cells (509; see 580).
- The content of NSE in neurons can be used as a molecular marker of axonal injury, regeneration and target reinnervation (427).
-NSE decreased during ageing (502; see 608).
- Treatment of cultured pheochromocytoma cells with nerve growth factor (NGF) increased the level of NSE (766).

In addition, the appearance of NSE in neurons and neuro-endocrine cells may further be correlated with their differentiation (396; 501; see review 501; 506; see 506; 509; see 580; see 581; see 584; see 667; 670; see 689; see 766). In conclusion, NSE may be regarded as an indicator for the differentiation of the nervous system (506; see 506; 511; see 584; 670).

NSE is composed of 2 gamma subunits (γ–γ homodimer) and is thought to be strictly localized in neurons and neuro-endocrine cells (see 17; see 72; see 129; 169;

197; 216; see 217; 396; see 420; see review 501; 502; 508; 510; 512; 521; see 581; 625; see 667; 669; 670; 671; see 689; 740). In 1978, Schmechel et al. reported that in the rat NSE is only present in APUD cells and neurons, but not in glial cells (see 169; see 506; 590; 669). In consequence, the gamma subunit was considered as a useful common marker for both neurons and neuro-endocrine (peptide containing) cells of the APUD series (169; see 169; see review 501; 502; see 584; 671; 696; 739).

Radioimmunoassay has showed that the highest levels of NSE occur in the brain, intermediate levels are present in the peripheral nerves and the various neuro-endocrine glands and very low levels occur in non-nervous tissue, serum and the cerebrospinal fluid (see review 501). APUD cells compared to neurons, have low levels of NSE and, unlike mature neurons, they also contain NNE (see 501; 506). Nevertheless, in the upper gastrointestinal tract of the human foetus NSE appears much earlier in endocrine cells than in neurons and the developmental pattern of NSE can be correlated to the appearance of the gastric neuropeptides (gastrin and somatostatin) (588). NSE is, in addition, present in non-neuronal cells and tissues such as platelets, erythrocytes, lymphocytes, smooth muscle cells of the visceral organs and blood vessels, and in the juxtaglomerular organ. Quantitative analyses, however, show that all these non-neuronal tissues contain very low NSE concentrations (420; 501; see 501; see 581).

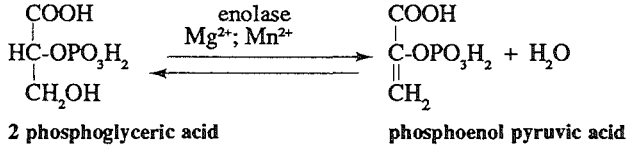
Immunohistochemistry has demonstrated NSE, in addition to the neurons, as a diffuse cytoplasmic stain in neuro-endocrine cells and as a good marker for the whole neuro-endocrine system (696). In addition, NSE immunoreactivity (NSE-IR) has been seen very early in the maturation of the gastrointestinal tract (329). Indeed, NSE-IR cells and nerves can be detected in the upper gastrointestinal tract of foetuses of 10 weeks and it appears that there is a simultaneous production of peptides, neurotransmitters and NSE (608). Moreover, NSE has been found to be present in neuro-endocrine cells in non-nervous tissue such as pinealocytes, parafollicular cells, adrenal medullary chromaffin cells, Langerhans cells, endocrine cells of the lungs, gut and the pancreas, APUD tumours and small cell carcinomas of the lung (see 17; 133; 169; see 169; 197; 302; see 302; see review 501; 506; 508; 547; 671; see 689; see 739; 788). In the nose skin of cats and rats, final NSE-IR cells have also been observed. These cells have all the morphological features of Merkel cells (specialized receptor cells in the skin closely associated with nerve terminals). Consequently, there is strong evidence that these cells too belong to the diffuse neuro-endocrine system (318).

In conclusion, NSE protein is not limited to the nervous structures alone, but appears in other cell types which are most probably derived from the neural crest (169).

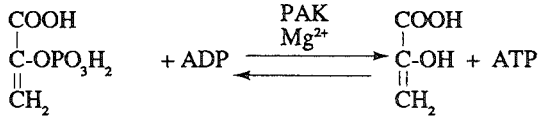
NNE, the α–α homodimer, is exclusively found in glial cells. The hybrid form, α–γ, appears to exist in partially differentiated neurons (see 504; 505; see 506; see 667; 670).

II.1.2. METABOLIC FUNCTION

Enolase (2 phospho-D-glycerate hydro-lyase, phosphopyruvate hydratase (EC 4. 2. 1. 11)) is involved in the dehydration of 2 phosphoglyceric acid yielding to the phosphatic ester of the enol tautomer of pyruvic acid. At pH 7 the heteroform is strongly favoured. The co-factor, Mg2+ or Mn2+, is tightly bound to the enzyme and participates in the binding of the substratum to the enzyme and, presumably, in the subsequent electronic rearrangements (49; see 63; 789).

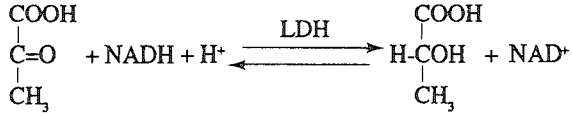


2 phosphoglyceric acid **phosphoenol pyruvic acid**



enol pyruvic acid

Under aerobic conditions the metabolism of pyruvic acid proceeds by oxidative decarboxylation with the formation of acetyl CoA, whereas in anaerobic conditions lactic acid is formed (49; see 64; 789).



pyruvic acid **lactic acid**

II.1.3. PRESENCE OF NSE IN THE GASTROINTESTINAL TRACT

The ENS is a complex and mainly independent entity of the ANS and is composed of neural and non-neural (glial and Schwann cells) elements. A variety of neurons, producing a multitude of classical and putative neurotransmitter, are recognized in this system (610). Radioimmunoassay has shown that the human gastrointestinal tract holds large amounts of NSE, particularly in the non-epithelial layers (217). Throughout the length of the human gut NSE is distributed equally in every segment i.e. 16 % in the mucosa, 12 % in the submucosa and 71 % in the smooth muscles (218). Thus, the major amounts of NSE in the gut occur in the muscle layer containing Auerbach's plexus (72; 217). In the mucosa and submucosa and in the epithelium the lowest quantities of NSE are found (72; 197; 214; 216). The loose network of Meissner's plexus is related to the low NSE concentrations in the submucosa (72; 217). Nerve fibres form a relative rich network in the lamina propria (258) which explains the higher NSE content in this layer compared with the epithelium (72; 217). Indeed, the very low NSE levels in the entero-endocrine cells of the epithelium, indicate that these cells are most probably the only NSE source within the epithelium (72). Furthermore, the concentrations of NSE in the endocrine cells are significantly lower than in the enteric neurons (72; 214; 216; 217). In rat and man NSE epithelial cells have only been observed in the upper part of the gut (stomach and duodenum) (197), although other investigators have found few NSE-IR epithelial cells in the rat and human distal intestine. These endocrine cells showed, in addition, a lower immunostaining intensity as compared to the small intestine (72; 217).

Thus, it may be said that the distribution of NSE in the human gut basically parallels the nervous elements (72; 217) and that the lower NSE concentrations in the mucosa (epithelium and lamina propria) reflect both the nerve supply and the neuro-endocrine cells, while the high content in the muscle layer only reflects the nerve supply (218).

Although in the peripheral nervous system NSE levels are relative low, NSE-IR has been found in the cytoplasm of virtually all types of neurons in the central and peripheral nervous systems (502; 506; see 506; see 608). As a consequence, all autonomic nerve fibres i.e. cholinergic, noradrenergic and the newly recognized serotoninergic and peptidergic (mainly VIP and SP) nerves (69; 72; 197; 608; 610) as well as all intrinsic ganglion cells of the normal gut have been found to contain NSE-IR (69; 197; 613; 614; 740) (cfr. NNE exclusively localized in non-neuronal components). In man and the rat a fraction of the NSE-IR nerve fibres has a very similar distribution to those containing amines (72). In all segments of the reticulo-rumen and the reticular groove of the calf and cow NSE-IR nerve fibres and neurons are predominantly present and are evenly scattered in the inner and outer muscle layers. Where the circular muscle is thicker (ruminal pillar) more NSE-IR have been observed. In the lips of the reticular groove NSE-IR fibres are abundant in suckling and weaning calves and less abundant in the weaned calf and cow, although they are always more abundant than in the reticulo-rumen. In younger calves only a few NSE-IR fibres are found in the lamina propria and there are none in the epithelium. Ganglionated NSE-IR plexuses have never been detected in the submucosal region (432).

In the intestine NSE-IR nerve fibres and endocrine cells have been seen in close contact. NSE-IR nerves run through the lamina propria up to the epithelium where they often appear to terminate on or near immunoreactive and non-immunoreactive mucosal cells (72).

In all regions of the gastrointestinal tract, including the pancreas, NSE immunostaining reveals a massive system of endocrine cells and neuro-endocrine complexes (DNES) (72; see 475; 690). Virtually all currently identifiable peptide/amine (gastrin, somatostatin, serotonin, motilin, secretin, gastric inhibitory peptide (GIP), enteroglucagon, neurotensin, CCK, PYY)-containing cells as well as enterochromaffin-like cells, enterochromaffin (APUD) cells and hitherto uncharacterized cell types contain NSE and react with NSE-antibodies (72; see 72; see 214; 216; see review 501; see 502; 547; 585; 586; 587; 608; 610; 614; 671; see 739; 740). Most of the NSE-IR cells of the fundus have been identified as enterochromaffin-like, while some cells in the antrum and duodenum, are identical with cells containing peptide hormones such as gastrin, somatostatin, neurotensin and GIP (614). Thus, NSE has been considered as a convenient marker of the peptide hormone producing cells of the gastrointestinal tract and of tumours originating from these cells (see 169; 738). Consequently, all neuro-endocrine tumours of the gut reacted strongly to NSE antibodies regardless of the amount of peptide present or the degree of malignancy (613). Additionally, the gut and lung har-

bour, besides NSE peptide containing cells, cells that displayed NSE immunoreactivity but that did not stain for any of the known peptides. Probably they represent uncharacterized members of the diffuse neuro-endocrine system (see 133; 502). In the upper regions of the bovine bowel, for example, NSE staining has revealed NSE positive (endocrine?) cells in the lamina propria and submucosa (72; 610). These cells are innervated and, on the basis of their morphological appearance, seem related to Fujita's paraneuron as well as Pearse's APUD system (72).

II.1.4. CLINICAL SIGNIFICANCE of NSE

As the isoenzyme NSE has been "exclusively" found in the central and peripheral nervous systems and in the central and peripheral divisions of the DNES, one may postulate that NSE is present in and relatively specific to neuro-endocrine tumours (see 475; 501; see review 501; 625; 671; 696; 739). This hypothesis has been confirmed by the following findings.

- NSE was found in all kinds of human endocrine tumours including neuroblastomas, paragangliomas, parathyroid adenomas and oat cell carcinomas of the lung (509; 547; see 547; 580; see 580; see 581; 739; 778).
- The NSE content of endocrine tumours is significantly higher than in non-endocrine tumours (608). In most patients with endocrine tumours the NSE plasma levels are elevated, reduced during effective chemotherapy and again elevated during recurrence (see 608). Nevertheless, plasma NSE levels are not always elevated in all patients with pancreatic islet cell carcinoma and intestinal carcinoid tumours (624).
- NSE is produced by and found in relatively high amounts in APUD-omas (502; 739). Thus, NSE may be a specific marker for this type of tumour (671; 739). Furthermore, the plasma NSE levels are elevated in some patients with neuro-endocrine or APUD tumours (580; see 580; see 623; 625). The elevated serum NSE seems to be related to the amount of neuro-endocrine tissue present since it decreased to normal or near normal levels after resection of the tumour or adequate chemotherapy (625). Thus, NSE may prove to be useful in the diagnosis and staging of APUD-omas in man (671).
- Tumour cells in human neuro-endocrine tumours reacted strongly with NSE -antibodies regardless of the amount of stored peptide (580; 740).
-In patients with small cell carcinoma of the lung there is a correlation between the amount of tumour and the elevation of the serum NSE (580; see 625).
- In man NSE has been found to be a reliable marker for partially differentiated neuroblastic tumours, while NNE (=S-100) may be used to trace poorly differentiated neuroblastic tumours (115). Consequently, NSE is useful in distinguishing endocrine and non-endocrine neoplasias (625; 739).
- Merkel cell tumours can be specifically identified by NSE immunostaining (317; 318).

Finally, the fact that NSE and NNE represent functional markers for neuronal and glial cells makes it possible that both enolases can be measured in biological fluids such as serum and cerebrospinal fluid (CSF). Indeed, in several neuronal pathologies e.g. Huntington's, Parkinson's and Alzheimer's disease and in cerebral infarctions elevated levels of NSE in the serum and CSF can be present (501; see review 501; 506). Furthermore, NSE is reduced by 45% in the basal ganglia in patients with Huntington's disease while the NNE levels are not significantly altered. In contrast, the NSE levels in the cerebral cortex remain unchanged and the NNE levels are significantly increased. So, NSE and NNE levels appear to be specific biochemical indicators of neuronal and glial cell number and their viability (504).

II.1.5. CONCLUSIONS

Neuron specific enolase, the γ–γ homodimer of the glycolytic dimeric cytoplasmic enzyme enolase, seems to be "strictly" localized to the cytoplasm of differentiated neurons in both the central and peripheral nervous system and, in the cells of the central and peripheral divisions of the DNES (APUD cells, paraneurons as described by Fujita). Very low concentrations have, however, also been found in non-neuronal cells and tissues (see 17; see 72; 133; see 169; 197; 216; see 217; 420; see 420; 501; see 501; 502; 508; see 508; 512; 513; 521; see 521; 581; 588; 625; see 625; 669; 670; 671; 739; 740). In consequence, this enzyme may be used as a marker for all type of neurons and virtually all neuro-endocrine or paraneuronal cell types. In addition it is a

good index for the maturation, and the functional and dynamic status of neurons (69; 501; see review 501). Consequently, NSE antibodies may be considered as a common and powerful marker to map the neural and endocrine components of all parts of the peripheral DNES in health and disease (72; 318; 501; see 501; 608; 740), while NSE immunostaining has allowed the simultaneous demonstration of endocrine cells and the whole nervous network in the gut and pancreas (humans and rats) (72).

Chapter 2

THE INTRAMURAL NEURO-ENDOCRINE SYSTEM OF THE RUMINANT STOMACH OF THE SHEEP

II.2.1 INTRODUCTION

The functional anatomy of the ruminant stomach obviously demonstrates that the normal functioning of this gastrointestinal segment takes place under direct and/or indirect nervous control (see introduction). As each ruminant primarily subsists on microbial fermentation, a process requiring rigorous physiological conditions and, not least, controlled and coordinated motility, an intact innervation of the ruminant stomach is an absolute prerequisite for the normal functioning of this organ. In other words, the intramural nervous system, as it is involved in the local reflexes and in the modulation of the efferent impulses from the CNS to the gut, is an essential link in the preservation of a ruminant's life.

From the review of the literature concerning NSE, it was concluded that NSE antibodies offer the possibility to specifically visualize the whole nervous network and the endocrine system of the gut. Consequently, in Part II of the study an NSE antiserum was used in order to visualize the intramural neuro-endocrine system in the different parts of the stomach of the sheep. The findings ensuing from this part of the study will, finally, serve as a springboard to the last part (Part III) of this work in which the presence, distribution and function of different substances, involved, at least partially, in the nervous control of the ruminant stomach, are studied.

II.2.2 MATERIAL AND METHODS

Material

Eight foetuses ranging from 12 to 36 cm C-R length (see Addendum/ Part II/ table 4) and five adult sheep of different age, sex and race (mostly Suffolk and Texel) were used in this study.

Immediately after slaughtering the stomach was dissected out through a midline incision and the following parts were taken:

Reticular Groove (RG)
Reticulum (RET)
Ruminal Dorsal Sac (RDS)
Ruminal Ventral Sac (RVS)
Ostium Reticulo-Omasicum (ORO)
Omasum (OMA)
Abomasum (ABO)
Antrum Pyloricum (AP)
Pylorus (PYL)

The foetal stomach on the other hand was at first fixed and rinsed undissected. Thereafter the different segments of interest were dissected out.

Methods

The preparation of the buffers and fixative as well as the technical procedure used in this study are described in the addendum (see Addendum/ Part II/ pag. 22 and fig. 8).

Neuron specific enolase (Dakopatts a/s Denmark, Lot 105, undiluted) (antigen source cow; antiserum source rabbit) was diluted in PBS buffer at a finally concentration of 1/1000 or 1/4000 for paraffin or cryostat sections respectively.

II.2.3. IMMUNOHISTOCHEMISTRY

Basic principles

The immune system protects an individual from molecules which normally do not exist in the individual (foreign invaders, toxic substances). But, substances that normally occur in a species can, if they are modified by coupling to larger carrier molecules through which they function as an antigen, stimulate antibody production. In this protection system both cellular and humoral mechanisms are involved. Antibody formation is known to be one of the most specific biologic interaction systems. Consequently, specific antibodies can be used as a tool for the recognition of molecules of different characteristics (227). One mechanism of protection is the production of specific large serum proteins, immunoglobulins (IgG), by plasma cells. The IgG can bind the foreign substances (antigens). The immune system is capable of recognizing surface structures of macromolecules with a molecular weight of over the 5000 daltons. However, the portion of the antigen against which the antibody is directed, the antigenic determinant, comprises only a minor part of the molecule (227; 759).
IgG recognizing a specific antigenic determinant consist of a complex of four polypeptides forming a symmetric Y-like structure (see Addendum/ Part II/ fig. 9). Two identical low molecular (light chain) and two identical high molecular (heavy chain) parts are joined by disulfide bridges. At the N-terminus each chain exhibits a variable portion where the surface recognition for the antigen is located and where in consequence the antigen-antibody reaction takes place. Papain or pepsin can degrade antibodies into smaller fragments. In this way it is possible to isolate and to obtain smaller molecular fragments, containing the complete univalent (Fab fragment) or divalent (F(ab')2 fragment) binding sites of the antibodies. Hence the Fc fragment which may produce non-specific interactions in immunohistochemical reactions can be eliminated (227; 759).

Immunohistochemical reactions

The antibody-antigen complex is not directly visible. Thus for the localization and visualization of the specific antibody-antigen complex on tissue sections a labelling system has to be combined with the antibody. For that purpose various direct and indirect labelling systems have been developed.
In the direct labelling methods the label is coupled to the specific primary antibody.
In the indirect methods the primary antibody is unlabelled. Consequently, the antibody-antigen complex is detected by means of a labelled secondary antiserum (antibody) directed against the specific primary antibody (227). In essence, the primary unlabelled antibody obtained from species A is applied to the tissue and the excess is washed off. A second labelled antibody obtained from species B and directed against the IgG of species A is then applied. The first antibody now acts as an IgG antigen. In this way the primary antigenic site is revealed (759). The indirect staining procedures are regarded as more sensitive than the direct ones. It is today generally accepted that among the indirect methods the peroxidase-antiperoxidase (PAP) technique is the most sensitive one (see 620). Thus, the same amount of antigen can be visualized by a lower concentration of primary antibody (759). Indirect methods have further the advantage that the same procedure can be used in combination with different (primary) antisera provided they are all raised in the same species (227; 620; 759).
For labelling of the antibodies fluorochromes, colloidal gold and enzymes are the most frequently used.

Fluorochrome-Labelled Antibodies

The fluorochrome-labelled antibody technique has the advantage of a higher resolution at the light microscopical level (227; 620; 759). In this method fluorochromes, such as fluorescein isothiocyanate (FITC) (giving a green fluorescence) or rhodamine B isothiocyanate (RITC) (giving a red fluorescence), are used to label the antibody and, in consequence, to detect the specific antibody-antigen binding. Depending on the methodology a direct immunofluorescence (primary antibody labelled with the fluorochrome) or a indirect immunofluorescence (secondary antibody, directed against the IgG of the unlabelled specific primary antibody, is labelled with the fluorochrome) may be distinguished.

Colloidal-Labelled Antibodies

Protein A (pA), a protein of the cell wall of Staphylococcus aureus, has a characteristic ability to interact with the Fc fragment of IgG molecules from several

species. It has therefore been introduced for the localization of different antigens at the light and electron microscopic level by combining the pA technique with ferritin, peroxidase and especially with colloidal gold as optic markers (see 227). In the case of colloidal gold labelled antibodies pA is added to the colloidal gold suspension and forms a non-covalent binding complex due to the interaction of the positively charged groups of pA with the negatively charged surface groups of the gold particles (see 227; 759).

Enzyme-Labelled Antibodies

The enzyme-labelled antibody methods correspond basically to the direct and the indirect fluorescence methods, but the fluorochrome is replaced by an enzyme, mainly horseradish peroxidase (HRP) (227). Hence, a direct and an indirect enzyme-labelled antibody technique may be distinguished.
The indirect enzyme-labelled antibody technique implies a bridging (unlabelled) antibody between the primary antibody and the final unconjugated antibody raised against HRP in the same species as the primary antibody. This layer is followed by HRP that is then bound by an antigen-antibody reaction. The bounded HRP is then histochemically visualized. Indeed, hydrogen peroxidase (H2O2) functions as a substratum for HRP, while a polymerizing diamine i.e. 3 3'-diaminobenzidine tetrahydrochloride (DAB) acts as an electron donor. In this way an insoluble brown reaction product is formed at these sites where the antibody-antigen complex is formed (227; 759).
The enzyme-labelled antibody technique has the important advantages that the result (=insoluble reaction product) is permanent and that the method can be applied for both light and electron microscopic studies. However, the sensitivity of this technique is not higher than that of the immunofluorescence.technique (227; 620; 759).

As the unlabelled antibody **Peroxidase-Anti-Peroxidase (PAP)** technique is considered to be the most sensitive indirect immunohistochemical method, this method will be discussed in some more detail.
The PAP- technique is in fact a modification of the indirect enzyme-labelled antibody technique. This method, developed by Sternberger in 1969, is much more sensitive (20-125 times) than the other immunohistochemical techniques (720). Sternberger combined the peroxidase (HRP) with anti-peroxidase antibody before the application of HRP to the tissue. This combination produces a very stable cyclic complex with three peroxidase molecules to two antibody molecules. The species A PAP complex will only react with the anti-species A IgG of the second layer. Hence, the PAP-complex acts as a third layer antigen. This increased amount of labelling has the advantage that it allows a higher dilution of the primary antibody, it reduces unwanted background staining, allows the preferential attachment of high affinity antibodies and, finally, decreases the possibility of a dissociation of the antibody from the tissue antigen since both combining sites of each antibody molecule are bound. In consequence, provided there is no unwanted binding of the second layer to the tissue, the PAP method is a very specific and background-free technique (227; 759; 760).
In essence, the PAP- technique comprises the following four steps (see Addendum/ Part II/ fig. 10.) (227; 759; 760).

Step I

Incubation of the tissue with a high dilution of the specific primary antiserum raised in species A and directed against the molecule of interest. The antiserum is diluted in tris buffer saline (TBS) containing 1 % normal goat serum (759). An excess of the primary antibody is usually applied on the tissue preparation. This results in preferential binding of the antibodies with the highest affinity while the low-affinity antibodies remain in solution. Hence, dissociation of the primary antibody from the tissue antigen during the washing of the sections is minimized.
The amount of primary antiserum to be used depends on several parameters such as tissue antigen concentration; the titre, avidity and dilution of the primary antiserum; the incubation time; and temperature as well as the accessibility of the antigen. Tissue antigen concentration itself also depends on the physiological condition of the animal, and the fixation and embedding procedure (760). So, a serum dilution appropriate for one tissue may not be so for another. Consequently, in an initial experiment the appropriate dilution of the primary antiserum has to be determined.
The highest possible dilution of the primary antibody is recommended for several reasons. It reduces the concentration of unwanted antibodies in the solution, it increases the ratio of specific staining/background staining and it allows the primary antibody to bind with both antigen binding sites minimizing thereby the dissociation during washing (227; 759; 760). Moreover, a

high concentration of primary antibodies in the tissue sections might result in the binding of both antigenic sites of the second (linking) antibody to the primary antibody. Hence, no antigen binding site is free to combine with the third layer i.e. PAP complex. Consequently, no specific staining is achieved due to a failure in the binding of the PAP complex (620; 759; 760).
Finally, a long incubation time is recommended since this allows the antigen-antibody reaction to reach its maximum (759).

Step II

Incubation of the tissue with an excess of the secondary unconjugated species B/anti-species A antiserum. It is important to use this antiserum in excess, since in this manner one of the two identical binding sites (Fab portion) remains free after reaction with the primary antibody. This free antigen binding site can then combine with the third layer i.e. the species A PAP complex. The choice of the dilution depends on the antibody titre and on the final background which increases with the concentration of the IgG solution (759; 760).

Step III

Incubation with a peroxidase-antiperoxidase (PAP) complex. The PAP complex consists of HRP bound to its antibody (anti-HRP) obtained from HRP-immunized species A.

Step IV

Incubation of the tissue with H2O2 as a substratum for HRP in the PAP-complex and DAB as the electron donor. HRP reduces H2O2 and, as a consequence, DAB is oxidized resulting in the formation of a brown insoluble polymeric oxidation product of DAB over the antigenic sites of the tissue section (759; 760).

The cutting out of one staining step by use of the PAP complex and the high degree of purification and specific binding of this final layer makes the PAP method, as compared to the original indirect enzyme-labelled antibody technique, a very clean and sensitive technique (759).

However, apart from the four basic steps, other additional procedures are used in the PAP technique. These will be, very briefly, considered below.

Fixation

The antigen to be localized must be insoluble or must be made insoluble by being fixed in the tissue. On the other hand it must be available to the antibody in an immunoreactive form. Almost any form of tissue processing (fixation, embedding) will change the immunoreactivity of the antigens, while in unfixed or mildly fixed tissue the embedding, as well as the long immunohistochemical incubation procedures, will harm the morphology.
Cryostat sections usually offer the best preservation of immunoreactivity. However, cell membranes are normally still intact in such sections and, consequently, hamper the penetration of antibodies. The use of a detergent, such as Triton X-100 during the antiserum incubations improves the penetration but causes, especially in unfixed sections, in an appreciable loss of tissue structure (620; 759).
The best morphological preservation is usually seen after fixation by routine fixatives (formaldehyde or glutaraldehyde) that produce inter-and intramolecular cross-linkages between proteins in the tissue. However, it used to be thought that the antigenic immunoreactivity would survive only very gentle fixation such as a low concentration of formaldehyde or specially developed weakly cross-linking reagents such as diethyl pyrocarbonate and p-benzoquinone (227; 759).
For the light microscopical localization of peptides good results can, with respect to the maintenance of the immunoreactivity and morphology, be obtained by using buffered fixatives based on formalin (with or without picric acid) or glutaraldehyde. The duration of the fixation seems likewise to be an important variable. Furthermore, embedding of the tissue in paraffin or Epon usually results in a decrease of the immunoreactivity (see 620).

Rinse in TTBS

Triton X-100 (0.1-2.0 %) allows a better and deeper penetration of the antibodies into the tissue. An additional advantage of this detergent is the diminished background staining, which is due to a less non-specific adherence of the antibodies to the tissue (620; 759).

Removal of the endogenous peroxidase activity

The presence in the tissue of enzymatically active peroxidases such as catalase and haemoprotein, which are capable of reacting with hydrogen peroxide and

hence can reduce diaminobenzidine (DAB), could confuse the final location of the antigen. Blocking the endogenous peroxidase with H2O2 at the start of the procedure is therefore usually performed (759; 760).

Incubation with Normal Goat Serum (NGS)

This is an essential step in all the methods unless affinity-purified or monoclonal antibodies are exclusively used. The principle is that non-immune serum, derived from the same species as that donating the second layer antibody, is applied at the beginning of the immunohistochemical procedure. NGS sticks to protein-binding sites either by non-specific adsorption or by binding of specific, but unwanted, serum antibodies to the antigens in the tissue. Since the non-specific adsorption reactions are likely to be of low affinity, the serum is not washed off before the primary antibody is applied. Moreover, NGS prevents the non-specific or unwanted specific attachment by the primary antiserum (759).

Addition of a protein to the antibody solution

Inclusion of 1% normal serum (from the same species that gives the second layer antibody) or 1% inert protein (e.g. albumin) in the primary antibody solution may also help by competing with the primary antiserum for non-specific binding sites in the tissue and by absorbing any antibody to albumin that may be present in the primary antiserum in order to prevent non-specific staining (759).

The ultimate goal of each immunohistochemical method would be the restricted localization of a tissue antigen by a highly specific antiserum directed against the antigen under study. Thus one of the major problems in immunohistochemistry is the specificity of localization.
Indeed, false-negative and false-positive results (pigment that resembles the DAB precipitate, pseudoperoxidase and endogenous peroxidase activity, autofluorescence by lipofuschin, etc.) can occur in each immunohistochemical study. Causes for such false results may be found in the tissue section, the immunohistochemical procedure used to detect the first antibody, and in the primary antiserum itself (620). Serum taken from an immunized animal contains numerous natural antibodies as well as antibodies to any carrier protein and conjugating molecules used in the immunization procedure. These will result in unwanted antibodies that can bind to the tissue during the immunohistochemical procedure. Although a high enough dilution of the primary antibody makes the unwanted reactions practically negligible and purification of the antibody in fact not necessary, purification of the antiserum by affinity absorption, as well as different specificity tests, are recommended (620; 759).
Moreover, antibodies are directed against the antigenic sites of the immunogen and not against the molecule as an entity. Related (but different) molecules in the tissue may share antigenic sites with this immunogen and therefore be a cause for a serum aspecific reaction. There is thus always the chance of a reaction to a similar sequence in another substance (cfr. the many "families" of different regulatory peptides related by their molecular structure) (620; 759). Monoclonal antibodies provide an answer to the specificity problems encountered in immunohistochemistry. But cross-reactivity of a monoclonal antibody cannot be excluded and moreover, if present, will involve the entire antibody population (620).
Since false immunological and non-immunological reactions may lead to a misinterpretation of the localization, several specificity tests are normally advised and it is recommended to use the term peptide-like immunoreactivity.

Negative control tests

From the negative reaction seen after dilution of the primary and secondary antisera; after the replacement of steps in the immunohistochemical procedure; and after the absorption control, it can be concluded whether or not the initial reaction is specific.

Dilution test

At low dilutions the specific antiserum may be suppressed. Reactions with low dilutions of the primary antisera mostly stem from non-immunologic, non-specific binding (see 759). Thus, if the immunohistochemical reaction appears stronger above a relative high dilution (1/2000) then the real dilution effect may become evident. The dilution factor of the primary antisera depends on the method used. Dilution tests of secondary antibodies are of less importance.

Replacement of steps in the immunohistochemical reaction

This control method is of importance in checking the appropriate immunohistochemical reaction, particularly when testing endogenous enzyme (peroxidase) reaction or non-specific binding of dye. In the case of the replacement of single steps in the PAP procedure this part of the reaction is either entirely omitted or carried out using PBS. All the reactivity still appearing afterwards is not related to the specific immunohistochemical reaction. Thus, a negative control section should also be prepared with non-immune serum or an inappropriate antiserum replacing the primary antiserum at the same dilution. The negative control section is stained the same as the test section. This could be due to inadequately blocked endogenous peroxidase or to non-specific uptake of immunoglobulins from the serum of from the second antibody (227; 759).
Omission of the first step in the PAP- technique (i.e. incubation of the tissue with the primary antiserum) has been systematically used as a specificity test in this study.

Absorption control

The principle is that the primary antibody is removed or bound by a reaction with the antigen initially used for immunization. It is doubtful whether it can be concluded that an absorption control discriminates between specific and non-specific reactions. The amount of substance to be added depends on the dilution of the primary antiserum (227; see 227).

Positive control tests
(For more detailed information about these techniques see 227; 759; 760).

- Parallel reference incubation
In the same run a tissue, in which the presence of the molecule of interest was already established, was incubated with the primary antiserum.
- Control by RIA
- Control by chromatographic methods
- Control by high pressure liquid chromatography (HPLC).

II.2.4. RESULTS

For the sake of clarity the results of this immunohistochemical study are represented very schematically. Hence, in discussing the results, the following scheme will be used:
layer (tunica)
specimen (foetal or adult sheep)
segment
As frozen tissue offered the best preservation of the immunoreactivity, and as the cryostat sections are considerable thicker (10 times), more and intenser reacting NSE-IR nervous elements were, compared with paraffin sections, encountered in cryostat sections. Consequently, the following discussion is largely based upon results observed in cryostat sections. It is only when a notable discrepancy in the results of both techniques was encountered that the results seen in paraffin sections will be described.

Tunica Mucosa

Lamina Epithelialis

FOETUSES

Reticular Groove

(see Addendum/ Part II/ photos 2; 4-5)

Mainly in the older foetuses (mean C-R length > 21 cm) were several epithelial cells showing a weak NSE immunoreactivity (NSE-IR) seen in the basal layer of the stratified, multilayered, aglandular epithelium. Moreover, NSE-IR cells were principally found at the reticular side of the groove and only sporadically in the epithelium of the reticular groove itself. The NSE-IR cells had the typical morphological appearance of an entero-endocrine cell i.e. they were triangular or pear shaped cells resting on the basal lamina. At the base of these cells a slender process was regularly observed.
In paraffin sections no NSE-IR was found either within or between the epithelial cells.

Ruminal Dorsal Sac

(see Addendum/ Part II/ photos 7-10)

NSE-IR was observed in many epithelial cells of the basal layer. These cells have also an endocrine-like aspect. Moreover, some cells were seen to have two basal processes giving them a U-like appearance. Just as in the reticulum single NSE-IR nerve fibres were seen to branch off from the submucosal intrapapillar NSE-IR nervous network and to come close to these NSE-IR epithelial cells.
In paraffin sections of the foetuses, NSE-IR was never observed either in the epithelial cells or in any structure between the epithelial cells.

Ruminal Ventral Sac

(see Addendum/ Part II/ photos 12-14)

In all specimens studied several epithelial cells, with an endocrine-like morphology, showed a distinct NSE-IR. Delicate NSE-IR nerve fibres, originating from the intrapapillar plexus, were seen to run just underneath the basal layer and hence close to the NSE-IR epithelial cells.
In paraffin sections, however, NSE-IR cells were never noticed in the epithelium.

Reticulum

(see Addendum/ Part II/ photos 2; 4; 16-17; 20)

The basal layer of the reticular epithelium was seen to hold various NSE-IR cells. As a rule their number increased in the older foetuses as compared with the younger ones. On some occasions a contact between the basal process of a NSE-IR cell and a single NSE-IR nerve fibre originating from the submucosal plexus can be distinguished.
In paraffin sections a rather weak NSE-IR was seen in a very few cells of the stratum germinativum.

Ostium Reticulo-Omasicum

(see Addendum/ Part II/ photos 22-24)

Neither in the paraffin sections or in the cryostat sections obtained from the foetuses used in this study, were NSE-IR epithelial cells observed at the level of this orifice.

Omasum

(see Addendum/ Part II/ photos 26-31)

Cryostat sections of a good quality were rather difficult to obtain from this segment. Nevertheless, various epithelial cells, displaying a distinct NSE-IR, were regularly distinguished in the basal layer of the omasal leaves.
In paraffin sections of the oldest foetus (C-R length 36.5 cm) were, several epithelial cells, scattered in the germinative layer, seen to have an apparent NSE-IR.

Abomasum

(see Addendum/ Part II/ photos 32-33)

Numerous NSE-IR epithelial cells were seen in all the foetuses studied. The majority of the NSE-IR cells were encountered in the basal part of the gastric glands.
In paraffin sections of some foetuses a feeble NSE-IR was found in a few epithelial cells.

Antrum Pyloricum

(see Addendum/ Part II/ photos 35-37)

In all foetuses studied several NSE-IR epithelial cells were found in the surface epithelium of this gastric segment.
In paraffin sections of the younger foetuses few epithelial cells showed a relative weak NSE-IR. These cells were mainly present in the surface epithelium. In older foetuses, in contrast, no positive reacting epithelial cells were found.

Pylorus

(see Addendum/ Part II/ photos 41-43)

Various epithelial cells showing a distinct NSE immunoreactivity were normally seen along the epithelium of the pylorus.

However, in paraffin sections of the younger foetuses NSE-IR epithelial cells were only sporadically seen. In the older foetuses, in contrast, the pyloric epithelium showed no NSE-IR.

ADULT SHEEP

In sharp contrast to the results observed in the foetuses, no NSE-IR was ever found in the epithelium of any segment of the ruminant stomach irrespective of the technique used (see Addendum/ Part II/ photos 11; 39)

Lamina Propria

FOETUSES

As a rule this layer is poorly developed in the forestomach and this is particularly the case in smaller (younger) specimens. Furthermore, due to the absence of a complete lamina muscularis mucosae in most forestomach compartments, except the OMA, it is often impossible to clearly separate the lamina propria from the underlying tunica submucosa. As a consequence, one can not always ascertain whether a NSE-IR nerve fibre actually lies in the basal part of the propria mucosae or in the upper part of the tunica submucosa. Generally speaking, in the younger foetuses no NSE-IR was noticed in paraffin sections of the different compartments of the stomach. In older foetuses, however, a very few thin NSE-IR nerves were observed close to the basal layer of the epithelium. Due to this topography it may be claimed that these NSE-IR nerve fibres lie, most probably, in the propria mucosae.

Reticular Groove

(see Addendum/ Part II/ photos 2; 4-5)

Although the lamina propria is difficult to delineate a single, very fine NSE-IR nerve fibres were distinguished on the reticular side of the groove in sections of the older foetuses. In the lamina propria of the reticular groove itself, there were no NSE-IR fibres just beneath the epithelium.

Ruminal Dorsal Sac and Ruminal Ventral Sac

(see Addendum/ Part II/ photos 7-10; 13-14)

In the centre of the future ruminal papillae several fine NSE-IR nerve fibres were seen to form a very delicate intrapapillar NSE-IR nerve plexus. Single NSE-IR nerve fibres branch off from this plexus and run just underneath the epithelium. Hence, it is possible that some of them lie in fact in the lamina propria.

Reticulum

(see Addendum/ Part II/ photos 2; 4; 16-17; 19-20)

Occasionally a delicate NSE-IR nerve fibre, running close to the basal lamina, was identified at the onset of the primordia of the reticular folds.

Ostium Reticulo-Omasicum

(see Addendum/ Part II/ photos 23-24)

Likewise in this segment no clear transition between the propria and the underlying submucosa could be observed. Thus, it is possibly that some of the fine NSE-IR nerve fibres, encountered in the upper part of the submucosa actually lie in the lamina propria. Interestingly, in the core of the claw-like papillae unguiculiformes a very dense and distinct NSE-IR nervous network was always observed.

Omasum

(see Addendum/ Part II/ photos 27-31)

Infrequently, delicate NSE-IR nerves were found in the lamina propria of the larger omasal leaves.

Abomasum, Antrum Pyloricum and Pylorus

(see Addendum/ Part II/ photos 32-33; 35-37; 41-43)

The results found in the glandular stomach were in sharp contrast with those seen in the forestomach. Moreover, as the lamina muscularis mucosae forms a constant part of the tunica mucosa throughout the

"true" stomach, a clear delineation between the lamina propria and the tunica submucosa can always be made. As compared to the forestomach the picture changes abruptly. Distinct ganglia lying between the base of the glands and the muscularis mucosae were observed. Particularly around the base of the gastric glands several NSE-IR nerve fibres were found. They contributed to the formation of extensive periglandular and interglandular plexuses. These NSE-IR nerves seem to originate both from the submucosal plexus and from small ganglia situated between the base of the glands and the lamina muscularis mucosae. Numerous, fine NSE-IR nerve fibres followed the propria protrusions and constituted a delicate NSE-IR nervous network. Delicate NSE-IR nerve bundles or single NSE-IR nerve fibres, finally, ascended in the propria up to the surface epithelium.

ADULT SHEEP

Reticular Groove

Several, isolated, fine NSE-IR nerve bundles, without any particular course, were normally distinguished in this segment. Ganglia were never observed.

Ruminal Dorsal Sac and Ruminal Ventral Sac

(see Addendum/ Part II/ photo 11)

Very fine NSE-IR nerve fibres were sometimes encountered in the transitional zone between the lamina propria and the submucosa. In some ruminal papillae fine NSE-IR nerve fibres were found to run just underneath the stratified, aglandular epithelium.
In paraffin sections no structure, establishing a clear NSE-IR, was found.

Reticulum

In the borderzone between the lamina propria and the submucosa several delicate NSE-IR nerves were seen. These were only seen in some paraffin sections.

Ostium Reticulo-Omasicum

Some NSE-IR nerve fibres were distinguished in the lamina propria of this transitional segment.

Omasum

In most omasal laminae sparse, irregular NSE-IR nerve bundles were discerned in the connective tissue layer separating the epithelium from the muscularis mucosae.
In paraffin sections fine, scattered NSE-IR nerve fibres were rather exceptionally observed.

Abomasum, Antrum Pyloricum and Pylorus

(see Addendum/ Part II/ photo 39)

Basically, a uniform distribution pattern in the NSE-IR nervous network was observed in the lamina propria along the whole glandular stomach. Generally several thin NSE-IR nerve fibres formed a definite and dense periglandular NSE-IR nerve plexus. NSE-IR nerve fibres entering in this formation originated either from the mucosal ganglia situated between the base of the glands and the underlying muscularis mucosae, or from the submucosal ganglia. In addition, fine NSE-IR nerve fibres, constituting a delicate NSE-IR nervous network, ascended in the lamina propria towards the epithelium.
However, it must be stressed that at the level of the pylorus this arrangement was not always clearly seen.

Lamina Muscularis Mucosae

FOETUSES

Reticular Groove

(see Addendum/ Part II/ photos 2; 5)

At the level of the reticular groove the muscularis mucosae consists of isolated groups of smooth muscle cells. In consequence, NSE-IR was found in these "islands". On the reticular side of the groove smooth muscle bundles were observed in the primordia of the

primary reticular folds. In these bundles a distinct intramuscular NSE-IR nervous network was always encountered.
In the groove itself scattered bundles of smooth muscle fibres were distinguished. All of them were innervated by several, relative thin NSE-IR nerve fibres.

Ruminal Dorsal Sac and Ruminal Ventral Sac

A muscularis mucosae was never seen in the ruminal dorsal sac or the ruminal ventral sac in any of the foetuses studied. In consequence, no NSE-IR nervous elements were detected at this level.

Reticulum

(see Addendum/ Part II/ photo 17)

The muscularis mucosae was present in all primary reticular folds. Hence, a clear and relative dense intramuscular NSE-IR plexus was usually distinguished in the smooth muscle bundles of these protrusions.

Ostium Reticulo-Omasicum

(see Addendum/ Part II/ photos 22-24)

At the level of this orifice is a partially developed muscularis mucosae. Nevertheless, the different isolated groups of smooth muscle cells were always clearly innervated by several NSE-IR nerve fibres.

Omasum

(see Addendum/ Part II/ photos 27-29; 31)

The omasum except for the tertiary and quaternary leaves is the only part of the ruminant stomach complex in which the muscularis mucosae is entirely developed (cfr. microscopic anatomy). In addition, in the centre of each omasal leaf an extension of the inner part of the circular muscle layer protrudes. At the top of the leaves these extensions fuse with the muscularis mucosae forming in this way a triple-layered smooth muscle band. Particularly in the primary and secondary leaves numerous, distinct NSE-IR fibres formed a dense intramuscular NSE-IR nervous network in this band. In addition, in the oldest foetuses the smaller i.e. the tertiary leaves were likewise supplied by NSE-IR nerve fibres.

Abomasum, Antrum Pyloricum and Pylorus

(see Addendum/ part II/ photos 32-33; 35-36; 41-43)

In all foetuses investigated several NSE-IR nerve bundles originating from the underlying Meissner's plexus were observed in the smooth muscle layer. Most nerve bundles ran parallel to the muscle fibres, while other NSE-IR nerves penetrated the muscularis mucosae obliquely and reached the lamina propria (see above). It seems that the muscularis mucosae is innervated in a similar way along the whole glandular stomach. However, the number of NSE-IR nerve fibres seen is most probably related to the development of the target organ (the muscularis mucosae) that is more developed at the level of the pylorus. In addition, at the submucosal side of the muscle layer several larger NSE-IR nerve fibres and ganglia were present.

ADULT SHEEP

Reticular Groove

Isolated groups of smooth muscle bundles, instead of a true muscle layer, were usually seen. In these bundles several intramuscular NSE-IR nerve fibres of varying dimensions were always observed.

Ruminal Dorsal Sac and Ruminal Ventral Sac

Just as in the foetal specimens, the muscularis mucosae is lacking in these compartments.

Reticulum

The muscularis mucosae was only seen in the upper part of the primary reticular folds. The number and size of the intramuscular NSE-IR nerve fibres seemed in proportion to both the thickness and the extent of the muscle layer.

Ostium Reticulo-Omasicum

The islands of smooth muscle bundles were always infiltrated by different and intensely reacting NSE-IR nerve fibres.

Omasum

Compared with the other parts of the forestomach, the muscularis mucosae is entirely developed in this compartment. Consequently, numerous NSE-IR nerve fibres, most of which have the same orientation as the smooth muscle cells themselves, were always found. As might be expected, their number and caliber seemed to be related to the extension and the thickness of the smooth muscle layer.

Abomasum, Antrum Pyloricum and Pylorus

(see Addendum/ Part II/ photo 39)

The muscularis mucosae was present in all parts of the glandular stomach. Hence, numerous NSE-IR nerve bundles originating from the submucosal plexus were seen to penetrate this layer. Most of them run very often for a considerable distance parallel to the smooth muscle cells. Furthermore, at the level of the pylorus the muscularis mucosae is stronger developed and has an irregular appearance. As a consequence, more intramuscular NSE-IR nerve fibres were normally distinguished at this level. However, some submucosal fibres were found to run through this muscle layer and enter the lamina propria and contribute to the formation of the periglandular plexuses. Furthermore, several ganglia, lying beneath the gastric glands, exhibit an intense NSE-IR. Relative large NSE-IR nerve fibres leave these ganglia and split into smaller bundles. The majority of them are involved in the formation of the periglandular plexuses. Other fibres however, ascend in the lamina propria, to contribute to the formation of a delicate NSE-IR nerve plexus and, finally, reach the surface epithelium.

Tunica Submucosa

FOETUSES

Reticular Groove

(see Addendum/ Part II/ photos 2; 4-5)

Although several, single NSE-IR nerve fibres were normally seen, no real submucosal plexus could be distinguished in the reticular groove itself. The different isolated groups of smooth muscle cells, representing the muscularis mucosae in this region were infiltrated by various fine NSE-IR nerve fibres. In addition, several medium to small NSE-IR nerve fibres were seen in the reticular part of the groove. They formed a delicate wide-meshed NSE-IR nervous network that extended in nearly all the submucosal protrusions (reticular folds). In the nodes of this meshwork, ganglia containing some NSE-IR neurons were sporadically found. A clear and compact NSE-IR nervous network was discerned in the future primary reticular folds. This network seemed principally related to the presence of the muscularis mucosae. Occasionally, medium NSE-IR nerve bundles were seen to rest upon the inner side of circular muscle layer.
Furthermore, tubulo-acinar glands were normally encountered in the submucosa of the reticular groove. These glands were always associated with a delicate but distinct periglandular NSE-IR nerve plexus.
Submucosal blood vessels, surrounded by a clear perivascular NSE-IR plexus, were invariable present.

Ruminal Dorsal Sac and Ruminal Ventral Sac

(see Addendum/ Part II/ photos 7-10; 12-14)

Small to medium NSE-IR nerve fibres were encountered in the submucosa. The medium NSE-IR nerves were seen to originate either from Auerbach's plexus or from the thick intramuscular NSE-IR nerve bundles, innervating the circular muscle layer. Small NSE-IR nerves branching off from the medium nerve bundles formed a delicate NSE-IR nervous network at the onset of the primordia of each papilla. Few NSE-IR neurons were detected in the nodes of this plexus. Furthermore, very fine NSE-IR nerve bundles originated from this network and ascend into the future ruminal papillae. In

this manner an extremely delicate intrapapillar NSE-IR nervous network is formed in the centre of the primordium of each papilla. In this intrapapillar NSE-IR nerve plexus ganglia were, just like in paraffin sections, not clearly delineated although a single neuron could be distinguished along the largest fibres. Single NSE-IR nerve fibres branch off from the intrapapillar plexus and run underneath the epithelium. In this way the subepithelial NSE-IR fibres come close to the NSE-IR epithelial cells.
Extensive perivascular NSE-IR plexuses were often seen. On some occasions fine NSE-IR nerve fibres left these plexuses and ascended into the centre of the future ruminal papillae.

Reticulum

(see Addendum/ Part II/ photos 16-20)

Medium to fine NSE-IR nerve fibres, some of which seemed to originate in Auerbach's plexus, were usually seen in the submucosa. A delicate submucosal plexus was always found at the base of the primordia of the reticular cells. In the primary reticular folds, in addition, a dense and distinct NSE-IR nervous network was noticed. From this plexus fine nerve fibres branched off and followed the smaller submucosal folds. Hence, in all reticular folds a clear NSE-IR plexus was distinguished. Moreover, numerous varicosities were observed along the single NSE-IR nerve fibres constituting this plexus. Some of these fibres were seen in close proximity to the NSE-IR epithelial cells. Only in very rare occasions some NSE-IR neurons were encountered.
Submucosal blood vessels, as well as their corresponding perivascular NSE-IR plexus, were normally present. Principally, this plexus was in proportion to the calibre of the vessel.

Ostium Reticulo-Omasicum

(see Addendum/ Part II/ photos 22-25)

A few, slender, isolated submucosal NSE-IR nerve bundles were found at the level of the ORO. However, in the developing claw-like papillae a very dense NSE-IR nerve plexus was seen as well in the primary reticular folds. A delicate NSE-IR nervous network was observed in the other reticular folds.
Several blood vessels, accompanied by a clear perivascular NSE-IR plexus, were also seen.

Omasum

(see Addendum/ Part II/ photos 27-31)

As the OMA forms part of the forestomach complex the microscopic picture largely corresponded to that described for the other forestomach compartments. Thus, some NSE-IR nerve fibres, distinct perivascular NSE-IR plexuses, but usually no ganglia could be distinguished in the omasal submucosa. Commonly in the submucosal protrusions, which form the core of the omasal leaves, NSE-IR nervous elements were found to constitute a delicate nervous network.
Blood vessels were, as a rule, accompanied by a perivascular NSE-IR nervous network.

Abomasum, Antrum Pyloricum and Pylorus

(see Addendum/ Part II/ photos 32-33; 35-37; 41-43)

The surprisingly scarcely innervated tunica submucosa of the forestomach changes drastically at the onset of the glandular stomach. Numerous NSE-IR nerve fibres of a variable diameter (medium and small) were plainly seen and ran throughout the length and width of the submucosa. Moreover, neurons, grouped into small ganglia, were always present. Both small ganglia and numerous NSE-IR nerve fibres, of different calibre, formed a distinct (Meissner's) plexus. In addition, it seemed that in the older foetuses there were more submucosal NSE-IR neurons per section. Most submucosal ganglia were observed just underneath the muscularis mucosae. Furthermore, small NSE-IR nerve bundles leave these ganglia and either penetrate into the muscularis mucosae, where an intramuscular NSE-IR nerve plexus is formed, or pierce it and enter the lamina propria where they apparently participate in the formation of a periglandular plexuses. Occasionally, larger NSE-IR nerve fibres and ganglia, both lying on the inner aspect of the circular smooth muscle layer, were seen. These NSE-IR nervous elements, most probably, belonged to deep part of Meissner's plexus.
At the height of the pylorus the submucosal NSE-IR nervous network become even more compact and relatively numerous irregular ganglia were always observed. Moreover, the different extensions of the circu-

lar muscle layer, contributing to the formation of the torus pyloricus, were characterized by a dense intramuscular NSE-IR nervous network.
In each section several submucosal blood vessels were distinguished and these were always accompanied by a manifest perivascular NSE-IR plexus.

ADULT SHEEP

Reticular Groove

Several widely separated NSE-IR nerve fibres, but no NSE-IR neurons, were detected in the submucosa of this segment.
Particularly around arterioles and larger veins perivascular NSE-IR plexuses were always seen.

Ruminal Dorsal Sac and Ruminal Ventral Sac

(see Addendum/ Part II/ photo 11)

The microscopic anatomy of the submucosa showed no basic differences between both compartments. Thus, no neurons and only a few NSE-IR nerve fibres were observed. Small NSE-IR nerve fibres as well as NSE-IR nerve plexuses, surrounding the submucosal blood vessels, were normally seen. Some submucosal NSE-IR nerve fibres seemed to originate from the perivascular NSE-IR plexus accompanying the submucosal blood vessels. Additionally, on several occasions, and in the preparations of the RVS in particular, NSE-IR nervous elements (fibres, neurons) were found lying upon the submucosal side of the circular muscle layer. Perivascular plexuses were usually observed.

Reticulum

No neurons, but a few delicate NSE-IR nerve fibres were seen throughout the whole submucosa. In the core of each reticular fold a delicate NSE-IR nervous network was observed. No submucosal NSE-IR neurons were ever observed in the reticulum of the adult sheep. All the blood vessels were enveloped in a perivascular NSE-IR plexus.

Ostium Reticulo-Omasicum

More and larger NSE-IR nerve fibres, perivascular NSE-IR plexuses, but no neurons, represented the submucosal nervous elements at this level. Moreover, small isolated smooth muscle bundles innervated by several intramuscular NSE-IR nerve fibres were usually seen. Thus, due to the presence of this intramuscular NSE-IR nervous network, it appears that the innervation of the submucosa is substantially denser than in the rumen in which the muscularis mucosae is absent. Perivascular NSE-IR plexuses also are present in the submucosa.

Omasum

The results seen in sections of this segment basically correspond to the findings observed in the other compartments of the forestomach. In consequence, relative few fine NSE-IR nerve fibres were seen, there were no NSE-IR neurons, but there were perivascular NSE-IR plexuses which ascend together with the blood vessels into the omasal leaves.

Abomasum, Antrum Pyloricum and Pylorus

As already described in the foetuses the innervation of the submucosa of the glandular stomach differs greatly from that of the aglandular forestomach.
Numerous, strong reacting NSE-IR nerve fibres and neurons were seen. Many isolated NSE-IR nerve bundles of varying diameter ran in different directions throughout the submucosa. Neurons, principally grouped into relative small ganglia, were usually seen to lie close to or in contact with the muscularis mucosae and on the inner side of the circular smooth muscle layer. From the submucosal ganglia NSE-IR nerve fibres originated, penetrated into the muscularis mucosae and ran parallel to the smooth muscle fibres wherein they formed an intramuscular NSE-IR nerve plexus. A few NSE-IR fibres, however, reached the lamina propria and in this way contributed to the formation of the periglandular plexuses. Furthermore, at the level of the pylorus the submucosa had several, isolated bundles of smooth muscle cells (submucosal extensions of the circular muscle layer). These bundles were always innervated by a considerable number of small NSE-IR nerve fibres. The largest part of these intramuscular NSE-IR fibres ran parallel to the longi-

tudinal axis of the smooth muscle cells.
Principally in the body of the ABO several thick NSE-IR nerve fibres and neurons were encountered on the innermost surface of the circular muscle layer. These NSE-IR nervous elements probably represented the morphological substratum for the external part of Meissner's plexus. However, this was not a constant finding in all specimens and in all sections studied.
As it might have been expected, the numerous submucosal vessels were always accompanied by a corresponding perivascular NSE-IR nervous network.

Tunica Muscularis

FOETUSES

Reticular Groove

Circular muscle layer

(see Addendum/ Part II/ photos 2-6)

A very dense intramuscular NSE-IR nerve plexus, consisting of large, medium and small NSE-IR nerve fibres, was encountered throughout the thickness of the circular smooth muscle layer. The thick NSE-IR nerve bundles were normally seen in the connective tissue septa separating the different groups of smooth muscle bundles. The medium and small NSE-IR fibres on the other hand ran within the smooth muscle bundles. Varicosities were regularly identified along the course of the smaller NSE-IR nerves. Most NSE-IR nerves ran parallel to the muscle cells, although some of the thicker NSE-IR nerves extended in an oblique or perpendicular direction. The density of the intramuscular NSE-IR nerve plexus in the circular muscle layer was substantially higher than in the longitudinal muscle. Moreover, the inner muscle layer thickened in the lips of the reticular groove. Consequently, there was a proportional increase in the number of intramuscular NSE-IR nerve fibres.

Auerbach's plexus

(see Addendum/ Part II/ photos 2-4)

Several ganglia, NSE-IR neurons, and intra- and interganglionic NSE-IR nerve fibres form the myenteric plexus. In older foetuses the majority of the neurons was localized at the periphery of the ganglia, while the intraganglionic NSE-IR nerve fibres were usually observed in the central part of the ganglia. NSE-IR nerve bundles of a variable diameter originated from Auerbach's plexus (ganglia or interganglionic nerve strands), penetrated into the adjacent musculature and formed a distinct intramuscular NSE-IR nervous network. Occasionally, smaller NSE-IR nerve bundles were seen to perforate the circular smooth muscle layer and to contact the submucosal plexus (see above).

Longitudinal muscle layer

(see Addendum/ Part II/ photos 2-4)

Several medium and small NSE-IR nerve fibres constituted a distinct intramuscular NSE-IR nerve plexus. As a rule the larger NSE-IR nerves course between the smooth muscle bundles while the smaller fibres run within them. As mentioned earlier, the intramuscular NSE-IR innervation of the outer muscle layer was not as dense as the NSE-IR network in the circular muscle layer. However, one has to take into account that the longitudinal muscle layer is much thinner than the circular muscle layer. Moreover, in the lips of the reticular groove the longitudinal layer increases in thickness and as a consequence ganglia and larger interganglionic NSE-IR nerve bundles contributed to the enhanced density of the intramuscular NSE-IR nerve plexus.
Blood vessels, often noticed in the delicate connective tissue layer between both smooth muscle layers, were always enveloped by a perivascular NSE-IR plexus.

Ruminal Dorsal Sac and Ruminal Ventral Sac

Circular muscle layer

(see Addendum/ Part II/ photos 7-8; 10; 12-15)

The inner muscle layer of both segments is characterized by an abundant innervation. Numerous medium and fine NSE-IR nerve fibres were observed within the smooth muscle bundles, while the large nerves were mainly situated in the connective tissue strands separating the bundles. As a rule the NSE-IR nerve bundles, except the largest ones, ran parallel to the longitudinal axis of the muscle cells. Especially in the RDS, individual neurons were regularly encountered along the course of the largest NSE-IR nerve fibres. Varicosities on the other hand were, as a rule, distinguished along the smallest nerve fibres. Furthermore, at the level of the ruminal pillar, a muscular dam separating the reticulum from the RVS, the circular muscle layer became much thicker. A compacter intramuscular NSE-IR nervous network was distinguished at this level. Thus, it seems that in this compartment the density of the intramuscular NSE-IR plexus is likewise related to the thickness of the musculature to be innervated.

Auerbach's plexus

(see Addendum/ Part II/ photos 7; 10; 12-13; 15)

All the NSE-IR nervous elements assembled between the two parts of the tunica muscularis, showed an intense but variable NSE-IR. Ganglia of various dimensions, holding several NSE-IR neurons and nerve fibres as well as thick NSE-IR nerve bundles interconnecting the ganglia (internodal strands), were always seen. These different NSE-IR elements formed a distinct plexus. Large ganglia of variable dimensions were particularly perceived in the RVS. Numerous smaller NSE-IR nerve fibres split off from the internodal strands and innervated, by forming a distinct intramuscular NSE-IR plexus, the adjacent musculature. However, not all NSE-IR nerve fibres, entering the circular muscle layer, participated in the formation of the intramuscular nervous network. Indeed, some of them ran through the musculature and reached the submucosal plexus. Moreover, on some occasions delicate NSE-IR nerve fibres branched off from larger NSE-IR nerves, perforated the inner musculature, and reached the perivascular NSE-IR plexus surrounding the submucosal blood vessels.

Longitudinal muscle layer

(see Addendum/ Part II/ photos 7; 10; 13; 15)

Although there were individual variations, the results seen in the different foetuses under investigation were fairly comparable. Indeed, the longitudinal muscle layer of the rumen is substantially thinner than the longitudinal muscle layer of the preceding compartment (reticulum). As a consequence, only a few small NSE-IR nerves were found in the outer muscle layer. Nevertheless, a clear-cut innervation through medium and small NSE-IR nerve fibres was always noticed. Nearly all intramuscular NSE-IR nerves ran parallel to the longitudinal axis of the smooth muscle cells.
In all foetuses, but particularly in the older ones, distinct perivascular NSE-IR plexuses were observed.

Reticulum

Circular muscle layer

(see Addendum/ Part II/ photos 16-21)

Principally, the same arrangement as in the reticular groove was found. Numerous strong reacting NSE-IR nerve bundles of varying diameter supplied this layer throughout its full thickness. They formed a compact and well-marked intramuscular NSE-IR nervous network. The large NSE-IR nerves of this network were seen between the smaller fibres in the smooth muscle bundles. Moreover, the thickness of the circular musculature and, in consequence the density of the muscular innervation, increased with the increasing age of the foetus. Furthermore, in one foetus (28.5 cm C-R length) NSE-IR neurons grouped into relatively small ganglia were found within the circular muscle layer. Medium-sized NSE-IR nerve bundles connected these ganglia with the myenteric plexus. Analogous to the situation in the reticular groove the NSE-IR nervous network in the inner muscle layer was much denser than in the longitudinal muscle layer.

Auerbach's plexus

(see Addendum/ Part II/ photos 16-19; 21)

In all the foetuses investigated the same picture was seen. In the space between both smooth muscle layers large and/or small aggregations of neurons formed, together with the corresponding numerous NSE-IR nerve fibres, irregular ganglia that were interconnected by large interganglionic NSE-IR nerve strands. The majority of the NSE-IR nerve bundles, originating from the ganglia and/or interganglionic NSE-IR bundles, infiltrated both muscle layers to form an intramuscular NSE -IR nerve plexus. Some immunoreactive fibres ran, however, to the submucosa.

Longitudinal muscle layer

(see Addendum/ Part II/ photos 16-19; 21)

The outer muscle layer of the reticulum is relatively thick compared to the longitudinal muscle layer of the other forestomach compartments. Consequently, its intramuscular NSE-IR nervous network seemed more extended than in the corresponding musculature of the other forestomach compartments. Moreover, it appeared that very often the density of the intramuscular plexus in the outer muscle layer equalled the density of the NSE-IR nervous network seen in the inner muscle layer.
All the blood vessels were accompanied by a perivascular NSE-IR plexus.

Ostium Reticulo-Omasicum

Circular muscle layer

(see Addendum/ Part II/ photos 22-23; 25)

Numerous large, medium and small NSE-IR nerve fibres supplied the circular muscle fibres and were involved in the formation of a dense intramuscular NSE-IR nervous network. The large NSE-IR nerves were usually seen between the muscle bundles and generally ran transversely to the longitudinal axis of the smooth muscle cells.

Auerbach's plexus

(see Addendum/ Part II/ photos 22-23; 25)

All components of the myenteric plexus i.e. NSE-IR neurons and nerve fibres, both grouped into ganglia, and interganglionic NSE-IR nerve bundles were always seen. From the myenteric plexus smaller NSE-IR nerve bundles originated. The majority of these fibres formed an intramuscular NSE-IR nervous network in the adjoining musculature. Some NSE-IR fibres which were usually smaller reached the submucosa and ended in the submucosal or perivascular NSE-IR plexus.

Longitudinal muscle layer

(see Addendum/ Part II/ photos 22-23; 25)

Compared to the reticulum the longitudinal muscle is relatively thin at the level of this ostium. Nevertheless, several medium to small NSE-IR nerve fibres composed a relative dense intramuscular NSE-IR nerve plexus.
Perivascular NSE-IR plexuses were always seen.

Omasum

Circular muscle layer

(see Addendum/ Part II/ photos 27; 30)

Many NSE-IR nerve fibres of various diameters left Auerbach's plexus, infiltrated the inner smooth muscle layer and formed an extensive and compact intramuscular NSE-IR nervous network. The largest NSE-IR nerve fibres of this plexus ran between the smaller ones within the smooth muscle bundles. From the innermost part of the circular muscle layer small smooth muscle bundles split off, ascended in the centre of the omasal leaves and fused at the top of the leaves with the muscularis mucosae from both sides. In these extensions of the circular muscle layer various relative thin NSE-IR nerve fibres were always observed .

Auerbach's plexus

(see Addendum/ Part II/ photos 26-27; 30)

In the omasum of all foetuses studied the myenteric plexus was strikingly well developed. Thus, numerous NSE-IR neurons and nerve fibres, as well as large interganglionic NSE-IR nerve bundles, were observed. The ganglia and/or the interganglionic NSE-IR nerve bundles supplied NSE-IR nerve fibres to the muscle layers (intramuscular plexuses) and to the submucosal plexus.
Taken as a whole, the myenteric plexus is the densest innervated part of the forestomach.

Longitudinal muscle layer

The OMA has an extraordinary thin longitudinal muscle layer. Hence, at a low magnification this smooth muscle layer was often not clearly delineated, although at higher magnification it was always seen. Consequently, a sparse, delicate intramuscular NSE-IR nervous network was formed by thin NSE-IR nerve fibres. Blood vessels, regularly seen at the serosal side of the circular muscle layer, were as a rule accompanied by a clear perivascular NSE-IR plexus.

Abomasum, Antrum Pyloricum and Pylorus

Circular muscle layer

(see Addendum/ Part II/ photos 32; 34-36; 38; 41; 44)

There was no evidence for any basic differences in the innervation of the circular muscle layer of the three gastric segments investigated. Small, medium and large NSE-IR nerve fibres built up a compact intramuscular nervous network. Most NSE-IR nerve fibres of this plexus ran parallel to the longitudinal axis of the smooth muscle cells. In older foetuses the circular muscle layer was thicker and at the level of the pylorus it increased, to form the pyloric sphincter. In both situations the density of the intramuscular NSE-IR plexus was substantially increased. Thus, it may be maintained that the density of the intramuscular network is related to the thickness of the muscle layer which is innervated. Moreover, in the pyloric region various extensions of the circular muscle layer form isolated bundles of smooth muscle cells in the submucosa. Hence, various, densely innervated, smooth muscle bundles were seen in the submucosa. Furthermore, in the older foetuses NSE-IR nervous elements of Meissner's plexus, lying upon the mucosal side of the circular muscle layer, were observed.

Auerbach's plexus

(see Addendum/ Part II/ photos 34-36; 38; 41; 44)

Between both smooth muscle layers large nerve bundles, showing an intense NSE-IR, were found to constitute a dense NSE-IR nervous network. At the nodes of this network ganglia, containing several NSE-IR nerve cell bodies and many NSE-IR nerve fibres, were present. The intensity of the NSE-IR in the different neurons showed considerable variations within the same ganglia, between the ganglia, and between the sections. Starting from the myenteric plexus NSE-IR nerve fibres infiltrated the adjacent muscle layers and built up a well-developed intramuscular NSE-IR nerve plexus.

Longitudinal muscle layer

(see Addendum/ Part II/ photos 34-35; 38; 41; 44)

Despite the fact that in the glandular stomach the longitudinal muscle layer is relatively thin, medium-

sized to fine NSE-IR nerves formed a distinct intramuscular plexus within this external smooth muscle layer. However, in the youngest foetuses the outer muscle layer was so thin that it was often difficult to find NSE-IR nerve fibres.
The blood vessels were surrounded by a perivascular NSE-IR plexus.

ADULT SHEEP

Reticular Groove

Circular muscle layer

Large, medium and small NSE-IR nerve fibres were seen throughout this inner muscle layer. All intramuscular NSE-IR nerves contributed to the formation of a distinct, compact intramuscular NSE-IR nervous network. Simultaneously with the increase in thickness of the inner (longitudinal) muscle layer in the lips of the reticular groove, the density of the intramuscular NSE-IR nerve plexus was increased.

Auerbach's plexus

Surprisingly, few components of a myenteric plexus were seen in this region. Instead there were a few large ganglia and thick interganglionic NSE-IR nerve bundles. Moreover, in some sections the classical picture of Auerbach's plexus was even not found.

Longitudinal muscle layer

Various medium to fine NSE-IR nerve fibres, forming a distinct intramuscular NSE-IR nervous network, were noticed in the longitudinal muscle layer. Most NSE-IR nerves ran in the same direction as the muscle fibres. However, the plexus was not as dense as the NSE-IR plexus encountered in the circular muscle layer.
Blood vessels were, as usually, accompanied by a clear perivascular NSE-IR plexus.

Ruminal Dorsal Sac and Ruminal Ventral Sac

Circular muscle layer

(see Addendum/ Part II/ photo 11)

No essential differences were found in the intramural innervation of the circular muscle layer in both segments. Thus, several, intense reacting NSE-IR nerve fibres built up a dense plexus in the circular muscle layer of both segments. Large NSE-IR nerves (interconnected by smaller NSE-IR nerve fibres), medium and small NSE-IR nerve bundles were always noticed in every section examined. Most NSE-IR nerve fibres, except the largest ones, were seen to run parallel to the longitudinal axis of the circular muscle fibres.

Auerbach's plexus

(see Addendum/ Part II/ photo 11)

In addition to the classical picture, as described for the other ruminant stomach segments, the myenteric plexus of the rumen was characterized by some peculiarities. Indeed, most neurons, showing a variable NSE-IR, were localized in the periphery of the ganglia, while the centre of the ganglia was filled with intraganglionic NSE-IR nerve fibres. In addition dots, characterized by their very intense NSE-IR, were seen around the perikarya of some NSE-IR myenteric neurons. These structures were not seen in the foetal specimens. However, due to the very compact organization of the myenteric ganglia in the foetuses and, in addition, because of the intense NSE-IR of their nervous elements these spots were, most probably, very difficult to distinguish under the experimental conditions of this study.

Longitudinal muscle layer

(see Addendum/ Part II/ photo 11)

Although the ruminal longitudinal muscle layer is relatively thin, a definite intramuscular NSE-IR nerve plexus, showing no striking differences between the RDS and the RVS, was found. This intramuscular NSE-IR nervous network was, however, not as dense as that observed in the inner musculature.
All the blood vessels encountered, were accompanied by a perivascular NSE-IR plexus.

Reticulum

Circular muscle layer

As already seen in the foetuses the circular musculature of this segment was infiltrated by abundant medium and small NSE-IR nerve fibres. These fibres ran throughout the full thickness of the circular muscle layer and became involved in the formation of a dense intramuscular NSE-IR nervous network. Smaller NSE-IR nerves were distinguished within the smooth muscle bundles, while the larger bundles coursed, as a rule, often obliquely between the smooth muscle groups.
Auerbach's plexus

A ganglionated plexus, showing a strong NSE-IR, was always found. From the ganglia, characterized by the peripheral arrangement of the NSE-IR neurons, and/or from the thick interganglionic NSE-IR nerve bundles smaller bundles split off, pierce into both muscle layers and constituted an intramuscular NSE-IR nerve plexus. Some fibres, however, perforated the inner musculature and invade the submucosa.

Longitudinal muscle layer

The relatively thick longitudinal muscle layer of the reticulum was supplied by numerous small intramuscular NSE-IR nerve fibres. Hence, a well-marked and compact innervation of this smooth muscle layer was always found.
Perivascular NSE-IR plexuses were normally seen.

Ostium Reticulo-Omasicum

Circular muscle layer

The results, seen in this segment, correspond basically to the findings described for the reticular groove. As the circular muscle layer forms a sphincter-like structure at the level of this orifice, numerous and intensely reacting large, medium and thin NSE-IR nerve fibres constitute a compact intramuscular NSE-IR nerve plexus. Thus, the intramuscular NSE-IR innervation of the inner musculature of this ostium seems more pronounced than in the adjacent compartments (reticulum, omasum). The NSE-IR nerve fibres of this plexus followed, as a rule, the rather irregular course of the muscle fibres.

Auerbach's plexus

All components of the myenteric plexus i. e. ganglia, NSE-IR neurons and their nerve fibres, showed a strong immunoreactivity. Only in a few sections could elements of Auerbach's plexus not be observed.

Longitudinal muscle layer

A distinct and dense intramuscular innervation which seemed more pronounced than in the corresponding paraffin section, characterized the longitudinal muscle layer of this segment.
All blood vessels displayed a clear-cut NSE-IR plexus.

Omasum

The laminae, the innervation pattern of the circular muscle layer, and the myenteric plexus give the OMA a typical morphological appearance.

Circular muscle layer

Contrary to the longitudinal muscle layer, the circular muscle of this compartment is relatively thick. Hence, a dense intramuscular NSE-IR innervation was always seen.
Moreover, from the innermost part of the circular musculature small muscle bundles split off and ascended the centre of the omasal leaves. As these muscle bundles originated from the densely innervated circular muscle layer numerous flimsy NSE-IR nerve fibres were always seen in these muscular protrusions.

Auerbach's plexus

The OMA is characterized by a well-developed and compact myenteric plexus. In the large, irregular ganglia the NSE-IR neurons, some of which were surrounded by intense reacting NSE-IR spots as well as numerous NSE-IR nerve fibres, were always observed. Large, interganglionic NSE-IR bundles united the ganglia and formed, together with them, the myenteric plexus. Many smaller NSE-IR nerve fibres branched off from the ganglia or the internodal strands and, usually, penetrated the adjacent circular muscle layer to form an extensive intramuscular NSE-IR nerve plexus. Most of the NSE-IR fibres were not involved in the formation of the intramuscular plexus, few of them reaching the submucosa and fusing with the submu-

cosal plexus. As in the foetal sheep, the OMA has the densest myenteric plexus of all the forestomach compartments.

Longitudinal muscle layer

Even in the adult sheep the outer muscle layer is extremely thin in this compartment. Consequently, only a few NSE-IR nerve fibres were seen in it.
Distinct perivascular NSE-IR plexuses accompanied the blood vessels.

Abomasum, Antrum Pyloricum and Pylorus

In this study no evidence was found for differences in the innervation pattern of the tunica muscularis between the different parts of the glandular stomach.

Circular muscle layer

(see Addendum/ Part II/ photo 40)

As in the forestomach the circular muscle layer of the stomach is innervated by a dense and extensive intramuscular NSE-IR nervous network consisting of large, medium and thin nerve fibres. The larger NSE-IR nerves course in principal obliquely to the longitudinal axis of the muscle fibres, while other NSE-IR nerves ran parallel to the muscle cells. In the pyloric region, the circular muscle increased in thickness resulting in more inter- and intramuscular NSE-IR nerve bundles. Additionally, several extensions of this smooth muscle layer extended into the submucosa at the level of the pylorus and were supplied by different NSE-IR nerve fibres of a varying dimensions.

Auerbach's plexus

(see Addendum/ Part II/ photo 40)

Ganglia, NSE-IR neurons and intra/interganglionic nerve bundles of different calibre formed a well-developed and strong-reacting myenteric plexus. Many NSE-IR neurons were surrounded by spots demonstrating a very intense NSE-IR.

Longitudinal muscle layer

(see Addendum/ Part II/ photo 40)

Several medium to small NSE-IR nerve bundles, constituting a clear intramuscular NSE-IR nerve plexus, were noticed in the relative thin longitudinal muscle layer. Most intramuscular fibres ran parallel to the muscle cells.
In all segments distinct perivascular NSE-IR plexuses were always observed around arteries and larger veins, mainly between the smooth muscle layers .

Tunica Serosa

(see Addendum/ Part II/ photos 12-13; 16; 22; 26-27)

Neither in the foetuses nor in the adult sheep was the plexus serosus always distinguished in every section from the different parts of the ruminant stomach.
Blood vessels, accompanied by a well-marked perivascular NSE-IR plexus and, large NSE-IR nerve fibres (some of which came into contact with Auerbach's ganglia), were usually distinguished. Subserosal ganglia, in contrast, were only sporadically seen and even then almost exclusively in the OMA. Despite the presence of some NSE-IR nervous elements in the tunica serosa, morphological evidence for the existence of a true plexus serosus was not obtained from this study.

II. 2. 5. CONCLUSIONS, COMMENTS and SUMMARY

Conclusions, Comments

From this immunohistochemical study the following conclusions may be drawn.

Although no quantitative data were produced no evidence emerge from this study to maintain that a NSE-antiserum stained more intramural nervous elements than the classical silver impregnation techniques. However, most silver staining methods are time-consuming, capricious and often unreliable. The NSE immunohistochemistry, on the contrary, is fast, easy to perform and is repeatable. Thus, NSE antibodies may be considered to be a very powerful tool in the study of the distribution of the neuro-endocrine system as well as in the arrangements of the intramural innervation.

The use of foetal material is very useful since it gives a clear insight into the basic principles of the intramural innervation of the ruminant stomach of the sheep.

Compared with paraffin sections, cryostat sections give a better and more clear-cut overall picture of the intramural innervation of the ruminant stomach both in foetuses and adult sheep. Cryostat sections therefore, were mainly used to describe the results of this study. Although whole mount preparations have the advantage in giving a clear overall picture, this method was not used in this study. Stripped specimens, especially those from adult sheep, are relatively thick, more antiserum at a lower dilution has to be used and NSE antiserum is rather expensive. Moreover, whole mount preparations were not suitable to gain a clear insight into the fine texture of the intramural innervation throughout the intestinal wall.

NSE-IR epithelial cells were normally observed in the different compartments of the foetal stomach. They were, on the contrary, never detected in the epithelium of the forestomach of the adult sheep. Moreover, in the adult sheep no neuronal structures, which may be regarded as sensory receptors, were found in the forestomach wall. As it was claimed that NSE antibodies react with all elements of the neuro-endocrine system, it is most probably that these NSE-IR epithelial cells in the foetal stomach represent entero-endocrine cells the functioning of which is unclear. However, in the foetuses delicate NSE-IR nerve plexuses were always observed in the submucosal protrusions. Single NSE-IR nerve fibres split off from these plexuses and came to lie very close to the basal processes of the NSE-IR epithelial cells. This relationship may represent the morphological basis of the sensory system of the ruminant stomach wall.

Elements of the classical intramural nervous system i.e. nerve cell bodies and nerve fibres were observed in all segments of the ruminant stomach in both foetuses and adult animals. However, between the different segments, between the different layers of the ruminant stomach wall and, between foetuses and adults considerable variations in the presence and distribution of these nervous elements were observed.

In both foetuses and adults the intramural NSE-IR nervous network in the true stomach and in the tunica muscularis of the forestomach was similar to that described for the intramural nervous system of the gut in part I.

The morphology of the submucosal and mucosal plexus of the forestomach, as pointed out in this study, however, did not fit in with the classical concept. This is particularly the case in the adult sheep where, despite the numerous sections studied, only few NSE-IR nerve fibres and no ganglionated plexus could be demonstrated in the submucosa. In the foetuses, in contrast, several submucosal NSE-IR nerve fibres formed a delicate but distinct NSE-IR nervous network in the core of the main submucosal protrusions. In the lower submucosal folds small NSE-IR nerve bundles, which branch repeatedly, were noticed. Interestingly, only in a few were NSE-IR neurons, observed in the nodes of the submucosal plexuses. Thus, in the foetuses submucosal NSE-IR fibres were more abundant as compared to the adult sheep, especially in the reticulo-rumen. Based on the embryology of the forestomach (see Introduction) it could be said that the tremendous growth of the forestomach, during the neonatal life, rather than an actual decrease of neural elements is responsible for the apparent lack of a "classical" submucosal plexus in the forestomach of the adult. Apart from the intramuscular NSE-IR nerve fibres in the muscularis mucosae, a mucosal plexus was never observed in the forestomach either in the foetus or in the adult sheep. Interestingly, the aberrant morphology of the mucosal and submucosal plexus in the forestomach of the sheep referred to the findings of the submucosal plexus of the esophagus as described in part I. Moreover, the findings of this NSE study supported the hypothesis, formulated in Part I Chapter 1, that the forestomach is most probably developed from a part of

the primitive foregut just in front of the gastric primordium and not from the gastric primordium itself. Indeed, the mucosa and submucosa of the true stomach in the sheep were, despite the remarkable growth of this segment during the foetal and neonatal life, always characterized by a well-developed and dense plexus.

In the foetus, as well as in adult sheep, notable differences in the density of the intramural innervation of the different parts of the forestomach were found in the following sequence: omasum > reticulum > rumen. In contrast, no basic differences were observed in the different parts of the same functional unit (cfr. RDS and RVS forming the rumen). Thus, a correlation between the function(s) of a segment and the density of its intramural innervation might be hypothesized.

Sphincters (ORO and PYL) and sphincter-like structures (OG) are characterized by the fact that the submucosa holds more NSE-IR nervous elements and there is a denser intramuscular innervation.
In the papillae unguiculiformes at the omasal side of the ostium reticulo-omasicum in the foetus, particular dense intrapapillar NSE-IR nervous network was observed. Since it is thought that these papillae play a critical role in the passage of the ingesta from the reticulo-rumen complex to the omasum/abomasum, it is tempting to correlate this morphological feature with a "scanning" role of the claw-like papillae.

In adult sheep a peripheral arrangement of the NSE-IR perikarya was noticed in most myenteric ganglia. Although the precise functional significance of such an arrangement remains to be elucidated, it has been postulated that it may be related to the receptor role of the myenteric plexus (432) since in the adult specimens no sensory receptors have been demonstrated in the forestomach wall.

Taken as a whole, it may said that the intramural innervation of the glandular part of the ruminant stomach in foetuses and in adult sheep fits in with the classical concept of the intramural innervation of the gastrointestinal tract (see Part I/ Chap. 3). This is, however, obviously not the case in the aglandular part. Indeed, under the experimental conditions of this study, only an extremely delicate submucosal plexus was observed in the foetal specimens. Moreover, in the adult sheep only a few submucosal NSE-IR nerve fibres but no real submucosal plexus was seen. Nevertheless, based on the results collected from the foetuses, a submucosal plexus appears to be present in the forestomach of the adult sheep but that, due to the substantial growth of the forestomach complex after birth, it is almost impossible to find all the classical elements of the submucosal plexus in consecutive sections.
Finally, the findings resulting from this immunohistochemical study may provide a useful morphological basis for further functional, pharmacological and pathological research concerning the role of the intramural nervous system in the regulation of the different ruminant stomach functions.

Summary

In this study an antiserum against NSE was used in order to elucidate the presence and distribution of the NSE-IR nervous and/or endocrine elements in the wall of the different subdivisions of the ruminant stomach of both foetal and adult sheep.
The results of this study are schematically summarized in table 5 (see Addendum/ Part II/ pags. 51-52).
From this table the following is clear:

No (ORO), few (RG) or various NSE-IR *epithelial* cells were exclusively observed in the stratum basale of the **foetal** forestomach and principally in the basal part of the gastric glands of the glandular stomach. In the glandular part of the ruminant stomach, and in the AP in particular, the number of NSE-IR epithelial cells were increased compared with that in the forestomach complex. NSE-IR epithelial cells have an endocrine-like appearance i. e. a triangular or pear-shaped form and one or two slender basal processes.
Regardless of the technique used, no NSE-IR could be observed in the epithelium either in the forestomach or in the glandular stomach of the **adult sheep**.

In the **foetal** ruminant stomach NSE-IR nervous elements could hardly be distinguished in the *lamina propria* of the forestomach. In the "true" stomach, however, various NSE-IR nerve fibres were always encountered. Most of these fibres were seen around the bases of the gastric glands and were involved in the formation of periglandular and interglandular plexuses. Consistently single NSE-IR nerve fibres ascended the lamina propria and reached the surface epithelium although intraepithelial NSE-IR nervous elements were never identified. These ascending fibres formed a very delicate NSE-IR nervous network within the lamina propria. In addition several small ganglia, lying between the bases of the gastric glands and the muscularis mucosae were frequently observed.
In he **adult sheep** few, thin NSE-IR nerve bundles were

noticed in the lamina propria of the forestomach compartments. Furthermore, in those segments where the muscularis mucosae is absent (RDS & RVS), the NSE-IR nerve fibres seemed preferentially localized in the border zone between the lamina propria and the submucosa. The results found in the glandular part of the ruminant stomach strongly resembled the picture described in the foetuses. However, in the adult specimens the periglandular plexuses seemed comparably, more elaborated.

In the forestomach of both **foetuses** and **adult sheep** the *muscularis mucosae* is not always present. In the rumen (RDS and RVS) this slender muscle layer was never found, while in the OG, RET and ORO it is represented by isolated groups of smooth muscle bundles. The OMA is the only exception to this general rule since, even in the youngest foetuses, this muscle layer was, at least in the primary and secondary laminae, always seen. Nevertheless, wherever the muscularis mucosae is present, either completely or incompletely, different intramuscular NSE-IR nerve fibres were always found.

In the glandular stomach of the **foetal sheep** the muscularis mucosae is fully developed and, as a consequence, various NSE-IR nerve fibres were usually distinguished within this layer. In addition, several ganglia (mucosal ganglia), lying on the inner aspect of the muscularis mucosae, were seen. NSE-IR nerve fibres, originating from these ganglia, branched profusely and contributed to the formation of plexuses around and between the gastric glands and the plexus within the lamina propria.

In the **adult sheep** principally the same was seen. But, since the muscularis mucosae is strikingly more developed than in the foetus, more and thicker intramuscular NSE-IR nerve fibres were usually observed.

Thus, in the foetus as well as in adult sheep, the muscularis mucosae is, with the lamina propria of the glandular stomach, the densest innervated mucosal layer of the ruminant stomach.

Several *submucosal* NSE-IR nervous elements, forming a delicate submucosal plexus were seen in all compartments of the **foetal** forestomach (except the OMA). NSE-IR nerve fibres were sparse and, as a rule, concentrated in the different submucosal protrusions. Fine NSE-IR nerve fibres, branched off from the submucosal plexus, ascended the different submucosal protrusions and formed in the core of these protrusions a subtle NSE-IR nervous network. Moreover, in those submucosal folds where the muscularis mucosae is present, the submucosal NSE-IR nerve plexus seemed more developed. In the rest of the submucosa NSE-IR nerves were rather occasionally noticed. NSE-IR neurons were very rarely found. These microscopic findings contrasted with the classical description of the submucosal plexus that was given in Part I. Thus, convincing morphological arguments for the presence of a "true" submucosal plexus was not obtained from this study. Blood vessels, in contrast, were supplied by a distinct perivascular NSE-IR plexus.

The situation changes abruptly in the glandular stomach. Indeed, throughout the whole submucosa diverse NSE-IR nerve bundles were always discovered. Several small ganglia, the majority of which was localized just underneath the muscularis mucosae, were encountered. NSE-IR nerve fibres, originating from these ganglia, were regularly seen to penetrate the muscularis mucosae and to contribute to the formation of an intramuscular NSE-IR nerve plexus. Other nerves pierced the muscularis mucosae and became involved in the formation of plexuses (periglandular, interglandular) in the lamina propria.

The results obtained from the study of the forestomach of the **adult sheep** were in essence comparable to those described for the corresponding segments of the foetus. Thus, it may be claimed that in the adult sheep the submucosa of the forestomach holds almost no NSE-IR neurons and only a few NSE-IR nerve fibres, although in the ORO (transitional segment) more and somewhat larger NSE-IR nerve fibres were normally seen. In the glandular part of the ruminant stomach of the adult sheep all elements of the classical submucosal plexus i.e. ganglia, NSE-IR neurons and nerve fibres were found.

In the **foetal** ruminant stomach the intramuscular NSE-IR nervous elements followed principally the same distribution pattern.

In all the specimens investigated an almost uniform pattern of innervation of the circular muscle layer was found. A very dense intramuscular NSE-IR nerve network occurred throughout the entire length and width of the inner musculature. This NSE-IR plexus consisted of large, medium and small NSE-IR nerve fibres most of which ran parallel to the smooth muscle cells. Moreover, the density of the intramuscular NSE-IR nervous network seemed clearly related to functional demands since in those segments where the circular muscle layer increased in thickness (sphincters: ORO and PYL and sphincter-like regions: OG), a proportional increase in the density of the intramuscular innervation was ascertained.

In the narrow space between the circular and longitudi-

nal muscle layer, a broad band of intense reacting NSE-IR nervous elements, containing all elements of the "classical" myenteric plexus, was always encountered in every segment investigated. Consequently, NSE-IR neurons, demonstrating a variable NSE-IR intensity and grouped into ganglia of variable dimensions, were distinguished. In addition, numerous inter- and intraganglionic NSE-IR nerve fibres, large NSE-IR nerve bundles interconnecting the adjacent ganglia, as well as smaller NSE-IR nerves that originate from the ganglia and/or interganglionic NSE-IR nerve bundles were likewise seen. From the myenteric plexus many NSE-IR nerve fibres branched off, entered the adjacent smooth muscle layers and become involved in the formation of a typical intramuscular NSE-IR nervous network. Sometimes, NSE-IR nerves were found to pierce the inner musculature and to contact the submucosal plexus. Furthermore, the RET and the OMA were, unlike the rumen, characterized by a very compact Auerbach's plexus. In the true stomach on the contrary, such differences between the different parts were not seen.

The longitudinal muscle layer, which is always substantially thinner than the circular musculature, was nevertheless characterized by a clear intramuscular NSE-IR nervous network consisting of several medium and small NSE-IR nerve fibres. These run parallel to the longitudinal axis of the smooth muscle cells. In young foetuses, however, the longitudinal muscle layer was often so thin that, in consequence, the intramuscular NSE-IR nerve fibres were difficult to find. Thus, just as for the circular muscle, there appears to be a positive correlation between the thickness of the longitudinal muscle layer and the density of its intramuscular NSE-IR nerve plexus (cfr. RET).

Arterioles and larger veins, lying either between or within the smooth muscle layers, were always enveloped by a perivascular NSE-IR plexus.

The intramuscular innervation of the circular muscle of the ruminant stomach of the **adult sheep** is very similar to the innervation of the foetal specimens. Additionally, as claimed for the foetuses, a relationship seems to exist between the thickness of the inner muscle layer and the density of its innervation.

In the adult sheep the myenteric plexus was represented by all classical components (ganglia, neurons and nerve fibres). However, most NSE-IR nerve cell bodies were detected in the periphery of the ganglia, while the intraganglionic NSE-IR nerve bundles were mainly concentrated in the centre of the ganglia. Furthermore, around several myenteric NSE-IR neurons spots, exhibiting a very intense NSE-IR, were perceived.

Finally, the longitudinal muscle layer in the ruminant stomach of the adult sheep was characterized by a well-marked intramuscular NSE-IR nervous network. Here too, the density of the network was in proportion to the thickness of the muscle.

Notwithstanding the fact that various NSE-IR nervous elements were seen in the tunica serosa no evidence for the existence of a "real" subserosal plexus in the ruminant stomach of the sheep was obtained from this study.

PART III

NEUROTRANSMITTERS/NEUROMODULATORS INVOLVED IN THE MOTOR AND SECRETORY FUNCTIONS OF THE RUMINANT STOMACH OF THE SHEEP:

a histochemical, radioimmunological, immunohistochemical and functional approach

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III.1. INTRODUCTION

The gastrointestinal tract of all mammals is richly innervated by a remarkable complex and interconnected neuronal network, which is heterogeneous in morphology, histochemistry and function (605). The most important component in the neural control of the gut is the enteric nervous system (ENS). This integrative system, made up of extrinsic and intrinsic components, programmes and coordinates the varied functions of the gut (339).

The extrinsic component consists of two divisions i.e. the sympathetic and the parasympathetic systems, each made up of preganglionic and postganglionic neurons (34; 616; see Part I).

The major parasympathetic input of the gut comes from the vagal nerves, composed of sensory as well as motor fibres (339).

Cholinergic nerves are involved in the excitatory component of the peristaltic reflex and the excitatory vagal and pelvic pathways to the intestine (stomach and large intestine) (246). Hence, acetylcholine (ACh) certainly serves as the major excitatory transmitter to both muscle layers. Additionally, it also mediates the synaptic transmission ("fast" excitatory postsynaptic potential) between neurons within the myenteric and submucosal plexuses. This transmission was found to be essential for the normal peristaltic activity (567).

Ruminal motility can easily be examined and it is classically used as an index for digestive function in the ruminant (81). Analogous to the above-mentioned findings in other species, it may be claimed that ACh is most probably involved in the regulation of the ruminant stomach motility. As it is argued that the myenteric plexus is primarily concerned with motility, whereas the submucosal plexus is thought to control absorptive and secretory functions (325), the presence and distribution of ACh in Auerbach's plexus was studied along the different segments of the ruminant stomach.

However, several investigators have drawn attention to the fact that the vagal nerves contain distinct populations of efferent axons. These axons make connection with intramural cholinergic excitatory neurons and/or with non-adrenergic, non-cholinergic (NANC) inhibitory neurons. Indeed, postganglionic catecholamine-containing fibres, originating from some sympathetic ganglia (i.e. the superior cervical and the stellate ganglia), are found to join in the vagal nerve (700). Moreover, besides cholinergic and adrenergic axons, NANC axons also have been demonstrated in the vagus (616; see Part I). As a consequence, the response to vagal stimulation consists very often of a mixture of both excitation and inhibition induced by different neurotransmittres/modulators (642).

The splanchnic nerves (greater, lesser and smallest) represent the main sympathetic pathways to the gut. They terminate in the large abdominal autonomic plexuses in which relatively large ganglionic masses (prevertebral ganglia) are embedded. Thus, the main sympathetic supply to the gut (stomach and small intestine) arises from the celiac plexus surrounding the celiac and cranial mesenteric artery. From these plexuses subsidiary plexuses, found in the vicinity of the homonymous branches of the abdominal aorta, are given off. Hence, the postganglionic sympathetic fibres reach their target organ along these branches (see Part I). Histofluorescence and autoradiographic investigations have shown a concentration of noradren (NA)-ergic nerve terminals near the margins of the myenteric ganglia (thus the basal lamina forms the only barrier between the terminals and the connective tissue space) and a random distribution in the submucosal plexus (474; see 474). Varicose NA-ergic nerve terminals occur around nerve cell bodies, but axo-somatic synapses were found in neither plexus. However, in the myenteric plexus close contacts between NA axons and other axons have been observed. Thus, NA-ergic axons most likely act on cholinergic axons arising from myenteric neurons (see 110; 474; see 474; see Part I). Occasionally, individual varicose NA-ergic axons have been seen in the internodal strands and to pass through several ganglia. Hence, NA released from these varicosities can affect many nerve cell bodies in several ganglia. Furthermore, a few postganglionic NA fibres directly impinge on the intestinal smooth muscle. This has led to the idea that postganglionic cholinergic neurons, rather than smooth muscles, are the principal targets of the sympathetic nerves. These morphological data are consistent with the physiological role of NA-ergic nerves in the enteric plexuses. Indeed, postganglionic sympathetic fibres inhibit the ganglionic transmission by blocking the presynaptic release of ACh from the myenteric neurons. In this way NA-ergic axons may have a general suppressive action on the excitatory transmission in the myenteric ganglia (see 474). Additionally, there is good evidence that NA inhibits intestinal motility, at least in part, by inhibiting the release of substance P (Sub. P) from the intramural

nervous elements (173). The sparse intramuscular NA-fibres were shown to inhibit or excite smooth muscle activity through α- or β- adrenoreceptors. Relaxation of the gut in response to sympathetic stimulation has thus principally been conceived as resulting from the removal of a cholinergic excitatory tone by inhibition of ganglionic transmission (see 474; see Part I). Furthermore, NA-ergic nerves has been shown to inhibit the propulsion of material along the digestive tract by acting on intrinsic cholinergic nerves and by constricting the sphincters (246; see 246). Thus, adrenergic nerves are supposed to be largely concerned with modulating reflex activity (intestino-intestinal reflex, enterogastric reflex and reflex inhibition of gastric motility elicited from the gastric antrum) in the gastrointestinal tract mainly at the neuronal level and partially at the level of the smooth muscles (see 108; see 110). Based upon the foregoing morphological and functional data it was, in consequence, obvious to study the presence and the distribution of NA in the ruminant stomach and to examine the effect of NA on the gastric musculature.

Auerbach's and Meissner's plexus as well as their interconnecting fibres constitute the intrinsic component of the ENS. Because of the position of these nervous elements in the gut wall they, the myenteric plexus in particular, must be greatly affected by the mechanical activities of the musculature (256; see Part I).

Structurally the enteric ganglia are characterized by a very compact organization, by the absence of collagen, by supporting cells which resemble glial elements of the central nervous system, by axons which are not individually enveloped in mesaxons and, finally, by specialized blood vessels which form a blood-myenteric barrier analogous to the blood-brain barrier (see Part I).
The most striking feature of the ENS is, certainly, its remarkable **abundance of neurotransmitters** (putative and established) in the cell bodies and neuritic processes. Indeed, no other region of the ANS has yet been found to have such an abundance of neuroactive substances as the ENS (see 195). Several observations (morphological, electrophysiological and pharmacological) indicate the existence of other types of nerves (non-cholinergic, non-adrenergic) distinguishable from the classical adrenergic and cholinergic nerves (325; see 723; see Part I). The first hints for the existence of autonomic NANC nerves in the gut go as far as the end of the last century, when atropine-resistant contraction of the urinary bladder and relaxation of the stomach after pelvic and vagal nerve stimulation respectively were demonstrated (cfr. Langley, 1898; Bayliss and Starling, 1899). At that time these responses were usually thought to be due to the activation of sympathetic nerves running within the vagal trunks (55; see 110). However, the relaxation of the stomach was shown to be unaffected by adrenergic neuron blocking agents and to persist after sympathectomy. Moreover, a similar relaxation was demonstrated in the developing fetal rabbit intestine before adrenergic nerves appeared (see 110). In 1931, the presence of an identical peptide in the brain and the gut was observed. This discovery was the starting point for the finding of a whole series of peptides common to the brain and gut ("brain-gut" axis) and for a new concept on the fundamental importance of peptides ("neuropeptides") i.e. vasoactive intestinal polypeptide (VIP), neurotensin, bombesin, gastrin/cholecystokinin, enkephalins, somatostatin and substance P in nerves of the central and peripheral system and in endocrine cells as well. In addition to their original localization in the "brain-gut" axis, some of these peptides may be found in significant quantities in many other areas (urogenital tract, lung, salivary glands, pancreas, pituitary). Thus, the existence of a peptidergic component as part of the autonomic nervous system was established. In 1970 Baumgarten and his co-workers described a new type of nerve terminal in the gut. Ultrastructurally these nerves were characterized by their large electron-dense vesicles. In view of the similarity with peptide-containing fibres in the pituitary gland, this type of nerves was termed as "peptidergic or p-nerves". Later on, peptides have been observed in a variety of peripheral neurons: (1) primary sensory neurons, (2) preganglionic sympathetic and parasympathetic neurons (spinal cord and lower brain stem), (3) neurons intrinsic to the gastrointestinal wall and (4) sympathetic and parasympathetic ganglia (see 195; 615). Many, although not all, gut peptides are present in the intramural neurons of the myenteric and submucosal plexus (see 55; 615) and the topography of these neurons suggests that neuropeptides could influence smooth muscle cells directly or via neuro-neuronal interactions (55). Moreover, new techniques make it clear that the gut peptides are also present in the endocrine elements, emphasizing the unity of these two apparently separate systems (615; see Part I). These peptide-containing endocrine cells may secrete their content into the blood stream, whereby the peptides act as true hormones, or the peptides may act by means of

their secretion into and transport via the extracellular tissue fluid (paracrine secretion).
According to their different distribution, stimulation threshold and sensitivity to drugs, it is likely that excitatory and inhibitory NANC nerves represent distinct nerve populations in the gut. In mammals a NANC inhibitory nervous control of the stomach, via preganglionic vagal cholinergic nerves, and of the intestine, via cholinergic interneurons,was demonstrated. NANC excitatory nerves are a prominent feature in both the small and large intestines of amphibians, reptiles, and birds. Correspondingly, stimulation of the NANC nerves evoked in the intestinal muscle cell membrane hyperpolarization, depolarization or both. Moreover, quantitative differences in the distribution of NANC excitatory and inhibitory neuronal inputs have been found between different regions and between the different smooth muscle layers of the gut (55; see 55).
VIP, Sub. P (and enkephalins) have been shown to be the most important group of regulatory peptides, with powerful actions on most intestinal functions, in the gastrointestinal tract of all species studied so far. These peptides display a characteristic distribution pattern and architecture, providing anatomical support for a separate and well defined set of actions (605; see Addendum). For example, the well known actions of Sub. P on the gut are contraction of the musculature and stimulation of the salivary, intestinal and pancreatic secretion. Gastric acid secretion, in contrast, is inhibited by Sub. P (see Addendum/ Part III/ Review literature Sub. P).
Additionally, in different species strong evidence for the existence of a 5-HT-ergic system within the gastrointestinal wall has been collected and the effects of 5-HT on some important intestinal functions (motility, secretion/absorption and local blood flow) has been established (see Addendum/ Part III/ Review literature 5-HT).
In summary, the existence of NANC-peripheral neurons utilizing serotonin or polypeptide as their transmitter, the discovery of powerful biologically active peptides in the autonomic innervation of the gut, the discovery of a coexistence in and a co-release of different neurochemical substances by the same neuron together with the realization that a number of peptides may be acting either as classic circulating hormones or as local/paracrine hormones or as neurotransmitters (i.e. a direct effect on the target cells) or as neuromodulators (i.e. a modification of the effect of other transmitters on the target cells) profoundly altered the current trend of thoughts concerning the architecture and functioning of the ENS (see 55; see 110; 202; 615; see Part I).
Consequently, it may be of interest to study the presence, distribution and functional impact of some of these recently established ENS neurotransmitters (5-HT; VIP; Sub. P) in/on the ruminant stomach.

Functionally, the ENS is unique because it is the only part of the peripheral nervous system that can show reflex activity in vitro. This intrinsic reflex activity implies the presence of a complete reflex arch i.e. primary afferent neurons, interneurons and motor neurons within the gastrointestinal wall (282). Apparently, by this system sensory information from different types of visceral receptors is processed (Meissner's and Auerbach's plexus) resulting in an appropriate control of the effector system. These properties are normally associated only with the central nervous system (339). The physiological independence of the ENS from the central nervous system control is further emphasized by the considerable discrepancy between the number of efferent fibres and the number of intramural nerve cell bodies (638; see Part I). Consequently, only a small portion of the enteric ganglion cells makes a direct contact with central (vagal) nerve fibres and hence many enteric neurons do not receive any direct input from the CNS. Thus, there is only a small morphological basis for a direct control of the CNS (393; 638). As a consequence, it has been suggested that the autonomic input from the CNS to the gut serves only to modulate the activity of the ENS, which contains in its ganglia the "programmes" for regulation of the normal gut activity. However, the degree of the CNS control varies from region to region: esophagus, stomach and large intestine are more dependent on the CNS input for the normal function than the small intestine (339).
As discussed earlier, there are morphological and functional arguments to claim that the ruminant stomach represents, undoubtedly, the most important and critical part of the ruminant gastrointestinal tract. Consequently, this complex is frequently involved in a broad spectrum of pathologies including carbohydrate engorgement, acute and chronic ruminal tympany, vagal indigestion, parakeratosis, abomasal displacement, primary acetonaemie etc., resulting in substantial economical losses (low conversion of food, retardation of growth, premature slaughtering, long lasting expensive medication).
For its normal functioning the ruminant stomach has to perform far more complex motility patterns than

simple peristaltic reflexes alone. Indeed, several events are accomplished in complex but coordinated cycles of forestomach motility patterns cfr. mixing and retaining of the ingesta, regurgitation, eructation of gas and, finally, controlled and orderly transport of the ingesta within the forestomach complex (36). Furthermore, the different parts are closely associated anatomically and functionally. As a consequence, damage to one or other part causes interference with the normal stereotyped movements of the other compartments and of the ruminant stomach as a whole, resulting in the classical spectrum of pathologies (251). Thus, the various motility patterns of the ruminant stomach have to be appropriately controlled and coordinated with propulsive activity and, most certainly, this is one of the principal functions of the ENS (275).
Considering the fact that a structurally and functionally intact ENS is an absolute prerequisite for the normal digestive functions, and in consequence for the health, of the ruminants; that an impaired ruminant stomach function very often results from a disturbed motility, which is in turn normally the direct or indirect expression of an ENS dysfunction and that, finally, few basic data concerning the morphology and functions of the ENS in the ruminant stomach are available in the literature a multidisciplinary study (morphological, immunological and functional) was undertaken in order to elucidated the neurochemical nature of the ENS in the ruminant stomach and to point out the functional significance of the above-mentioned neurotransmitters on the ruminant stomach musculature.

III. 2. OBJECTIVES

The objectives of the *morphological part* of this study were as follows:

Firstly, to map out, largely on the basis of the morphological picture of the intramural nervous system of the stomach obtained from the study in Part II, the occurrence and typical distribution of the classical and recently discovered NANC neurotransmitters/neuromodulators in the ruminant stomach wall.

Secondly, to provide basic morphologic and quantitative data for further physiological and pharmacological research concerning the fundamental mechanisms controlling and coordinating the different motility patterns, the secretion/absorption process and the regional blood flow in the ruminant stomach.

Thirdly, to increase our understanding of the normal functioning of the ruminant stomach and the pathogenetic significance of abnormalities in the intramural innervation for a disturbed motility and the secretion/absorption process.

Fourthly, to provide further morphological elements for an appropriate therapy.

The aims of the *functional part* of this study were as follows:

Firstly, to show, by use of a specific blocker, the presence or absence of receptor-linked channels in the longitudinal and circular smooth muscles of the different compartments of the ruminant stomach in sheep.

Secondly, to show the action of different neurotransmitters/modulators on the muscle tone of the ruminant stomach in sheep.

III. 3. MATERIAL AND METHODS

Material

Six adult sheep (3 male and 3 female) and three foetuses (14, 26 and 37 cm Crown-Rump length) were used in this study.
From each animal samples of the different gastric segments to be studied, (see Addendum/ Part III/ photo 45) were dissected out and rinsed in an ice-cold buffer (0.1 M phosphate in 0.9% NaCl; 0.01% azide; pH:7.6).
For the radioimmunological approach samples were divided into two parts. One part was stripped (mucosa and submucosa separated from the muscularis), the other was left intact.
Samples, obtained from the foetuses were studied by enzymehistochemistry (in toto preparations) and immunohistochemistry.

Methods

An outline of the methodological approach of this study is given in the Addendum/ Part III/ fig. 11.
The different methods used in this study are only mentioned very briefly here and for more detailed information the reader is referred to the original articles (e.g. 43; 227; 251; 476).

Enzymehistochemistry

(see Addendum/ Part III/ fig. 12)

Acetylcholinesterase (AChE) (43)

The different specimens were first stripped and then fixed in 6% buffered paraformaldehyde solution (pH: 7, 4; 4°C; 48 h) or vice versa. After rinsing in PBS buffer (4°C; 48 h) the specimens were stripped and thereafter incubated in toto with acetylthiocholine and iso-OMPA (pseudocholinesterase inhibitor) at 37° C. After the appropriate incubation time (2 to 6 h) they were washed, cleared, stored in 100 % glycerin and, finally, photomicrographed.

Noradrenaline (NA) (251)

The tissue samples were first stripped and pieces of the muscularis were immersed in 1% glyoxylic acid dissolved in sucrose-potassium-phosphate. After 1 to 2 h of incubation the specimens were spread out, pinned up on a cork-plate, air dried (15 to 30 min) and transferred to a preheated oven at 100°C for 2 to 3 min.
Small pieces of the dried samples were then mounted, serosal side up, in Entellan and studied under the epifluorescence microscope (Leitz Orthoplan with Ploem Opak).

Immunohistochemistry (227)

Using the same procedure as described for NSE in Part II, the tissue samples were fixed and rinsed. Paraffin (5 to 10 μ m) as well as cryostat sections (20 to 50 μ m) were used (see Addendum/ Part III/ fig. 13).

Different antisera were applied (for dilutions see Addendum/ Part III/ table 6)
After the immunohistochemical procedure the paraffin sections were slightly counterstained with 1% cresylviolet; both paraffin and cryostat sections were studied under the light microscope (Reichert Univar) and photomicrographed.

Incubation of the tissue sections with normal instead of primary antiserum was the specificity test normally performed in this study. Absorption control test i. e. incubation of the specific primary antiserum saturated with its specific antigen for technical reasons was not systematically performed.

Radioimmunoassay (RIA)

Specimens used for the RIA were cut in two parts. One part was frozen immediately in 2-methylbutane (isopentane) cooled with dry ice (-70°C); the other part was stripped and then frozen. All pieces were stored at -80°C.
Sub. P and VIP were measured using the procedure described by Long and Bryant (476).
Labeled Sub. P and VIP was from New England Nuclear (6072 Dreieck, B.R.D.), antibody and porcine Sub. P and VIP standard were obtained from U. C. B. Bioproducts (B 1420 Braine-L'Alleud).
The assay system has a detection limit of O, 16 fmol/ tube.

Functional approach

Strips (10 x 10 cm) of the wall of the different compartments of the ruminant stomach obtained from sheep immediately after slaughtering were stored in a cooled (4° C) Ringer solution perfused with O_2. In the laboratory the mucosa was removed the longitudinal and circular muscle layers were separated and cut into pieces (1-2 mm diameter; 1-2 cm length) and mounted in organ baths containing 100 ml Ringer solution of the following composition (in mmol/l): NaCl 122; KCl 4.7; $CaCl_2$ 2.5; $MgCl_2$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 15.5; glucose 11.5. The solution was kept at pH 7.4 and aerated continuously with a mixture of 95% O2 and 5% CO_2.
Muscle tone was registered by using an isometric recording system.
Before adding the substances at different dose levels, the bath was rinsed twice and an equilibrium period of 10 min was allowed. The muscle tone during the last five minutes was taken as control value. The mean muscle tone of the 10 min period after adding the substances was calculated. The bath was again rinsed and the procedure was restarted. For calculations of the net effect of the substances tested the mean control values were substracted from the mean values registered. These data are presented graphically.

III. 4.
RESULTS AND COMMENTS

Acetylcholine (ACh)

1. Enzymehistochemical approach

Esterases are enzymes which are capable of hydrolyzing esters. There are many different types of esterases acting upon different substrates. Unfortunately, there is a considerable overlap between the different types. Thus, many esterases are capable of hydrolyzing the same substrate to some extent (44). Acetylcholinesterase (AChE) (acetylcholine hydrolase, E.C. 3.1.1.7) is a "specific" choline esterase that is characteristically found in high concentrations in brain, nerve cells, motor endplates and red blood cells. AChE preferentially converts the neurotransmitter ACh into choline and acetate, but it hydrolyzes a variety of acetic esters into acid and alcohol (48; 514; 816). In consequence, despite the fact that many enteric neurons and nerve fibres contain AChE, this enzyme can not be relied upon to be a specific cholinergic marker. Thus, the possibility cannot be excluded that other types of nerve fibres may stain for AChE without being cholinergic (see 275; 497; 514).
From the in toto staining of the different parts of the ruminant stomach of the foetal sheep the mesoscopic (the area between macroscopy and light microscopy) structure of Auerbach's plexus can be described as follows
The truncus vagalis dorsalis/ventralis contain very large AChE-positive nerves. During their course over the ruminant stomach complex, large AChE-positive nerve fibres branched off. They terminate partly by branching into medium-sized fibres either in the subserous plexus or the myenteric plexus.

Reticulum (RET)

(see Addendum/ Part III/ photo 46)

A dense AChE-positive nervous network consisting of large polygonal ganglia, thick interconnecting nerve strands and a distinct interlacing secondary and tertiary plexus was found. Compared to the rumen, the density of the myenteric plexus is considerably higher in the reticulum.

Ruminal Dorsal Sac (RDS)

(see Addendum/ Part III/ photos 47; 48)

Large polygonal ganglia alternate with more elongated ones. Large to medium-sized nerve bundles interconnected these ganglia and formed in this way an irregular nervous network. Along the course of the interconnecting bundles small ganglia were regularly encountered. In addition, smaller nerve bundles branch off from the internodal nerve strands and formed a clear interlacing plexus within the meshes of the network (secondary and tertiary plexuses).

Ruminal Ventral Sac (RVS)

(see Addendum/ Part III/ photos 47; 49)

Taken as a whole, the myenteric plexus of the RVS is not as dense as in the RDS. Larger elongated and smaller, more rounded, ganglia together with thin and thick interganglionic nerve bundles constituted Auerbach's plexus. The meshes of this network were wider and more angular than in the RDS. The secondary and tertiary plexuses were well developed.

Omasum (OMA)

(see Addendum/ Part III/ photo 50)

In this segment the densest AChE-positive nervous network of the forestomach and of the whole ruminant stomach complex was found
Large polygonal ganglia and thick internodal nerve bundles were seen, forming a remarkably dense AChE-positive nervous network characterized by small, rounded meshes. Smaller nerve bundles, branching off from the large interganglionic bundles, formed distinct secondary and tertiary plexuses.

Abomasum (ABO)

(see Addendum/ Part III/ photos 51; 52)

Ganglia of variable dimensions were interconnected, in proportion to their size, by thick or small nerve bundles. In this way an uninterrupted nervous network was formed, characterized by more or less elliptical meshes of different dimensions. Secondary and tertiary plexuses were, however, not always as clearly observed as in the previous segments.

Pylorus (PYL)

(see Addendum/ Part III/ photos 53; 54)

In essence the picture is the same as described for the ABO. However, relative dense and well-developed secondary and tertiary plexuses were always seen and this may be the most striking feature of this segment. Moreover, there was no discontinuity in the plexus between the pylorus and the duodenum.

***In summary**, this mesoscopic study has indicated that the arrangement of Auerbach's plexus in the different segments of the ruminant stomach follows an essentially similar pattern throughout the whole complex. However, clear differences in the prominence of the plexus, in the size and shape of the ganglia, in the thickness of the interganglionic nerve bundles, and in the density and distribution of nerve fibres between the muscle bundles were observed.*
Passing from one ganglion mass to another, relative thick interconnecting nerve strands were noticed. They formed a continuous open two-dimensional nervous network, with ganglia lying at irregular intervals in the nodes of this meshwork. As a rule the densest AChE-positive plexus of the whole forestomach was found in the OMA followed by the RET and the rumen, respectively. In the latter no difference in the density of Auerbach's plexus between the dorsal and the ventral ruminal sac could be observed.
In the glandular part of the stomach the density of the myenteric plexus was higher at the level of the pylorus, which was further characterized by a distinct interlacing plexus.
In addition, no discontinuity in Auerbach's plexus was observed at the level of the transitional zones (reticular groove, ostium reticulo-omasicum, pylorus).
Within the ganglia some cells showed strong reactivity whereas others were moderately or very weakly stained. It has been suggested that sensory neurons may exhibit a low level of AChE activity. Thus, no or moderately stained neurons possibly represent sensory neurons.

2. Functional Approach and Comments

Functional Approach

In ruminants a considerable bacterial and protozoal metabolism is observed in the rumen. This segment is also important for its reservoir function and for the absorption of the end-products of fermentation. The reticulum functions as a cyclic pump, controlling the flow of contents between the esophagus, rumen and omasum in adult animals. The omasum is involved in the absorption of ions and fluid, while the abomasum has the classical functions of monogastric animals.
To fulfill all the functional requirements on motility during the evolution, smooth muscles of the different compartments have to be adapted to the specific function of the different compartments.
The ruminant stomach functions as a voluminous and complex reservoir, in which the largest part of the ingested food is fermented (see Part I). As a consequence this part of the gastrointestinal tract is continuously subject to considerable quantitative variations in contents and in intraruminal pressure (cfr gas production). These variations are normally, at least to a large extent, compensated by proportional adjustments in the tone of the ruminant stomach wall. Thus, for the maintenance of normal motility patterns, the muscle tone must fit in with the functional demands of the ruminant stomach.
The mucosa-free strips of ruminal smooth muscles of sheep, exhibit no (or limited) spontaneous electrical and contractile activity but show a spontaneous myogenic tone. The tone can be completely eliminated in Ca^{2+}-free solutions and also by sodium nitroprusside (SN) (574). Similar results have been obtained with SN on circular and longitudinal ruminal smooth muscle strips in cattle and calves.
These data indirectly suggest that muscle tone may be of importance for the adaptation to changing intraluminal volume and for the control of digesta flow in the sheep.
Smooth muscle membranes permeability usually affect changes in the membrane potential by allowing one or more ions to pass through the membrane more freely. Since in smooth muscle cells none of the ions are in equilibrium at a membrane potential of - 50 to - 60 mV, there will be a net flow of ions into and out of the cells, tending to move the membrane potential closer to equilibrium. Membrane depolarization usually results in a contraction, although membrane potential is not a prerequisite for induction of contraction in all smooth muscle cells (119). Three sources of Ca^{2+} can be distinguished: the free Ca^{2+} in the extracellular fluid, the Ca^{2+} bound to the surface membrane, and the intracellular Ca^{2+} in endoplasmatic reticulum and mitochondria. Ca^{2+} influx during receptor activation seems to be different from Ca influx induced by membrane depolarization (186).
Contractions of smooth muscles can be initiated by changes in membrane potential (voltage-dependent channel) by activation of membrane receptors (receptor-linked channel). Activation of the first results in a phasic contraction, whilst activation of the second induces a tonic contraction. In some muscles between both types of channels, there is an overlapping of the sensitivity for activation between both types of channels. In addition, two pathways of transmembrane flux of Ca^{2+} exist: a voltage-dependent and a receptor-linked Ca^{2+} channel. In either instance, electrical or chemical cell membrane signals indirectly activate contractile elements by releasing membrane-bound Ca^{2+}, by increasing Ca^{2+} influx or by both.
Receptor agonists induce contraction without changing membrane potential in smooth muscles that do not generate action potentials or in depolarized smooth muscles (703). All eukaryotic cells contain inositol lipids. Phospholipase C catalyses the breakdown of phosphatidylinositol 4, 5-biphosphate, a multiple-charged anion that has a very high affinity for Ca^{2+} (higher than that of EDTA), into inositol triphosphate (IP3) and the lipid soluble product 1, 2 diacylglycerol (DAG). Both act as second messengers in the cells (see 386; 428). The rise in IP3 may be the means by which the intracellular (non-mitochondrial) pools of calcium are mobilized (722). The less Ca^{2+} bounds to inositol the stronger the attraction of Ca^{2+}. DAG activates a specific, calcium-activated, phospholipid-dependent protein kinase C, which catalyzes the phosphorylation of a specific group of protein substrata. DAG is one of the sources of arachidonic acid (liberated by phospholipase A_2) which serves as the substratum for prostaglandin, leukotrine and/or thromboxane synthesis (564; 730).
The organic Ca^{2+} antagonists or Ca^{2+} channel blockers (e. g. Verapamil, Methoxyverapamil) are specific inhibitors of Ca^{2+} influx through the voltage-dependent Ca^{2+} channels and inhibit both the Ca^{2+} influx and contraction. Nitrocompounds, especially SN, are selective inhibitors of the receptor-linked Ca^{2+} channel (415; 530; 751; 781). Thus, SN offers the possibility to determine in which segments of the ruminant stomach

the muscle tone is principally regulated by receptor-linked Ca^{2+} channels.

Mapping of the receptor-linked Ca^{2+} channels along the ruminant stomach using SN

(see Addendum/ Part III/ figs. 14-18).

The longitudinal smooth muscles of the RET hardly responded, while those of the RDS (10^{-8} to 10^{-4} M) and the RVS (10^{-9} to 10^{-4} M) showed a decrease. The circular muscles responded less but again in the same order of magnitude: RDS (10^{-8} to 10^{-4} M), RVS (10^{-8} to 10^{-4} M). The circular smooth muscles of the RET also showed a dose-dependent effect (10^{-7} to 10^{-4} M).
Also some reduction of longitudinal smooth muscle tone of the OMA (10^{-5} to 10^{-4} M), of the ABO (10^{-7} to 10^{-4} M) was observed. The longitudinal muscle of the AP did not respond. Similar observations were also made for the circular smooth muscles: OMA (10^{-6} to 10^{-4} M), ABO (10^{-8} to 10^{-4} M). Some reduction in the circular smooth muscle tone of the AP was observed at 10^{-10} M.
Thus, the tonic type activity (inhibited by SN) is present on the longitudinal muscles of RDS, RVS, OMA and ABO and on the circular muscles of LES, RG, ORO, RDS, RVS, OMA and ABO. The decrease of muscle tone observed in these tissues were rather limited in both longitudinal and circular muscles of OMA and RET. In the longitudinal muscles of RDS and RVS and also in the circular muscles of the ORO, an important tonic component was found. The other tissues with a tonic component were in between.
On the circular smooth muscles of the sphincters SN reduced dose-dependently the muscle tone of the LES (10^{-7} to 10^{-4} M), of the RG (10^{-6} to 10^{-5} M) of the ORO (10^{-7} to 10^{-5} M). No change of muscle tone of the PYL was observed. The decrease in muscle tone was most pronounced in ORO, followed by LES and RG in decreasing order.

These data clearly showed that the tonic component is important in the rumen (adaptation to the changing volume and intraruminal pressure). In the ORO, the tonic component could be of importance for the control of passage of contents. In the ABO, the tonic component is again involved in the adaptation of the volume and in the control of intra-abomasal pressure (see also results 654).
In other species it was shown that excitatory agents contract fundus muscle by depolarizing the membrane, while inhibitory agents relax this muscle by producing a membrane hyperpolarization. In the corpus changes in tone are achieved through a similar mechanism. Antral muscle and pyloric ring muscle normally do not exhibit tonal contractions. Corpus, antral and pyloric ring muscles generate a spontaneous myogenic action potential. This action potential complex regulates the occurrence of the gastric peristaltic contractions and the strength of the moving peristaltic ring. In pyloric muscle an additional mechanism was suggested to regulate the strength of contraction (see 110).

Functional Approach of Acetylcholine

(see Addendum/ Part III/ figs. 19-23)

Under the experimental conditions of this study the effect of ACh (10^{-10} to 10^{-7} M) on the sphincters (LES, RG, ORO, PYL), the longitudinal and circular muscles of RET, the longitudinal muscles of OMA, ABO and AP and the circular muscles of AP, was limited to absent. However, on the longitudinal muscles of RDS and RVS, a clear increase of muscle tone was observed (maximal effect at 10^{-7} M). Also on the circular muscles of the RVS a comparable effect was demonstrated. The circular muscles of the RVS showed only a significant increase of the muscle tone at 10^{-7} M, while the circular muscles of OMA and ABO only responded with an increased muscle tone at 10^{-7} M.
These data show that ACh, at the dose levels used, is only to a limited extend involved in the regulation of the muscle tone of the sphincters in the ruminant stomach of the sheep. At 10^{-7} M ACh increases muscle tone of the longitudinal muscles of RDS and RVS and the circular muscles of RVS. At the lower dose levels, a limited increase of muscle tone is restricted to both the longitudinal and circular muscles of the RVS. Remarkably, in the non-storage compartments a distinct increase of muscle tone is only observed on the circular muscles of the OMA. The circular muscles of the ABO also showed some increase at 10^{-7} M in the acute stage (= 0 min).
Thus, in the ruminant stomach the effect of ACh on the smooth muscle tone seems to be limited to the ruminal smooth muscles and the circular smooth muscles of the ABO. In these compartments increased muscle tone, due to ACh, could help in the flow of contents induced by the phasic contractions (antegrade- and retrograde contractions on the rumen and antral contractions in the

abomasum). The significance of the increase in muscle tone of the circular muscles of the OMA is much more difficult to explain. Perhaps increase is involved in the fluid and ion uptake in the filtration of the large particles ?

Comments

Although clear differences in the architecture of Auerbach's plexus were observed along the ruminant stomach of the sheep, the basic acetylcholinesterase- positive innervation of this gastrointestinal segment, as shown in this study, is in general accordance with the picture of the intramural innervation of the gut described for other mammals in the literature (see Part I/ Chap. 3). This similarity in the basic morphologic arrangement of the intramural nervous network provided a solid basis to suggest that acetylcholine has similar effect(s) on the motility of the ruminant stomach .

Pharmacologic evidence for the action of enteric cholinergic neurons on intestinal muscles stems from the many observations that stimulation of the extrinsic and intrinsic nerves induces contractions of the intestinal musculature. These contractions were, just like those induced by acetylcholine, blocked by antagonists of the cholinergic (muscarinic) receptors and enhanced by anticholinesterases (see 239).

Axons, emanating from the motor nucleus of the vagal nerve extend to the wall of the gut and make, via nicotinic and cholinergic receptors, synapses with the ganglionic cells of the myenteric and submucosal ganglia (339). Stimulation of vagal fibres generally increases the contractile activity of the gut. Endings of the cholinergic nerves are at the surfaces of the enteric ganglia and the interganglionic fibre tracts (796; 797). Thus, cholinergic nerves are involved in the excitatory pathways to the gut and in the excitatory component of the peristaltic reflex (246). However, some vagal fibres terminate either on inhibitory neurons or on these interneurons in the circuitry that activate the inhibitory ganglion cells. It was found that the inhibitory pathways of the vagus act via nicotinic cholinergic and perhaps via serotoninergic synapses. Consequently, vagal stimulation produces two types of potentials (in the proximal colon of the rabbit): excitatory and inhibitory one (see 403).

In the enteric nervous system acetylcholine functions as the neurohumoral substance of all preganglionic sympathetic and parasympathetic fibres, many postganglionic parasympathetic fibres, in some cell bodies, and it seems also involved in the non-adrenergic, non-cholinergic transmission (629).

Two groups of cholinergic receptors i. e. nicotinic and muscarinic were established. Preganglionic fibres act on postganglionic neurons by activation of either nicotinic or a combination of both receptors. Postganglionic cholinergic nerves act primarily through muscarinic receptors on the gut smooth muscle (see 239; 629).

In the myenteric plexus acetylcholine was established as a neurotransmitter. Indeed, acetylcholine is released from the gut spontaneously and when enteric nerves were stimulated. The spontaneous acetylcholine release can be reduced, but not abolished, by ganglion-blocking agents (hexamethonium) or by agents that abolish nerve conductivity (tetrodotoxin). Moreover, about 40% of this acetylcholine release persists in the presence of these agents, suggesting that there are spontaneously active cholinergic interneurons in the myenteric plexus even when the gut is at rest. Accordingly, almost all spontaneously released acetylcholine originates from intrinsic neurons (275; see 275). At least two types of cholinergic neurons are suggested to exist within the myenteric plexus of the guinea-pig ileum. One type sends off long processes to other distant nodes. Synapses are then made with other cholinergic neuron(s) through muscarinic receptors. The other type of cholinergic neurons receives cholinergic inputs from mechanosensitive neurons through nicotinic receptors. The processes of these neurons are relatively short. Both types of neurons may be excited following distension (731). Thus, cholinergic neurons are without doubt involved in the control of intestinal motility (see 239; see 438). Many, if not all, of the final excitatory neurons to the muscle coat are cholinergic (275; see 275), since the principal neurotransmitter released by the excitatory enteric neurons is acetylcholine. Cholinergic excitatory neurons have been shown to supply the circular musculature of the esophagus, the stomach, the circular and longitudinal muscle of the small and large intestine, the muscles of some sphincters and the biliary system (see 239). Moreover, blockade of cholinergic synapses releases the muscle from ongoing inhibition. Thus, cholinergic transmission between at least two different neurons may be involved in the tonically-active inhibitory pathways to the gut musculature (see 799). Nevertheless, there is good evidence that other substances (opioids, Substance P) also contribute to the excitatory responses (239).

After release acetylcholine traverses relatively long diffusion pathways to the intestinal musculature. As a rule the longitudinal muscle of the small intestine is

dominated by an excitatory cholinergic innervation. The circular muscle layer, on the contrary, by a non-adrenergic, non-cholinergic inhibitory innervation, although cholinergic junction potentials were also recorded in some of the circular muscle cells but these are rare and species dependent. The excitatory cholinergic component of the circular muscle may be associated with tonic contractile activity (35; 698; 796; 797). Overall there is a slow transition, down the gastrointestinal tract, from dominantly cholinergic excitatory innervation proximally to inhibitory innervation in the distal parts of the gut. Moreover, cholinergic activity was found to be dominant in the highly motile parts of the gut and is less dominant in others where motility is normally less pronounced. Nevertheless, intrinsic contractions of the smooth muscle, differences in the pattern of the intrinsic innervation and levels of various hormones are also important for the motility gradients (35).

As mentioned earlier, the extreme compartmentalization of the ruminant stomach may be associated with the functional specialization found within this complex (cfr. omasum: absorption of fluid and electrolytes as well as regulation transit of fluid and ingesta between the reticulo-rumen and abomasum; reticulum: cyclic pump; rumen: fermentation chamber). The ruminant stomach, the rumen in particular, is continuously subjected to considerable variations in the volume of ingesta and intraluminal pressure. Consequently, region specific adjustments in the muscle tone are essential for the maintenance of the normal motility patterns and, since the different compartments are anatomically and functionally linked to each other, also for the normal functioning of the ruminant stomach as a whole. Thus, it was supposed to find a considerable tonic component in those ruminant stomach compartments (rumen, abomasum) where large variations in volume and pressure normally occur.

Tonic contractility of the gastrointestinal smooth muscle is largely controlled by receptor-linked Ca^{2+} channels. Logically sodium nitroprusside, a receptor-linked Ca^{2+} channel blocker, was found to induce a drastic decrease in the muscular tone of the rumen, the abomasum and the ostium reticulo-omasicum, confirming in this way our hypothesis. Acetylcholine on the other hand was found to increase the muscular tone in the rumen, the abomasum and the omasum. Thus, based upon the morphological and functional results of this study, it may be claimed that the muscle tone of the rumen and the abomasum is, at least partially, regulated by acetylcholine receptor-linked Ca^{2+} channels. The substantial effect of acetylcholine on the omasal muscle tone was rather unexpected since sodium nitroprusside provided no evidence for the presence of a tonic component in this compartment. Likewise, the direct relaxant effect of acetylcholine on the reticular groove (see Addendum) remains unexplained until now.

Noradrenaline (NA)

1. Histochemical Approach

(see Addendum/ Part III/ photos 55-57)

In the wall of the stomach of the adult sheep most adrenergic nerves terminate in the myenteric plexus, while nerve fibres in the smooth muscles are not well visualized. Intensely green fluorescent varicose adrenergic terminals were abundant in the myenteric ganglia and formed networks around non-fluorescent ganglion cells. It seems that the largest part of the myenteric cells have varicose NA-ergic axons close to them, suggesting a close topographic relationship between adrenergic varicose fibres and neurons in the Auerbach's plexus. In the different segments of the ruminant stomach no basic difference in the adrenergic supply of the myenteric ganglia could be observed, although the size and shape of the ganglia did differ.
As in the small and large intestines of other mammals there are practically no adrenergic fibres within the longitudinal and circular muscle layers (see 252). Thus, little morphological evidence for a direct innervation of the smooth muscle by these fibres was found.
Furthermore, few beaded fluorescent fibres were observed in the internodal strands. Intramural arteries and arterioles were well supplied with perivascular NA-ergic axons.
Thus, the primary adrenergic innervation of the different segments of the ruminant stomach seems to be contained almost exclusively within the myenteric plexus.

2. Immunohistochemical Approach

(see Addendum/ Part III/ photos 58-67)

The techniques using glyoxylic acid or several aldehydes for the visualization (fluorescence) of the catecholamine-containing nerves, can now be replaced by the use of specific antibodies to converting enzymes involved in the catecholamine synthesis. This technique includes antibodies to tyrosine hydroxylase and dopamine-b-hydroxylase (DBH). DBH is present within catecholamine storage vesicles in the adrenal medulla and sympathetic nerves. There it catalyzes the conversion of dopamine to noradrenaline. The discrete localization of DBH makes this enzyme an ideal marker for the study of sympathetic neurons (764).
The results of the immunohistochemical study, using an antibody against DBH, are summarized in table 7 (see Addendum/ Part III/ pag. 87).
From the results of this immunohistochemical approach it may be concluded that:

- The same basic distribution pattern is found along the ruminant stomach complex i.e. abundant DBH-IR in Auerbach's plexus and a clear reaction around the blood vessels
- In the mucosa of the forestomach no DBH-IR was observed neither in the adult sheep nor in the foetus. In the glandular stomach DBH-IR was exclusively found around blood vessels
- DBH-IR was noticed in the submucosa of both the adult and foetal sheep. In adult specimens DBH-IR was clearly observed around blood vessels but not in Meissner's plexus. In all foetuses a DBH-IR network, most probably associated with blood vessels, was encountered in all submucosal protrusions. In the glandular stomach of the foetal sheep similar results were seen to that observed in the adult sheep.
- In the circular muscle layer varicose DBH-IR nerve fibres were regularly seen running parallel to the smooth muscle fibres. Remarkably, more intramuscular DBH-IR nerves were found in the sphincter regions i.e. the lips of the RG, in ORO and PYL.
- By far the most DBH-IR was found in the myenteric plexus. In the periphery of Auerbach's plexus numerous varicose DBH-IR nerve terminals were noticed around non-immunoreactive perikarya.
- The longitudinal muscle layer contained several DBH-IR nerve fibres particularly in the RET where multiple fibres were found.

From the histochemical and immunohistochemical data it may be ***concluded*** *that the NA-ergic innervation of the ruminant stomach strongly resembles the situation in other species. Consequently, this study has provided a solid morphological basis which allows us to speculate on the possible influence of NA on ruminant stomach functions. Indeed the presence of dense pericellular NA-ergic networks in the myenteric plexus and the general sparseness of adrenergic nerves in the ruminant stomach musculature may be in accordance with the view that the adrenergic effects on the mammalian gastrointestinal motility are mainly indirect.*

3. Functional Approach and Comments

Functional approach

(see Addendum/ Part III/ figs. 24-28)

From the in vitro experiments it has become clear that the effect of NA (10^{-10} to 10^{-7} M) on the smooth muscles of the sphincters i. e. LES, RG, ORO, PYL, on the longitudinal and circular muscles of RET, OMA, and AP, on the longitudinal muscles of RDS and, finally, on the circular muscles of RVS is limited to absent. Only the longitudinal muscles of the RVS (increase) and circular (decrease) muscles of the RVS showed a clear response.
Thus, NA seems much less involved than ACh in the direct regulation of the smooth muscle tone of the ruminant stomach.

Comments

Sympathetic fibres to the gut originate from the spinal cord and the prevertebral ganglia. Electrophysiological studies show that prevertebral ganglia possess a nervous circuitry that is able to integrate inputs both from the gut and central nervous system and to mediate both inhibitory inputs to the gut and extramural intestinal reflexes (161; see 573; 642). Moreover, the spinal cord is most probably the source of the neural inhibition of the gut since spinal cord lesions, ventral root section or spinal cord removal induces intestinal hypermotility (see 642).
Both, the histochemical and immunohistochemical results of the present study clearly demonstrate that the intramural plexuses, particularly Auerbach' s plexus, and the vasculature are the main target organs for the noradrenergic fibres. The ruminant stomach musculature receives, except the sphincters, practically no direct noradrenergic innervation. These findings correspond very well with the data found in the literature. Thus, it may be postulated that noradrenaline has, just like in other species, little or no direct effect on the smooth muscles of the ruminant stomach complex and that noradrenaline controls the motility of this complex principally through its modulatory action on the neurotransmission within the intramural ganglia.
Indeed, similar anatomical observations i.e. that noradrenergic fibres extensively ramify in Auerbach's ganglia, that there is a dense accumulation of noradrenergic fibres around the enteric perikarya and that few noradrenergic fibres run in the intestinal musculature itself have been made in other species and largely suggest that the primary adrenergic innervation of the gut is contained within the myenteric plexus. As a consequence, the noradrenergic inhibitory mechanism principally takes place in Auerbach's plexus (see 56; 108; 236; 275; 632) where noradrenaline may directly act on perikarya, nerve terminals or both to inhibit nerve impulse transmission (108; see 237; 275; 387; 796). Moreover, electron microscopy has revealed noradrenergic sympathetic terminals, making reciprocal axo-axonic synapses with cholinergic terminals, and adrenergic varicosities located close to the somata of the intrinsic neurons (56; 237; 275; see 275). The numerous noradrenergic synapses on enteric ganglion cells suggest an extensive influence of these sympathetic nerves on the functioning of the enteric neurons.
Clear pharmacological and physiological evidence showed that the postganglionic sympathetic nerves running to the intestine inhibit peristalsis by releasing noradrenaline. Noradrenaline acts presynaptically on receptors of cholinergic neurons to reduce the acetylcholine output from parasympathetic nerve terminals and enteric neurons (108; 237; see 239; see 246; 275; see 275; 339; 387; see 688; see 767; 796; see 796). In addition, in the myenteric plexus/longitudinal muscle preparation of the guinea-pig ileum presynaptic (prejunctional) inhibitory (α_2), postsynaptic (postjunctional) inhibitory (α_2 and β) and excitatory (α_1) adrenoreceptors have been demonstrated (372; 558). The inhibitory effects of noradrenaline on cholinergic transmission have been suggested to be mediated either by presynaptic α_2 -receptors on the myenteric neurons or by inhibiting the spontaneous activity via β-receptors (237; see 237; 573; see 642; see 688; 705). Thus, the sympathetic inhibition of the gut seems principally carried out at the ganglionic level largely by activation of the presynaptic α-adrenergic receptors. This activation leads to an inhibition (=hyperpolarization) of the acetylcholine release from presynaptic nerve terminals, to an inhibition of the excitatory intramural neurons and to a suppression (inhibition) of the firing of cholinergic nerves (159; see 164; 237; 403; 404; 629; 642; see 688). Hence, noradrenaline most probably reduces the basal parasympathetic activity in the myenteric ganglia and, in consequence, affects the smooth muscle cells indirectly (56; 237; 275; see 275). Furthermore, noradrenaline has been shown to augment the excitability in AH/type 2 neurons, to prevent the release of serotonin, to suppress both cholinergic

and serotoninergic synaptic transmission within the enteric interneuncial circuitry and, finally, to reduce the release of acetylcholine in response to polypeptides. This may result in ileus by inactivating the networks that synaptically inhibit the tonically active inhibitory neurons (see 767; 796; see 796). As noradrenergic nerves to the gut exert a major part of their inhibitory action on enteric cholinergic neurons some degree of cholinergic tone is, in consequence, necessary to demonstrate these effects (see 237).

Consequently, stimulation of noradrenergic nerves inhibits the propulsion of food along the digestive tract (peristalsis) by constricting the sphincters and by acting on intrinsic cholinergic nerves involved in the peristaltic reflex (246). Thus, activity of the noradrenergic nerves paralyzes the peristaltic mechanism, contracts sphincters and relaxes the non-sphincteric muscles. The normal churning activity of the gut is abolished and, since there is a decrease in mucosal blood flow, secretion and absorption are impaired (35; see 35; 150; 237; 629). In addition, adrenergic nerves interact with cholinergic nerves and the two transmitters, noradrenaline and acetylcholine, can modulate each other's release. Indeed, noradrenaline, released from sympathetic nerve terminals, reduces the release of acetylcholine from cholinergic nerves in the gut, thereby inhibiting gastrointestinal motility. Exogenous acetylcholine interferes with the release of noradrenaline from stimulated perivascular nerves (muscarinic mechanism) (see 110). Furthermore, the anatomy of the myenteric plexus shows that there is an adrenergic-cholinergic mutually (antagonistic) axo-axonic junction. Thus, the mutual modulation of the release of acetylcholine and noradrenaline appears to be a significant component of the enteric action of these two classical transmitters. This modulation augments and sharpens the antagonistic effects of the two compounds on smooth muscle. Adrenergic inhibition is accompanied by inhibition of cholinergic excitation, and the reverse appears to be true as well (see 110). Thus, it may be claimed that the classical view of sympathetically released noradrenaline as having a role only as a neuromuscular transmitter requires modification.

Adrenergic nerves present in the intrinsic ganglia are usually inactive under resting conditions (237). Hence, sympathectomy is generally without significant effect on bowel function probably due to the lack of important tonic influences of sympathetic nerves on the resting gut (237; 629). The discharge of noradrenergic nerves is evoked either through extra-intestinal chemical and mechanical stimuli, mediated by somatic/autonomic afferents, adrenergic efferents and mesenteric vascular reflexes or through reflex pathways originating within the alimentary tract itself (35; see 35; 150; 237; 629). Noxious stimuli, such as rough handling of the viscera or peritoneal infection, cause the reflex firing of adrenergic nerves and if this reflex inhibits the peristaltic mechanism for an extended time adynamic ileus may develop (237).

Taking together, the primary action of sympathetic nerves to the gut is to inhibit ganglionic transmission in the excitatory neural pathway (35; see 237; see 275). This indicates that the intestine is subject to a tonic sympathetic inhibition that is essentially related to a depression of synaptic transmission within the myenteric plexus and to some direct depression of smooth muscle as well. Firing in the sympathetic inhibitory fibres is generated in part by the central nervous system and in part via peripheral reflex pathways in prevertebral ganglia (161; 797). Nevertheless, it seems that only the electrical discharge patterns of single-spike neurons are altered by exogenously applied noradrenaline, while in vitro experiments prove that noradrenaline reduces the rate of discharge to less than 20% of the single-spike cells (see 799).

But, not all sympathetic action on the gut rests entirely on an inhibition of ganglionic transmission since postganglionic noradrenergic axons have been demonstrated within the circular muscle layer (237; see 688) and were found to have a direct action on intestinal smooth muscle (150; 237; see 275). Moreover, histochemical studies have revealed a noradrenergic innervation of the sphincters that is often substantially more dense than the adjacent non-sphincter regions. This is, nevertheless, not a general rule since in the rat, cat and monkey in contrast to the guinea-pig, few noradrenergic fibres (no more than in the adjacent non-sphincteric area) were seen in the lower esophageal sphincter musculature. In addition, there is no significant increase in the density of the noradrenergic innervation of the ileo-colic sphincter of the guinea-pig, whereas in rats and rabbits this sphincter is characterized by a denser noradrenergic innervation than the adjacent musculature. Finally, in the guinea-pig, cat and dog the internal anal sphincter is richly supplied by noradrenergic nerve fibres, but it is less heavily innervated in the rat and man (239; see 239). The origins of the noradrenergic fibres to the gastrointestinal sphincters seem to correspond with these sources to the adjacent non-sphincteric muscles.

Stimulation of the sympathetic supply to the sphincter musculature has a constrictor effect that is mimicked by

adrenaline and noradrenaline. The excitatory effect of noradrenergic nerves at the level of the sphincters is a direct effect of noradrenaline on receptors in the muscle (239). It is interesting to note that no fluorescent nerves have been observed in Auerbach's plexus of the toad or teleost fish (237). In consequence, there is most probably no modification of the cholinergic transmission of the type suggested for mammals. However, the denser adrenergic innervation of the circular muscle of the large intestine in both species suggests that direct adrenergic effects upon the muscle may be relatively more important than in mammals (324; 632).

Exogenous noradrenaline applied to an antral strip in vitro reduces the strength of contraction by reducing the ionic currents. Thus, exogenous noradrenaline mimics sympathetic nerve stimulation although this in vitro action of noradrenaline is primarily through α-adrenoreceptors located on the smooth muscle cells. Indeed, the direct inhibition (hyperpolarization) of the intestinal musculature by noradrenaline involves postsynaptic α- and β-receptors (237; see 237; see 239; 573; see 642; see 688; 705). In the guinea-pig this action seems to be mediated through β-adrenoreceptors, and in the rabbit through both α- and β-adrenoreceptors (150; 237; see 275). Postsynaptic β- adrenoreceptors are nearly always inhibitory (by limiting the availability of Ca^{2+} for interaction with contractile proteins), while postsynaptic α-receptors often mediate excitation in response to catecholamines (237; see 237; 629; see 688). However, stimulation of α-receptors normally results in an inhibition of gastric and intestinal motility (58). Postsynaptic α_1-receptors have been shown to mediate the contraction in response to catecholamines (237; see 237; 629; 688). The functional significance of these adrenoreceptor types depends largely on the topographical localization of the smooth muscles within the gastrointestinal tract (237; see 237; see 573). In sphincteric regions both α excitatory and β inhibitory receptors are present on the smooth muscle (629). It has been suggested that in the guinea-pig ileum and the ileo-cecal sphincter of the cat, however, the direct adrenergic inhibition is mediated via β-adrenoreceptors (see 164). Thus, direct inhibition of the muscle is most likely mediated through both α- and β-receptors (403; 404; 629). The direct inhibitory action of noradrenaline on the intestinal musculature involves contraction of the sphincters and, to a slight extent, relaxation of the non-sphincteric smooth muscle. In addition, there is some contribution to the direct inhibitory action on the muscle by catecholamines which are often released into the circulation from the adrenal glands when the adrenergic nerves to the gut were reflexly activated (159; see 164; 237; see 237; see 239; see 246; see 275; 403; 404; 629; 642; see 688; 767).

In conclusion, the inhibitory effect of sympathetic nerves seems to be present both at the level of the intestinal musculature as well as at the level of the intramural plexuses. As exceptions are the sphincters where they may be excitatory (403; 404; 629).

In the abomasum of the sheep stimulation of α-adrenoreceptors enhances, while stimulation of the β-adrenoreceptors inhibits the motor activity. In addition, the sympathetic nervous system seems to act on the pyloric motor activity mainly through β-adrenoreceptors (780).

In the in vitro experiments of this study some direct action of noradrenaline upon the muscular tone is found only in those segments where a clear tonic component has been demonstrated (see Addendum). Based upon the pharmacological evidence for a direct action of noradrenaline upon the intestinal musculature in other species, it may be anticipated that the inhibitory effect of noradrenaline on the circular muscle tone of the ruminal dorsal sac is mediated through α_2/β-adrenoreceptor-linked Ca^{2+} channels. The tonic contractile effect of noradrenaline upon the longitudinal muscle cells of ruminal ventral sac is on the other hand most likely mediated through α_1 adrenoreceptor-linked Ca^{2+} channels. Thus, despite the sparse direct innervation of the ruminal musculature by noradrenergic fibres, it is most likely that in vivo noradrenaline is, besides its action Auerbach's neurons, also to some extent directly involved in the control of the tone of the ruminal wall.

B-adrenoreceptor Ca^{2+} channels most probably mediated the relaxing effect of noradrenaline upon the lower esophageal sphincter, whereas noradrenaline induced a tonic contraction of the reticular groove possibly through α_1-adrenoreceptors. Nevertheless, the overall effect of noradrenaline upon the ruminant stomach sphincters is rather limited and is, as compared to other species, less pronounced. But, due to the dense noradrenergic innervation of the sphincter musculature, stimulation of noradrenergic nerves may be relative important in vivo and most likely induces relaxation of the lower esophageal sphincter and a tonic contraction of the reticular groove. These findings may further explain why under stress conditions ruminal motility is reduced and the eructation and regurgitation reflex are blocked.

Moreover, recent investigations have provided evidence for a coexistence of noradrenaline with one or more peptides in the extramural ganglion cells project-

ing to the gastrointestinal tract. These findings were as yet not confirmed in the sheep, but it may be hypothesized that in vivo the noradrenergic effects upon the ruminant stomach tone may, at least in part, be mediated through the co-stored and co-released non-adrenergic, non-cholinergic neurotransmitters/modulators.

Although not studied in the sheep, synapses between axons and gut endocrine cells have been demonstrated in other species, indicating a nervous control of the release of secretory products from these cells (12). In addition, functional evidence for a nervous control of the duodenal enterochromaffin cells has been obtained. Indeed, stimulation of the cervical vagal trunk induced a decrease in serotonin content of the guinea-pig duodenum. This decrease can be directly correlated to the enterochromaffin cells since a clear reduction in the serotonin content in individual enterochromaffin cells following such stimulation has been demonstrated in the cat (12). Further, denervation experiments and pretreatment with adrenergic blocking agents has strongly suggested that an adrenergic neuronal pathway is involved. This hypothesis is in agreement with previous investigations reporting a release of serotonin from the small intestine upon stimulation of periarterial sympathetic nerves or after intra-arterial infusion of noradrenaline (12; 101; 102; 490; 737). Furthermore, the substances released from enterochromaffin cells due to noradrenergic fibres stimulation may in turn stimulate local nerves, and from there the different intestinal reflex arches (see 246).

Taking the different mechanisms of action of noradrenaline together it may be suggested that the adrenergic reflexes arising from within the intestine act as buffers to regulate gastric motility, gastric emptying and intestinal peristalsis. If the contents of the upper intestine become hyperacidic or hypertonic (chemoreceptors) then an entero-gastric reflex, mediated through adrenergic nerves, reduces the rate of gastric emptying. Similarly, if regions of unusually high pressure develop within the intestine (mechanoreceptors) an adrenergic intestino-intestinal inhibitory reflex suppresses the activity in distension-sensitive cholinergic neurons the continued excitation of which could aggravate an obstruction. Thus, intestinal adrenergic reflexes are protective and may prevent the development of hyperacidity and hypertonicity in the upper small intestine as well as excessive intraluminal pressure in the bowel. In ischemic injuries they may reduce the metabolic requirements of the intrinsic neurons (and secondarily of the muscle and mucosal cells) when a reduction in blood supply is imminent or present (237; see 237). Indeed, neurons are more sensitive than smooth muscle to the lack of an adequate blood supply. Thus, it may be significant that noradrenergic nerves supply the ganglion cells rather than the muscle of the gastrointestinal tract. This means that when the blood supply is reduced the activity and hence the metabolic requirements of the enteric neurons also falls. Stimulation of the extrinsic adrenergic fibres results in vasoconstriction, and it is possible that, in some circumstances, the function of the noradrenergic nerves is primarily to protect the ganglion cells and that suppression of peristalsis is incidental (159; see 164; 237; see 239; see 688).

Finally, intestinal vasomotor nerves participate in cardiovascular and metabolic homeostasis (237; see 237).

Serotonin (5-HT)

1. Review of the literature

(see Addendum/ Part III/ pags. 93-105)

2. Conclusions from the literature

In the gut of all species examined 5-HT has a dual localization primarily in the enterochromaffin (EC) cells and in smaller quantities in the intramural nervous elements (neurons and nerve fibres). It has been estimated that 65% of the total 5-HT body is stored in the 5-HT-IR EC cells. Thus, in mammals the gastrointestinal tracts seems to be the most important source of 5-HT. The number and distribution of the EC cells varies in different parts of the gastrointestinal tract and also from one species to another (it may depend on the development of the adrenergic innervation) (314). 5-HT neurons in the gut have their cell bodies in the myenteric ganglia while their varicose axons are distributed in a distal direction within the myenteric and submucosal plexuses. Most likely 5-HT neurons are interneurons controlling the descending neural pathways and possibly the descending motor programmes in the gut (see 576). It is most interesting to mention that there is evidence for a co-storage of 5-HT with some regulatory peptides e.g. enkephalins, motilin and Sub. P within these cells.

A variety of stimuli either acting from the mucosal side (food, fat, change of intraluminal pH, osmotic pressure, pressure, motility, mechanical stimulation, cholera toxin, mechanical obstruction) or from the serosal side (transmural electrical stimulation, vagal and splanchnic nerve stimulation, vagotomy, drugs) cause a release of 5-HT from the mucosal and/or intramural neural pool.

At the level of the gastrointestinal tract 5-HT may influence motility and the secretion/absorption process as well. Depending on the localization in the gastrointestinal tract 5-HT has direct as well as indirect excitatory and inhibitory actions on the smooth muscle of the gut.

5-HT is able to control (modulate) the peristaltic reflex and the gastrointestinal motility by increasing the tone of the smooth muscle, inducing contractions, increasing electrical activity (of the small intestine), inducing the release of ACh from Auerbach's plexus, increasing the sensitivity of the smooth muscle for ACh, inhibiting AChE, and finally by modulating the electrical activity and synaptic behavior of the myenteric plexus. However, 5-HT-IR neurons do not appear to project to the intestinal muscle but to other enteric ganglia. Hence, 5-HT neurons appear to behave as interneurons (791).

Likewise, the secretion/absorption process may be controlled by 5-HT. 5-HT may stimulate secretion and suppress absorption because of the inhibition of electrogenic Na+ transport and the stimulation of Ca^{2+} influx into the epithelial cells, which induces a local release of VIP from the submucosal pool. Finally, 5-HT causes a vasodilation of the mesenterial and intramural arterioles and a vasoconstriction of venules which results in a congestion of the intestinal mucosa. The action of 5-HT on the mesenteric blood flow appears to be the subject of the dose, preparation, species and intestinal motor activity (see 529; see 576). In this respect it has been suggested that, when 5-HT does increase visceral motor activity, mechanical compression of the mesenteric microcirculation manifests itself as a vasoconstriction (see 576).

The stimulatory action of 5-HT on the intestinal secretion is primarily on crypt cells and seems not to be mediated via a change in the cellular levels of c-AMP. In addition, it has been shown that the intestinal secretory action of 5-HT is mediated by a neuronal mechanism (see 576).

3. Immunohistochemical Approach

(see Addendum/ Part III/ photos 68-73)

The results of the immunohistochemical study concerning 5-HT are summarized in tables 8 and 9 (see Addendum/ Part III/ pags. 109-110).

This study shows that there are no basic differences in the presence and distribution of 5-HT within the ruminant stomach wall of both the foetal and adult sheep except that more and stronger reacting 5-HT-IR elements were seen in the cryostat sections.

In the adult sheep 5-HT can only be demonstrated in the glandular part of the ruminant stomach i. e. in the abomasum and in the antrum pyloricum. Within these segments 5-HT-IR cells are encountered in the lamina epithelialis, mainly in the base of the gastric glands and less frequently in the surface epithelium. The morphological appearance of these 5-HT-IR epithelial cells resembles the picture described in other species, i. e. triangle to flask-shaped cells sometimes showing a long slender apical process directed toward the lumen

and/or a short basal process probably making contact with adjacent structures.
Contrary to the findings in the adult sheep, the distribution pattern of the 5-HT EC cells in the foetal stomach seems to be different. Indeed 5-HT-IR epithelial cells were found in all segments of the ruminant stomach, the pylorus excepted (see Addendum/ Part III/ pags. 109-110). In the forestomach these cells were exclusively seen in the stratum germinativum. In the glandular part of the stomach, on the contrary, they were, although preferentially found in the basal part of the gastric glands, encountered along the whole gastric epithelium.
Neither in the foetus nor in the adult sheep 5-HT-IR nervous elements were noticed in the ruminant stomach wall. However, it may be of interest to note that in the small intestine (duodenum, jejunum) of the oldest foetuses 5-HT-IR was, in addition to its epithelial localization, also observed in both intramural plexuses (neurons and nerve fibres).

Conclusions from this study:
Based upon the morphology and distribution of the 5-HT-IR epithelial cells in the ruminant stomach of the adult sheep, there is good evidence to assume that these "taste cells of the gut" belong, at least in part, to the intramural data-processing system modulating and controlling motility as well as the secretion/absorption process. Although it has been suggested that 5-HT may be involved in the genesis of the spontaneous movements of the foetal rumen (see Review literature), the exact functional significance of the presence of 5-HT-IR epithelial cells in every segment (except the pylorus) of the ruminant stomach of the foetus remains unknown.

4. Functional Approach and Comments

Functional approach

(see Addendum/ Part III/ figs. 29-32)

In the in vitro experiments of this study 5-HT increased muscle tone, especially of the longitudinal and circular muscles of the RDS and RVS at 10^{-7} M. A decrease of muscle tone has been observed in the longitudinal muscle of the RVS at 10^{-9} to 10^{-8} M and in the circular muscle of the RVS at 10^{-9} M and in the OMA (10^{-7} M). The effect on the other smooth muscles studied were limited to some increase or decrease of muscle tone.
The present data showed that, to a major extent, 5-HT is involved in the control of the smooth muscle tone of the rumen.

Comments

Serotonin-immunoreactive epithelial cells are observed in the whole ruminant stomach of the foetus, but in the adult sheep they were limited to the glandular stomach only. Neither in foetuses nor in the adult sheep serotonin-immunoreactivity is, in contrast to other species, found in the intramural nervous elements of the ruminant stomach. In most species studied so far, serotonin has a dual localization within the gastrointestinal tract i.e. primarily in the entero-endocrine cells and secondly in smaller quantities in the intramural nervous elements. However, the intramural serotonin containing neurons and nerve fibres have only been seen if the animals are pretreated with tryptophan or a MAO inhibitor. The fact that the animals, used in the present study, are not pretreated, may explain the discrepancy between both results. Hence, it may be anticipated that serotonin is present in the intramural nervous system of the ruminant stomach as well, but in quantities below the detection limit of our immunohistochemical approach. This idea is further strengthened by the observation that in the foetal sheep serotonin-immunoreactivity is regularly seen in the intramural nervous elements (Auerbach's plexus in particularly) of the duodenum (see Addendum/ Part III/ table 8). However, the inconsistency between the morphological data emerging from this study and those from the literature renders the functional significance of serotonin in the forestomach of the sheep speculative.
When introduced into the intestinal lumen, serotonin

initiates peristalsis (744). Furthermore, transmural stimulation of the proximal colon of the guinea-pig causes sustained contractions of the longitudinal muscle, while distension induces (pharmacologically) similar contractions in the circular muscle of the distal colon. In both cases these contractile effects are antagonized by serotonin blocking drugs (methysergide) and by previous exposure of the intestine to high doses of serotonin (tachyphylaxis). Thus, the substance involved in these contractions might be related to serotonin, although the circular muscle of the distal colon is quite insensitive to serotonin (see 148; see 246). In addition, stimuli applied directly to the myenteric plexus cause excitatory responses in enteric neurons. These responses are abolished after desensitization of the serotonin receptors (tachyphylaxis) and are, in addition, absent in Ca^{2+}-free solutions. Hence, these responses are presumably due to the release of an excitatory substance (most probably serotonin) at a neuro-neuronal synapse (see 246). Furthermore, it has clearly been shown that serotonin activates enteric neurons which release acetylcholine. In this way serotonin can cause contraction of the intestinal musculature (275). The smooth muscles itself shows, depending on the region of the gut and the species examined, a variable response to serotonin. For example, the longitudinal muscle of the fundus of the rat is very sensitive to serotonin, while this of the small intestine is insensitive to this amine. Likewise, the circular muscles of the guinea-pig stomach, ileum and colon are virtually unresponsive to serotonin (see 275).

Taken together, there is now substantial morphological and functional evidence that serotonin, once released from its intramural pool, may function as a non-cholinergic excitatory transmitter that induces excitatory or inhibitory effects by acting either directly on the smooth muscle cells or indirectly on other cholinergic and non-adrenergic, non-cholinergic neuronal structures. Indeed, serotonin may be involved in the interneuronal transmission and, as a consequence, in the cholinergic and also the non-adrenergic, non-cholinergic control of the gut since serotonin has been found to activate non-adrenergic, non-cholinergic intrinsic inhibitory neurons (see 275; 629). Pharmacological data raise the possibility that bulbospinal serotoninergic pathways exert an inhibitory influence on the sacral autonomic outflow to the colon (161).

As intestinal serotonin is primarily pooled in the epithelial endocrine cells, several stimuli from the mucosal side (e.g. food, changes in intraluminal pH and osmolarity, mechanical stimulation, etc.) can induce a release of serotonin from this pool. Once released, serotonin can influence other epithelial cells (paracrine action) and/or intramural nervous elements (neurocrine action). In this way serotonin containing epithelial cells may translate intraluminal changes to changes in the intestinal secretion/absorption process, as well as in changes in the intestinal motility and local blood flow. The morphological findings of this study (serotonin-immunoreactive epithelial cells, dense submucosal plexus) provide much evidence to postulate that in the glandular stomach of the sheep (foetus and adult) epithelial serotonin containing cells are indeed involved in the above-mentioned control system. In the aglandular stomach, however, serotonin-immunoreactive cells are absent in the adult sheep, while in the foetus the link (i.e. the submucosal plexus) between these epithelial cells and the "motor apparatus" (musculature and myenteric plexus) is absent or poorly developed (see results Neuron Specific Enolase). Thus, morphological evidence for a serotoninergic control of the motility patterns in the foetal forestomach did not emerge from this study. However, it has been shown that 5-HT has also a trophic effect upon the gastrointestinal epithelium. Thus, it is likely that in the foetus serotonin containing epithelial cells are, at least in part, involved in the proliferation of the forestomach epithelium (stratified, multilayered).

Nevertheless, the morphological observations of this study (virtual absence of serotonin in the adult forestomach) disagree with the distinct effects of serotonin on the muscular tone of the forestomach complex observed in the functional approach of this work. Therefore, it may be anticipated that in vivo "exogenous" serotonin reaches the forestomach via the vasculature to exert its tonic contractile effect directly (D-receptors) and/or indirectly upon the smooth muscle cells. In this way serotonin increases ruminal muscle tone disturbing the normal ruminal motility, which in turn deregulates the motility patterns in the other segments of the ruminant stomach. The inhibitory effect of serotonin on the tone of the circular muscle of the omasum is difficult to explain. Possibly,serotonin exerts its direct action on the omasal muscles through an as yet unknown receptor-type.

It has been shown that serotonin activates the receptor-linked channel resulting in a breakdown of phosphatidylinositol 4,5-P2 by activation of phospholipase C. 1, 2 Diacylglycerol and inositol triphosphate are the end-products of this process. Inositol triphosphate releases Ca^{2+} from intracellular pools, while diacylglycerol activates the protein kinase C. Both are involved to-

gether in the phosphorylation of endogenous proteins and result in an increase of muscle tone. In this way, serotonin may be involved in the development of bloating: increased muscle tone results in a decreased number of phasic contractions and a dyscoördination between ante- and retrograde contractions. These findings may explain why intravenous (IV) administration of serotonin (6 mg/kg expressed as the base) in sheep results in a short-lived contraction followed by a sustained and long-lasting increase in muscle tone and a concomitant inhibition of the extrinsic reticulo-rumen contractions. The short-lived contraction is probably due to presynaptic neural stimulation (on presynaptic cholinergic neurons) and the long-lasting increase of muscle tone seems to be due to a direct smooth muscle action. The corresponding blockades produced by atropine (0.05 mg/kg), serotonin$_2$-receptor antagonists (Ketanserin) and the chemical sympathectomy suggest the involvement of a peripheral cholinergic mechanism in the initial contractile response and a direct muscle action in the second response (219). The first component can be blocked by morphine, the second persists in both reticulum and rumen after atropine or morphine. The increase in muscle tone of the rumen due to serotonin (5 mg/kg) under atropine blockade is inhibited by serotonin$_2$ blockers. In sheep it has also been shown that a specific serotonin$_2$ (S_2)-receptor antagonist at a dose level of 0.05 mg/kg significantly increases the volume of eructated gas when the intraruminal pressure is maintained at 2 mm Hg and increases the frequency of primary and secondary contractions of the rumen with 41.5 and 24.3 % respectively. Also spontaneous bloating can be prevented or treated with a serotonin$_2$ antagonist (see 575).

At an intraruminal pressure of 4 mm Hg, Ketanserin (O.1 mg/kg) significantly increases the volume of eructated gas and also the frequency of both primary (23.6 %) and secondary contractions (23.7 %) (575).

In conclusion, all these experimental data point to the supposition that serotonin is indeed involved in the subtile mechanism regulating the tone in the ruminant stomach of the sheep.

Vasoactive Intestinal Polypeptide (VIP)

1. Review of the literature

(see Addendum/ Part III/ pags. 115-123)

2. Conclusions from the literature

In the peripheral nervous system VIP occurs in sympathetic ganglia, vagal nerves and autonomic nerves supplying exocrine glands, the walls of blood vessels, non-vascular smooth muscle cells and in ganglionic neural cell bodies that provide the "intrinsic" VIP innervation of the gut (see 110; 660; see 660; see Addendum).
The gut contains large quantities of VIP throughout its entire length. Most, if not all, VIP in the gastrointestinal tract is contained within the nervous tissue. A substantial fraction of these VIP-ergic nerves is probably intrinsic to the gut wall. Thus, apparently VIP fibres constitute quantitatively the (most) important peptidergic nerve population. In addition, VIP-IR epithelial cells have been demonstrated in the esophagus of the cat and human foetus and in the small and large intestine of the quail, dog, pig and baboon.
Numerous VIP positive perikarya seem to be present in the gastrointestinal wall, where they are found in both plexuses. These neurons innervate all layers of the gut. Varicose VIP fibres have been seen to ramify extensively around non-immunoreactive nerve cell bodies in Auerbach's and Meissner's plexuses.
VIP-IR nerve fibres are abundant in the muscular coat and especially in regions having a sphincter function. However, there seems to be a difference in the density of the VIP-ergic innervation of both muscle layers, it being very rich in the circular muscle layer and sparse (or absent in some species) in the longitudinal layer.
In the mucosal layer VIP nerves form a network around the gastric and intestinal glands. In the small intestine they extend into the core of the villi where small terminals have been demonstrated just beneath the epithelium. This morphological arrangement is suggestive of the control of secretory and/or absorptive functions by VIP nerves.
A rich perivascular VIP-ergic innervation has also been found in the wall of intramural blood vessels.
VIP is released from its principal source under a variety of experimental conditions i. e. electrical stimulation of the vagal and pelvic nerves, transmural electrical stimulation, intraluminal perfusion of the intestine with various chemicals, gastric distention, mechanical stimulation of the intestinal mucosa, intestinal ischaemia, IV injection of ACh etc.
The biological actions of VIP include a potent relaxant effect (being slow both in onset and recovery) on the gut muscle especially sphincters, a potent vasodilation effect promoting blood flow to splanchnic and other organs, a suppression of the gastric acid secretion and a stimulation of the intestinal secretory activity (water & ions) which is most probably mediated by an increase in the intracellular cyclic AMP.
Finally, the fact that VIP-IR fibres are numerous beneath the intestinal epithelium, around perikarya in both plexuses, and in autonomic ganglia outside the gut, as well as the fact that VIP stimulates the ACh release from myenteric neurons suggests additional roles for VIP including a neuromodulatory one which may be the coordination of the functions of both plexuses (393; 792; 809). In addition, intrinsic VIP-containing neurons found throughout the gut may also be interneurons, since they have been shown to innervate nerve cells in both Meissner's and Auerbach's plexuses.

3. Radioimmunological Approach

(see Addendum/ Part III/ table 10)

The results from the radioimmunological assay show a number of features:

- VIP is present in all the segments of the ruminant stomach studied.
- VIP concentration is always higher in the muscular coat as compared with the mucosa, suggesting that in the ruminant stomach wall most of the VIP is pooled in the tunica muscularis.
- With exception of the OMA, over the whole ruminant stomach comparable VIP concentrations seem to be present in the tunica muscularis .
- In contrast to data from the literature, there is, at least under our experimental conditions, no evidence for a higher VIP concentration in the tunica muscularis of sphincters (PYL) or sphincter-like regions (RG).
- The mucosa contains as a rule only small quantities of VIP. However, in the glandular stomach the VIP concentration tends to be higher than in the aglandular part.

This finding may, at least in part, be explained on morphological grounds. Indeed, in the glandular stomach the tunica mucosa is relatively thick and contains a well developed lamina propria and lamina muscularis mucosae. Additionally, one has to realize that "the mucosa" in the stripped specimens also included the tunica submucosa. In the glandular part of the ruminant stomach this tunica contains a considerable number of blood vessels and a well developed neural network (Meissner's plexus). In all these structures a distinct VIP-IR has been observed.
The higher VIP concentration in the pyloric mucosa, as compared with the rest of the gastric mucosa, may partly be explained by the fact that in this region the submucosa holds a relative large number of smooth muscle bundles which are derived from the internal circular muscle layer. These bundles were found to be innervated by numerous VIP-IR nerve fibres.
Nevertheless, in the mucosa of some forestomach segments (RG, OMA) relative high VIP concentrations were also noticed. This finding may likewise have a morphological basis. In the reticular groove the muscularis mucosae, which is continuous with that of the esophagus, is especially prominent along the margins of the sulcus. In the OMA, on the other hand, the mucosa is characterized by numerous leaves. The thick muscularis mucosae forms within the principal laminae a double smooth muscle layer. In addition, from the innermost part of the circular muscle layer small muscle bundles split off and ascend into the omasal folds. Thus, each omasal leave may contain a considerable number of smooth muscle fibres. In the immunohistochemical study the muscularis mucosae of both the esophagus and OMA has been seen to be innervated by numerous VIP-IR nerves.

4. Immunohistochemical Approach

(see Addendum/ Part III/ photos 74-96; tables 11-12)

From the immunohistochemical approach one may reach the following conclusions:

- The results of the immunohistochemical study are in fairly good agreement with the quantitative data obtained from the RIA.
- VIP-IR was always found in all segments of the ruminant stomach studied.
- VIP-IR was exclusively encountered within the nervous tissue.
- VIP-IR nerves are far less abundant than the NSE-IR fibres.
- In paraffin sections in the longitudinal muscle layer VIP-IR varicose nerve fibres are sparse, or absent. In cryostat sections, in contrast, a clear and relative dense intramuscular VIP-IR nervous network was always seen both in the adult sheep and in the foetus. Additionally, the number of intramuscular VIP-IR fibres seems to be in proportion to the thickness of the longitudinal muscle layer and as a consequence a denser VIP-ergic innervation of this layer was always found in the RET compared with the rest of the forestomach. However, no specific distribution pattern within the ruminant stomach wall could be distinguished.
- In paraffin sections varicose VIP-IR nerve fibres were clearly seen in Auerbach's plexus, where they ramify around some non-immunoreactive perikarya. This picture was particularly evident in the OMA. Sporadically VIP-IR neurons were encountered.
Comparable results were seen in cryostat sections, although the VIP-IR was more pronounced. VIP-IR nerve cell bodies were regularly noticed in the foetal ruminant stomach principally in the glandular part of the stomach, while in the forestomach compartments they were rather rarely observed.
- By far the densest VIP-IR network was always found in the inner (circular) muscle layer (especially in the RG, OMA, ABO, AP). Numerous VIP-IR fibres run parallel to the smooth muscle cells, following the delicate connective tissue strands separating the muscular bundles.
- In paraffin sections there was no clear evidence for a different distribution pattern of VIP containing nerve fibres in the inner circular layer of sphincter regions.
In cryostat sections, however, a very dense intramuscular VIP-ergic innervation was observed at the level of the sphincters (RG, ORO, PYL). The most striking example was found in the lips of the RG where abundant medium to small nerve fibres were noticed between and within the smooth muscle bundles respectively. Along a single nerve fibre running within a muscle bundle numerous varicosities were encountered. Thus, from cryostat sections it may be argued that in the sphincter regions the circular muscle layer is indeed characterized by denser VIP- ergic innervation.
- The VIP-ergic innervation of the submucosa is not uniform.
In paraffin sections obtained from the adult sheep a clear reaction was only noticed in the glandular part of the stomach. Especially in Meissner's plexus VIP-IR nerve fibres, as they do in Auerbach's plexus, ramify

around non-immunoreactive nerve cell bodies. Immunoreactive perikarya were not seen. In paraffin sections, but more frequently in cryostat sections, of the ABO and AP small ganglia (3 to 5 neurons) were frequently found between the base of the gastric glands and the muscularis mucosae. In these ganglia VIP containing fibres were also seen to surround some of the non-immunoreactive neurons. In the aglandular part of the stomach in contrast immunoreactivity against VIP could hardly be ascertained.
In cryostat sections, on the other hand, single varicose nerve fibres were sometimes encountered in the submucosa of the forestomach, although a real VIP-IR submucosal plexus was never observed. In the glandular part numerous VIP-IR nerves were normally found at the inner surface of the muscularis mucosae. Meissner's ganglia were characterized by several distinct VIP-IR fibres surrounding non-immunoreactive neurons. VIP-IR nerve cell bodies were, however, not observed.
In paraffin sections of the foetal ruminant stomach no VIP-IR was seen in any of the segments studied.
In cryostat sections, however, a VIP-IR nerve plexus could be detected in nearly all submucosal protrusions. This plexus was found to be in proportion to the extent of the submucosal fold. Thus, the plexus is delicate in the small submucosal protrusions and particularly prominent in the larger protrusions, where in addition groups of smooth muscles -innervated by fine VIP-IR nerves- are normally found.
A VIP-ergic innervation of the muscularis mucosae was particularly evident in those segments where this smooth muscle layer is well developed i. e. in the RG, OMA and the glandular stomach as a whole, while VIP-IR is of course absent in those segments where the muscularis mucosae is lacking (RDS and RVS).
In the ganglia found between the basis of the gastric glands and the muscularis mucosae VIP-IR nerve fibres were seen to surround non-immunoreactive perikarya.
Mainly in cryostat sections numerous delicate VIP-IR fibres could be seen in the lamina propria of the glandular stomach. VIP-containing nerve were found around the gastric glands. Some fibres run towards the luminal epithelium.
Finally, VIP-IR fibres are regular seen to form a perivascular plexus around the larger intramural blood vessels.

***Conclusions** from this study :*
Based upon the findings of the radioimmunological and immunohistochemical study it may be stated that the overall picture of the intramural VIP-ergic innervation of the ruminant stomach strongly resembles the observations made in different animals, including man, by other investigators (see Review literature VIP).
Although basic information concerning the functional significance of VIP on some ruminant stomach functions is still very scanty or lacking, based upon the above-mentioned morphological similarities it is tempting to speculate on a similarity in functions too. Beside its profound effect on gastrointestinal motility (relaxation) and the intestinal secretion/absorption process, VIP has an inhibitory effect on the gastric acid secretion in various animals and in man. This may fit in with the dense VIP-ergic innervation of the gastric glands.
Anatomically the ruminant stomach is a complex and highly compartmentalized intestinal segment in which specific functions may be correlated to specific compartments. Thus, it may be possible that in the ruminant stomach VIP is, at least partly, involved in the control and coordination of motility and in the critical control of the secretion/absorption process and intramural blood flow. Consequently, there is at least morphological evidence that VIP may be an essential link in the normal functioning of this by far most important part of the ruminant gastrointestinal tract.
In order to test this hypothesis the influence of VIP on the tone of the ruminant stomach wall was studied in in vitro experiments.

5. Functional Approach and Comments

Functional approach

(see Addendum/ Part III/ figs. 33-36)

In the functional approach of this study VIP showed a remarkable effect on the smooth muscle tone of the ruminant stomach of the sheep. Both the longitudinal and circular muscles of RDS (mainly the circular muscle) and RVS (mainly the longitudinal muscle) showed a clear decrease of muscle tone at 10^{-7} M. The longitudinal muscles of OMA, ABO and AP and the circular muscles of AP did not respond at the dose levels used, while the circular muscles of OMA and ABO showed some decrease. In the LES an initial increase (10^{-10} M) was followed by a steep decrease at 10^{-8} M. The RG also showed a clear decrease at 10^{-7} M. These data indicate that the effect of VIP on LES is similar to the observations made in other animals and man. Thus, VIP is probably involved in the regulation of LES tone in sheep. In the rumen (RDS and RVS) this peptide may also play a role in the control of muscle tone (more in longitudinal than in circular muscles). Also the circular muscles of the RG show a clear response.

Comments

In this study vasoactive intestinal polypeptide-immunoreactivity is exclusively found in the intramural nervous elements i.e. a dense intramuscular vasoactive intestinal polypeptide-immunoreactive nervous network, as a dense vasoactive intestinal polypeptidergic innervation of the sphincters, as vasoactive intestinal polypeptide-immunoreactive perikarya in Auerbach's plexus etc. (see Addendum). This intramural distribution pattern of vasoactive intestinal polypeptide-immunoreactivity is in agreement with the data obtained from other species in the literature. Thus, it is tempting to speculate on the fact that vasoactive intestinal polypeptide has a comparable functional impact (muscle relaxation, stimulation of the secretion, vasodilation) on the ruminant stomach musculature of the sheep.
In the cat strong evidence has been obtained that certain non-adrenergic, non-cholinergic gastrointestinal responses are attributed to the release of vasoactive intestinal polypeptide. This peptide is indeed released from a population of postganglionic parasympathetic neurons and this release is unaffected by splanchnic nerve stimulation (188; see 188). Throughout the gut vasoactive intestinal polypeptide occurs in nerve fibres originating from vasoactive intestinal polypeptidergic myenteric neurons that project in a caudal direction to innervate the circular muscles (see 188; see 239; 248; see 248; 325; see 514). Moreover, numerous vasoactive intestinal polypeptidergic fibres occur at the level of the sphincters (lower esophageal sphincter, pylorus), in the circular muscle layer, around intramural neurons, around intestinal blood vessels and in the lamina propria where they run immediately beneath the epithelium (see 188; see 239; see 325). Based upon this distribution pattern, the potential targets for vasoactive intestinal polypeptide-immunoreactive fibres seem to be the epithelial cells, the intestinal glands, the blood vessels, the smooth muscles and the neurons (325; see 325; 606). Vasoactive intestinal polypeptide-immunoreactive nerves form the main non-adrenergic inhibitory neural circuit in the gut (73; see 73) and therefore are, most probably, involved in the regulation of various intestinal functions i. e. motility, sphincter action (relaxation), secretory and absorptive processes, and the local blood flow (77; see 188; see 239; see 243; see 325; see 571; see 605; 723; see 723). Vasoactive intestinal polypeptide has been established as the inhibitory neurotransmitter of different regions of the gut inducing in this way relaxation of the intestinal smooth muscle and sphincters (lower esophageal sphincter, pylorus etc.). This potent relaxant effect of vasoactive intestinal polypeptide is slow both in onset and recovery (108; see 188; 202; see 239; 325; see 325; 605; 629; 723; see 723). Such slow relaxations to this peptide have likewise been reported in the canine gall bladder, stomach and small intestine (see 108). In addition, vasoactive intestinal polypeptide has also direct inhibitory actions on some smooth muscle preparations. Thus, there is evidence that vasoactive intestinal polypeptide is a neurotransmitter candidate involved in the descending inhibitory component of the peristaltic reflex mediating descending inhibition in the gut (see 121; see 239;248; see 248; see 379; 616). Nevertheless, an excitatory action of vasoactive intestinal polypeptide on some intestinal smooth muscle has been reported (see 571).
The stomach has two important functions: storage and grinding as well as mixing of contents. The former is accomplished in the fundus and corpus, while in ruminants this occurs mainly in the forestomach and to a limited extent in the fundus of the abomasum. In monogastric mammals mixing of contents is per-

formed by the fundus and the antrum,while in polygastric animals this function is likewise for the largest part completed in the forestomach. Changes in volume-capacity of the stomach and in the strength of the mixing and propulsive contractions are brought about by a complex interplay of inhibitory and excitatory neurotransmitters and by circulating gastrointestinal polypeptide hormones. In this respect non-adrenergic, non-cholinergic neurons may play an important role in the functioning of the stomach.

One of the most characteristic motor patterns of the fundus region of the stomach is receptive relaxation during swallowing. This response seems to be mediated by vagal efferents synapsing on intramural non-adrenergic, non-cholinergic neurons. Vagal nerve stimulation releases vasoactive intestinal polypeptide from intrinsic nerve terminals and, therefore, it has been suggested that vasoactive intestinal polypeptide might be a mediator of the atropine-resistant gastric relaxation (202). The vagal gastro-gastric reflex, whereby stimulation of gastric mechanoreceptors leads to relaxation of the stomach wall, also appears to be mediated by non-adrenergic, non-cholinergic inhibitory nerves. In addition, non-adrenergic inhibitory nerves are by opening sphincters, by increasing the stomach size and by expanding the intestine in front of an advancing bolus involved in inhibitory reflexes that facilitate passage of material through the alimentary canal. Thus, the non-adrenergic inhibitory neurons most probably compose "the efferent link in a cascade of descending reflexes extending from the esophagus to the anal sphincter" (see 110).

Additionally, it has also been suggested that intrinsic inhibitory neurons exert a continuous tonic suppression of gut motility, so that stimulation of the gut, leading to increased activity may be due to suppression of inhibitory nerve activity rather than to activation of excitatory nerves (see 110; see part I).

Further research has proved that vasoactive intestinal polypeptide inhibits gastric motor activity in two distinct ways. First, it uncouples electromechanical coupling during spontaneous and acetylcholine-induced electrical and mechanical activity. Secondly, it antagonizes gastric-induced increases in mechanical and electrical activity (see 110). In the gut neuronal vasoactive intestinal polypeptide has a direct excitatory effect on about half of the myenteric neurons and it stimulates acetylcholine release from the plexus (excitatory transmitter) (see 108; see 121; 188; see 188; see 239; see 325; see 379; see 571). However, vasoactive intestinal polypeptidergic neurons not only operate as motor neurons but, most probably, act as interneurons as well since they have been shown to innervate nerve cells in both Meissner's and Auerbach's plexuses (see 108; see 188; see 239; 325). Furthermore, vasoactive intestinal polypeptide has also been identified in extramural autonomic ganglia, suggesting additional roles for vasoactive intestinal polypeptidergic neurons including that of neuromodulation (see 188; see 325).

In the functional approach of this study, a distinct direct relaxant action of vasoactive intestinal polypeptide on the ruminal musculature and on the sphincters, the lower esophageal sphincter in particular, is evinced. The fact that vasoactive intestinal polypeptide has little or no direct effect on the muscular tone of those segments (abomasum, ostium reticulo-omasicum) in which a considerable tonic component and a dense vasoactive intestinal polypeptide-immunoreactive innervation is observed (see Addendum), may indicate that in these segments nerves are involved in the effects of vasoactive intestinal polypeptide upon the smooth muscle cells. In this respect it is interesting to note that other investigations have shown that vasoactive intestinal polypeptide stimulates the release of acetylcholine from myenteric neurons in the small intestine of the guinea-pig. In addition, vasoactive intestinal polypeptide/Neuron Peptide Y neurons, projecting from extramural ganglia to the gut, have been found in all species examined so far (not in the sheep). Thus, the possibility remains that in the above-mentioned segments vasoactive intestinal polypeptide-immunoreactive nerves act indirectly on the musculature through the release of acetylcholine, noradrenaline and/or Neuron Peptide Y. Furthermore, in contrast to the glandular stomach, the apparent lack of a plexus mucosus and the poorly developed submucosal plexus in the forestomach did not fit in with the peptide's stimulatory action on secretion. As a consequence, the morphological and functional data emerging from this study largely suggest that in the forestomach vasoactive intestinal polypeptide is mainly concerned with the regulation of the tone of the sphincters and with the "receptive relaxation" of the rumen, omasum and abomasum; that vasoactive intestinal polypeptide is involved in the relaxation of the lower esophageal sphincter after swallowing and that the peptide likewise plays a crucial role in the regulation of these reflexes e.g. eructation and regurgitation, which are of vital importance for the ruminant.

Substance P (Sub. P)

1. Review of the Literature

(see Addendum/ Part III/ pags. 145-156)

2. Conclusions from the Literature

- Sub. P resides in all layers of the gut.
- In the gut this peptide has a dual localization i. e. in some mucosal endocrine cells (principally in the small and large intestines) and in nerve fibres and cell bodies along almost the entire length of the gut. It is, however, least prominent in the upper proportion of the gastrointestinal tract (esophagus, stomach).
- Sub. P-IR nerve cell bodies are mainly localized in Auerbach's plexus.
- A very dense network of Sub. P-IR nerve terminals is found around the perikarya of both intramural plexuses particularly in the myenteric plexus. These fibres are also seen to extend from the plexus in between the bundles of smooth muscle cells. By so doing the circular muscle layer becomes richly innervated by Sub. P-IR nerves, whilst the longitudinal muscle layer is poorly innervated.
Some Sub. P-IR nerve fibres are also seen in the lamina propria.
- The distribution of Sub. P-IR nerves within the gut wall suggests that the smooth muscle cells and neurons are the main target organs. The contracting action of Sub. P on the non-vascular smooth muscle might be twofold: a direct and an indirect effect. The latter involves the stimulation of afferent nerve fibres of the peristaltic reflex arc, the induction of ACh release from the myenteric neurons and the release of Sub. P from non-cholinergic interneurons in the myenteric plexus.
- In the ruminant stomach of cattle Sub. P-IR are rarely observed in the subepithelial region and in the myenteric plexus. Sub. P-IR fibres are predominantly distributed in the muscle layer: they are more abundant in the reticulum than in the rumen and are numerous in the lips of the reticular groove. In addition, differences in the density of the Sub. P innervation are observed between young, weaned calf and cow.
- The majority of the Sub. P containing axons are intrinsic to the gut, though the intestine is also innervated by extrinsic Sub. P-IR axons, which run principally along the blood vessels.
- Evidence has been provided that Sub. P is present in the dorsal root of the spinal cord suggesting that it is involved in the transmission of sensory information. Thus, it may be possible that some Sub. P nerves in the gut wall are processes from sensory neurons coming from visceral receptors. Even the morphological features of Sub. P fibres in ganglia outside the gut wall may evoke the idea that these fibres may be involved in some type of visceral sensory function or special types of reflex arches.
- It has also been shown that Sub. P stimulates the secretion of intestinal, pancreatic and exocrine glands, while the intestinal absorption is reduced and the gastric acid secretion is inhibited.
- Finally, Sub. P is a potent vasodilator in different vascular beds including the splanchnic area.

3. Radioimmunological Approach

(see Addendum/ Part III/ table 13)

The data obtained from the radioimmunological study lead to the following conclusions:

- Sub. P is present in all layers of the ruminant stomach wall although in considerable lower concentrations than VIP.
- Considering the aglandular part (RG, RET, RDS, RVS, OMA) it seems that the tunica muscularis contains the highest Sub. P concentration while in the mucosal-submucosal strips relatively small quantities of Sub. P are found. Within the forestomach low Sub. P concentrations are found in the rumen (RDS and RVS), while higher values are demonstrated in those segments (RG, RET, OMA) where the lamina muscularis mucosae is a well developed.
- In the glandular stomach (ABO, AP, PYL) the reversed situation exists. The Sub. P content of the mucosa tends to be higher as compared to the muscularis and is considerably higher than in the aglandular mucosa. This finding may, at least partially, be explained by the presence of a well developed lamina muscularis mucosae, a well developed Meissner's plexus (Sub. P-IR neurons and fibres) and the presence of Sub. P-IR nerve fibres in the lamina propria. However, as compared to the other segments of the "true" stomach, more Sub. P was isolated from the muscularis of the pyloric sphincter. In this respect the pylorus follows the trend seen in the aglandular part.

4. Immunohistochemical Approach

(see Addendum/ Part III/ photos 97-113; tables 14-15)

The immunohistochemical study shows that

- The immunohistochemical observations, are in good agreement with the results obtained from the RIA
- Sub. P-IR was found in all ruminant stomach segments investigated
- Sub. P-IR was exclusively observed in the intramural neural elements
- Within the same segment Sub. P-IR was far less abundant as compared to the NSE-IR and the VIP-IR
- In paraffin sections of the ruminant stomach of the adult sheep as well as in cryostat sections of the forestomach of both adult and foetal sheep Sub. P-IR was not seen in the epithelium or in lamina propria. However, in those segments where the muscularis mucosae is entirely or partially present (RG, RET, ABO, AP, PYL), and particularly in the OMA, varicose Sub. P-IR nerve fibres were normally demonstrated in the muscle layer.
No Sub. P-IR was observed in paraffin sections of the foetal ruminant stomach in any of the segments studied. This is in contrast with the results seen in cryostat sections. In the glandular stomach of both the adult and the foetal sheep numerous, very delicate Sub. P-IR nerve fibres were observed throughout the lamina propria. They run mainly close to the luminal epithelium forming delicate loops. In addition, Sub. P-IR fibres were seen to surround non-immunoreactive perikarya of the small ganglia localized between the basis of the gastric glands and the muscularis mucosae. The muscularis mucosae of all segments studied was seen to hold various Sub. P-IR nerve fibres.
- Submucosal Sub. P-IR structures were seen with difficulty in paraffin sections from the forestomach of the adult sheep. However, in the glandular stomach, and in ABO in particular, Sub. P-IR neurons and Sub. P-IR fibres, surround non-immunoreactive neurons, were regularly seen in the submucosal plexus. On the contrary, in cryostat sections a few isolated Sub. P-IR nerves were encountered in the submucosa, while in the glandular stomach Sub. P-IR fibres as well as Sub. P-IR neurons are normally present.
In cryostat sections from the foetal forestomach a clear Sub. P-IR nervous network was observed in all submucosal protrusions. The extent of this submucosal plexus seems to be related to the size of the submucosal folds. Thus, prominent plexuses are seen in the large submucosal protrusions e. g. RET and ORO, while delicate plexuses are encountered in the smaller folds e. g. ruminal papillae.
In the submucosa of the glandular stomach of the foetus isolated Sub. P-IR nerves were easily observed. A clear and relatively dense Sub. P nervous network was seen just beneath the muscularis mucosae, while Sub. P-IR varicose nerve fibres were noticed in the submucosal ganglia sometimes containing Sub. P-IR neurons.
- In paraffin, as well as in cryostat, sections from both the adult and foetal sheep along the ruminant stomach most Sub. P-IR was principally encountered in the circular muscle layer. Although numerous medium-sized, varicose Sub. P-IR nerve fibres, running parallel to the smooth muscle fibres, were always seen, the density of the intramuscular plexus seems to be proportional to the thickness of this layer. Consequently, a particular dense intramuscular Sub. P-IR nervous network was observed at the level of the RG, ORO and PYL.
- Although not noticed in every paraffin section of the forestomach of the adult sheep, varicose Sub. P containing nerve fibres were seen to encircle non-immunoreactive neurons in Auerbach's plexus. Immunoreactive neurons were a rare observation in the myenteric plexus.
In cryostat sections from both the adult sheep and the foetus basically the same picture was observed. However, more and stronger reacting Sub. P-IR nerve structures were found.
- The longitudinal muscle layer seems to be moderately innervated by Sub. P-IR nerves. In fact, in paraffin sections of most segments of the adult sheep Sub. P-IR could hardly be demonstrated. However, in cryostat sections obtained from both groups in the longitudinal muscle layer Sub. P-IR nerve fibres, forming an intramuscular nervous network, were always found. As the density of the plexus also seems to be related to the thickness of the muscle, a prominent plexus was established in the relative thick longitudinal muscle layer of the RET, while a delicate nervous network was found in the extremely thin longitudinal muscle layer of the OMA.
Regularly very fine Sub. P immunoreactive fibres were seen in the wall of some intramural blood vessels.

Conclusions from this study:
In the adult, as well as in the foetal, sheep Sub. P could be demonstrated in the wall of every segment of the ruminant stomach studied, although clear differences in intensity and density were observed between the adult and the foetal sheep and among the different segments investigated. In addition, after examining consecutive sections of the same segment, the impression exists that the immunohistochemical reaction for Sub. P was not as pronounced as for VIP.
Generally, the microscopical picture of the distribution of Sub. P-IR arising from this study, seems to be in fairly good agreement with the picture described in other species. This similarity in morphology may, as a consequence, also refer to similar functions i. e. induction of contractions, stimulation of secretion, reduction of the absorption, vasodilation and as a sensory transmitter. Thus, it may be possible that in the ruminant stomach Sub. P is involved, together with VIP and probably with other substances, in these functions. In this respect it is interesting to note that the most striking Sub. P-IR was found in "decisive" segments (RG, RET, OMA, ABO).
In addition, it has been suggested that Sub. P may act as a sensory transmitter in primary afferent neurons. The present study shows, particularly in the foetus, Sub. P-IR nerve plexuses in all submucosal protrusions of the forestomach as well as multiple Sub. P-IR nervous elements in the lamina propria and submucosa of the glandular stomach. It may be interesting to note that in the forestomach a distinct Sub. P-IR was seen in "sensitive" areas (cfr. the dense Sub. P innervation of the lips of the RG and the prominent Sub. P nerve plexus in the claw-like papillae of the ORO). Even the proximity of Sub. P-IR fibres to the luminal epithelium in the glandular stomach may be suggestive of a sensory function of Sub. P in this area as well.
The presence of Sub. P-IR nerve fibres in the muscularis mucosae may further provide the morphological basis for the contractile effect of Sub. P on this smooth muscle layer. In addition, it has been suggested that the muscularis mucosae, by compressing the intestinal glands and by altering the unstirred layer adjacent to the villi, may affect absorption.

5. Functional Approach and Comments

(see Addendum/ Part III/ figs. 37-39)

Functional approach

The in vitro effects of Sub. P (10^{-10} to 10^{-8} M) on the muscle tone of the different parts of the ruminant stomach of the sheep was rather limited. The LES (10^{-10} M) showed a slight decrease in muscle tone, while an increase in the tone of the RG was noted. The tone of the longitudinal and circular muscles of RDS and RVS decreased significantly.
Thus, one might postulate that, since Sub. P is a mediator especially involved in sensory neurons, the variable effect observed on the longitudinal and circular muscle of the RDS and the RVS is suggestive of a modulatory function of Sub. P on the smooth muscle tone.

Comments

Although under the experimental conditions of this study no substance P-immunoreactive epithelial cells and no substance P-immunoreactive submucosal neurons are observed in the ruminant stomach and the forestomach respectively, the distribution of the intramural substance P-immunoreactive nervous elements in the ruminant stomach of the sheep basically resembles the morphological data obtained from the literature (see Addendum).
Consequently, based upon this conformity in morphology, it was thought that substance P has analogous effects on the ruminant stomach musculature as those observed on the gastrointestinal muscles from other species.
The rich supply of varicose substance P-containing nerve terminals, often surrounding ganglion cells in a basket-like manner, may indicate that these fibres play a functional role in special types of reflex arches within the ganglia (see 108; see 239; 325; 354). Substance P, released in the close vicinity of cholinergic neurons, induces an acetylcholine release and in this way effectively modulates cholinergic transmission. In the dog it was found that low concentrations of substance P only activate cholinergic neurons, whereas higher concentrations contract the smooth muscle directly. The fact that substance P, but no serotonin, is capable of facilitating the transmission of nerve impulses within the myenteric plexus further emphasizes the role of sub-

stance P in the communication between myenteric neurons (52; see 52). Consequently, some actions of substance P on the gut may be cholinergically mediated (see 605; 629).

Substance P is a stimulant of the non-vascular smooth muscles (29; 325). The peptide is present in the enteric nervous system in much lower concentrations than acetylcholine, although it is more potent in contracting the intestinal muscle (139). In general, large quantitative and qualitative differences in the contractile effects of substance P have been encountered both between different segments and layers of the alimentary canal and between different species. Qualitatively, different patterns of contractile responses to substance P can be distinguished i.e. facilitation of spontaneous phasic contractions, initiation of phasic contractions, facilitation of phasic contractions plus tonic contraction, tonic contraction with inhibition of the spontaneous phasic contractions and tonic contractions by themselves. Which of these effects is observed, depends not only on the species and on the segment and layer of gastrointestinal tract examined, but also on the concentration of substance P tested and on the recording conditions as well (52).

Electrophysiological recordings have shown that substance P can modulate excitatory junction potentials in the longitudinal muscle as well as inhibitory junction potentials in the circular muscle of the guinea-pig ileum. Practically all nerve-mediated contractions of the longitudinal muscle of the guinea-pig small intestine can be explained by the release of acetylcholine and substance P. Although the predominant effect of substance P on the longitudinal muscle of the small intestine is a tonic contraction in many species, there are species differences with respect to the effect of substance P on the spontaneous phasic contractions. In the rat, cat and pig the spontaneous phasic contractions are generally reinforced by substance P, whereas in the rabbit only low concentrations of substance P, which do not themselves cause tonic contractions, facilitate the phasic contractions. Other effects of longitudinal muscle contraction (e.g. changes in the position of stretch receptors triggering peristalsis) may also be considered. In the circular muscle of the guinea-pig small intestine substance P produces a series of neurally mediated phasic contractions superimposed on an increased tone, whereas in the circular muscle of the dog ileum this peptide causes a tonic contraction which is associated with the inhibition of spontaneous phasic contractions (52; see 52).

In the lower esophagus, substance P produces a tonic contraction of each of the three muscle layers : the muscularis mucosae (opossum, guinea-pig); the circular muscle (opossum, cat) and the longitudinal muscle (opossum). Substance P also has a stimulating effect on the lower esophageal sphincter. In the opossum and cat in vivo this effect involves the activation of nerves, whereas in the rat and guinea-pig in vitro it seems to reflect a direct action on the muscle.

From a number of studies it is evident that the muscle layers from different regions of the guinea-pig and canine stomach differ greatly in their sensitivity and type of contractile response to substance P. The response of the fundus, corpus and pylorus is predominantly a tonic contraction which is often accompanied by a suppression of spontaneous phasic contractions. However, the canine antrum, which is less sensitive to substance P than other regions of the stomach, responds with purely phasic contractions. Reinforcements or initiation of phasic contractions, in addition to a tonic contraction, is also produced by substance P in preparations of sheep, bovine and human stomach and intestine (52; see 52). Thus, substance P has been found to increase the tone and amplitude of the phasic contractions of the intestinal musculature (29) and to increase the basal colonic intraluminal pressure as well as the frequency and amplitude of phasic changes in the pressure (166; 446; 573). These effects may be exerted via two different pathways: a direct effect on the smooth muscle cells and a neurotrophic effect by which substance P is released from excitatory myenteric interneurons, modulating in this way the cholinergic and adrenergic transmission. A neurotrophic effect is further suggested by the presence of varicose substance P fibres around most ganglion cells in both the submucosal and myenteric plexuses and by its role as an excitatory transmitter in motor neurons of the gut. It further involves stimulation of afferent nerve fibres in the peristaltic reflex arch (108; see 108; see 239; 325; see 605; 616; 629; 662; 723).

The ascending excitatory and the descending inhibitory reflexes of peristalsis are mediated by an ascending excitatory and a descending inhibitory nervous pathway, respectively. A third pathway (excitatory and descending) enables excitation in the enteric nervous system to spread caudally (see 52). Each of these pathways seems to contain intrinsic sensory neurons, afferent fibres, an undetermined number of interneurons and final efferent neurons to the intestinal muscle (52). There is ample evidence to suggest that substance P is involved in both the segmental and peristaltic movements of the alimentary canal. This is because

substance P is contained in a considerable proportion of the enteric neurons, because it is released on stimulation of these neurons, because it can activate both other neurons and intestinal smooth muscle cells and because there seems to be an arrangement of enteric substance P neurons which is capable of maintaining propulsive motility after cholinergic transmission through muscarinic cholinoreceptors has been blocked (52; see 52). In addition, it is assumed that acetylcholine and substance P coexist in and are co-released from the same neurons and that both transmitters control the release of each other (52; see 52). Moreover, noradrenaline released from sympathetic nerve endings will inhibit not only cholinergic transmission in the enteric plexus but possibly also transmission mediated by substance P (see 52). In theory this peptide could thus be involved in the pathogenesis of gastrointestinal atonia in a dual way i.e. as a transmitter of extrinsic sensory neurons activated by irritation, trauma or infection of the bowel and as an enteric transmitter, the release of which is inhibited by the reflexly activated sympathetic system (52).

In this study however, substance P has a rather limited direct effect on the tone of the ruminant stomach musculature and its sphincters (see Addendum). Moreover, contrary to our expectations, substance P decreases the tone of the ruminal smooth muscles preparations. Nevertheless, it must be stressed that in other species (guinea-pig, dog) the smooth muscle of the different gastric segments differs considerably in its sensitivity and tonic contractile response to substance P. Additionally, coexistence of substance P with acetylcholine, serotonin, calcitonin gene related peptide, somatostatin and met-Enkephalin has been observed in the enteric nervous system of several other species. Whether this is also the case in the sheep is, until now, not elucidated. However, it remains possible that likewise in the sheep substance P has primarily an indirect modulatory effect on the control of the muscular tone in the ruminant stomach complex.

Moreover, substance P has been discovered in spinal ganglion cells. Thus, it may be an excitatory neurotransmitter involved in reflexes mediated via primary sensory neurons (see 73; 88; see 88; see 108; see 239; 325; see 356; 616). Hence, peripheral substance P nerve fibres in the gut may represent the branches of primary sensory neurons (354). Capsaicin releases substance P from sensory terminals. Consequently, depletion of the peptide, after application of this drug provides a simple marker for substance P-containing sensory fibres (see 73; see 239; see 519). In the gut capsaicin does not alter the substance P content suggesting, in consequence, that there are no substance P-containing sensory fibres here (see 73). However, recently a number of capsaicin-sensitive substance P fibres were observed around intestinal blood vessels (73; 519; see 519) and in the submucosal plexus of the guinea-pig intestine. These capsaicin-sensitive nerve fibres, most probably, originated from the perivascular substance P plexuses (519). Thus, it may be possible that in the gut substance P is likewise localized in sensory fibres probably involved in peristalsis (see 108; see 239; 325; 354; see 605; 629). Since substance P modulates the cholinergic release of enteric neurons and since there is evidence that the peptide is a neurotransmitter in afferent or sensory fibres in the gut as well, one might postulate that the peptide is an excitatory transmitter of interneurons with their peripheral branches located in the mucosa and lamina propria. This idea is further supported by the presence of substance P-immunoreactivity in Meissner's plexus (616; 629) and by the fact that substance P has been observed in autonomic ganglia where it was shown to increase the excitability of the prevertebral neurons (88; see 88; see 356; 616). Finally, evidence is accumulating that the peptide is also involved in peripheral nociception. In addition, immunohistochemical studies show that substance P-like immunoreactivity is present in sensory neurons with thin unmyelinated/myelinated axons forming free nerve endings in the connective tissue close to or even within the gut epithelium. The possibility therefore exists that the intraganglionic substance P nerve terminals may be involved in nociceptive mechanisms at the level of the gastrointestinal tract (see 73; 88; see 88; 354; see 356; 616).

Since in this study animals are not pretreated with capsaicin and since substance P-immunoreactive nerve fibres are not seen in the mucosa and only to a limited extent in the submucosa of the foetal ruminant stomach, there is as yet no solid morphological basis to postulate that in the ruminant stomach of the sheep substance P is present in intramural sensory fibres or that substance P is, contrary to other species, directly involved in nociceptive mechanisms and/or in the control of the secretion/absorption process within the forestomach.

III. 5. GENERAL CONCLUSIONS AND SUMMARY

General Conclusions

From this multidisciplinary study, concerning the presence, specific distribution and functional significance of the classical (acetylcholine and noradrenaline) and putative (serotonin, vasoactive intestinal polypeptide and Substance P) neurotransmitters in the enteric nervous system, it may be concluded that :

Morphological Approach

(see Addendum/ Part III/ fig. 40)

After in toto staining for acetylcholinesterase no principal differences in the basic morphology of Auerbach's plexus have been seen in the different segments of the ruminant stomach of the foetal sheep. Nerve bundles of a varying diameter form, together with numerous irregular ganglia, situated at the transsection points of these nerve bundles an uninterrupted nervous network along the entire length of the ruminant stomach complex. Nevertheless, clear differences in the width of the meshes, the thickness of the nerve bundles as well as the size and the shape of the myenteric ganglia were found. In the forestomach the densest network was always noticed in the omasum, followed by the reticulum and the rumen. Along the glandular stomach the density of the myenteric plexus increased from the cardia (ostium omaso-abomasicum) to reach a maximum at the pyloric level. In addition, differences in the intensity of the AChE staining of the ganglion cells was frequently observed.

The glyoxylic acid induced fluorescence applied to whole mount preparations of the ruminant stomach of adult sheep, revealed no noradrenergic nerve cell bodies. In contrast, numerous noradrenergic nerve terminals were encountered within the interganglionic nerve bundles and within the myenteric ganglia where they surrounded non-fluorescent nerve cell bodies.
As a rule, few noradrenergic nerve fibres were seen within the muscular coat. As it may be argued from this morphological data that the myenteric ganglia represent, most probably, the main target organ for the noradrenergic fibres. A correlation between the density of Auerbach's plexus and the density of the noradrenergic innervation of the ruminant stomach may exist. Hence, the densest noradrenergic innervation in the forestomach was seen in the omasum and in the glandular stomach at the level of the pylorus. The results of the histochemical approach were to a large extent confirmed and completed by the immunohistochemical study. Indeed, in all parts of the ruminant stomach dopamine-B-hydroxylase-immunoreactive fibres were encountered in Auerbach's plexus around non-immunoreactive perikarya, in some fibres in the smooth muscle layers (except in the sphincters where numerous dopamine-B-hydroxylase-immunoreactive fibres were seen). Finally, in each layer of the wall perivascular dopamine-B-hydroxylase-immunoreactive plexuses were detected.
Neither in foetuses nor in the adult sheep were any dopamine-B-hydroxylase-immunoreactive fibres observed in the tunica mucosa of the forestomach. In the mucosa of the glandular stomach, on the other hand, dopamine-B-hydroxylase-immunoreactive was exclusively seen around blood vessels (arterioles).
In the submucosa of the ruminant stomach complex of both foetal and adult sheep dopamine-B-hydroxylase-immunoreactivity was principally seen around the submucosal blood vessels. In the glandular stomach a few dopamine-B-hydroxylase-immunoreactive fibres sometimes supplied, in addition, Meissner's plexus. Isolated dopamine-B-hydroxylase-immunoreactive fibres, running in the submucosa, were never observed.
In the muscular coat dopamine-B-hydroxylase-immunoreactive varicose fibres were regularly noted. As a rule, most intramuscular dopamine-B-hydroxylase-immunoreactive nerve fibres were discovered in the circular muscle layer and this was especially the case in the sphincteric regions. The noradrenergic innervation of the longitudinal muscle layer was generally sparse, although in those segments where the longitudinal layer is thickened (cfr. reticulum) various intramuscular dopamine-B-hydroxylase-immunoreactive nerve fibres were seen. In consequence, it may be maintained that in both muscle layers the density of the intramuscular dopamine-B-hydroxylase-immunoreactive nervous network was in proportion to the thickness of the musculature.

Except in the small intestine of the foetal sheep where intramural serotonin-immunoreactive nervous elements supplied the stomach wall, serotonin-immunoreactivity was exclusively seen in the epithelium. This finding may be due to the fact that the intramural

nervous elements of the ruminant stomach of the sheep have, in contrast to the small intestine and gastrointestinal tract of other species, no serotonin, or to the fact that they contain a very low concentration (below the detection limit of the immunohistochemistry) of serotonin or, finally, to the fact that in this study no drugs blocking the transport of serotonin were used.
In the ruminant stomach of the foetus serotonin-immunoreactive epithelial cells were distinguished throughout the whole complex. In the multilayered epithelium of the forestomach serotonin-immunoreactive cells were only encountered in the basal (germinative) layer (most serotonin-immunoreactive cells seem to be present in the omasum), while in the glandular part of the stomach these cells were observed along the epithelium but preferentially in the basal part of the gastric glands.
In the adult sheep serotonin-immunoreactive epithelial cells were exclusively established in the glandular stomach, principally in the fundus of the gastric glands.

Vasoactive intestinal polypeptide-immunoreactivity was exclusively noticed in the intramural nervous elements along the enteric ruminant stomach. However, clear differences in the density of the vasoactive intestinal polypeptide-ergic innervation could be found.
An intramuscular vasoactive intestinal polypeptide-immunoreactive nervous network was normally seen in the longitudinal muscle layer, especially in those segments (reticulum) where the outer muscle layer is relatively well developed. Some vasoactive intestinal polypeptide-immunoreactive neurons and numerous vasoactive intestinal polypeptide-immunoreactive fibres ramifying around some non-immunoreactive perikarya were noticed in Auerbach's plexus. Throughout the whole complex the densest vasoactive intestinal polypeptide-ergic innervation was always observed in the circular muscle layer, but in the sphincteric and sphincter-like regions in particular. Clear differences between the adult and foetal sheep were encountered in the vasoactive intestinal polypeptide-immunoreactive innervation of the submucosa. In the adult sheep submucosal vasoactive intestinal polypeptide-immunoreactive nerve fibres were sporadically distinguished in the forestomach. In the true stomach, on the contrary, vasoactive intestinal polypeptide-immunoreactive nerve fibres were detected just underneath the muscularis mucosae and, within Meissner's plexus where they surround non-immunoreactive neurons. Vasoactive intestinal polypeptide-immunoreactive neurons were not seen in the submucosal plexus.
In the foetus a distinct vasoactive intestinal polypeptide-immunoreactive plexus supplied nearly every submucosal protrusion. The extent of this plexus seems to be in proportion to the extent of the submucosal fold. In addition, the isolated groups of smooth muscle cells situated in the largest protrusions were characterized by a relatively dense intramuscular vasoactive intestinal polypeptide-ergic nervous network.
As a rule, the mucosa of the forestomach holds no or few vasoactive intestinal polypeptide-immunoreactive nervous elements. However, a particularly evident vasoactive intestinal polypeptide-ergic innervation of the muscularis mucosae was discovered in those segments where this smooth muscle layer is relatively thick (omasum, glandular stomach). In the rest of the tunica mucosa no vasoactive intestinal polypeptide-immunoreactive elements could be found. In the glandular part of the stomach ganglia, situated between the base of the gastric glands and the muscularis mucosae, have various vasoactive intestinal polypeptide-immunoreactive nerve fibres surrounding non-vasoactive intestinal polypeptide-immunoreactive perikarya. In addition, numerous delicate vasoactive intestinal polypeptide-immunoreactive nerves were seen to run in the lamina propria and some of them reached the surface epithelium. In all layers of the wall vasoactive intestinal polypeptide-ergic nerve fibres formed a perivascular plexus around the arteries.
The results of the immunohistochemical approach were largely supported by the results of the radioimmunoassay. However, this quantitative method gave, in contrast to the immunohistochemical results, no evidence for a higher VIP content in the sphincteric and sphincter-like regions of the ruminant stomach complex.

Substance P, present in all segments of the ruminant stomach, was only perceived in the intramural nervous elements. Both radioimmunoassay and immunohistochemistry proved that this peptide is far less abundant in the ruminant stomach complex as compared to vasoactive intestinal polypeptide.
Neither in the foetus nor in the adult sheep was substance P-immunoreactivity seen in the tunica mucosa of the forestomach, apart from the muscularis mucosae. In the glandular stomach, however, scanty, delicate varicose substance P-immunoreactive fibres ran in the lamina propria often close to the epithelium. In the "mucosal" ganglia, lying on top of the muscularis mucosae, a few substance P-immunoreactive fibres

surrounding non-immunoreactive ganglion nerve cell bodies were discovered.
In the forestomach of the adult sheep very few substance P-immunoreactive nerve fibres supplied the submucosa. In the foetus, in contrast, a delicate substance P-immunoreactive nervous network was seen in all submucosal folds.
In the glandular stomach of both foetal and adult sheep various substance P-immunoreactive nerve terminals were normally distinguished in the submucosa and in the submucosal plexus, which also contained substance P-immunoreactive neurons. In both foetuses and adults the densest substance P-immunoreactive innervation was throughout the entire ruminant stomach complex always encountered in the circular muscle layer. The density of this intramuscular nervous network is most probably related to the thickness of the muscle layer to be innervated. In consequence, a particularly dense substance P-immunoreactive nervous network was detected in the sphincter areas (reticular groove, ostium reticulo-omasicum and pylorus).
Several substance P-immunoreactive nerve terminals, surrounding non-immunoreactive perikarya, as well as a few substance P-immunoreactive neurons were always observed in Auerbach's plexus in all segments investigated. The longitudinal muscle layer, finally, is characterized by a moderate substance P-immunoreactive innervation but the density of the intramural substance P-immunoreactive nervous network increased with the increased thickness of the smooth muscle layer (cfr. reticulum).
Perivascular substance P-immunoreactive nerve fibres represented a constant finding throughout all layers of the ruminant stomach wall.

Functional Approach

(see Addendum/ Part III/ figs. 41-43)

Sodium nitroprusside, the receptor-linked calcium channel blocker demonstrates the existence of a considerable tone in the longitudinal muscle layer of the rumen (ruminal dorsal sac and ruminal ventral sac) a moderate tone in the circular muscle layer of the ruminal ventral sac, abomasum and ostium reticulo-omasicum, a negligible tonic component in the lower esophageal sphincter, in both muscle layers of the reticulum, in the circular muscle layer of the ruminal dorsal sac, in the longitudinal muscle layer of the abomasum and in the circular muscle layer of the antrum pyloricum. Under the experimental conditions of this study, finally, no muscle tone could be demonstrated in the reticular groove, omasum and pylorus. These findings are in full agreement with the earlier discussed functional specialization within the ruminant stomach complex.

Despite the clear morphological evidence for a dense acetylcholinesterase innervation of all compartments of the ruminant stomach, the in vitro excitatory effect of acetylcholine on the smooth muscle tone of the ruminant stomach musculature seems limited to the ruminal muscles and the circular muscle layer of the omasum. It may be postulated that in the former compartment acetylcholine is involved in the control of the flow of ingesta, induced by phasic contractions. In the latter segment acetylcholine may be involved in the squeezing and grinding of food.

Exogenously applied noradrenaline seems, to a limited extent, directly involved in the control of the smooth muscle tone of the ruminant stomach. Indeed, noradrenaline increased the tone of the longitudinal muscle and decreased the tone of the circular muscle of the ruminal dorsal sac and ruminal ventral sac, respectively. Since the direct effect of exogenous noradrenaline is principally mediated through a- adrenoreceptors, which are located on the smooth muscle cell membrane, it is most probable that the excitatory effects on the ruminal ventral sac and the inhibitory effect on the ruminal dorsal sac are both mediated through a-adrenoreceptors. Thus, just like in other species the functional significance of a- adrenoreceptors seems to depend largely on the topographical organization of these receptors along the ruminant stomach complex.

Serotonin seems mainly involved in the control of the smooth muscle tone of the rumen (increased tone) and the omasum (decreased tone). The other segments investigated were insensitive or less sensitive to the direct action of serotonin on the smooth muscle cells. This may indicate that only in the rumen serotonin increased the tone through receptor-linked channels in the smooth muscle membrane. The inhibitory effect of serotonin on the tone of the circular muscle of the omasum is difficult to explain. Possibly, serotonin exerts its direct effect on the omasal muscles through an as yet unknown receptor-type.

Under the experimental conditions of this study vasoactive intestinal polypeptide was found to decrease significantly the tone of the ruminal musculature and the circular muscle of the sphincters (lower esophageal sphincter, reticular groove). The response of the

circular muscle layer of the omasum and abomasum was limited, while the longitudinal muscle layer of the omasum, abomasum and antrum pyloricum did not react to the exogenously applied vasoactive intestinal polypeptide at the dose levels used. Thus, vasoactive intestinal polypeptide has a direct inhibitory effect on the tone of the above-mentioned musculature and in this way it may, just like in other species, induce relaxation of the muscle of some ruminant stomach compartments and the sphincters in particular. Storage (receptive relaxation) mixing, grinding as well as controlled and coordinated transport of the food within the ruminant stomach is of crucial importance for the normal functioning of this gastrointestinal segment. Thus, it may be postulated that vasoactive intestinal polypeptide is an important element in the complex interplay of the different neurotransmitters that regulate the spectrum of the various motility patterns managing the ruminant stomach of the sheep.

In the in vitro experiments only a limited effect of substance P on the muscle tone of the ruminant stomach was demonstrated. The sphincters reacted differently i.e. a slight decrease in the muscle tone of the lower esophageal sphincter and a slight increase in the muscle tone of the reticular groove were observed. In the rumen, in contrast, substance P has an inhibitory effect on the tone. These findings contrast with the results obtained in other species in which substance P was found to have principally a direct stimulatory effect on the tone and the amplitude of the phasic contractions of the intestinal musculature (in this respect substance P is more potent than acetylcholine). However, the different reactions in the different muscle layers of the various ruminant stomach segments may refer to the large quantitative and qualitative interspecies (e.g. rat, guinea-pig, cat, dog, opossum) differences in the contractile effect of substance P that have been observed between the different layers and segments of the alimentary canal. In consequence, the results from this study indicate that substance P most probably has a rather neurotrophic effect in that it modulates the interneuronal transmission, principally in the myenteric plexus.

Summary

In this study the presence and distribution of different neurotransmitters/ neuromodulators in the intrinsic nervous system of the ruminant stomach was studied using histochemistry, radioimmunoassay and immunohistochemistry.

Acetylcholine, biogenic amines (noradrenaline, serotonin) and regulatory peptides (Vasoactive Intestinal Polypeptide, Substance P) having a characteristic and region specific distribution within the stomach wall were demonstrated. Based upon these findings it is most likely that, besides the classical cholinergic and noradrenergic innervation, the ruminant stomach is also richly innervated by a complex non-adrenergic non-cholinergic nervous system. Although this system seems to be distributed throughout the length and width of the ruminant stomach, marked differences in the localization (possibly compatible with a well-defined set of actions) within the various layers of the ruminant stomach wall were noted.

Peptidergic nerves occur in both plexuses and in the smooth muscle layer (mainly the circular), implying that they take part in the regulation of smooth muscle activity (723). The peptide-containing neurons seem further to be involved in the communication between the myenteric and submucosal plexus since vasoactive intestinal polypeptide nerve fibres may run from the submucosal neurons to the myenteric plexus where they surround and innervate neurons and since a substance P pathway may run in the opposite direction. The system of peptide-containing neurons may therefore provide the morphological basis for functional coordination and autonomy of the gut (325; see 325). Furthermore, vasoactive intestinal polypeptide and substance P are found in the submucosal plexus and the mucosal layer in the glandular part of the ruminant stomach where they may be involved in the regulation of epithelial functions (secretion/absorption) (723).

Furthermore, it is generally believed that serotonin and the regulatory peptides, besides the classical neurotransmitters (acetylcholine, noradrenaline), function within the context of the autonomic nervous system. Since intramural neurons most likely modulate the responses to the extrinsic nerve impulses, intramural peptidergic neurons can conceivably mediate and modulate the responses to extrinsic nerve impulse flow according to the local needs. Due to the relative independence of the enteric nervous system from the central nervous system control, the different type of neurons in the enteric plexuses are most likely involved in local

reflex mechanisms either as sensory neurons, interneurons or efferent neurons (723).
The morphological data concerning the texture of the enteric nervous system in the ruminant stomach as established in this study broadly agree with the morphology of this system reported in other species. Thus, there is a solid morphological basis to speculate on a similarity in functions i. e. the impact of the enteric nervous system on motility, secretion/absorption and the intramural blood flow of the stomach. However, based upon the results of this study it may be claimed that the intramural innervation of the ruminant stomach seems to be far more complex than originally stated and our classical morphological and functional picture of a bipartite innervation of the ruminant stomach has to be reconsidered.
Although morphological studies may contribute significantly to our current understanding of the neuronal topography in the ruminant stomach wall, the functions of most of these substances remain to be adequately defined. However, their localization in the intramural nervous elements and endocrine cells has given rise to reasonable speculation about the role they may play (e. g. neurotransmitter, neuromodulatory, endocrine and paracrine). The in vitro experiments of this study demonstrate a manifest tonic component in these compartments (rumen, abomasum) of the ruminant stomach which are normally, due to their topography in the ruminant stomach, continuously subjected to considerable variations in the volume of their contents and their intraluminal pressure. All neurochemical substances found in the wall of the ruminant stomach play most probably a rule in modifying and regulating the tonic type of activity and the phasic contractions. Both are of importance for the control of each compartment (e. g. adaptation of volume, sphincter activity, pump mechanism, absorption of fluid and metabolites) and the coordination between the different compartments (e. g. between reticulum and the reticulo-omasal orifice). Thus, it may be claimed that the tone, and hence indirectly all motility patterns, within the ruminant stomach is under the indirect/direct control of a subtle interplay of various neurochemical substances.
Taken into account the striking conformity in the results emerging from this study and the data from the literature it may be finally concluded that the ruminant stomach motility is managed by a large variety of neurotransmitters/modulators which control and modulate each others release and functions within the complexity of the enteric nervous system. Logically, even minor disturbances in the wheel-work of the enteric nervous system may lead to a more or less severe deregulation of the stereotyped motility patterns and in consequence in the normal functioning of the ruminant stomach.
Hopefully, the results of this study may serve as a spring-board for further morphological and functional research in order to elucidate the true significance of the different neurochemical substances for the governing of this anatomically and functionally most remarkably gastro-intestinal segment.

GENERAL SUMMARY

This morphological and functional study, related to the enteric nervous system in the ruminant stomach of the sheep, comprises three parts.

In the first two chapters of Part I some opening remarks have been given concerning the morphology and functions of the ruminant stomach and the autonomic nervous system respectively. In Chap. 3 attention has been paid to the ontogeny, the microscopic anatomy, the ultrastructure and the functional and clinical significance of the enteric nervous system in order to provide a solid theoretical basis for the second and third parts of this study.

Embryologically the enteric neuroblasts originate from well-defined levels of the neural crest. Their commitment to a neuronal lineage occurs before or during their migration to the primitive gut. Their continued capacity to divide after arrival in the gut wall together with the sequential changes in the intestinal microenvironment are responsible for the large number and the ultimate neurotransmitter phenotype of the enteric neurons in the adult species.

Depending on its topographical localization the intramural nervous network has classically been divided into different plexuses, the principal of which are the myenteric plexus (found between the muscle layers) and the submucosal plexus (lying within the connective tissue of the submucosa). Apart from the morphological differences between both plexuses considerable variations in the shape of the meshwork, the dimensions of the meshes, the size of the ganglia, the neuronal density as well as the neuronal morphology have been observed within each plexus. These variations depend on the species, the topography in the gut and the age of the animal under investigation. Nevertheless, the basic arrangement of the intramural plexuses has been found to follow essentially a similar pattern throughout the digestive tract in all species studied.

The characteristics of the enteric ganglia e.g. numerous neurons and glial elements, dense synaptic neuropil, virtual absence of an intraganglionic extracellular space and connective tissue, absence of blood vessels etc. refer to an intrinsic system with an integrative capability (cfr. central nervous system) and distinguish the enteric ganglia from the other autonomic ganglia which do not share these characteristics.

Modern techniques have revealed an abundance of putative and established neurotransmitter substances both in the enteric nerve cell bodies and their processes and in the brain (brain-gut axis). Consequently, besides the classical neurotransmitters (acetylcholine, noradrenaline) serotonin and different peptides (vasoactive intestinal polypeptide, substance P, somatostatin, enkephalins etc.) have been identified in the extrinsic and intrinsic autonomic nervous supply of the gut. Thus, the gastrointestinal tract seems to be innervated by a heterogeneous and complex autonomous nervous unit which harbours a large non-adrenergic, non-cholinergic system. This system is present throughout the entire length and width of the gut and demonstrates highly ordered projections as well as a characteristic distribution. This arrangement provides anatomical support for the existence of an enteric "minibrain" and is compatible with a well-defined set of actions. Moreover, numerous co-existence and co-release situations of neurotransmitters have been observed in the peripheral parts of the autonomic nervous system. Such a multi-messenger system provides the means for obtaining differential responses and for increasing the capacity for information transfer in the nervous system.

The considerable discrepancy between the number of enteric neurons and the number of central nerve fibres, reaching the gut, supports the physiological independence of the enteric nervous system from the central nervous system. In addition, this morphological finding provides much evidence to claim that all elements of the classical reflex arch (i.e. receptors, afferents, "central" neurons, efferents) are present within the gastrointestinal wall and further subscribes the hypothesis that the central nervous system has only a modulatory action on the enteric nervous system. The functional significance of the different elements of the intramural reflex arches is clearly demonstrated by the dysfunctions brought about when one or more of these elements are lost during the embryonic or neonatal life.

As immunostaining of neuron specific enolase allowed the simultaneous demonstration of endocrine cells and the whole nervous network of the gut, a polyclonal antiserum for neuron specific enolase was used in Part II of this study in order to gain an overall picture of the intramural neuro-endocrine system in the ruminant stomach of the sheep.

Epithelial endocrine cells have normally been observed in all compartments of the foetal stomach. In the forestomach of the adult sheep, on the contrary, these cells have never been found.

Apart from some local differences, the basic morphology of Auerbach's plexus along the whole ruminant stomach as well as the morphology of the submucosal

plexus in the glandular stomach fits within the general concept of the intramural innervation of the gut both in foetuses and adult sheep. This is, however, not the case in the forestomach where a mucosal plexus has never been observed either in the foetus or in the adult sheep. In addition, only an extremely delicate submucosal plexus has been found in the foetus, while in the adult sheep some neuron specific enolase immunoreactive fibres have been identified instead of a true submucosal plexus. These findings refer to the appearance of both plexuses in the esophagus of different species (see Part I) and provide, therefore, a solid morphological argument to claim that the forestomach develops from the distal part of the primitive foregut and not, as it was generally believed, from the gastric primordium itself.

The **third part** of this study intends a further chemical differentiation of the results emerging from Part II. Using histochemical, radioimmunological and immunohistochemical techniques the neurochemical nature of the enteric nervous system has been screened based upon the data deriving from Part I (neurochemistry of the enteric nervous system). At the same time in vitro experiments have been performed in order to elucidate the functional impact of the different substances on the muscle tone within the different compartments of the ruminant stomach.
Contrary to serotonin, which could not be demonstrated in the intramural nervous elements, the basic distribution pattern of the classical neurotransmitters (acetylcholine and noradrenaline) and of the regulatory peptides (vasoactive intestinal polypeptide, substance P) within the wall of the ruminant stomach strongly resembles the observations made in different animals. This allows us to speculate on a similarity in the effects of these neurotransmitter substances on fundamental gastrointestinal functions such as motility.
The functional approach of this study has clearly demonstrated a muscle tone (controlled by receptor-linked Ca^{2+} channels) in the "storage" compartments of the ruminant stomach i.e. in the segments which are continuously liable to considerable variations in the volume of their contents. The results emerging from this functional study largely suggest that the tone within the ruminant stomach wall is, either directly or indirectly, controlled and regulated by a complex interplay of several neurochemical substances. Since the different compartments of the ruminant stomach are anatomically and functionally linked to each other it may be argued that the different motility patterns, identified along the ruminant stomach complex, are, at least partially, controlled and coordinated by different neurotransmitters/neuromodulators.

For a more detailed synthesis of this study the reader is referred to the subsummary formulated at the end of each topic.

SAMENVATTING

Het autonome zenuwstelsel verzorgt de innervatie van de viscera. Een structureel en functioneel apart onderdeel van dit zenuwstelsel, het enterische zenuwstelsel, controleert en coördineert voor zover bekend de functies van het intestinum. Om die reden werd in deze morphologisch-functionele studie getracht de aanwezigheid, de specifieke spreiding evenals de functionele betekenis van deze neurochemische substanties (acetylcholine, noradrenaline, serotonine, substance P, vasoactive intestinal polypeptide), waarvan bij andere diersoorten bekend is dat ze én aanwezig én werkzaam zijn in het enterische zenuwstelsel, in de herkauwersmaag aan te tonen. Voor deze doelstelling werd het onderzoek in drie stappen (deel I, II, III) gepland. Het schaap werd daarbij als prototype van het herkauwende huisdier gekozen.

In het *eerste deel* van deze studie wordt in een eerste hoofdstuk het uitgangspunt en daaraan gekoppeld de objectieven van het onderzoek geformuleerd. Vervolgens werd de embryologie en de functionele morphologie van de herkauwersmaag kort toegelicht. Uit dit eerste hoofdstuk moge blijken dat herkauwers een "huis"dier-groep vormen met een zeer vergaande socio-economisch impact. Juist omwille van de buitengewone constructie van hun maagcomplex (groot volume, ver doorgedreven compartimentalisatie met daaraan gekoppeld een functionele specialisatie) en de talrijke vitale functies (bacteriële voedselafbraak, energieproduktie, eiwitsynthese, vocht-en electrolietenuitwisseling) die ze te vervullen heeft, is de herkauwersmaag aan talrijke stoornissen onderhevig en vertegenwoordigt de pathologie van de herkauwersmaag veruit één van de belangrijkste diergeneeskundige problemen.
Talrijke, zoniet alle, functies van de herkauwersmaag staan, hetzij direct, hetzij indirect, onder de controle van het enterische zenuwstelsel. Derhalve mag gesteld worden dat, naar alle waarschijnlijkheid, dit zenuwstelsel, hetzij als oorzaak, hetzij als gevolg, steeds rechtstreeks of onrechtsreeks betrokken is in nagenoeg iedere pathologie van de herkauwersmaag. Vermits het enterische zenuwstelsel een apart onderdeel van het autonome zenuwstelsel vormt, wordt in het tweede hoofdstuk de oorsprong, de basisstruktuur (die bij alle bestudeerde zoogdieren hetzelfde lijkt te zijn) en de functionele betekenis ervan toegelicht.
Deze studie handelt essentieel over het enterisch zenuwstelsel in de herkauwersmaag van het schaap. Derhalve wordt in het derde en laatste hoofdstuk van het eerste deel speciale aandacht besteed aan een aantal morphologische, functionele en klinische aspecten van de maag-darmbezenuwing op zich. Steunend op de gegevens van deze literatuurstudie werd het originele deel van deze studie (Part II en III) gepland, uitgevoerd en de bekomen morphologische en functionele resultaten geïnterpreteerd.
Embryologisch gezien, ontstaan de belangrijkste elementen (neuronen en neurogliacellen) van het enterische zenuwstelsel uit welbepaalde gebieden (vagale en lumbosacrale regio) van de neurale lijst. Cellen uit deze regio's migreren, wellicht langs vastgestelde banen, naar de primitieve darm en trekken, althans bij de kip, in de darmwand verder caudaalwaarts. Hoewel deze cellen, hetzij voor, hetzij tijdens hun migratie, hun neuronale bestemming verwerven, vertoeven ze een relatief langere periode in de darmwand alvorens hun "neuron" phenotype tot expressie te brengen. Tegelijk blijven de enterische ganglioblasten zich in de wand van de primitieve darm verder delen zelfs nadat ze reeds als neuron herkenbaar zijn. Dit gedragspatroon heeft twee heel belangrijke consequenties. Vooreerst worden er op die manier zeer veel enterische ganglioblasten, en dus toekomstige neuronen, aangemaakt en dit in zulke mate dat het uitgerijpte enterisch zenuwstelsel bij sommige onderzochte diersoorten nagenoeg evenveel neuronen als het ruggemerg bevat. Belangrijker is wellicht nog het feit dat de ganglioblasten door en tijdens hun proliferatie blootgesteld worden aan het zich voortdurend wijzigend micromilieu van de primitieve darm. Talrijke, elegante experimenten, leveren voldoende argumenten om te stellen dat de plasticiteit van het intestinale micromilieu van fundamentele belang is voor het uiteindelijke neurotransmitter phenotype van het enterische neuron. Deze hypothese wordt daarenboven kracht bijgezet door het proximo-distale verloop in de expressie van het neurotransmitter type van de enterische ganglioblasten langsheen de primitieve darm. Dit verschijnsel werd zowel bij de kip als bij de zoogdieren, waar nochtans geen proximo-distale colonisatie van de primitieve darm kon worden aangetoond, waargenomen.
Het intramurale enterische zenuwstelsel manifesteert zich als een ononderbroken driedimensioneel netwerk dat zich uitstrekt van het uiteinde van de slokdarm tot aan de interne anale sphincter. Vertrekkend van de serosa naar het darmlumen toe onderscheidt men, naargelang de topographie in de darmwand, vijf verschillende plexussen.

De plexus subserosus, die als het ware de brug vormt tussen de extrinsieke en de intrinsieke maag-darminnervatie, bestaat uit een aantal met elkaar in verbinding tredende zenuwbundels die tussen het serosale mesotheel en de longitudinale spierlaag verlopen. De weinige ganglia, die deze plexus kenmerken, worden daarenboven bij voorkeur gevonden aan de mesenteriale pool van de darm.
Tussen beide lagen (de inwendig circulaire en uitwendig longitudinale) van de spiermantel in, lokaliseert zich de myenterische of Auerbach's plexus. Deze plexus is van alle darmplexussen het sterkst uitgebouwd. Hij bestaat uit talrijke ganglia en min of meer dikke interganglionaire zenuwbundels. Van deze interganglionaire bundels splitsen zich fijnere zenuwbundels af, die zich binnen de mazen van het oorspronkelijke netwerk gaan verenigen tot een secundaire en tertiaire plexus. De topografische ligging langsheen de maag-darmtractus, de diersoort, evenals de ouderdom van het onderzochte dier bepalen mede de specifieke morphologie van deze plexus. Dit wil zeggen de vorm van het netwerk, de vorm en de grootte van de mazen, de vorm en de grootte van de ganglia evenals de densiteit en de morphologie van de ganglionaire neuronen. Als algemene regel kan gesteld worden dat de plexus van Auerbach sterker ontwikkeld is ter hoogte van de sphincters of sphincterachtige regio's, dat de neuronale dichtheid per oppervlakte eenheid darm rechtevenredig is met de grootte van het dier. Het lichaamsgewicht lijkt rechtevenredig te zijn met het aantal neurogliacellen, met het volume percentage van de neuropil en de grootte van het neuron. De laatstgenoemde parameter schijnt daarenboven ook gecorreleerd met de dikte van de te innerveren intestinale spiermantel. Gebruikmakend van verschillende histologische technieken werd de morphologie van de enterische zenuwcellen bestudeerd. De resultaten van deze onderzoekingen werden door verschillende auteurs aangewend om deze neuronen te classificeren. De meest courante classificatie is deze van Dogiel (type I, II en III neuronen). Slechts voor type I en type II konden de synaptic en de after hyperpolarization neuronen als respectievelijk neurophysiologisch correlaat aangetoond worden.
Talrijke zenuwbundels van verschillend kaliber ontspringen aan de plexus myentericus en dringen in de intestinale musculatuur ter vorming van een intramusculair zenuwnetwerk. In de regel ontvangt de circulaire spierlaag de dichtste innervatie en dit is vooral opvallend ter hoogte van de sphincters. De longitudinale spierlaag evenals de lamina muscularis mucosae kenmerken zich, in vergelijking met de circulaire spierlaag, door een matige bezenuwing, hoewel ook hier speciesverschillen konden worden aangetoond.
Niet alle zenuwbundels, die in de circulaire spierlaag dringen, dragen bij tot de vorming van de intramusculaire plexus. Inderdaad, sommige van hen trekken gewoon doorheen de inwendige spierlaag op weg naar de plexus submucosus of Meissner's plexus. Deze plexus, die de verschillende niveau's van de wijde tunica submucosa omspant, ontwikkelt zich doorheen de gehele breedte van de submucosa en kenmerkt zich, vergeleken met de Auerbach plexus, door wijde zeer onregelmatige mazen, door kleinere onregelmatige ganglia en door een lage neuronale dichtheid. Zoals dit voor de myenterische plexus werd vastgesteld, kenmerkt de morphologie van de Meissner plexus zich eveneens door aanzienlijke regionale en interspecies verschillen.
Verschillende zenuwvezels van de plexus submucosus sluiten na de penetratie van de lamina muscularis mucosae, waarin sommige van hen een bescheiden intramusculaire plexus vormen, aan op de plexus mucosus. Dit is een in principe cellichaam-vrij zenuwvlechtwerk opgebouwd uit zeer talrijke fijne zenuwbundels. Afhankelijk van de topographie van deze plexus in de mucosa zelf spreekt men van een subglandulaire, periglandulaire en intravilleuze plexus. Zoals mocht verwacht worden, is de morphologie van deze plexus in zijn geheel ook onderhevig aan aanzienlijke regionale en diersoort verschillen.
Het gastrointestinale epitheel zelf is zenuw-vrij, hoewel bij verschillende species morphologische en physiologische contacten tussen naakte zenuwuiteinden en hoofdzakelijk epitheliale entero-endocriene cellen konden worden waargenomen. Aldus wordt in de maag-darmtractus het neuro-endocriene complex geconstrueerd.
De lichtmicroscopische en eerste physiologische argumenten om het enterische zenuwstelsel als een apart en uniek onderdeel van het autonome zenuwstelsel te beschouwen, werden kracht bijgezet door de resultaten van het electronenmicroscopische onderzoek. Hierdoor kwam vast te staan dat de ultrastructurele organisatie van de enterische ganglia sterk gelijkt op deze van het centrale zenuwstelsel. Met andere woorden op een systeem dat gekenmerkt wordt door een verregaande intrinsieke integratieve capaciteit. Enterische ganglia bezitten inderdaad een groot aantal neurogliacellen en neuronen van een verschillend type. Beide elementen zijn in de enterische ganglia zo dicht op elkaar gepakt dat er een dense neuropil ontstaat en

dat tegelijk de intraganglionaire extracellulaire ruimte nog enkel virtueel voorkomt. Bindweefsel en bloedvaten ontbreken in de enterische ganglia, terwijl de neuronen niet of slechts zeer ten dele omhuld worden door een endo-en perineurale schede. Ganglia tenslotte isoleren zich in de darmwand van hun omgeving op zulk een wijze dat er een bloed-enterische plexus barrière wordt gevormd die, functioneel gesproken, zeer sterk gelijkt op de bloed-hersen barrière.
Recentelijk hebben nieuwe onderzoeksmethoden, in hoofdzaak de immunohistochemie en de radioimmunoassay, een ware revolutie teweeg gebracht in onze kennis en inzichten omtrent de neurochemie van het enterische zenuwstelsel. Ze hebben ons ertoe genoopt te accepteren dat dit zenuwstelsel, voor het uitoefenen van zijn normale functies, zich naar alle waarschijnlijkheid naast acetylcholine en noradrenaline van een brede waaier aan neurochemische substanties bedient. Geprikkeld door dit vernieuwd morphologisch inzicht, kende het neurophysiologisch onderzoek van het darmzenuwstelsel een nieuw elan. Wat de typische morphologische spreiding van een bepaalde substantie in het enterische zenuwstelsel functioneel kon laten vermoeden, werd in een aantal gevallen (cfr. noradrenaline, serotonine, substance P, vasoactive intestinal polypeptide) physiologisch bevestigd.
Acetylcholine, van oudsher de transmitter in het enterische zenuwstelsel, wordt bij alle onderzochte dieren over de gehele lengte en breedte van het maag-darmkanaal aangetroffen. Het lijkt er zelfs op dat nagenoeg iedere structuur uit de darmwand door cholinerge zenuwvezels in meerdere of mindere mate wordt geïnnerveerd.
Dat noradrenaline een verregaande invloed kon uitoefenen op menig intestinale functie was reeds lang bekend. Hoe dit precies gebeurde, bleef echter nog voor lange tijd een vraagteken. Gebruikmakend van de formaldehyde geïnduceerde fluorescentie, de fluorescentie-microscopie en later de immunohistochemie kwam men tot de eerder verrassende vaststelling dat de maag-darmwand massaal door noradrenerge-terminalia geïnfiltreerd wordt hoewel er, met uitzondering van het proximale colon van de cavia, geen noradrenerge neuronen konden worden waargenomen. Ondanks het feit dat de overgrote meerderheid van de noradrenerge zenuwvezels de myenterische ganglia als eindbestemming lijkt te hebben, konden een gering aantal noradrenerge zenuwen in de spierlaag, de plexus submucosus, de mucosa en rond de intramurale bloedvaten weergevonden worden. Uitgaande van deze morphologische bevindingen werd verondersteld dat het sympatische zenuwstelsel het intestinum in hoofdzaak indirect, d.w.z. via een beïnvloeding van de neuronale transmissie in de myenterische ganglia, controleerde. Physiologisch en pharmacologisch onderzoek toonden aan dat noradrenaline in de myenterische ganglia, via presynaptische alfa-adrenoreceptoren, de release van acetylcholine onderdrukt en derhalve de excitatorische intramurale neuronen inhibeert. Aldus werd de op morphologische gronden geformuleerde hypothese functioneel bevestigd. Niettegenstaande, met uitzondering van de sphincters, slechts een relatief gering aantal noradrenerge zenuwvezels in de verschillende spierlagen van de maag-darmtractus werd gezien, lijkt noradrenaline eveneens een directe werking op de intestinale musculatuur te bezitten. Dit effect wordt middels alfa- en beta-adrenoreceptoren, waarvan de physiologische betekenis verschilt naargelang de diersoort en het darmsegment, gerealiseerd. In de mucosa tenslotte werden nauwe functionele contacten tussen noradrenerge zenuwuiteinden en secretorische en entero-endocriene cellen aangetoond. Morphologisch kon aldus een nerveuze controle van de release uit de entero-endocriene cellen worden verwacht. Physiologisch onderzoek kwam deze veronderstelling bevestigen. Immers, stimulatie van de N. vagus in het cervicale gebied induceerde de vrijstelling van serotonine uit de serotonine-houdende entero-endocriene cellen van de dundarm. Deze vrijstelling staat onder controle van de in de N. vagus verlopende noradrenerge zenuwvezels. Gelet op deze nauwe functionele relatie tussen de intramurale noradrenerge zenuwvezels en het entero-endocriene systeem, kan het sympatische zenuwstelsel op deze wijze wellicht een brede waaier aan intestinale functies indirect beïnvloeden.

Al heel vroeg in het physiologisch onderzoek naar de besturing van de maag-darmmotiliteit bleek dat niet ieder gedragspatroon van de intestinale musculatuur op rekening van de al lang gekende neurotransmitters acetylcholine en noradrenaline kon geschreven worden. Aldus werd het bestaan van niet adrenerge, niet cholinerge excitatorische en inhibitorische substanties in het enterische zenuwstelsel vooropgesteld. Aanvankelijk werden purines (adenosine tri-,di- en monophosphaat) als niet-adrenerge inhibitorische neurotransmitters in het enterische zenuwstelsel aanzien. Terwijl deze veronderstelling nog steeds ter discussie staat, werden er een hele reeks substanties (thans een twintigtal) in de darmwand aangetroffen waarvan de meeste zich, juist omwille van hun spreiding en topographie in de maag-darmwand, als potentiële

transmitters/ modulatoren aanmelden. Slechts de belangrijkste en best bestudeerde van deze substanties worden in deze studie verder besproken.
Serotonine kent een dubbele localisatie in het maag-darmstelsel te weten in sommige entero-endocriene cellen en in het intramurale zenuwstelsel. Serotonine bevattende myenterische neuronen projecteren caudaalwaarts hetzij in de plexus van Auerbach zelf, hetzij naar de spieren van de darmwand of naar de plexus van Meissner.
Peptiderge zenuwen vormen zowel kwalitatief als kwantitatief een heel belangrijk onderdeel van de extrinsieke en intrinsieke enterische bezenuwing.
Substance P wordt, net als serotonine, aangetroffen in de entero-endocriene cellen en in de intramurale nerveuze elementen die zich in alle lagen van de darmwand situeren. Substance P immunoreactiviteit wordt voornamelijk teruggevonden in de myenterische neuronen, in zenuwvezels rondom enterische neuronen (voornamelijk Auerbach) en in de tunica muscularis. Derhalve kunnen de plexus myentericus en de spierlaag als belangrijkste targetorganen voor substance P aanzien worden. Nochtans worden substance P-houdende vezels eveneens waargenomen in de tunica mucosa en rond de intramurale bloedvaten zodat, naar alle waarschijnlijkheid, ook deze structuren onder controle van dit peptide staan. Tenslotte is substance P ook aanwezig in sensorische neuronen en bestaat de mogelijkheid dat het peptide als een transmitter in de sensorische informatieoverdracht vanuit de maag-darmtractus fungeert.
Vasoactive intestinal polypeptide vertegenwoordigt kwantitatief ongetwijfeld het belangrijkste peptide uit de maag-darmtractus en het kan als dusdanig in alle lagen van de darmwand worden teruggevonden. De meeste vasoactive intestinal polypeptide-houdende neuronen localiseren zich in de plexus submucosus van waaruit talrijke projecties gebeuren naar de mucosa, de gastrointestinale klieren, de circulaire spierlaag (voornamelijk de sphincters) en de plexus van Auerbach. Binnen de enterische ganglia zelf maken de vasoactive intestinal polypeptide-houdende zenuwvezels ringvormige netwerken omheen de neuronen. Ook de intramurale perivasculaire plexussen bevatten talrijke vasoactive intestinal polypeptide-immunoreactive zenuwvezels. Uit dit intramurale distributiepatroon van vasoactive intestinal polypeptide mag blijken dat nagenoeg iedere structuur van de maag-darmwand (epitheel, klieren, spieren en neuronen) door dit peptide kan beïnvloed worden.
Somatostatine wordt voornamelijk aangetroffen in de entero-endocriene cellen en in het intramurale zenuwstelsel. Somatostatine-houdende neuronen worden enkel in de plexus submucosus teruggevonden. Zij projecteren in de enterische plexussen meestal caudaalwaarts en eindigen korfvormig rondom andere ganglioncellen. In de spierlaag worden sporadisch somatostatine-houdende zenuwvezels gezien. Vandaar dat de ganglionaire neuronen het belangrijkste effectororgaan van deze zenuwvezels uitmaken en dat het peptide weinig of geen direct effect op de maag-darm musculatuur bezit. Physiologisch onderzoek komt deze morphologische bevindingen op een elegante manier aanvullen, vermits kon worden aangetoond dat somatostatine in de eerste plaats de acetylcholine output van de enterische neuronen onderdrukt en daarenboven sommige inhibitorische neuronen stimuleert.
Enkephalines tenslotte situeren zich uitsluitend in het intramurale zenuwstelsel. Enkephaline-houdende neuronen worden alle, op enkele uitzonderingen na, in de plexus van Auerbach gevonden. Zij projecteren hoofdzakelijk in de plexus zelf en naar de circulaire spierlaag. In tegenstelling met wat uit deze morphologie kan verwacht worden, lijken de enkephalines nagenoeg geen direct effect op de intestinale gladde spieren te bezitten. Hun werking lijkt zich eerder tot een hyperpolarisatie (= acetylcholine output verminderen) van de geactiveerde enterische neuronen te beperken.
De tot op heden gevonden grote diversiteit in het aantal en type transmitters/modulatoren in het enterische zenuwstelsel vormt slechts één facet van de enorme complexiteit van dit stelsel. Inderdaad, naast de reeds eerder aangehaalde ultrastructurele gelijkenis tussen het centraal zenuwstelsel en de enterische ganglia, doen recente bevindingen de grens tussen beide systemen nog meer vervagen.
Zo werden er, naast acetylcholine en noradrenaline, talrijke non-adrenerge, non-cholinerge substanties hetzij eerst uit de darmwand geïsoleerd en nadien in het centrale zenuwstelsel (ruggemerg en hersenen) teruggevonden, of omgekeerd. Op grond hiervan werd het begrip "brain-gut" as ingevoerd.
Daarenboven zijn er nu talrijke aanwijzingen om het historische axioma "één neuron één transmitter" zowel voor het centrale als voor het enterische zenuwstelsel aan de nieuwe bevindingen en inzichten aan te passen. Inderdaad werden er, zowel in de extrinsieke als in de intrinsieke maag-darmbezenuwing, talrijke structurele en functionele voorbeelden van co-storage en co-release (klassieke/non-adrenerge, non-cholinerge;

non-adrenerge, non-cholinerge/ non-adrenerge, non-cholinerge neurotransmitter) binnen en door éénzelfde neuron gevonden. Hierbij ligt het voor de hand te veronderstellen dat deze substanties elkaars vrijstelling en/of effect(en) in deze of gene zin kunnen controleren en/of beïnvloeden.
De dubbele localisatie van verschillende peptiden in zowel entero-endocriene cellen als in intramurale nerveuze elementen, pleit ervoor om in de toekomst beide systemen op functionele gronden niet meer van elkaar te onderscheiden doch als één enterisch neuroendocrien systeem te beschouwen. Het feit dat een aantal epitheliale endocriene cellen hun inhoud na stimulatie van het enterische zenuwstelsel vrijstellen, kan deze gedachte alleen maar ondersteunen.
De aanzienlijke discrepantie tussen het aantal enterische neuronen en het aantal vanuit het centraal zenuwstelsel vertrekkende gastrointestinale efferenten (> 20.000/1) wijst op de grote physiologische onafhankelijkheid van het enterische zenuwstelsel ten aanzien van een centrale controle. Deze veronderstelling wordt verder gestaafd door talrijke in vitro experimenten waarin geïsoleerde darmstukken in staat blijken te zijn (stereotype) reflexactiviteit uit te oefenen. Dit impliceert dat het intramurale zenuwstelsel alle sleutelelementen van een reflexboog in zich draagt en dat het centraal zenuwstelsel een eerder modulerende dan wel een beslissende invloed op de onderscheiden darmfuncties uitoefent. Derhalve moeten in de darmwand receptoren, afferente neuronen en zenuwvezels, een centrale verwerkingseenheid evenals efferente neuronen en zenuwvezels terug te vinden zijn, die daarenboven in harmonie met elkaar moeten kunnen functioneren. Het verstoren van deze harmonie of het niet of onvoldoende functioneren van slechts één schakel uit de reflexboog is voldoende om de talrijke functies van het enterische zenuwstelsel ernstig te ontregelen.
Hoewel het definitieve morphologische bewijs voor de aanwezigheid van receptoren in de maag-darmwand nog niet geleverd is, heeft de neurophysiologie het bestaan van chemo-, mechano-, pijn- en thermoreceptoren in de darmwand op overtuigende wijze aangetoond.
Chemoreceptoren reageren op veranderingen in pH en de osmolariteit alsook op de aanwezigheid van glucose en bepaalde vetten in het opgenomen voedsel.
Mechanosensitieve neuronen, reagerend op uitrekken en beklemmen van de darmwand evenals op de door de peristaltiek geïnduceerde vervorming van het ganglion, werden in de plexus van Meissner en Auerbach teruggevonden. Gebaseerd op de huidige resultaten van het neurophysiologisch onderzoek kunnen drie typen mechanosensitieve neuronen worden onderscheiden met name traag en snel adapterende neuronen en tonic-type neuronen.
Pijn vanuit de buik- en bekkenstreek wordt, naar alle waarschijnlijkheid, uitsluitend via sympatische afferenten vervoerd. Hoewel specifieke pijnreceptoren in de maag-darmtractus lijken voor te komen, wordt algemeen aangenomen dat pijngevoel, onder adequate omstandigheden, kan opwekt worden vanuit de meeste en dus ook niet-specifieke pijnreceptoren.
Thermoreceptoren werden voornamelijk in het maag-darmtractus van de kat teruggevonden. Zoals dit voor de viscerale pijnperceptie het geval lijkt te zijn, reageren ook niet-specifieke thermoreceptoren op overdreven warmte-prikkels.
Na registratie van de veranderingen, die in het darmmilieu zijn opgetreden, wordt de sensorische informatie naar de verschillende centrale verwerkingseenheden doorgespeeld. Deze zijn in eerste instantie de intramurale plexussen (plexus van Meissner en Auerbach) vervolgens de extramurale (prevertebrale) ganglia en tenslotte naar het centrale zenuwstelsel (ruggemerg en hersenstam) voor de integratie van de informatie op "supraintestinaal" niveau. De input naar de verschillende extra-intestinale reflexcentra is waarschijnlijk zeer aanzienlijk vermits de N. vagus voor ongeveer 80% uit viscerale afferenten bestaat terwijl de Nn splanchnici eveneens grote hoeveelheden afferente zenuwvezels bevatten. Electrophysiologisch lijken de extramurale ganglia daarenboven informatie uit de darm en het centraal zenuwstelsel met elkaar te integreren. Verschillende neuropeptiden (vasoactive intestinal polypeptide, substance P, somatostatine, etc.) lijken in deze informatiestroom functioneren. Immers na neurectomie of onderbinden van de N. vagus en de Nn. splanchnici kan men vaststellen dat deze neuropeptiden normaliter in de prevertebrale ganglioncellen aanwezig zijn (co-existence) en van daar naar de maag-darmtractus worden getransporteerd. Met andere woorden ook het verwerken van afferente viscerale informatie lijkt veel complexer dan aanvankelijk verondersteld.
Tot de afferente informatie, die naar de intramurale informatieverwerkende centra wordt aangevoerd, moet echter ook de informatie vanuit de prevertebrale ganglia, het ruggemerg en de hersenstam gerekend worden. Deze bereikt de excitatorische en inhibitorische enterische neuronen via parasympatische (N. vagus) en sympatische (Nn. splanchnici) vezels. De

klassieke begrippen parasympatisch en sympatisch moeten echter in het licht van de recente onderzoeksresultaten veel ruimer begrepen worden vermits zowel de N. vagus als de Nn. splanchnici verscheidene non-adrenerge, non-cholinerge efferenten bevatten.
Informatie uit de enterische receptoren evenals deze uit de extra-intestinale reflexcentra, wordt aangevoerd naar de talrijke intramurale ganglia die als locale minicomputers functioneren. Onderling, door zeer vele interganglionaire zenuwbundels met elkaar en met hogere viscerale centra verbonden, vormen ze samen het enterische "brein" waarin inkomende en uitgaande informatie met elkaar worden geïntegreerd en gecoördineerd. Dit laat althans de opvallende gelijkenis in de ultrastructuur en de neurochemie tussen het centraal zenuwstelsel en de enterische ganglia vermoeden. Enterische neuronen bedienen zich daarenboven voor hun gegevensverwerking, naast de klassieke neurotransmitters acetylcholine en noradrenaline, eveneens van een hele gamma non-adrenerge, non-cholinerge substanties (purines, serotonine, substance P, vasoactive intestinal polypeptide, somatostatine, enkephalines, etc.) en deze substanties lijken daarenboven binnen eenzelfde enterisch neuron in meerdere combinaties voor te komen.
Extracellulaire registraties hebben de enterische neuronen, op basis van hun electrophysiologisch gedrag ten aanzien van intestinale stimulatie, opgedeeld in twee groepen te weten de single-spike en burst-type neuronen. De laatste groep kan, naargelang de aan- of afwezigheid van een vaste periode tussen de bursts in, opgedeeld worden in erratische bursters en steady bursters. Uit het gedrag van de steady bursters kan men opmaken dat zij fungeren als endogene oscillatoren die geen synaptische input van andere neuronen ontvangen. De erratische bursters daarentegen worden bestuurd door de steady bursters onder wiens impuls zij hun inhibitorische transmitter(s) vrijstellen ten einde op een tonische wijze de spontane activiteit van de intestinale musculatuur te onderdrukken.
Intracellulaire registraties delen de enterische neuronen op in drie groepen met name de after hyperpolarization/type 2; de synaptic/type 1 neuronen en tenslotte een intermediaire groep neuronen die kenmerken van beide voorgaande groepen in zich dragen (electrophysiologisch gedrag als after hyperpolarization neuronen, doch een serotoninerge synaptische input). After hyperpolarization neuronen ontvangen noch morphologisch noch physiologsich enige synaptische input en ze kunnen derhalve als equivalent van de extracellulair geregistreerde steady bursters (cfr. tonic-type enteric neurons) aanzien worden. Morphologisch vertonen de after hyperpolarization/type 2 neuronen veel gelijkenis met de Dogiel type II perikarya. 2/3 van de Auerbach en nagenoeg alle Meissner neuronen werden als synaptic neuronen geïndentificeerd. Synaptic neuronen kennen een duidelijke synaptische input en gelet op hun electrophysiologsich gedrag kan men sommige als interneuronen anderen dan weer als excitatorische/inhibitorische motoneuronen bestempelen. Synaptic neuronen vertonen een duidelijke Dogiel type I morphologie. Binnen de plexus myentericus lijken, en dit in tegenstelling tot de plexus submucosus, zowel de after hyperpolarization als de synaptic neuronen op een zeer geordende wijze over meerdere mm zowel naar oraal als caudaal als circumferentieel te projecteren. Door deze laatste wijze van projectie treedt de plexus myentericus onder andere in verbinding met de plexus submucosus.
De intramurale motoneuronen, hun efferenten en de effectororganen vormen de laatste schakel van de intramurale intestinale reflexboog.
De maag-darmmusculatuur, één van de belangrijkste eindorganen van het enterische zenuwstelsel, kenmerkt zich door een aparte bouw en gedrag welke beide aanleiding geven tot een heel apart innervatiepatroon. Intestinale gladde spiercellen groeperen zich in bundels van meerdere honderden spiercellen. Binnen iedere bundel gaan de membranen van de naast elkaar liggende gladde spiercellen op meerdere plaatsten fusioneren en aldus electrisch aan elkaar gekoppeld worden. Zodoende kan zich een verandering in de membraanpotentiaal van één gladde spiercel binnen het electrisch syncytium (gladde spierbundel) naar alle niet direct geïnnerveerde spiercellen verspreiden.
De membraan van de gladde spiercel ondergaat spontaan cyclische veranderingen in haar permeabiliteit voor ionen. Daardoor zal de gladde spiercel en dus een bundel van gladde spiercellen ritmisch en geheel onafhankelijk van enige innervatie contraheren (myogene activiteit).
Beide specifieke eigenschappen d.i. het electrisch gekoppeld zijn en de spontane ritmische contracties van de intestinale musculatuur, impliceren dat de nerveuze controle van de maag-darmtractus slechts kan berusten op een tonisch neurogene inhibitie van een myogeen systeem dat inherent geëxciteerd is. Met andere woorden in het enterische zenuwstelsel zijn de intrinsieke inhibitorische neuronen tonisch actief en het is enkel ter hoogte van de sphincters dat deze neuronen onder normale (rust) omstandigheden inactief zijn. Mede hierdoor zijn de sphincters tonisch

gecontraheerd. Vermits zowel excitatorische als inhibitorische neuronen in het enterische zenuwstelsel voorkomen, ligt het voor de hand aan te nemen dat de circulaire spierlaag voornamelijk onder inhibitorische (non-adrenerge, non-cholinerge) en de longitudinale spierlaag onder excitatorische (cholinerge) controle staan. Niet alleen neurophysiologisch onderzoek van de darmwandneuronen (het meerendeel van de myenterische neuronen vertoont een continue ontlading) heeft het functioneren van zulk'n innervatiesysteem bewezen, doch ook ander experimenteel en klinisch onderzoek hebben deze hypothese gestaafd. Immers een algehele of gedeeltelijke uitval van het enterische zenuwstelsel geeft aanleiding tot een tonische hyperexcitatie van de circulaire spierlaag en aldus een ernstige verstoring van de normale maag-darmmotiliteit.

De talrijke in de darmwand voorkomende substanties kunnen daarbij hetzij direct hetzij indirect d.i. via een modulatie van de activiteit van de ganglionaire neuronen op de gladde spiercelmembraan inwerken.

Acetylcholine en noradrenaline grijpen in zowel op de ganglionaire transmissie (acetylcholine via nicotine receptoren; noradrenaline onderdrukt hoofdzakelijk de acetylcholine release via presynaptische alpha receptoren) als direct op de gladde musculatuur via respectievelijk muscarine en adrenoreceptoren (alfa en beta).

Non-adrenerge, non-cholinerge inhibitorische zenuwen spelen naar alle waarschijnlijkheid een zeer belangrijke rol in de controle van de normale peristaltiek (receptieve relaxatie van de maag, descenderende inhibitie in de peristaltische reflex) en dus in de normale voedselpassage.

ATP werkt inhiberend op de ganglionaire cholinerge neurotransmissie en als dusdanig indirect inhiberend op de maag-darmmotiliteit.

Serotonine induceert darmcontracties door zijn excitatorisch effect op de intraganglionaire cholinerge en non-adrenerge, non-cholinerge structuren. Serotonine stimuleert immers de vrijstelling van acetylcholine uit enterische neuronen en activeert non-adrenerge, non-cholinerge inhibitorische neuronen. Het direct effect van dit indolamine op de gladde spieren is variabel en onderhevig aan regionale en diersoort verschillen.

Meerdere enterische neuronen bevatten substance P van waaruit het peptide na stimulatie wordt vrijgesteld. Daarenboven worden de meeste enterische neuronen door substance P houdende zenuwvezels omgeven en werd er een co-storage en een co-release van acetylcholine en substance P in en door eenzelfde enterische neuron aangetoond. Substance P heeft naast zijn modulatie van de cholinerge (inductie acetylcholine release) en adrenerge ganglionaire transmissie ook een direct effect op de gladde spieren van de darmwand (cfr. dichte intramusculaire substance P innervatie van de circulaire spierlaag). Dit directe myogene effect gaat van een fascilitatie van de spontane fasische contracties tot een inductie van tonische contracties. Welk effect substance P finaal op de maag-darmmotiliteit induceert is van meerdere factoren zoals diersoort, maag-darmsegment, gebruikte concentratie en registratieomstandigheden afhankelijk. In de meeste gevallen echter induceert substance P een tonische contractie van de intestinale musculatuur.

Vasoactive intestinal polypeptide localiseert zich in enterische neuronen (motor en interneuronen), in zenuwuitlopers rond enterische neuronen en in talrijke zenuwvezels die de circulaire spierlaag innerveren (dit is zeer opvallend ter hoogte van de sphincters). Het vasoactive intestinal polypeptide kan de maag-darmmotiliteit op twee manieren inhiberen d.i. enerzijds via zijn effect op de ganglionaire transmissie (stimuleert acetylcholine release) en anderzijds via zijn directe inhibitorische invloed op de gladde musculatuur.

Somatostatine en enkephalines lijken geen direct doch enkel een indirect inhibitorisch effect (somatostatine en enkephalines onderdrukken de release van acetylcholine, noradrenaline en andere neurotransmitters) op de gladde spieren van de darmwand uit te oefenen.

Het intestinale epitheel vormt een tweede belangrijk effectororgaan voor het enterische zenuwstelsel. Zo vallen niet enkel de secretie/absorptie van water en electrolieten doch ook de mucussecretie en de release uit de entero-endocriene cellen onder nerveuze controle. Dit kan men afleiden zowel uit de zeer dichte innervatie van de mucosa als uit de talrijke physiologische experimenten waarbij stimulatie van enterische zenuwen aanleiding geeft tot hetzij het vrijstellen van verschillende maag-darmhormonen, die op hun beurt weer het secretie/absorptieproces kunnen beïnvloeden, hetzij tot actieve secretie van ionen waardoor water passief volgt.

Secretomotor neuronen zijn zowel cholinerg als niet-cholinerg en in beide typen werden daarenboven verschillende peptiden (vasoactive intestinal polypeptide, somatostatine, neuropeptide Y, cholecystokinine, calcitonine gene related peptide, etc.) teruggevonden.

Noradrenaline onderdrukt de intestinale water- en electrolietensecretie middels stimulatie van inhiberende alfa adrenoreceptoren aanwezig op de submucosale secretomotor neuronen enerzijds en anderzijds via

adrenerge receptoren op de epitheliale cellen zelf. Daarbovenop reduceert noradrenaline in aanzienlijke mate de mucosale bloedvloei waardoor, logischerwijze, het secretieproces wordt onderdrukt.
De talrijke projecties van de substance P houdende neuronen naar de submucosa en mucosa laten toe om te veronderstellen dat dit peptide eveneens betrokken is in de controle (stimulatie) van het intestinale secretie/ absorptieproces. De invloed van substance P op dit proces wordt daarenboven verder in de hand gewerkt door het intense vasodilatatorische effect van substance P op de intramurale bloedvaten.
Een zeer dicht vasoactive intestinal polypeptide-houdend zenuwnetwerk kenmerkt de intestinale mucosa (klieren en villi). Verder werden receptoren voor dit peptide over nagenoeg de gehele lengte van het intestinale slijmvlies aangetoond. Deze morphologische bevindingen verklaren mede het zeer krachtige stimulatorische effect van vasoactive intestinal polypeptide op de vocht- en electrolietensecretie. Twee klinische situaties, met name cholera en het Verner-Morrison (WDHA) syndroom, vormen een zeer duidelijke illustratie van de functionele consequenties van deze morphologische bevindingen .
Somatostatine-houdende zenuwvezels omhullen het basale deel van de intestinale klieren en kunnen zodoende de verschillende secretorische processen van de darm inhiberen en de absorptie stimuleren. Enkephalines onderdrukken de intestinale bicarbonaat- en enzymsecreties.
De mucussecretie vanuit de intestinale slijmbekercellen valt niet alleen onder controle van intrinsieke cholinerge neuronen, doch ook vasoactive intestinal polypeptide-houdende neuronen kunnen naar alle waarschijnlijkheid in dit proces betrokken zijn.
Talrijke zenuwvezels in de mucosa verlopen net onder het epitheel en kunnen aldus in zeer nauwe relatie met de entero-endrocriene cellen treden. De in de N. vagus verlopende noradrenerge zenuwvezels induceren, na stimulatie van de betrokken zenuw, een vrijstelling van serotonine uit de entero-endocriene cellen van de dundarm. Cholinerge neuronen inhiberen de vrijstelling van het epitheliale somatostatine, non-adrenerge, non-cholinerge neuronen deze van gastrine. Bombesine, als universele releaser, en somatostatine, als universele inhibitor, kunnen tenslotte de vrijstelling van talrijke maag-darmhormonen moduleren.
De splanchnische circulatie vertegenwoordigt nagenoeg 20 % van het totale bloedvolume. Daarom moet de intestinale vasculatuur eveneens als een belangrijk eindorgaan voor het enterische zenuwstelsel aanzien worden. Nagenoeg alle intramurale bloedvaten (voornamelijk arterieel) worden omgeven door een sterk ontwikkeld perivasculair netwerk dat naast acetylcholine en noradrenaline ook non-adrenerge, non-cholinerge zenuwvezels bevat. Zowel extrinsieke als intrinsieke factoren zijn in staat gebleken de intramurale bloedvloei hetzij via een directe hetzij via een indirecte wijziging van de vasculaire diameter aan de algemene en/of locale behoeften aan te passen. Noradrenaline induceert vasoconstrictie via alpha adrenoreceptoren. Serotonine, vrijgekomen na stimulatie van de mucosa uit de entero-endocriene cellen of uit de interneuronen, induceert een locale hyperaemie (vasodilatatie). In deze reflex zou een vasoactive intestinal polypeptide-houdend neuron als laatste schakel fungeren. Substance P en vasoactive intestinal polypeptide houdende zenuwvezels helpen de intramurale perivasculaire plexussen mede uitbouwen. Beide peptiden veroorzaken een uitgesproken vasodilatatie.
Het hoofdstuk omtrent het enterische zenuwstelsel wordt, tenslotte, afgesloten door de klinische betekenis van dit zenuwstelsel, aan de hand van een aantal maagdarmpathologieën waarin het enterische zenuwstelsel direct of indirect betrokken is, kort toe te lichten.

In het *tweede deel* van deze studie werd, gebruik makend van een polyclonaal antiserum tegen neuron specifieke enolase, het neuro-endocriene systeem in de herkauwersmaag van het schaap bestudeerd.
Uit een voorafgaande literatuurstudie omtrent neuron specifieke enolase blijkt dat dit glycolytisch isoenzyme in relatief hoge concentraties voorkomt in gedifferentieerde neuronen van het centrale en perifere zenuwstelsel en in cellen van het diffuse neuro-endocriene systeem. Zeer lage (bijna verwaarloosbare) neuron specifieke enolase concentraties werden teruggevonden in niet-neuronale cellen en weefsels. Vandaar dat een antiserum tegen NSE zich aandient als een zeer krachtig instrument om het gehele intramurale neuro-endocriene systeem te bestuderen.
In dit onderzoek werd gebruik gemaakt van zowel dunne paraffine als van dikke cryostaat coupes afkomstig van foeten en volwassen schapen, dit om een zo volledig mogelijk totaalbeeld van het neuro-endocriene complex in de herkauwersmaag te verkrijgen.
Talrijke neuron specifieke enolase-immunoreactieve cellen konden in het epitheel van alle onderzochte segmenten van de foetale herkauwersmaag worden teruggevonden. Aangezien uit de literatuur blijkt dat neuron specifieke enolase- antilichamen reageren met alle elementen van het neuro-endocriene systeem,

kunnen deze neuron specifieke enolase-positieve epitheliale cellen als entero-endocriene cellen bestempeld worden. Ter ondersteuning van deze hypothese kan de opvallend gelijkenis in morphologie van de neuron specifieke enolase-immunoreactieve cellen (driehoekig tot peervormig, smal toelopende apex naar het lumen, brede basis met één of meer basale uitlopers) met de klassieke beschreven entero-endocriene cellen aangevoerd worden. Daarenboven werden, voornamelijk in cryostaat coupes, bij alle onderzochte foeten en in alle onderscheiden segmenten meerdere voorbeelden gevonden van dunne zenuwvezels die de plexus submucosus verlaten om net onder het oppervlakte epitheel te gaan verlopen. Aldus komen deze zenuwvezels zeer dicht bij de basale uitloper(s) van de neuron specifieke enolase-immunoreactieve cellen. Deze lichtmicroscopische bevinding verwijst dus duidelijk naar het bestaan van een neuro-endocriene eenheid in de herkauwersmaag van het foetale schaap. Vermits nu de entero-endocriene cellen functioneel kunnen beschouwd worden als gastro-intestinale sensoren, wordt de veronderstelling geopperd dat het waargenomen neuro-endocriene complex als een onderdeel van het sensorische systeem in de herkauwersmaag zou functioneren. In het voormagencomplex van het volwassen schaap daarentegen kon geen enkele neuron specifieke enolase-immunoreactieve epitheelcel worden teruggevonden. Verder lijken de plexus submucosus en mucosus, in de originele betekenis van het woord als tweede luik van het neuro-endocriene systeem eveneens te ontbreken.

Hoewel neuronen, ganglia en zenuwvezels als de basiselementen van de intramurale bezenuwing in alle segmenten van alle onderzochte species werden teruggevonden, werden toch, zowel reeds uit de voorkennis omtrent het enterische zenuwstelsel kon verwacht worden, een aantal opmerkelijke verschillen in het onderzochte materiaal waargenomen.

In de regel lijkt de intramurale innervatie van de herkauwersmaag bij de foetus veel dichter dan bij het volwassen schaap. Dit is naar alle waarschijnlijkheid geen absoluut gegeven doch veeleer het gevolg van het feit dat bij het volwassen dier een vergelijkbare hoeveelheid zenuwweefsel over een aanzienlijk grote oppervlakte uitgespreid ligt. Logischerwijze lijkt hierdoor de innervatiedichtheid per preparaat geringer.

Zowel bij de foetus als bij het volwassen schaap werden duidelijke regionale en topografische verschillen tussen de plexus subserosus, Auerbach en muscularis vastgesteld. Binnen het voormagencomplex werd de dichtste plexus myentericus en intramusculaire innervatie teruggevonden in de boekmaag, gevolgd door de netmaag en tenslotte de pens. Waarschijnlijk zijn ook hier deze densiteitsverschillen in sterke mate gecorreleerd aan het verschil in oppervlakte van de onderscheiden segmenten. In het klierige maagdeel werden dergelijke regionale verschillen in de intramurale innervatie niet geconstateerd, hoewel meer zenuwweefsel voorkomt ter hoogte van de pylorus en het stuk van het antrum pyloricum grenzend aan de pylorus. Deze bevinding lijkt echter een algemeen principe te zijn vermits dit ook werd waargenomen ter hoogte van de sphincters (slokdarmsphincter, ostium reticulo-omasicum) of sphincterachtige structuren (slokdarmsleuf of reticulaire groeve) van het voormagencomplex.

Zowel bij de foetus als het volwassen dier is de longitudinale spierlaag, algemeen gesproken, slechts matig geinnerveerd, behalve in deze segmenten (netmaag, reticulaire groeve) waar deze spierlaag sterker ontwikkeld is.

Doorheen de ganse herkauwersmaag beantwoordt de morphologie van de myenterische plexus aan het klassieke beeld van deze plexus geschetst in deel I. Dit impliceert derhalve duidelijke verschillen in grootte en vorm van de mazen, de ganglia, de interganglionaire zenuwbundels, etc.

In overeenstemming met wat voor de andere diersoorten werd beschreven, ontvangt zowel bij de foetus als bij het volwassen schaap de circulaire spierlaag over de gehele herkauwersmaag de dichtste innervatie. De dichtheid van deze intramusculaire bezenuwing lijkt daarenboven in verhouding te zijn tot de dikte van de circulaire spierlaag zodat ter hoogte van de sphincters de densiteit van de plexus duidelijk toeneemt (vide supra).

Tot dusver konden in het morphologische grondplan van de plexus subserosus, Auerbach en muscularis van de herkauwersmaag geen wezenlijke verschillen tussen foetus en volwassen schaap, noch met het klassieke beeld van deze plexussen zoals beschreven in deel I, worden vastgesteld. Dit kan echter niet gezegd worden voor de plexus submucosus en mucosus.

In de "kliermaag" werden tussen foetus en volwassen specimens geen opmerkelijke verschillen in de morphologie van beide plexussen gevonden. Tegelijk beantwoordt hun miscroscopisch beeld, zoals waargenomen in deze studie, aan de beschrijving van deze plexussen bij andere diersoorten. Heel anders is het gesteld met het voormagencomplex. Inderdaad, bij het volwassen schaap worden slechts sporadisch immunoreactieve zenuwvezels aangetroffen doch geen perika-

rya of ganglia. Op grond hiervan kan gesteld worden dat een plexus submucosus, in de oorspronkelijke betekenis van het woord, in de voormagen van het volwassen schaap ontbreekt. Bij de foetus echter vormen meerdere submucosale neuron specifieke enolase-immunoreactieve zenuwvezels een delicaat vlechtwerk dat het duidelijkst wordt gezien aan de basis van alle toekomstige submucosaplooien en voor de grote (toekomstige) plooien tevens in het centrum van deze plooien. Kenmerkend voor de Meissner's plexus in het foetale voormagencomplex is verder het gering aantal ganglia die daarenboven in de regel weinig neuronen bevatten. Het zoëven geschetste beeld van de plexus submucosus wordt echter niet in gelijke mate in alle segmenten van het voormagencomplex teruggevonden. Netmaag en pens beantwoorden het best aan dit beeld. Op de overgang netmaag-boekmaag ontwikkelen zich normaliter klauwvormige papillen in wiens submucosaplooi, die deze papillen ondersteunt, een relatief zeer dichte (de dichtste van het hele voormagen complex) plexus submucosus wordt vastgesteld. Deze bevinding kadert goed in de "aftastende" rol die deze papillen zouden spelen in de voedselpassage van de netmaag naar de boekmaag. Noch aan de basis noch in het centrum van de boekmaagbladen kon met neuron specifieke enolase een zenuwvlechtwerk in de submucosa waargenomen worden.
De aanwezigheid van een delicate maar duidelijke plexus submucosus in de voormagen van de foetus en het blijkbaar ontbreken van deze plexus bij het volwassen dier hoeft niet per se contradictorisch te zijn. Wellicht is het zo dat de toch al matig ontwikkelde plexus submucosus van de foetus door de enorme postnatale groei van het voormagencomplex, en in nog sterkere mate van diens epitheel, dermate uitgerokken en dus ijl wordt dat deze plexus in coupes van de volwassen voormaag niet meer als plexus doch slechts als enkele sporadische zenuwvezels herkenbaar is.
Een plexus mucosus kon noch bij de foetus noch bij het volwassen schaap worden gevonden. Hoewel in deze segmenten waar de lamina muscularis mucosae geheel of gedeeltelijk ontwikkeld is, al naargelang de dikte van de spierlaag, meer of minder neuron specifieke enolase-immunoreactieve zenuwvezels aanwezig zijn.
Samenvattend kan dus gesteld worden dat de intramurale innervatie van de herkauwersmaag, met uitzondering van de plexus submucosus en mucosus van het voormagencomplex, in essentie beantwoordt aan de klassieke beschrijving van de intramurale bezenuwing van de maagdarmtractus bij andere diersoorten. Dit impliceert tevens duidelijke regionale en topografische verschillen in de morphologie van deze innervatie. De afwijkende morphologie van de plexus submucosus en de afwezigheid van de plexus mucosus in de voormagen van het schaap vormen wellicht de meest relevante bevindingen van het tweede gedeelte van dit onderzoek. Inderdaad, refereren ze niet alleen naar het "afwijkend" gedrag van deze plexussen in de oesophagus van andere diersoorten (cfr. deel I) doch ondersteunen ze tegelijk de bij de embryologie van de herkauwersmaag geformuleerde hypothese dat het voormagencomplex zich niet, zoals algemeen wordt aangenomen, ontwikkelt uit een deel van de maagaanleg maar veeleer uit een stuk primitieve darm (slokdarm) gelegen voor de eigenlijke maagaanleg.

De resultaten voortvloeiend uit het tweede deel vormden de springplank tot het *derde en laatste deel* van deze studie waarin nader wordt ingegaan op het voorkomen, de spreiding en het effect op de spiertonus van een aantal substanties waarvan vaststaat dat ze aanwezig zijn in en functioneren in het intramurale enterische zenuwstelsel. In deze optiek werden de klassieke neurotransmitters acetylcholine en noradrenaline evenals enkele van de non-adrenerge, non-cholinerge transmitters met name serotonine, substance P en vasoactive intestinal polypeptide in de herkauwersmaag bestudeerd. Voor het morphologische luik werd gebruik gemaakt van zowel de enzymhistochemie, de immunohistochemie als van de radioimmunoassay. In het functionele gedeelte werd, in in vitro experimenten, het effect van de bovengenoemde substanties op de spiertonus van de verschillende segmenten van de herkauwersmaag bestudeerd.
De aanwezigheid van acetylcholine werd enkel op gestripte totaalpreparaten enzymhistochemisch onderzocht. Uit dit onderzoek blijkt dat -in volledige overeenstemming met het onderzoek uit deel II- de plexus van Auerbach hetzelfde grondplan, met daarop weliswaar de nodige variaties, in alle segmenten van de herkauwersmaag vertoont. Zoals reeds bij de studie met neuron specifieke enolase vermeld, wordt ook met de acetylcholinesterasemethode het dichtste zenuwnetwerk van de voormagen waargenomen in de boekmaag gevolgd door de netmaag en tenslotte de pens. In het klierige maagdeel vertoont de acetylcholinesterasereactie geen specifiek distributiepatroon, behalve ter hoogte van de pylorus waar, naar analogie met het neuron specifieke enolase-onderzoek, de dichtheid van het acetylcholinesterase zenuwvlechtwerk duidelijk toeneemt.
Functioneel gesproken lijkt acetylcholine weinig

direct effect te hebben op de sphinctertonus van de herkauwersmaag. In de pens, de boekmaag en de lebmaag daarentegen verhoogt acetylcholine de tonus van één of beide spierlagen. In de segmenten waar het voedsel wordt opgeslagen (pens en lebmaag) is acetylcholine dus naar alle waarschijnlijkheid mede betrokken in de verplaatsing van de voedselmassa geïnduceerd door phasische contracties.
Het noradrenerge innervatiepatroon in de wand van de herkauwersmaag werd zowel enzymhistochemisch (totaalpreparaten) als immunohistochemisch (cryostaat coupes) onderzocht. Noradrenerge neuronen konden met geen van beide onderzoeksmethoden worden aangetoond. Het meerendeel van de intramurale noradrenerge zenuwvezels houdt zich op in de plexus myentericus waar een opvallend dicht netwerk rond niet-noradrenerge neuronen wordt gevormd. Vermits op grond van deze bevinding de plexus van Auerbach de belangrijkste eindbestemming van de noradrenerge vezels lijkt te zijn, lag het in de lijn van de verwachtingen dat -en dit wordt door onderzoeksresultaten bevestigd- de waargenomen topografische verschillen in de noradrenerge innervatie van de herkauwersmaag zich zouden weerspiegelen in de reeds eerder vastgestelde topografische verschillen in de architectuur van de Auerbach plexus. Slechts enkele fluorescerende noradrenaline-houdende zenuwvezels werden in de uitwendige spierlaag van de totaalpreparaten teruggevonden. Ieder waargenomen bloedvat lijkt begeleid te zijn door een noradrenerge perivasculaire plexus. De immunohistochemie (antiserum tegen dopamine-beta-hydroxylase een enzyme uit de synthese van noradrenaline) bevestigde de enzymhistochemische resultaten en kwam daarenboven het beeld van de intramurale noradrenerge-innervatie van de herkauwersmaag vervolledigen. Zowel in de longitudinale als in de circulaire spierlaag werden dopamine-beta-hydroxylase-immunoreactieve zenuwvezels aangetroffen en hun aantal lijkt daarbij duidelijk gekoppeld aan de dikte van de te innerveren spierlaag (cfr. sphincters). In de submucosa werden zowel bij het volwassen schaap als bij de foetus dopamine-beta-hydroxylase-houdende zenuwbundels gezien. In het voormagencomplex van de foetus wordt in alle uitstulpingen van de submucosa een zeer fijn dopamine-beta-hydroxylase-immunoreactief zenuwnetwerk gevonden. Gelet op het geringe aantal ganglia en neuronen in deze tunica (cfr. deel II) kon niet uitgemaakt worden of deze structuren dopamine-beta-hydroxylase-immunoreactieve zenuwvezels ontvangen. Door het "ontbreken" van een plexus submucosus in de voormagen van het volwassen schaap kon een gelijkaardig beeld, met uitzondering van een dopamine-beta-hydroxylase-immunoreactieve perivasculaire plexus, niet worden waargenomen. De dopamine-beta-hydroxylase-innervatie van de kliermaag lijkt zich bij de foetus en het volwassen dier tot de submucosale bloedvaten te beperken, hoewel sporadisch immunoreactieve zenuwvezels in de plexus van Meissner werden vastgesteld. In de tunica mucosa van het voormagencomplex kon noch bij de foetus noch bij het volwassen schaap enige dopamine-beta-hydroxylase-immunoreactiviteit teruggevonden worden. In de kliermaag daarentegen werden dopamine-beta-hydroxylase-houdende perivasculaire zenuw-vezels waargenomen.
Het directe myogene effect van noradrenaline op de herkauwersmaag lijkt zich tot de longitudinale (stijging) en de circulaire (daling van de tonus) spierlaag van respectievelijk de ventrale en dorsale penszak te beperken. Met andere woorden alpha- en beta-adrenoreceptoren komen waarschijnlijk voor op de membraan van de gladde spiercellen van de pens. Nochtans zal, zoals de bovengeschetste morphologie suggereert en zoals dit bij andere diersoorten kon worden waargenomen, noradrenaline de motiliteit van de herkauwersmaag eerder indirect d.i. via zijn modulerend effect op de ganglionaire transmissie in de plexus van Auerbach beïnvloeden.
In deze studie kon, in tegenstelling tot de literatuur, noch in de herkauwersmaag van de foetus noch in deze van het volwassen schaap een dubbele localisatie van serotonine (d.i. in het epitheel en in de intramurale plexussen) worden vastgesteld. Inderdaad werd enkel in sommige epitheliale cellen serotonine-immunoreactiviteit waargenomen. Bij de foetus werden zulke epitheelcellen in alle bestudeerde segmenten gezien. In de voormagen localiseren deze cellen zich uitsluitend in de germinatieve laag van het meerlagige, klierloze epitheel. Vooral de boekmaag lijkt relatief veel van deze cellen te bevatten. In het klierige maagdeel liggen de serotonine-houdende cellen langsheen het ganse epitheel uitgespreid hoewel ze zich blijkbaar concentreren in het basale deel van de maagklieren. Bij het volwassen schaap doen serotonine-immunoreactieve epitheliale cellen zich enkel en alleen voor in de lebmaag en dit, net zoals bij de foetus, bij voorkeur in het fundusdeel van de klieren.
Uit de in vitro experimenten blijkt dat serotonine een direct effect op de spiertonus van de pens (toename) en de boekmaag (afname) uitoefent, doch slechts een matige of geen invloed op de overige segmenten heeft. Het stimulerend effect van serotonine op de spiertonus

van de pens ligt in de lijn van de resultaten gevonden bij andere diersoorten. Hoewel de functionele betekenis van het relaxerend effect van serotonine op de boekmaag vanuit deze studie niet direct kan verklaard worden, blijkt eveneens uit de literatuur dat de intestinale musculatuur naargelang species en regio verschillend op serotonine kan reageren.

Het voorkomen van de peptiden vasoactive intestinal polypeptide en substance P in de herkauwersmaag van het schaap werd zowel radioimmunologisch als immunohistochemisch onderzocht.
Radioimmunoassay van vasoactive intestinal polypeptide bewees de aanwezigheid van dit peptide in de gestripte preparaten van alle onderzochte segmenten. De hoogste concentratie werd daarbij steeds teruggevonden in de spierlaag die, met uitzondering van de boekmaag, op zich geen opvallende regionale verschillen vertoont. In zijn geheel genomen bevat de mucosa beduidend minder vasoactive intestinal polypeptide dan de spierlaag. Daarenboven konden hier, in tegenstelling tot de muscularis, duidelijke regionale verschillen worden aangetoond. De mucosa van de voormagen bevat het minste vasoactive intestinal polypeptide. Binnen dit complex werden de hoogste concentraties in deze segmenten gevonden waar de lamina muscularis mucosae, die immunohistochemisch een duidelijke vasoactive intestinal polypeptide-houdende innervatie ontvangt, het sterkst ontwikkeld is. Relatief hoge waarden werden in het klierig maagdeel aangetoond. Dit is naar alle waarschijnlijkheid toe te schrijven aan de goed ontwikkelde plexus mucosus, aan de door vasoactive intestinal polypeptide-houdende zenuwvezels geïnnerveerde lamina muscularis mucosae en aan de goed uitgebouwde plexus submucosus. De resultaten van het immunohistochemisch onderzoek naar het voorkomen en de spreiding van vasoactive intestinal polypeptide in de herkauwersmaag van het schaap, bevestigden en vervolledigden de resultaten van de radioimmunoassay. Vasoactive intestinal polypeptide-immunoreactiviteit werd daarbij uitsluitend in de intramurale nerveuze elementen van alle onderzocht segmenten gevonden.
In de longitudinale spierlaag van de herkauwersmaag werd, voornamelijk in cryostaatcoupes, een duidelijke vasoactive intestinal polypeptide-immunoreactive intramusculaire plexus waargenomen. De densiteit van deze plexus lijkt gecorreleerd aan de dikte van de betreffende spierlaag.
In de plexus van Auerbach werd altijd vasoactive intestinal polypeptide-immunoreactiviteit waargenomen. Talrijke intraganglionaire vasoactive intestinal polypeptide-houdende zenuwvezels doen zich over de gehele lengte van de herkauwersmaag voor. Vasoactive intestinal polypeptide-immunoreactieve neuronen manifesteren zich vooral in het klierige maagdeel en wat minder frequent in de voormagen.
Het dichtste vasoactive intestinal polypeptide-houdend zenuwvlechtwerk doet zich steeds voor in de circulaire spierlaag en dit in opvallend sterke mate ter hoogte van de sphincters of de sphincterachtige regio's.
De vasoactive intestinal polypeptide-houdende innervatie van de submucosa en de mucosa vertoont een aantal opmerkelijke regionale verschillen, die vooral in vriescoupes tot uiting komen. Voor het volwassen schaap wordt de duidelijkste reactie gezien in het klierig maagdeel met vasoactive intestinal polypeptide-immunoreactieve zenuwvezels in de plexus van Meissner, in de lamina muscularis mucosae, in de "mucosale" ganglia en in de lamina propria. Alleen in cryostaatcoupes van het voormagencomplex konden enkele losse vasoactive intestinal polypeptide-houdende zenuwvezels in de submucosa worden aangetoond. In alle voormaagsegmenten wordt de lamina muscularis mucosae door meerdere, fijne vasoactive intestinal polypeptide-houdende zenuwvezels geïnnerveerd. In de mucosa tenslotte werd geen immunoreactiviteit aangetroffen.
De resultaten vastgesteld in vriescoupes van de foetale lebmaag zijn goed vergelijkbaar met deze beschreven voor het volwassen schaap.
In alle submucosa plooien van het foetale voormagencomplex wordt, in tegenstelling tot het volwassen dier, steeds een delicaat vasoactive intestinal polypeptide-houdend zenuwnetwerk gevonden. Net zoals voor de volwassen species bevat de lamina muscularis mucosae vasoactive intestinal polypeptide-houdende zenuwvezels.
Vasoactive intestinal polypeptide-immunoreactieve zenuwvezels werden in nagenoeg alle intramurale perivasculaire plexussen opgemerkt.
In de doorgevoerde in vitro experimenten heeft vasoactive intestinal polypeptide, algemeen gesproken, een duidelijk direct relaxerend effect op de gladde musculatuur van de herkauwersmaag. Inderdaad induceert het peptide een daling van spiertonus in de pens, de boekmaag en de lebmaag evenals in de sphincters (slokdarmsphincter en reticulaire groeve). Nochtans schijnen zowel binnen éénzelfde segment als tussen de onderscheiden segmenten de circulaire en de longitudinale spierlaag verschillend te reageren.

Substance P, tenslotte, kon -hoewel in beduidend lagere concentraties dan het voorgaande peptide- radioimmunologisch in alle segmenten van de volwassen herkauwersmaag worden aangetoond. In het klierige maagdeel worden de hoogste substance P waarden in de tunica muscularis teruggevonden, terwijl de mucosa relatief geringe hoeveelheden substance P bevat met evenwel iets hogere concentraties in deze compartimenten waar de lamina muscularis mucosae aanwezig is. In het klierige maagdeel daarentegen bevat de mucosa, met uitzondering van de pylorus, de hoogste substance P gehaltes. Wellicht kan dit verklaard worden door de aanwezigheid van het peptide in de lamina propria, de lamina muscularis mucosae en de Meissner plexus.
Zoals dit voor vasoactive intestinal polypeptide het geval was, werd een opvallende overeenstemming tussen de resultaten van de radioimmunoassay en de immunohistochemie gevonden. In tegenstelling echter tot de literatuur, localiseert deze studie substance P-immunoreactiviteit uitsluitend in het intramurale zenuwnetwerk en is, in zijn geheel genomen, de intramurale substance P innervatie van de herkauwersmaag zwak vergeleken met deze van het vasoactive intestinal polypeptide.
De longitudinale spierlaag bevat in de regel een weinig substance P-houdende zenuwvezels. Hun aantal lijkt echter evenredig te stijgen met de toename in dikte van de spierlaag (cfr. reticulum).
De plexus myentericus bevat meerdere substance P-houdende zenuwvezels doch slechts enkele substance P-immunoreactieve neuronen.
Zoals voor vasoactive intestinal polypeptide werd geconstateerd, bevat de circulaire spierlaag de hoogste concentratie aan substance P -houdende zenuwvezels. Hoewel geen opvallende regionale verschillen in de dichtheid van deze innervatie werden vastgesteld, lijkt ook hier -zoals dit tevens voor de overige bestudeerde neurotransmitters het geval was- de densiteit van het intramusculaire substance P-immunoreactieve zenuwvlechtwerk gekoppeld aan de dikte van de circulaire spierlaag.
In de submucosa van de voormagen van het volwassen dier werden nagenoeg geen (in vriescoupes enkele) substance P-houdende zenuwvezels teruggevonden. In de plexus submucosus van de "echte" maag doen zich zowel substance P-immunoreactieve neuronen als meerdere zenuwvezels voor.
Zowel in het klierloze als in het klierige maagdeel van de foetale herkauwersmaag kan men een duidelijke substance P-immunoreactiviteit vaststellen. In nagenoeg alle submucosaplooien van de voormagen wordt een delicaat maar duidelijk substance P-immunoreactief zenuwvlechtnetwerk teruggevonden. Hoe groter de plooi, hoe geprononceerder diens begleidende plexus. Het voorkomen alsook de spreiding van substance P in de submucosa van het klierige maagdeel valt volkomen te vergelijken met de resultaten van het volwassen schaap.
De lamina muscularis mucosae wordt over de gehele lengte van de herkauwersmaag zowel bij de foetus als bij het volwassen dier door meerdere substance P-houdende zenuwvezels geïnnerveerd. Met uitzondering van de kliermaag kon geen substance P-immunoreactiviteit in de lamina propria worden aangetoond. Substance P-immunoreactieve perivasculaire zenuwvezels werden in alle segmenten teruggevonden.
Functioneel gesproken heeft substance P slechts een matig direct effect op de spiertonus van de herkauwersmaag. Het peptide reduceert de tonus van de pens en de slokdarmsphincter, terwijl enkel in de reticulaire groeve substance P een toename van de spiertonus induceert. Het lijkt er dus op dat substance P eerder een indirect modulerend dan wel een direct effect de spiertonus van het voormagencomplex uitoefent.
Als meest relevante conclusie van het laatste deel van dit onderzoek kan naar voorgebracht worden dat de klassieke opvatting van een tweeledige innervatie (sympatisch, parasympatisch) van de herkauwersmaag niet langer houdbaar is. Inderdaad, werden in deze studie naast acetylcholine en noradrenaline ook andere neurotransmitters met name vasoactive intestinal polypeptide en substance P in het intramurale zenuwnetwerk aangetoond. Op grond van deze gegevens lijkt de neurochemische bouw van de herkauwersmaag zich deels te weerspiegelen in de in deel I beschreven chemische complexiteit van het enterische zenuwstelsel.
Daarenboven heeft de functionele benadering van dit onderzoek op klare wijze aangetoond dat de verschillende morphologisch geïdentificeerde neurotransmitters direct of indirect betrokken zijn in de regulatie van de spiertonus van de herkauwersmaag en zodoende in de besturing van de motiliteit van het hele complex.
Maar naar alle waarschijnlijkheid licht deze studie slechts een tip van de sluier, die de nog veel complexere chemische bouw van het intramurale zenuwvlechtwerk in de herkauwersmaag bedekt, op. Immers met de literatuurgegevens en de voorliggende onderzoeksresultaten voor ogen kan gesteld worden dat het enterische zenuwstelsel in de herkauwersmaag zich hoogst

waarschijnlijk eveneens van een brede waaier aan neurochemische substanties bedient om zijn "vitale" opdracht uit te voeren. Wellicht zullen ook hier de verschillende neurotransmitters/modulatoren hetzij afzonderlijk hetzij in combinatie met elkaar ingeschakeld worden om de diverse, vaak aan elkaar gerelateerde deelopdrachten, binnen de taakomschrijving van de maag-darminnervatie te helpen realiseren.
Juist omwille van zijn complexiteit is het enterische zenuwstelsel in zijn functioneren een relatief kwetsbaar systeem. Derhalve kunnen subtiele veranderingen, welke op zich beschouwd onbelangrijk lijken, in dit complexe nerveuze raderwerk toch verstrekkende functionele stoornissen voor de herkauwersmaag met zich meebrengen. De klinische relevantie van deze stelling wordt ten overvloede bewezen door het uitgebreide spectrum aan en het vaak voorkomen van voormaagpathologieën.

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CURRICULUM VITAE

De auteur van dit proefschrift werd als tweede telg uit een gezin van zes kinderen op 12 december 1947 te Bonn (W. Duitsland) geboren. De lagere school doorliep hij te Mechelen. Zijn middelbare studies (Latijn-Wetenschappen) sloot hij in 1966 af aan de Scheppers Normaalschool te Alsemberg alwaar hij een jaar later het diploma van onderwijzer behaalde.
In 1967 vatte hij aan de Katholieke Universiteit Leuven de studies in de Diergeneeskunde aan. In 1974 promoveerde hij aan de Rijksuniversiteit van Gent tot Doctor in de Diergeneeskunde. Reeds tijdens zijn doctoraalopleiding was hij als student-assistent werkzaam op het Laboratorium voor Anatomie van de Huisdieren van de Rijksuniversiteit Gent en was hij actief betrokken in het begeleiden van de practische oefeningen Anatomie.
In 1974 trad hij als wetenschappelijk medewerker in dienst bij het Laboratorium voor Anatomie en Embryologie van de Huisdieren (diensthoofd Prof. Dr. P. Krediet) van het Rijksuniverstair Centrum Antwerpen. In deze hoedanigheid werd hij ingeschakeld in het theoretisch en praktische onderricht aan de kandidaatsstudenten Diergeneeskunde. Van 1974 tot 1977 begeleidde hij mede de praktische oefeningen in de microscopische anatomie aan de studenten Diergeneeskunde, Geneeskunde en Tandheelkunde. In 1978 werd hij aangesteld als eerstaanwezend assistent in de dienst Anatomie en Embryologie van de Huisdieren. In 1983 tenslotte, werd hij benoemd tot geassocieerd docent en toegevoegd aan de Leerstoel Diergeneeskunde.
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Hij is lid van meerdere wetenschappelijke verenigingen waaronder :
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