COLON
Structure and Function

Edited by
Luis Bustos-Fernández

TOPICS IN GASTROENTEROLOGY • SERIES EDITOR: HOWARD M. SPIRO

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TOPICS IN GASTROENTEROLOGY

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PLENUM MEDICAL BOOK COMPANY
NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Main entry under title:

Colon, structure and function.

(Topics in gastroenterology)
Includes bibliographical references and index.
1. Colon (Anatomy) I. Bustos-Fernández, Luis. II. Series.
QP156.C64 1982 612'.36 82-20413

ISBN 978-1-4757-0309-2 ISBN 978-1-4757-0307-8 (eBook)
DOI 10.1007/978-1-4757-0307-8

233 Spring Street, New York, N.Y. 10013

Plenum Medical Book Company is an imprint of Plenum Publishing Corporation

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Preface

The functional and organic alterations of the colon constitute one of the leading reasons why patients consult gastroenterologists. The irritable colon is one of the most common causes of discomfort in human beings. The organic pathology of the large bowel (malignancy and chronic inflammatory disease) contributes, particularly among Occidental peoples, to discouragingly high levels of morbidity and mortality.

One realizes the importance of having a thorough physiologic knowledge of the colon in order to scientifically plan the functional treatment of organic colonic diseases.

If we consider the large amount of material published on the physiology of the esophagus, stomach, small bowel, pancreas, and liver, we realize that the colon has been relatively neglected. The chapters in this book have been written by people who have done their utmost to alter this imbalance.

I want to thank all the contributors for their generous collaboration that allows me to present in one volume virtually all the information known about the structure and function of the colon, and to record my deep graditude to Dr. Howard Spiro for his willingness to include this volume in his series. I would also like to express my sincere appreciation to Plenum Publishing Corporation for making this book possible. A special thanks goes to Dr. Isable Ledesma de Paolo for her cooperation in selecting the material to be published, to my daughter María Angélica Bustos-Fernández de Aragone for her diversified help in the preparation of the work, and also to my secretary Marcela Claudia Alvarez for her correspondence with the authors, as well as her diligent processing and retyping of some of the revised chapters.

My wife Susana was once more an important supporter of my work.

Luis Bustos-Fernández

Contents

10. Nerves of the Colon

Ove Lundgren and Mats Jodal

11. Circulation of the Colon

Ove Lundgren and Mats Jodal

1

Colon Structure

Gregory L. Eastwood

I. DEVELOPMENT OF THE COLON

During the 5th week of human gestation, the midgut begins to elongate rapidly and extrudes into the umbilical cord. The subsequent enlargement and return of the midgut loop to the abdomen, and the rotation of the bowel during this process, determines the adult configuration of the colon.

The rotation and final placement of the midgut occurs in three stages. The first stage marks the extrusion of the midgut into the umbilical cord, where it remains between the 5th and 10th weeks. This process produces a 90-degree-counterclockwise rotation of the midgut loop, when the embryo is viewed from the ventral side.

The second stage coincides with the return of the midgut to the abdomen. The small intestine enters first, to the right of the superior mesenteric artery, but as more gut returns, the first folds are pushed behind the artery into the left abdomen. These loops of small bowel, in turn, displace the dorsal mesentery of the hindgut to the left so that the descending colon occupies the left flank. The cecum and ascending colon are the last to be reduced into the abdomen and lie at first in the right upper quadrant in front of the small bowel. The colon subsequently elongates, pushing the cecum downward by the 11th week, and completing a 270-degree rotation of the midgut loop.

During the third stage, which continues until shortly before birth, the cecum descends further into the iliac fossa. The mesenteries of the cecum, ascending colon, hepatic flexure, splenic flexure, and descending colon become fused to the posterior abdominal wall, leaving only the transverse colon and sigmoid colon on free mesenteries. The final placement of the colon within the abdomen is thus achieved.

In the rat, between the 16th and 20th days of a 22-day gestation, the mucosa of the colon changes from a simple tube with a tiny lumen lined by stratified epithelium (Fig. 1) to a much more complex structure with a large lumen and well-developed crypts

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lined by a single layer of columnar epithelium (Fig. 2).[1] Further, proliferating cells are present throughout the early stratified epithelium. However, when the crypts develop, the proliferating cells are confined to the lower half of the crypts.[1] An increase in glycoprotein synthesis and the development of the adult pattern of intracellular migration of newly synthesized glycoproteins parallel the changes in cellular differentiation.[2]

Nearly 70 years ago, Johnson described the development of the mucosa of the large intestine in human embryos.[3] He found that the large intestine initially is a simple tube of epithelium, similar to the findings in the rat, and is the last portion of the digestive tract to show distinctive changes in the mucosa. The first change to occur is the formation of longitudinal ridges, which appear first in the rectum and then extend proximally. The ridges also arise in the region of the cecum and subsequently extend distally. Thus, the transverse colon is the last portion of the colon to develop these structures. Villi then form from the longitudinal ridges by segmentation of the ridges and budding of new growths. The mucosal crypts develop from knoblike protuberances of epithelium which extend into the underlying mesenchyme. As the crypts enlarge, the villi diminish in size, until the mature pattern of a flat mucosa which contains only closely spaced crypts, without villi, is achieved.

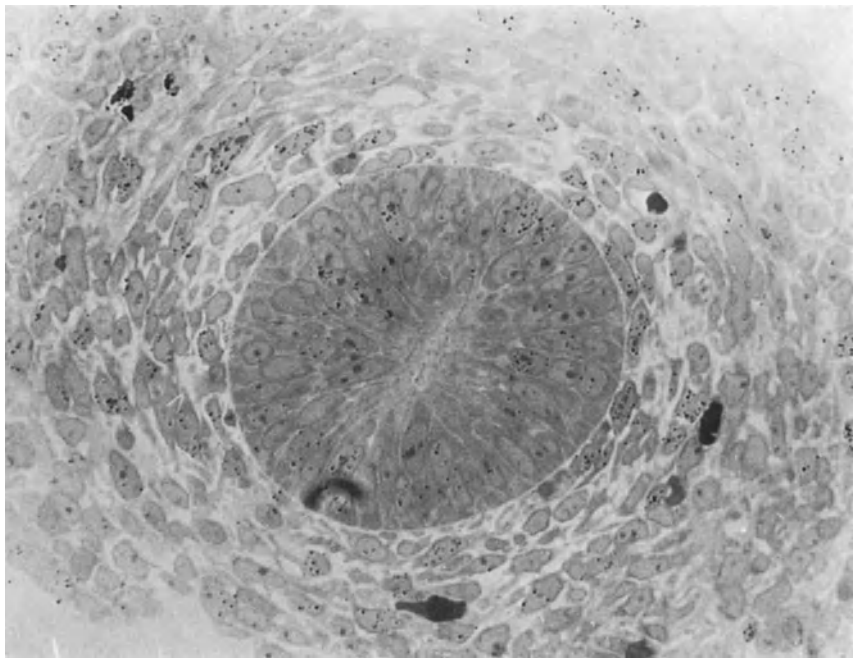

Figure 1. Light microautoradiograph of a cross-section of the colon from a 16-day-gestation fetal rat. The tubular gut consists of a tiny lumen surrounded by 2- to 3-cell-thick layer of stratified epithelium, which in turn is surrounded by a concentrically arranged mesenchyme. Labeled nuclei, identified by accumulations of black granules, are randomly scattered throughout the epithelium and the mesenchyme. (Toluidine blue, × 600)

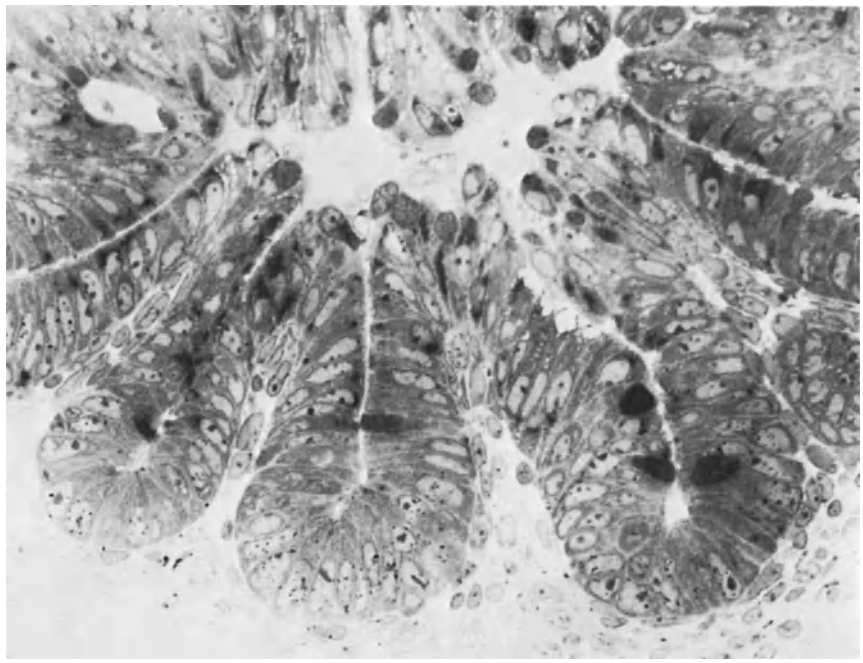

Figure 2. Light microautoradiograph of the colon from a 20-day-gestation fetal rat. Labeled nuclei are confined to the lower portions of well-formed crypts separated by mesenchymal lamina propria. (Toluidine blue, × 600)

The development of gut epithelium may be related intimately to the underlying mesenchymal tissue. During fetal and early postnatal development of the rat duodenum, numerous epithelial cell cytoplasmic processes project through gaps in the basement membrane to make contact with underlying mesenchymal cells.[4] By 4 weeks after birth the number and size of the processes have decreased to resemble the epithelio-mesenchymal interface in adults, where only rare epithelial processes are found. What role the mesenchyme plays in eipthelial development is unclear, but the observation that cultured or grafted rabbit gastric epithelium cannot develop when deprived completely of mesenchyme[5] suggests that the mesenchyme is vitally important during organogenesis of fetal tissue.

II. MACROANATOMY

The large intestine extends from the ileocecal junction to the anus (Fig. 3). In adults, the large intestine is about 150 cm (5 ft) in length, but variations occur, primarily due to lengthened, redundant sigmoid colon.

The cecum, to which the vermiform appendix attaches, is located in the right lower abdomen. It forms a blind pouch below where the terminal ileum inserts into the

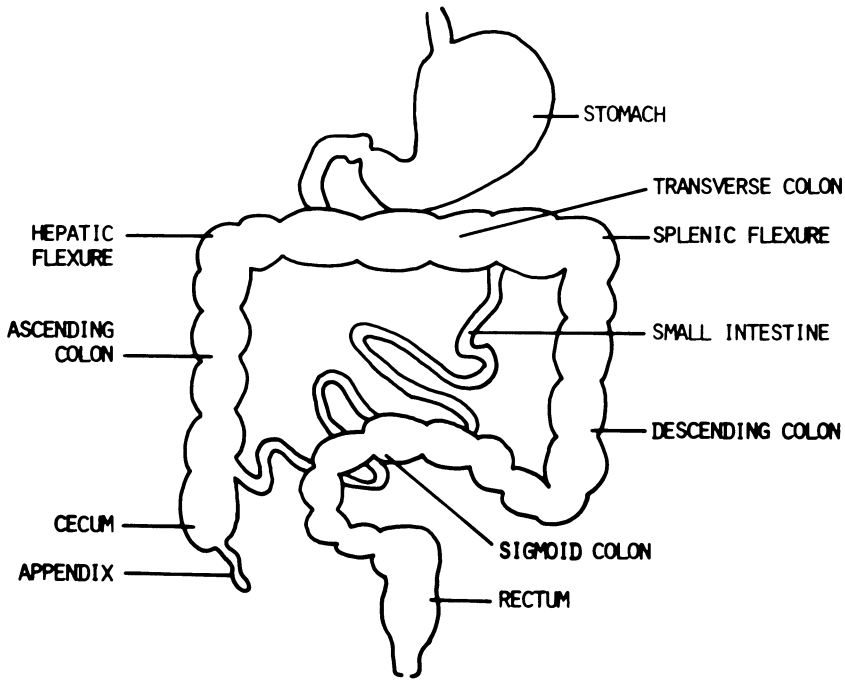

Figure 3. Diagram of the gastrointestinal tract.

colon medially. The ascending colon continues cephalad to the right upper abdomen, where it turns at the hepatic flexure to become the transverse colon. The transverse colon then extends anteriorly across to the splenic flexure in the left upper abdomen, which marks the beginning of the descending colon. In the left lower abdomen, the descending colon connects with the sigmoid colon, which, by a series of one or more S-shaped loops in the pelvis, empties into the rectum. The rectum, which is about 15 cm long, terminates at the anus.

Although the muscular coat of the colon is composed of inner circular and outer longitudinal layers as are found elsewhere in the gut, in the colon the longitudinal muscle fibers are concentrated into three flat, long bands called taeniae coli. The taeniae coli run the entire length of the colon and are equally spaced around the circumference. The posterior taenia runs along the border, which is attached to the mesentery; the anterior taenia is located one-third of the circumference around on the anterior aspect of the colon; and the lateral taenia extends along the medial side of the ascending and descending colon and on the under aspect of the transverse colon. Because the taeniae are shorter than the other coats of the colon, they cause the wall to contract and form sacculations called haustrae.

The anatomy of the anal canal and lower rectum is of special interest. In the upper anal canal a number of longitudinal folds are produced by an infolding of the mucosa and muscle layer. These folds are called the rectal columns of Morgagni. They are separated from one another by rectal sinuses that end distally in small valvelike folds

called anal valves. Above, in the rectum at several levels, are three or four transverse semilunar folds known as Houston's valves. The muscle coat which surrounds the anal canal has a specialized sphincter function. The inner circular smooth muscle becomes thickened to form the internal anal sphincter. Around this is a circular ring of striated muscle called the external sphincter.

III. MICROANATOMY

A. The Four Layers of the Colon

The four layers of the colon are mucosa, submucosa, muscularis externa, and serosa (Fig. 4).

The *mucosa,* which lines the lumen of the colon, is divided further into epithelium, lamina propria, and muscularis mucosae (Fig. 4). The architectural plan of the mucosa of the large intestine is simple compared with that of the stomach or small bowel. The surface of the mucosa is flat, and numerous, closely spaced crypts extend down into the mucosa (Fig. 5). A continuous, single-cell-thick layer of *epithelium* lines the crypts and covers the mucosal surface. The various epithelial cell types will be discussed in detail below.

The lamina propria occupies the space between and beneath the crypts. It is composed of loose connective tissue and contains lymphatics, blood vessels, nerves, and smooth-muscle fibers. Further, plasma cells and lymphocytes are found within the lamina propria in varying numbers.

Beneath the crypts and perpendicular to them lies a continuous sheet of smooth muscle, the muscularis mucosae.

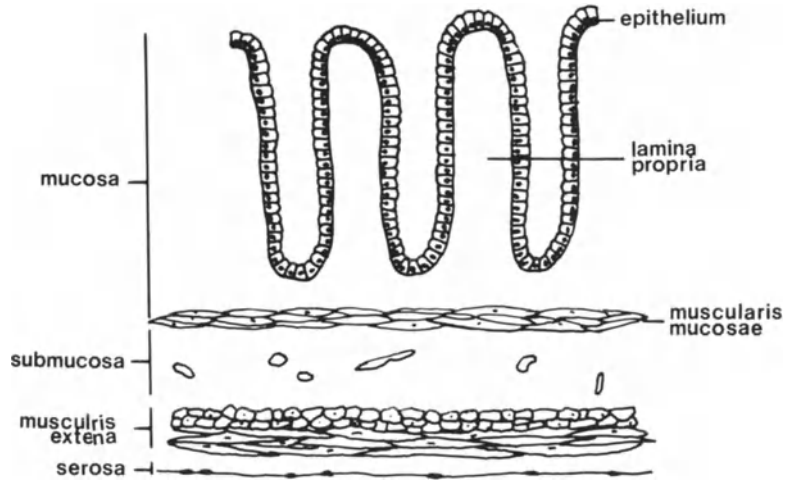

Figure 4. Diagram of the four major layers of the large intestine and the three components of the mucosa.

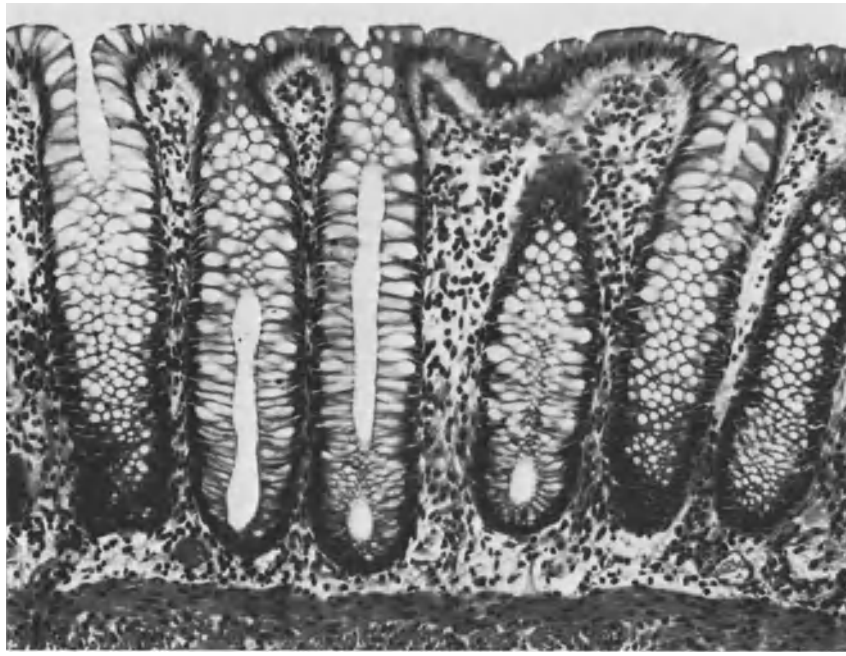

Figure 5. Light micrograph of a normal human rectal biopsy. The columnar epithelium, composed primarily of goblet cells and absorptive cells, lines the crypts and covers the surface of the mucosa. The lamina propria, which lies between the crypts, contains connective tissue, round cells, blood vessels, lacteals, and smooth-muscle fibers. The muscle fibers which run horizontally at the bottom of the figure compose the muscularis mucosae. (Hematoxylin and eosin, × 150)

The *submucosa* is a layer of connective tissue which lies immediately beneath the mucosa. It provides a loose framework for the passage of arteries, veins, lymphatics, and nerves.

The *muscularis externa* is the major muscle coat of the large intestine. An inner layer of circular fibers surrounds the bowel. However, the outer longitudinal layer is not continuous around the bowel but rather forms the taeniae coli as described above.

The *serosa,* the outermost coat of the large intestine, consists of areolar tissue covered by a single layer of squamous mesothelial cells.

B. Epithelial Renewal

The epithelium of the entire gastrointestinal tract undergoes constant, rapid renewal.[6] In the large intestine, this process begins within the lower two-thirds of the crypts, where undifferentiated cells divide to provide a constant source of new cells. The cells then migrate up the crypts and are lost into the gut lumen at the mucosal surface.

The crypts are surrounded by a sheath of fibroblasts within the lamina propria, which in the colon of the rabbit undergo proliferation and migration in synchrony with epithelial migration.[7] Whether these fibroblasts ultimately are sloughed into the lumen

of the bowel in a manner analogous to the fate of mature epithelial cells, or whether they are "recycled" to deeper layers of the lamina propria, is unknown. Similar experiments using the colon of rats have failed to confirm migration of the pericryptal fibroblasts, although the fibroblasts do incorporate tritiated thymidine ([^{3}H] − TdR). Thus, in the rat, pericryptal fibroblasts do constitute a slowly renewing cell population.[8]

Proliferating epithelial cells within the crypts undergo a sequence of phases called the cell renewal cycle (Fig. 6). Upon completion of mitosis (M phase), proliferating daughter cells enter the first portion of interphase, the postmitotic-presynthetic gap, or G_1 phase. DNA replication then ensues during the DNA synthesis (S) phase, followed by another interval, the postsynthetic-premitotic gap, or G phase, before mitosis occurs again. As indicated in Fig. 6, cells which have undergone mitosis do not necessarily continue the cyclic process of cell division. Some cells may enter a prolonged G_1 phase called G_0 phase, during which DNA synthesis and mitosis are temporarily suspended, but the potential for cell proliferation remains. Other cells, on completion of mitosis, enter a nonproliferative phase, migrate out of the proliferative zone, and differentiate into mature cells.

The events of the cell renewal cycle are accompanied by great metabolic activity.[9] Although DNA synthesis occurs only during a discrete period, synthesis of RNA and protein is active throughout the cycle except during M phase, when RNA synthesis stops and protein synthesis decreases. During G_1 phase, the replication of new DNA is anticipated by the synthesis of enzymes involved in nucleic acid metabolism. Indeed, proliferating cells have characteristic patterns of nucleic acid metabolic enzymes which change as the cells differentiate and migrate out of the proliferative zone. [10,11] Thymidine kinase activity, for instance, is high in the proliferating crypt cells of human and rat small and large intestine, but activity diminishes as the cells migrate up the crypts and onto the villi.

The durations of the phases within the cell renewal cycle are estimated by the autoradiographic method of counting the percentage of mitoses which are labeled at various times after a pulse label of [^{3}H]-TdR (Fig. 7). For example, if the first labeled mitosis does not appear until 1 hr after injection of the label, then the minimum duration of the G_2 phase is 1 hr. The reason for this is that in order for a mitotic figure to be labeled, the radioisotope must have been incorporated into the DNA during the synthesis of new DNA in that cell. In this example, the [^{3}H]-TdR was incorporated into newly synthesized DNA toward the end of the S phase; the cell then proceeded through

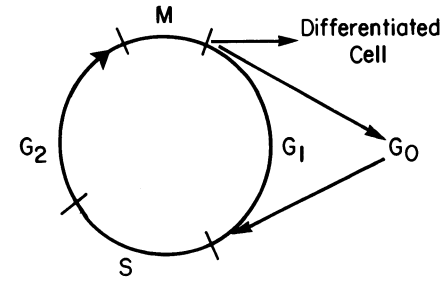

Figure 6. The cell renewal cycle. G_1, postmitotic-presynthetic gap; S, DNA synthesis; G_2, postsynthetic-premitotic gap; M, mitosis; G_0, prolonged G_1.

G_2 phase and finally entered M phase, when it became identified as a labeled mitosis 1 hr later. Similarly, other cells which were in mid or early S phase at the time of the [^{3}H]-TdR pulse label will manifest labeled mitoses at later times. Therefore, over a period of time, the fraction of labeled mitoses rises, then falls, then rises again as the events of the cell renewal cycle recur. Because the duration of the cell renewal cycle of all cells within a given gastrointestinal epithelial proliferative zone is not uniform,[12] the actual and theoretical curves of labeled mitoses differ. The actual curves are rounded, the first wave approaches but does not reach 100% labeled mitoses, and the second and subsequent waves become progressively wider and attenuated, deviating even further from the theoretical curves.[13] Nevertheless, using the reasoning explained in the legend under Fig. 7, estimates of the durations of the phases in the cell renewal cycle can be made from analysis of the configuration and duration of the first wave of labeled mitoses and the time between the first and second waves.

The total duration of the cell renewal cycle in human intestinal epithelium is on the order of 1–2 days. The durations of the phases of the cell renewal cycle vary from one region of the gastrointestinal tract to another, but in general, S phase requires about 10 h, G_2 phase requires 1–6 h, and M phase about 1 h.

C. Epithelial Cell Types

Three major cell types are found in the epithelium of the large intestine, namely, columnar absorptive cells, mucous (goblet) cells, and enteroendocrine cells.

The ultrastructure of a mature *columnar absorptive cell* is shown in Figs. 8 and 9. These cells are characterized by stubby, closely packed microvilli that protrude from the apical cell surface and are covered by a glycoprotein filamentous matrix called the "fuzzy coat" or glycocalyx. Within the microvilli are fine filaments which extend into and intermesh with a network of filaments across the apical aspect of the cell, called the terminal web.

Adjacent cells are attached to one another near their apical margins by a typical junctional complex, composed of a tight junction and intermediate junction.[14] A tight junction is formed when the outer leaflets of the trilaminar plasma membranes from two adjacent cells become fused to form a pentalaminar structure (Fig. 8). Immediately below the tight junction the lateral plasma membranes become uniformly separated for a short distance to form the intermediate junction. The junctional complex surrounds each cell like a belt, forming a tight apposition of the apical perimeter of each cell with its neighbors. In addition to the junctional complexes, disklike desmosomes are scattered throughout the lateral plasma membranes to provide additional intercellular attachments. The lateral plasma membranes of adjacent cells are redundant and interdigitate with one another. Because of this, the intercellular space may be quite variable. Within the columnar absorptive cell are the nucleus, which may have a smooth or scalloped margin, numerous mitochondria, lysosomes, endoplasmic reticulum, Golgi material, microtubules, and free ribosomes.

Columnar absorptive cells originate in the lower crypts as immature proliferative cells, differentiate as they migrate up the crypts, and finally are extruded from the mucosal surface into the bowel lumen.[15,16] This process requires 2–3 days in rodents. In human beings, estimates of migration range between 3 and 8 days.[17–20]

Mucous (goblet) cells contain abundant mucous globules in the apical cytoplasm (Fig. 10). The nucleus is depressed to the base of the cell by the mass of mucous gran-

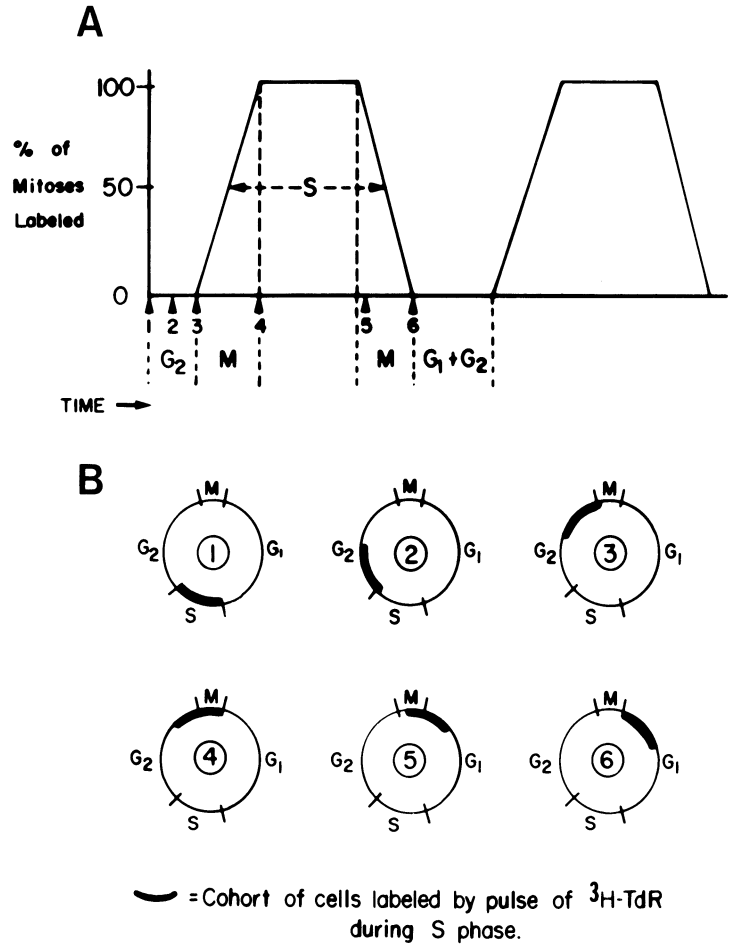

Figure 7. (A) Theoretical curve of percentage of mitoses labeled after exposure to a [^{3}H]-TdR pulse for a population of proliferating cells with a cell renewal cycle of uniform duration. The stages of the cell renewal cycle, G_1, S, G_2, and M, are identified in Fig. 6. Numbers along the time axis refer to events depicted in Fig. 7B. (B) Progression through the cell renewal cycle of a cohort of cells labeled by a pulse of [^{3}H]-TdR during the DNA synthesis (S) phase. (1) A pulse of [^{3}H]-TdR labels the cohort of cells currently synthesizing DNA. (2) The cohort of labeled cells has moved into G_2 phase. Inasmuch as none of the labeled cells has entered M phase, no mitotic figures will be labeled. (3) The leading cells of the cohort, that is, cells which had incorporated the [^{3}H]-TdR during the end of S phase, have begun to enter M phase. A small percentage of mitoses will be labeled. The time between the exposure of the cells to the [^{3}H]-TdR pulse and the appearance of the first labeled mitosis represents the duration of G_2. The percentage of mitoses labeled will increase as more labeled cells enter M phase. (4) The cohort of labeled cells has advanced so that all cells undergoing mitosis are in the cohort. Therefore, 100% of the mitoses will be labeled. The time between the appearance of the first labeled mitoses (3, above) and 100% labeling of mitoses is taken as the duration of M phase. (5) As the cohort proceeds through M phase, all mitoses will continue to be labeled until unlabeled cells trailing the cohort begin to enter M phase. The percentage of mitoses labeled then will fall progressively to zero over a period again equal to the duration of M phase. The duration of S phase is usually considered to be the time between the 50% points on the ascending and descending limbs of the curve. This avoids the inclusion of two M-phase periods as would occur if the total time under the curve were taken as S phase. (6) The entire cohort of labeled cells has entered G_1 phase. There will be no labeled mitoses. The time between the disappearance of labeled mitoses from the first wave and the reappearance of labeled mitoses in the second wave represents the duration of G_1 plus G_2 phase. This is because at the time of the disappearance of the labeled mitoses, the leading cells of the cohort of labeled cells have already been in G_1 phase for a time equal to the duration of S phase, leaving a time equal to the sum of G_1 + G_2 through which the leading cells must pass before they again enter M phase.

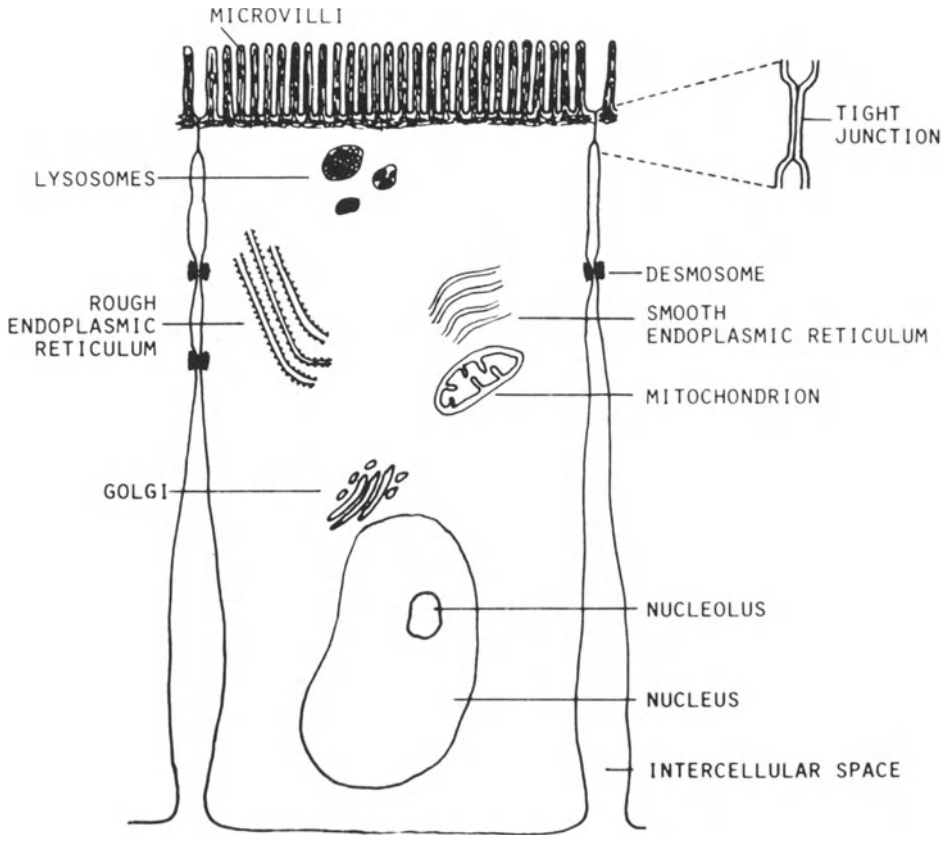

Figure 8. Diagram of the electron-microscopic appearance of a columnar epithelial cell in the large intestine. See text for further information.

ules. Above the nucleus, the Golgi material appears well developed. Granular endoplasmic reticulum and mitochondria are found in the few areas of the cytoplasm not occupied by mucous granules. Mucous cells appear to arise from the immature stem cells that proliferate in the lower portion of the crypt.

The epithelium of the colon contains a wide variety of *enteroendocrine* cells which have in common the intracytoplasmic accumulation of secretory granules, but which may be distinguished on the basis of granule morphology and fluorescent antibody staining. As is the case with the other major cell types, enteroendocrine cells in the rat also appear to arise from undifferentiated proliferating crypt cells.[16]

IV. CONDITIONS WHICH AFFECT COLONIC STRUCTURE

A. Food

Because of the intimate relationship between the gastrointestinal tract and ingested food, it is reasonable to expect that food might affect the form and function of

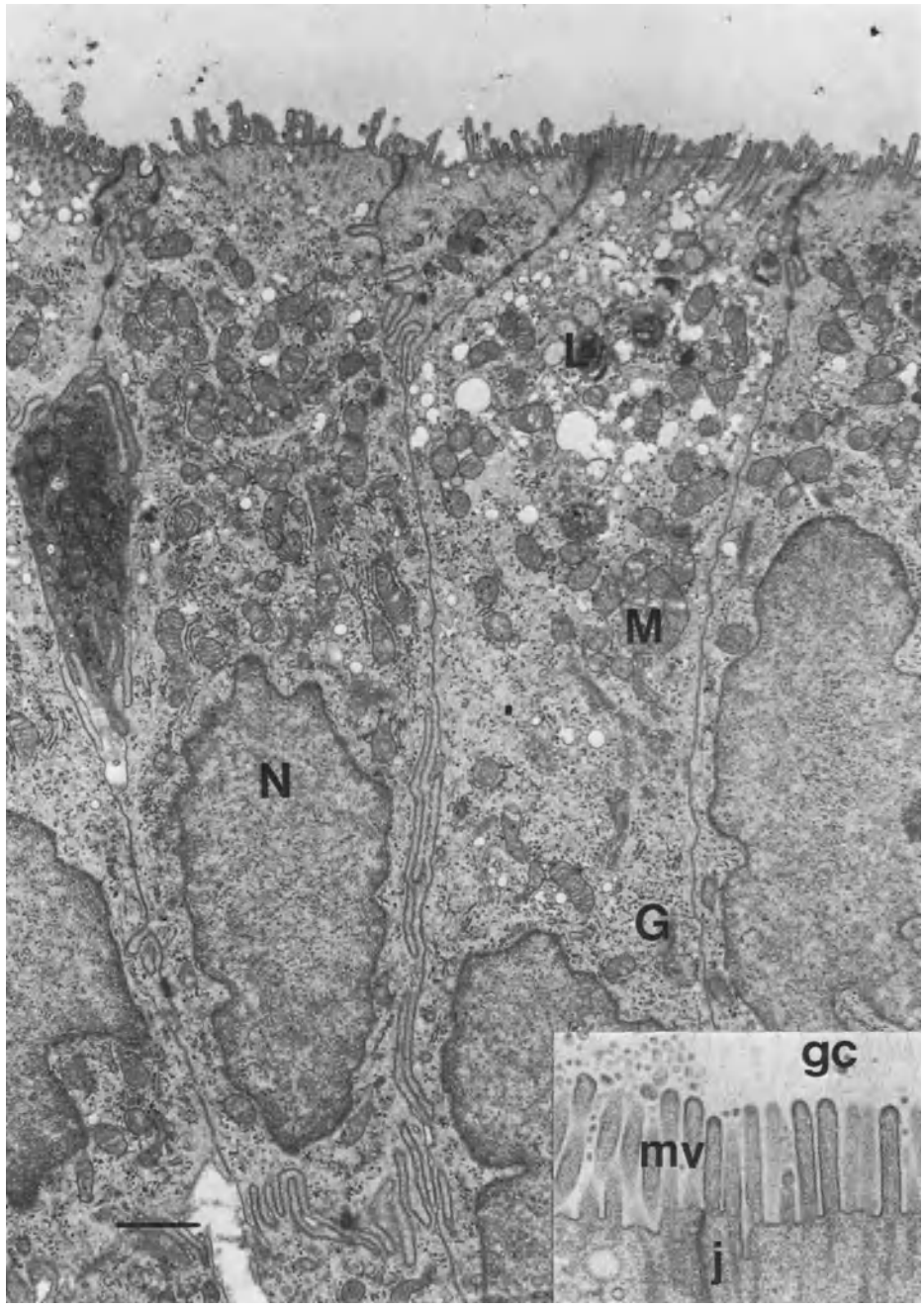

Figure 9. Electron micrograph of a human colonic columnar absorptive cell. N, nucleus; M, mitochondria; G, Golgi material; L, lysosome (× 5000). Scale bar, 2 mm. Inset: the glycocalyx (gC), microvilli (mV), and junctional complex (j) are seen at higher magnification (× 25,000)

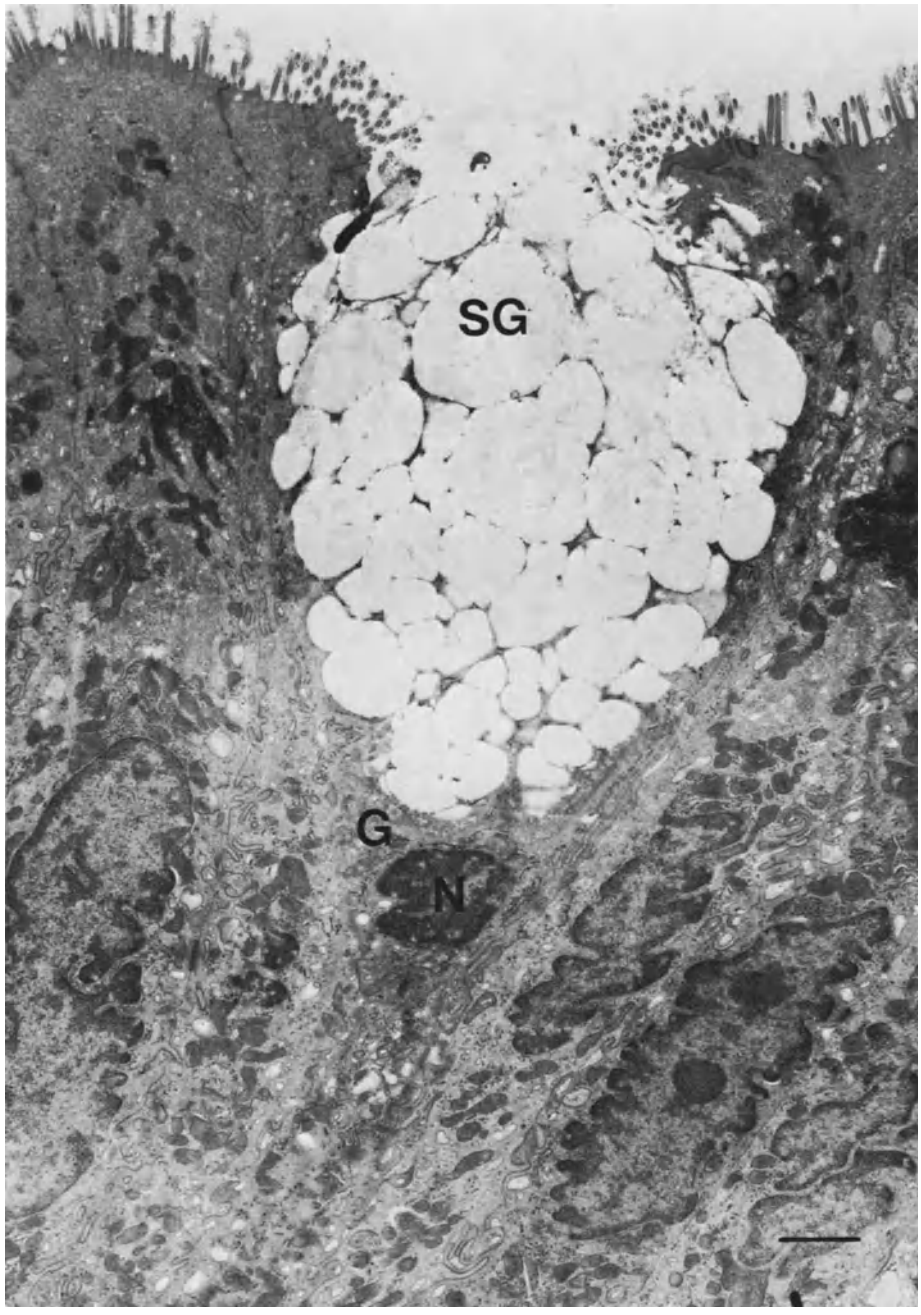

Figure 10. Electron micrograph of a human colonic goblet cell flanked by absorptive cells. Many mucous secretory granules (SG) pack the apex of the goblet cell cytoplasm. The nucleus (N) lies beneath the secretory granules. The Golgi material (G) is found between the nucleus and the mucous granules (× 5000). Scale bar 2 mm.

the bowel. Although the effects of starvation have been studied more extensively in the small intestine,[21–23] some information is available on the response of the colon to food deprivation and repletion. During fasting in mice, proliferation in the crypts of the colon becomes depressed.[24] On subsequent refeeding, however, there is a marked burst in proliferative activity accompanied by an increase in the cellularity of the crypts.[25,26]

The effect of food on intestinal structure and function appears to be, at least in part, hormonally mediated. Injection of pentagastrin into fasted mice stimulates colonic crypt cell proliferation and thus reverses the inhibitory effect on proliferation that is induced by fasting.[24] Starvation depresses antral and serum gastrin concentrations in the rat, and feeding of a high-bulk, nonnutritive diet does not prevent these changes.[27] Intravenous alimentation of rats also is associated with profound decreases in antral gastrin levels,[28] but the continuous infusion of pentagastrin into intravenously alimented rats in a dose equal to one-half the D_{50} for acid secretion prevents the expected decreases in both gastrointestinal tissue weights and disaccharidase activity.[29]

B. Bowel Resection

Extensive small-bowel resection in the rat produces compensatory changes in the remaining bowel, not only in the small intestine, but also in the colon.[30] When the ileum was resected from young rats, the subsequent RNA content and DNA content of the colonic mucosa were increased, indicating both epithelial hypertrophy and hyperplasia.[30] Further, experimental bypass of the colon in the rat results in a reduction of the number of cells per crypt of the colon 6 weeks after the bypass.[31]

C. Colonic Disease

Mucosal disease of the colon may cause profound changes in mucosal morphology. For example, chronic ulcerative colitis is characterized by a dense infiltration of inflammatory cells within the lamina propria, a reduction in the height of the surface columnar epithelial cells so that they become cuboidal or flattened, and changes in the crypts manifested by shortening, tortuosity, branching, or frank loss of crypts (Fig. 11). Further, epithelial proliferation and migration appear to be increased in active ulcerative colitis.[32,33] Perhaps of greater interest is the demonstration that the zone of cell proliferation in the epithelium is expanded upward toward the surface of the crypts in ulcerative colitis.[32,33] This finding links ulcerative colitis with certain other premalignant conditions of the bowel in which the same observation has been made.[34–38]

D. Irradiation

Irradiation produces changes in the epithelium of the large intestine which resolve after irradiation is stopped. In rectal biopsies of patients who had undergone radiation therapy, the epithelial cells lost their columnar appearance and became cuboidal.[39] A marked tissue eosinophilia and eosinophilic crypt abscesses accompanied these changes. One month after completion of radiotherapy, the rectal mucosa had reverted

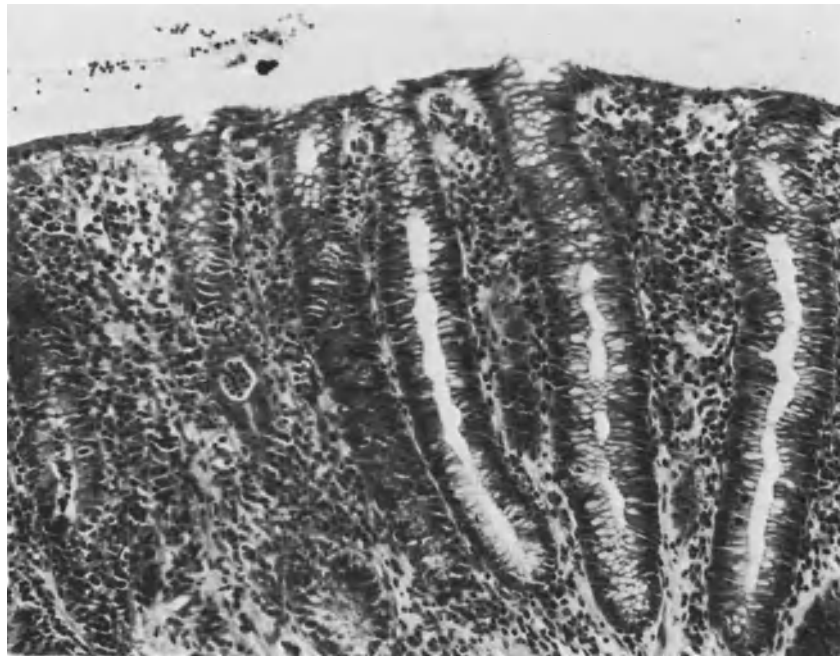

Figure 11. Light micrograph of a rectal mucosal biopsy from a patient with ulcerative colitis. The surface epithelium is cuboidal or flattened, the lamina propria contains numerous inflammatory cells, and a crypt abscess is seen toward the right (arrow). (Hematoxylin and eosin, × 150)

toward normal. Similar effects on large-bowel mucosa have been described during treatment of patients with 5-fluorouracil.[40,41]

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2

Absorption and Secretion of Electrolytes by the Human Colon

Paul Kerlin and Sidney Phillips

I. INTRODUCTION

A. Aims and Overview

An understanding of colonic function has developed more slowly than that of the stomach or small intestine. Although the recognition that the colon is "dispensable" in man no doubt contributed to this neglect, study of the colon *in vivo* has not been easy. Among the difficulties encountered are (1) a motor activity which is complex, which surely has important effects on absorption, but which is characterized poorly; (2) the existence in the large bowel of regions which differ in function; and (3) the billions of resident microorganisms whose identity and interrelationship with the host are unclear. However, it is clear that the metabolic actions of fecal flora modify colonic function in several important ways.

Our aim in this review is to present the current hypotheses for absorption and secretion of electrolytes and water by the human colon. We shall integrate information gained from *in vitro* and *in vivo* studies in man and, where helpful, add data from other mammals. Electrolytes most abundant in extracellular fluid will receive greatest attention, while little space will be devoted to calcium, magnesium, and zinc. Emphasis will be given to the concept now emerging that the colon absorbs many endproducts of intraluminal metabolism by the fecal flora. Finally, we shall discuss factors known to modify colonic function normally and to alter it in disease.

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Thus, in contrast to earlier and more simple views, there is increasing evidence that the mammalian colon is a versatile organ. Although the major absorptive function is to conserve electrolytes and water, recent animal and human studies indicate that short-chain fatty acids, ammonia, and other bacterial metabolites are also absorbed. Moreover, these bacterial biotransformations of dietary substrates are implicated in the pathophysiology of malabsorption, but little is known of comparable functions in normal man. The colon also secretes fluid under a variety of pathological conditions that have direct relevance to human disease. Finally, the colon is emerging as a potential source of hormones or neuropeptides. These compounds have the potential to influence colonic absorption, by acting as local messengers; or they might also modify remote events, by acting as humoral agents. The colon emerges as possessing diverse functions that, given the strategic location of the large bowel, may be involved intimately in the clinical expression of many diseases.

B. Comparison of Man with Other Species

A brief overview of comparative anatomy and physiology will focus attention on the large bowel of man in relation to other species. The size and complexity of the organ in other mammals is to some extent predicated by the nature and caloric density of their diets.[1] Carnivores like the dog have a simple tubular colon; their diets contain little fiber, and enzymatic digestion of food is completed in the upper gut. Herbivores are at the other end of the spectrum; their diets are bulky with fiber, which is resistant to mammalian enzymes, and microbial digestion is paramount. For example, the horse and rabbit have voluminous colons with extensive haustration and compartmentalization.[2] Long ago, Garry[3] proposed this spectrum of intestinal complexity from carnivores through omnivores to herbivores. Specialization of the herbivore's gut occurs in the colon (e.g., horse) or the complex stomachs of ruminants.[1] The importance of microbial digestion of nutrients is illustrated by the horse, in which it is estimated that production and absorption of volatile fatty acids in the colon supplies a minimum of 25% of basal energy requirements.[4] In addition, the colon of a pony weighing 160 kg absorbs up to 30 liters of fluid daily[5]; little wonder that acute diarrhea is often lethal in such species! Important studies in veterinary physiology by Argenzio, Stevens, and others have provided insight into human physiology. A comprehensive discussion of these animal studies is available elsewhere.[2] Man as an omnivore lies intermediate in complexity between carnivores and herbivores. However, while providing a useful concept, the existence of such a spectrum must not be overextended, as was pointed out by Christensen.[6]

C. Experimental Approaches to the Study of Colonic Absorption

Both *in vitro* and *in vivo* techniques have helped us understand colonic function, and we propose to discuss results obtained from both. In integrating data

from these different approaches, it is important to recognize certain merits and disadvantages, which are listed in Table 1. The most useful *in vitro* technique utilizes mucosa dissected free from submucosal tissue and mounted in an Ussing chamber[7,8] between perspex half-chambers. The mucosal membrane then separates the chamber into mucosal and serosal bathing fluids, the composition of which can be varied independently. Tracer probes can be used to quantify bidirectional movements. The spontaneous transepithelial electrical potential of colonic mucosa (10–50 mV in various mammals,[9] mucosal side negative) can be measured or it can be short-circuited by passing an external current between electrodes. Thus, electrochemical, osmotic, and hydrostatic gradients are easily imposed or negated. Known inhibitors or stimulants of ion movement can be utilized to dissect the processes of cellular transfer. The *in vitro* model is amenable to exquisite experimental manipulation but has important inherent shortcomings. The mucosa has limited viability, its surface area is small (usually < 2 cm^2), and regional differences are probable. Moreover, the normal milieu of blood supply, feces, and microorganisms is absent.

The *in vivo* approach, using luminal perfusion, studies the colon *in situ* by comparing the compositions of rectal effluents and fluids perfused into the cecum. The effect of differing ionic concentrations in the luminal fluids on absorptive function can be assessed. The major drawback of this approach is the inability to evaluate several important variables, e.g., blood flow, motility, transit, and surface area. However, it is probably correct to state that an ultimate understanding of colonic function in man requires the study of intact man.

Numerous other techniques have been utilized less frequently, including the use of everted sacs of colonic mucosa[10] and *in vitro* systems that have access to perfusates within both the vascular and luminal spaces.[11] Details of these approaches will not be discussed further, and readers are referred for further information to a comprehensive review.[12]

Table 1. Comparisons of in Vitro and in Vivo Techniques

Indices	*In vitro* approach	*In vivo* approach
Potential difference	Can be negated (short-circuited)	Spontaneous
Chemical, osmotic gradients	Can be varied precisely	Can be varied
Access to bathing fluids	Mucosal and serosal fluids readily accessible	Mucosal accessible only
Unidirectional fluxes	Measurable (2 isotopes)	Measurable
Metabolic inhibitors	Can be used	Nonapplicable (+−)
Stimulants	Can be used	Can be used
Vitability of tissue	+− present	Complete
Regional and species differences	Can be studied—frequently neglected	Can be studied—but other variables also present
Normal fecal milieu	Absent	Variably present

II. CONCEPT OF COLONIC EPITHELIUM AS A "TIGHT" MUCOSA

Plasma membranes of epithelial cells comprised a phospholipid bilayer enveloped by protein and containing specialized carrier molecules. Whereas lipophilic solutes permeate cell membranes readily by nonionic diffusion, hydrophilic solutes are unable to permeate membranes by the same mechanism. Some water-soluble molecules traverse lipid membranes by interacting with carrier proteins, specific for such transport. However, other aqueous solutes, which do in fact traverse lipid membranes freely, have no such carriers. This observation has required the postulation of water-filled "pores" through which such solutes move. The physical location of these pores is unknown; they have not been demonstrated unequivocally, but a portion of this aqueous permeability is thought to reside in the "tight junction" region between cells.

Extending this concept further, Diamond[13] has proposed that epithelial surfaces can be classified overall into those that are "tight" and those that are "leaky." Important to any understanding of absorption by the colon is the observation that the colon is a "tighter" epithelium than is the mucosa of the small bowel. This concept implies that passive fluxes of an actively transported ion, such as Na^+ or Cl^-, will be small, a property that fits well with the known biological functions of the colon. Following are several experimental expressions of the limited aqueous permeability of the colon that support this view.

A. Estimates of Pore Size

The effective pore size[14] of the colon is estimated to be smaller than the molecular size of urea (2.3 Å); comparable values calculated for the jejunum and ileum are 8 Å and 4 Å, respectively.[15] These calculations are based on the flow of water into the lumen of the intestine in response to equimolar solutions of urea and mannitol. In the colon, both substances attracted equal volumes into the lumen and neither was absorbed.[14]

B. Permeability of the Colon to Water and Hydrophilic Solutes

The human colon transports water against osmotic gradients of up to 50 mOsm/kg,[14] while transport in the small bowel is essentially isotonic.[15] This finding implies that an osmotic gradient of > 50 mOsm/kg is needed to drive water through channels of limited permeability. Another approach is to instill inert, hydrophilic solutes with a spectrum of molecular sizes, e.g., polyethylene glycol 400, into segments of small and large bowel; quantities of the probe molecules excreted subsequently in the urine are then compared.[16] Molecular sieving by the colon was shifted in the direction of small molecules, as compared with the ileum and jejunum.

C. Transepithelial Potential Difference

The functional consequence of active absorption of sodium ions and limited passive backflow of Na is a large spontaneous potential difference (PD) across colonic epithelium. Thus, the healthy human colon has a transepithelial PD of 30–40

mV,[17,18] with the mucosa negative, relative to blood or serosa. In contrast, PD's measured in jejunum and ileum[19] are low. That the PD arises in the mucosa is shown by its persistence after subepithelial tissues are stripped *in vitro*.[18]

The technique for measurement of the PD *in vivo* has been discussed in detail by Edmonds.[18] The probing electrode should be placed on the epithelium with the reference electode in peripheral blood or subcutaneous tisses.[18] A millivoltmeter with high-impedance input is the recorder. The PD of the colon *in vivo* is increased by exogenous and endogenous aldosterone. Indeed, measurement of PD has been proposed as a diagnostic test for mineralocorticoid excess,[20] this augmentation of PD reflecting the ability of mineralocorticoids to increase active Na^+ absorption. Functional "health" of the epithelium seems important in maintaining a normal PD, since mucosal disease is reflected by a decreased PD.[21–23]

D. Regional Differences

There are important differences in function among different regions of the large bowel. For instance, the proximal colon in man absorbs more Na than does the distal colon. Using colonic perfusion, Levitan[24] showed that more Na^+ Cl^-, and water were absorbed from cecum to transverse colon than were absorbed from the latter point to the rectum. A more direct observation was made by instilling labeled Na and water into different segments of the large bowel and noting the speed of their arrival in peripheral blood.[25] Fig. 1 shows initial rates of appearance of isotopes in the blood; arrival is most rapid from the cecum and slowest from the rectum. Thus, cecal mucosa is most permeable, and more like small bowel, and rectal mucosa is least permeable. Regional specialization should be kept in mind when comparing results from *in vitro* studies, since only small areas of mucosa can be studied by these techniques. In concert with the above is the clinical observation that diarrhea occurs more often following right hemicolectomy than after left hemicolectomy.[26]

III. ABSORPTION IN VITRO

Colonic absorption *in vitro* has been reviewed comprehensively[27,9]; much of the recent progress in our understanding has been due to the work of Schultz and his colleagues.[27] They use rabbit colon in Ussing chambers, and results with this approach will be stressed in this section. The rat has not been studied as extensively,[28,29] and experiments on human tissue have been relatively infrequent.[30–32] However, there is general agreement that among mammalian species colonic mucosa absorbs Na^+ and Cl^- actively and that bicarbonate accumulates on the mucosal side.[28,31–33]

A. Sodium Transport

In human and rabbit colon, Na^+ is absorbed by an active, electrogenic process (i.e., giving rise to a PD); moreover, Na^+ transport accounts for the entire PD and short-circuit current (I_{sc}).[32,33] This transcellular movement of Na^+ has two components. Microelectrode techniques in the rabbit have shown (1) that the cell interior is

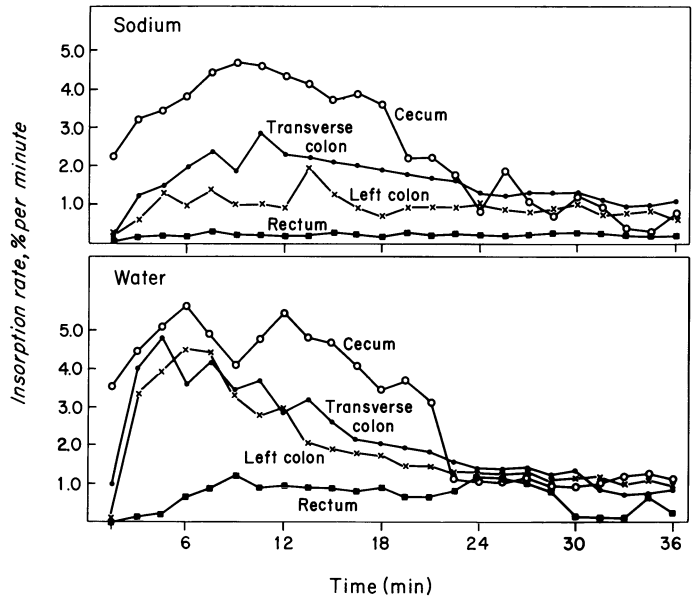

Figure 1. The rate at which labeled sodium (^{24}Na) and water (D_2O) enter blood is most rapid when solutions are instilled into the cecum (open circles) and slowest when solutions are placed in the rectum (solid squares). Studies in the transverse colon (solid circles) and descending colon (crosses) give intermediate results. Data, for one person at each site, are from Devroede, et al.[25]

negatively charged with respect to the mucosal solution and (2) that the intracellular concentration of Na^+ is low.[34] Thus, Na^+ moves into the mucosal cell down both electrical and chemical gradients. The second component of transepithelial movement is exit from the cell, and in all species, Na^+ exit at he serosal side is active. This active component includes an exchange of Na^+ for K^+ at the basolateral membranes, utilizing energy from a Na^+, K^+–ATPase, which thus can be considered as a "sodium pump." Na^+ exit is blocked by low serosal concentrations of K^+ (S. Schultz, personal communication) or by the addition of ouabain to the serosal solution. Ouabain also abolishes the PD and I_{sc} by inhibiting Na^+, K^+–ATPase.[32,33]

In contrast with their stimulatory effects in the small bowel,[35,36] mucosal glucose or l-alanine does not augment Na^+ uptake or PD in the colon.[30,32] Conversely, mineralocorticoids, when added to mucosal solutions, increase Na^+ absorption and the PD, an effect not seen in the small bowel.[37] The mechanism proposed for this action of mineralocorticoids is an increased permeability of the apical membrane to Na^+. The potent diuretic, amiloride, when added to mucosal fluids, abolishes the PD and I_{sc}, by blocking Na^+ insorption across the mucosal membrane.[38] In the rabbit, Na^+ movement into cells is not coupled to K^+ movement. When the PD is abolished experimentally, K^+ is not translocated, suggesting a passive movement of K^+ only.[33] Several workers, using human tissue, have found a discrepancy between net Na^+ transport and the I_{sc}.[28,29] On the basis of recent studies, Hawker and associates[32] proposed an Na^+–K^+ exchange at the mucosal surface to explain their finding

of a small secretion of K^+ by short-circuited human colon. Further studies with human tissue will be required to clarify this point. Binder's studies in the rat[28] point to other differences from the rabbit; he has reported an additional mechanism, the coupled, electrically silent entry of Na^+ and Cl^- at the luminal pole. However, the general hypothesis for mammalian colon is that Na^+ movement across the mucosal membrane is electrogenic and primarily if not completely uncoupled. From within the cell Na^+ is then pumped actively to the serosal (blood) side by Na^+–K^+–ATPase, as in the small bowel. A useful current model of colonic absorption is shown in Fig. 2.

B. Chloride

In most mammalian colons, Cl^- absorption is active but electrically silent. Anion exchange occurs, with bicarbonate (HCO_3^-) being secreted as Cl^- is absorbed.[9] In the rabbit, Cl^- absorption is unaffected when Na^+ movement across the mucosal membrane is inhibited by the diuretic amiloride[33] or when basolateral extrusion of Na^+ is blocked by ouabain.[32,33] A lack of coupling between Na^+ and Cl^- movements is also demonstrated by ion replacement experiments. Replacement of Na^+ with a nontransported cation (choline) does not affect Cl^- transport, nor does replacement of Cl^- with isothionate or other nontransported anions inhibit Na absorption.[28,33]

C. Bicarbonate

Neutral, electrically silent exchange of Cl^- and HCO_3^- at the luminal pole has been proposed in the rat, the rabbit, and man.[28,32,33] In support of this, the rate of

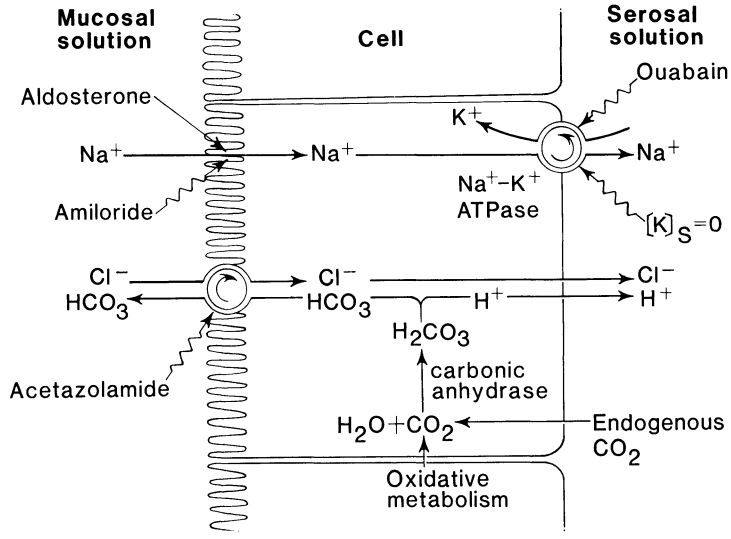

Figure 2. Model for electrogenic sodium transport and neutral Cl–HCO_3 exchange by rabbit colon. Straight arrows imply stimulation of a process, sine arrows an inhibition. Reproduced and modified from Frizzell and Schultz.[27]

HCO_3^- secretion is reduced markedly by removal of Cl^- from the lumen.[33] There is evidence that the HCO_3^- destined for secretion is generated within the cell, from the hydration of metabolic CO_2 as catalyzed by carbonic anhydrase. This enzyme is present in high concentrations in colonic mucosa.[39] Thus, secretion of HCO_3^- *in vitro* is independent of the HCO_3^- and CO_2 concentrations of serosal solutions[31]; moverover, the carbonic anhydrase antagonist, acetazolamide, simultaneously abolishes Cl^- absorption and HCO_3^- secretion *in vivo*.[40] However, Hubel[41,42] has stressed that movement of HCO_3^- from cell to lumen is equal electrically to movement of OH^- in the same direction, or to movement of H^+ from lumen to cell. Thus, a precise interpretation of studies involving HCO_3^- transport is not always possible.

D. Potassium

This ion has not received close scrutiny in *in vitro* models. In the rabbit and rat colon under short-circuit conditions, net K^+ transport is not observed,[33,43] suggesting the K^+ moves in this system by passive diffusion, along electrochemical gradients. However, Hawker[32] showed in short-circuited human colon a small net secretion of K^+, which was dependent on luminal Na^+. Edmonds and Nielsen[44] measured transmembrane potentials in the rat colon that are inconsistent quantitatively with a passive diffusion of K^+; further, in hyperkalemic rats, Bastl et al[43] found evidence for active secretion of K^+. These observations deserve follow-up, since the mechanism for translocation of K^+ is not clear.

E. Short-Chain Fatty Acids

Microbial digestion of carbohydrate generates large amounts of acetic, propionic, and butyric acids within the lumen of the colon. In fact, short-chain fatty acids (SCFA's) constitute the major anion of feces in man and most mammals. Concentrations in human feces of 100–200 meq/liter have been reported.[45–47] Earlier reports of high fecal concentrations of SCFA's in patients with carbohydrate malabsorption[48] or ingesting high-carbohydrate diets[49] led to the hypothesis that SCFA's were poorly absorbed and thereby responsible for osmotic diarrhea.[48–50] However, SCFA's are absorbed from the colon of many species, including goat[51] pony,[52] pig,[53] rat,[54] rabbit,[55] and man.[56,57] The nutritional importance of SCFA absorption is stressed by the observation that some ruminants obtain 70% of their energy requirements from SCFA production and absorption in the forestomach.[58,59] Moreover, in some animals, colonic transport of SCFA's equals or exceeds comparable absorption rates for rumen epithelium.[53] The role of SCFA's in man's requirements for energy is unknown.

Indeed, rather than being causes of diarrhea, SCFA's may actually facilitate Na and water absorption, as shown by *in vitro* studies with pig,[60] goat,[51] and rat colon.[61] Further, using human colon *in vitro,* W. E. W. Roediger (personal communication) noted that SCFA's are absorbed, and by a process that enhances Na absorption. They utilized a technique, previously used by Parsons and Powis in the rat,[11] that combined vascular and intestinal perfusion of fresh segments removed at surgery.

The mechanism of SCFA absorption, and the explanation for their stimulation of Na absorption, is as yet unclear. Argenzio[52] has suggested that the rate of absorption

of individual SCFA's is inversely related to their molecular weighs and, in addition,[60,62] that hydration of CO_2 in the lumen of the colon may provide a source of H^+ for protonation of organic anions, thereby facilitating absorption of the more permeable, undissociated acid (R–COOH). Ruppin's *in vivo* human studies,[57] to be discussed later, can also be interpreted as favoring the absorption of undissociated organic acids.

However, an exchange of organic anions with HCO_3^- at the luminal pole has been suggested also[61]; other reports incriminate Na–H exchange as a means of providing H^+ for protonation of organic anions.[60] An additional complexity to the interpretation of these observations is the potential metabolism of organic anions to ketone bodies by the mucosa,[63] though in one study this was shown to be small.[64] Important interim conclusions remain that SCFA's are absorbed and do not appear to impair absorption of Na and water.

IV. SECRETION IN VITRO

Interest in net fluid secretion as a pathogenic mechanism for diarrhea was sparked by studies of Asiatic cholera.[65] In this condition, large volumes of an isotonic ultrafiltrate of plasma accumulate in the small bowel due to the action of cholera enterotoxin, which is also a potent stimulus for secretion of ions *in vitro*[65]

Fluid would accumulate in the intestine when absorption was inhibited, if this inhibition unmasked a basal state of secretion. Conversely, fluid would accumulate if an active secretion of ions was stimulated.[66] The relative contribution, or indeed the existence, of either or both of these mechanisms is not clear. Moreover, studies done *in vivo* are limited by their capacity to distinguish between these possibilities; however, *in vitro* approaches can control many of the variables that complicate these phenomena *in vivo*. The list of stimuli known to elicit colonic secretion of fluid is large and clinically relevant; it includes several laxatives (ricinoleic acid,[67–69] bisacodyl,[70] dioctyl sodium sulfosuccinate[71,72]), dihydroxy bile acids[73–75] hormones,[76–78] cholera toxin,[79] and prostaglandins.[80,81]

A phenomenon fundamental to intestinal secretion is the active secretion of Cl.[80] Exogenous cyclic AMP (cAMP) has been shown to stimulate secretion of Cl by rabbit colon[80] and, when incubated with colonic mucosa *in vitro* each of the above stimuli, increases the mucosal concentration of cAMP. Moreover, the inhibitor of phosphodiesterase, theophylline, also causes a marked serosa-to-mucosa movement of Cl.[80] Frizzell and Schultz[27,37] have dissected the mechanisms of Cl secretion and proposed a model that is shown in Fig. 3.

For Cl^- to be available to the cell for secretion, it is assumed that its entry through the serosal pole is linked with Na^+ extrusion at the basolateral membrane. Thus, the Na^+ pump may provide the energy for Cl^- secretion. The Schultz and Frizzell model therefore explains why ouabain inhibits Cl^- secretion, presumably by blocking Na^+,K^+–ATPase, which provides the driving force. Moreover, secretion is inhibited by serosal furosemide, which is thought to block the entry of Cl^- at the basal pole.[80] However, secretion is independent of Na^+ entry at the luminal pole, since mucosal amiloride abolishes both the I_{sc} and Na^+ absorption, but Cl^- secretion is

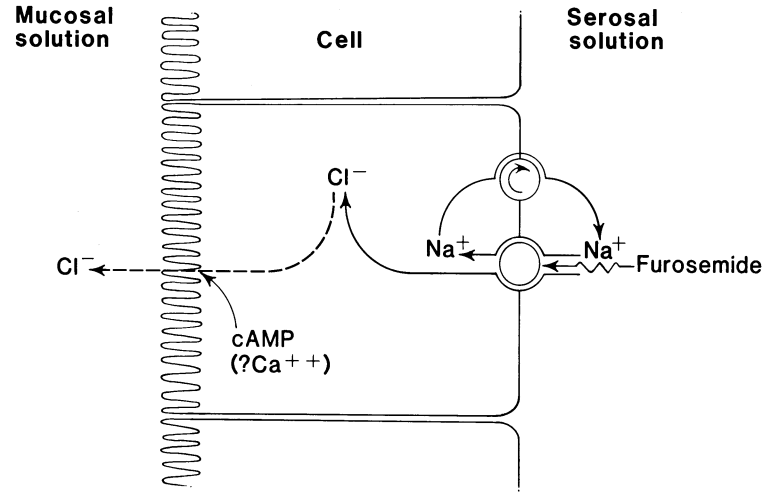

Figure 3. Model for chloride secretion in the rabbit colon. Straight arrows indicate stimulation, sine arrows indicate inhibition. Modified from Frizzel and Heintze.[80]

unaffected. [80] Supporting this concept, replacement of mucosal Na^+ with choline abolishes the I_{sc}, but the secretion of Cl^-, which is electrically neutral, is unaffected.

Although cAMP has received most attention as the potential intracellular mediator for Cl^- secretion, other possibilities need to be considered. For example, the divalent cation ionophore, A 23187, was found to elicit active Cl^- secretion by mobilizing calcium stores without affecting cellular levels of cAMP.[82] Therefore, it is possible that cAMP may increase intercellular levels of calcium and that calcium may be the cellular "messenger."

A role for prostaglandins in the cellular process of secretion is tantalizing but uncertain. The addition of prostaglandin E_2 at the serosal surface of rabbit colon elicits a secretory response.[80] The increased I_{sc} and augmented movement of Cl^- from serosal to mucosal bath is similar to the effect elicited by exogenous cAMP.[80] Serosal furosemide or ouabain abolishes the secretory response to prostaglandins, supporting the concept that serosal Na is involved in active Cl^- secretion. Recently, several groups have demonstrated increased mucosal levels of cAMP in rat[81] and human[83] colon after the addition of prostaglandins. Similar effects were described by Kimberg[84] in the ileum. Several laxatives known to modify electrolyte and water movement in the colon also increase mucosal levels of prostaglandin.[85,86] The significance of these findings remains unclear until relationships between prostaglandins and cAMP are clarified.

V. ABSORPTION BY THE COLON IN VIVO

A. Estimates of Ileal Input into the Colon

Since the water and electrolyte content of feces is known not to vary widely in health, colonic absorption could be quantified if input to the cecum were known. Previ-

ous indirect assessments of the volume and composition of ileal flow were based on the volumes collected from well-established ileostomies (400–800 ml daily). However, intestinal absorption after colectomy may be influenced by many factors, including resection or residual disease (e.g., regional enteritis) of the ileum and any intestinal adaptation that may occur after colectomy. The direct quantification of intestinal flow, by applying dye dilution techniques to the terminal ileum thus offered an attractive alternative approach. These studies suggested that ileal flows in health may be greater than the volumes of ileostomy effluents; they quantified fasting ileal flows of 0.1–1.7 ml/min [87-91] rising to peaks of approximately 5 ml/min at 1–2 hr. after a, meal.[88] By integrating such data over 24 hr., about 1500 ml is estimated to enter the colon each day; this volume contains 200 meq Na, 100 meq Cl^-, and 10 meq K.[88] In health, the stools contain up to 100–150 ml of water, 1–5 meq Na^+, 1–2 meq Cl^-, and 5–15 meq K^+ per day. Using these estimates, the capacity of the healthy colon to absorb water and electrolytes is shown in Fig. 4.

B. Colonic Absorptive Capacity

Given these basal values for colonic absorption, a logical extension is to ask what the colon can accomplish under stress. By infusing into the cecum an isotonic electrolyte solution that stimulates the composition of fasting ileal contents it is possible to determine the threshold at which the colon "overflows" and fecal volume increases. A daily load of 2 liters of isotonic saline did not increase stool weight, whereas a load of 4 liters induced mild diarrhea.[90] When the exogenous and endogenous "loads" are combined, almost 6 liters of fluid was delivered to the colon; over 5 liters of this was absorbed (see F. 4).

One intuits that the rate at which fluid is delivered to the colon should also be important. In fact, a 250 ml bolus of fluid did not influence fecal output whereas a 500 ml bolus overwhelmed the absorptive capacity of the colon and produced a liquid

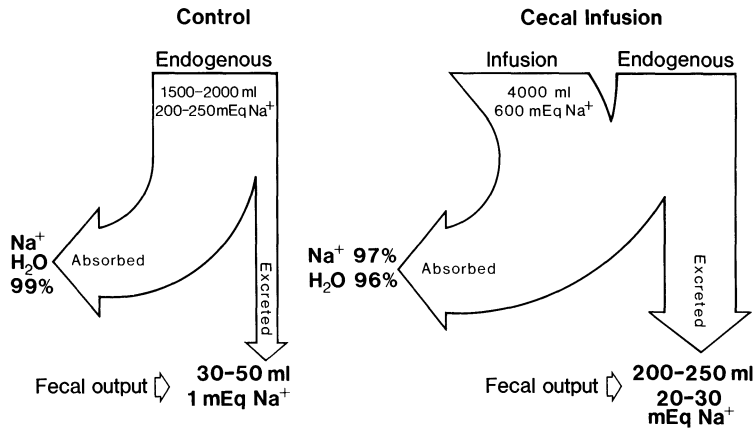

Figure 4. Net balance of water and electrolytes in the human colon, under control conditions and when colonic input is augmented by a 4-liter infusion into the cecum. Modified from Debongnie and Phillips.[90]

stool.[90] Thus, the colon has major reserves to handle fluid overload, provided the excess is presented to it slowly. However, it seems likely that brief episodes of "small-bowel diarrhea" can lead to colonic "decompensation."

C. Specifics of Electrolyte and Water Transport

Quantitation of absorption from the human colon has been possible since the description of colon perfusion in man by Levitan and Ingelfinger in 1962.[24] The technique was modified by others to allow more accurate assessment of endogenous ileal flow into the colon.[92] The essentials of the technique are (1) intubation of the cecum per os, (2) lavage of the colon, (3) pumping of solutions into the cecum at 10–15 ml/min, and (4) collection of effluent by a rectal tube. A "steady state" is necessary for the calculation of absorption rates, this is defined by minimal variation in the composition of fluids recovered from the rectum.[92] A nonabsorbable volume marker, usually polyethylene glycol 4000, is used to quantify changes in fluid volumes during perfusion. The results in man are essentially in agreement with (and potentially explained by) results of the more mechanistic approaches *in vitro* discussed earler.

1. Sodium and Water

Na^+ is absorbed against concentration and electrical gradients; absorption is therefore active.[14,93,94] There is a linear relationship between the luminal concentration of Na^+ (from 25 to 150 meq/liter) and the rate of Na^+ and water absorption.[14,93] No absorption of Na^+ occurs when its luminal concentration is below 25 meq/liter.[14,93] Moreover, Na^+ and water absorption are not enhanced by the addition of glucose,[14] in contrast to what is seen in small bowel.[95] Water absorption is thought to be passive and to follow osmotic gradients created by Na^+ movement. When hypertonic solutions containing mannitol are perfused, Na^+ absorption continues, even though water moves passively into the lumen.[14] These findings are in accord with an active transport of Na^+ and minimal passive "back flow," under the influence of bulk movements of solvent.

2. Chloride and Bicarbonate

Cl^- is also actively absorbed against large concentration gradients.[14,93] In fact, at equal luminal concentrations of Na^+ and Cl^-, Cl^- absorption exceeds that of Na^+.[14,93] Cl^- absorption continues when bulk flow of water into the lumen is induced osmotically.[14,93] When Cl^- is absorbed, HCO^- is secreted, and the presence of Cl^- in the lumen facilitates the secretion of HCO_3^-[14,93] These results suggest a coupled transport (anion exchange) mechanism between Cl^- and HCO_3^-, in which Cl^- is absorbed and HCO_3^- is secreted, similar to results obtained *in vitro*. Indeed the hyperchloremic acidosis following uretero-sigmoidostomy is readily understood on the basis of these studies.[96]

3. Potassium

K^+ is secreted into the lumen when perfusates contain less than 15 meq/liter K^+ but is absorbed when greater concentrations of K^+ are perfused.[93,94] These findings are consistent with passive movement of K^+ along electrochemical gradients.[94] In-

deed, when electrochemical gradients are calculated,[94] K^+ partitions in accord with passive movement. However, not all studies support this conclusion.

An alternative method of studying K^+ movement *in vivo* has used small dialysis bags[97,98] within which test solutions are introduced into the colon. Changes in the composition of these solutions can then be observed. With this approach, the rate of K^+ secretion was not affected by the rate of Na^+ absorption, by the luminal concentration of Na^+, or by the osmolality of the luminal fluids. K^+ was secreted when its concentration in the lumen was less than 40–50 meq/liter but absorbed when introduced at greater concentrations. The magnitude of the measured PD was insufficient in these studies for K^+ movement to be passive. Salas-Coll and co-workers[97] have used these data to conclude that K^+ secretion is active. Certainly, the results are in conflict with those from perfusion studies.

However, uncertainties about the movement of K^+ are compounded by several additional findings. Firstly, colonic mucus is rich in K^+, containing concentrations up to 100 mM/liter, and measurable amounts are secreted by the healthy or diseased colon.[94,99] Further, fecal bacteria also contain high concentrations of K^+. Moreover, Edmonds has proposed the presence of two mucosal pools of potassium, including one of small size but with rapid turnover, which he feels is the source of active secretion of K^+.[100] Currently, no simple or unifying hypothesis has been proposed to resolve the conflicts surrounding the mechanisms of K^+ movement.

4. Short-Chain Fatty Acids

McNeil and associates, using a dialysis bag technique,[56] have observed absorption of acetate, propionate, and butyrate from the human rectum, together with augmented Na absorption. More recently, colonic perfusion studies in man, using solutions containing propionate, acetate, and butyrate, have demonstrated that SCFA absorption is concentration dependent, with no evidence of a saturable process up to concentrations of 90 mmole/liter. [57] The rate of SCFA absorption exceeded that of other electrolytes, and absorption was accompanied by increased absorption of Na, K, and water; these absorptive events are accompanied by secretion of HCO_3^-.[57] Ruppin and co-workers[57] proposed two mechanisms for absorption of SCFA: (1) a major mechanism of nonionic diffusion of protonated SCFA, which this will involve consumption of luminal CO_2; and (2) a less important, ionic diffusion of the sodium and potassium salts of SCFA. These authors proposed that this second mechanism might be the process for enhanced Na absorption. It is clear that the byproducts of the bacterial biotransformation of carbohydrate can no longer be thought of as intrinsic cathartics. Indeed, the presence of SCFA's in a fecal milieu may explain why estimates of maximum colonic absorption are higher during slow perfusion of the bowel than they are during rapid perfusion of the cleansed colon.[90]

VI. INTRALUMINAL METABOLISM AND ABSORPTION

A. Dietary Fiber and Carbohydrates

With interest heightening in dietary fiber and its possible role in colonic function in health and disease, we shall consider in some detail the possible interactions be-

tween fiber and the colon. Also, since residues of simpler carbohydrates often reach the colon, certainly in malabsorptive disease and perhaps even in health, some of what is said about fiber applies also to other carbohydrates. These functions are summarized schematically in Fig. 5.

Holloway et al [101] assessed the digestion of dietary fiber in man by comparing the output of fiber components from ileostomies with amounts recovered from stools. Only 22% of ingested cellulose and 4% of hemicellulose were recovered in stools, compared with 85% and 28%, respectively, recovered from ileostomies. Lignin was recovered fully in both groups, suggesting that lignin is not digested in the colon, as has been reported in animals.[102] In other reports, only 43% of cellulose and no dietary pectin were recovered in human stools.[103,104] Thus, Southgate and Durnin[105] summarized by suggesting that 50–75% of dietary fiber is broken down in the bowel, mainly in the colon. In view of this and the demonstration that products of bacterial metabolism of carbohydrate (SCFA's) are well absorbed, the concept of fiber as an inert bulking agent requires modification. On the other hand, not all fiber is digested; for instance, Eastwood and Mitchell have discussed the physicochemical characteristics of undigested fiber, suggesting this as a basis for the binding and adsorption of ions, thus making them less available for absorption.[106] Many fiber-rich foods and some semipure components of fiber increase fecal bulk,[107] but quantitative effects vary, possibly depending on the degree to which different fibers are metabolized. Whether physicochemical binding of ions and water by fiber explains its bulking effects on feces or whether fiber has additional effects, such as an increase in fecal excretion of bacterial mass[108], is still unsettled.

Studies by Bond et al[109] in patients with intestinal bypass have shown that the human colon salvages more than 50% of simple sugars which escape absorption in the small bowel. Moreover, direct placement of ^{14}C-labeled glucose, acetate, and lactate

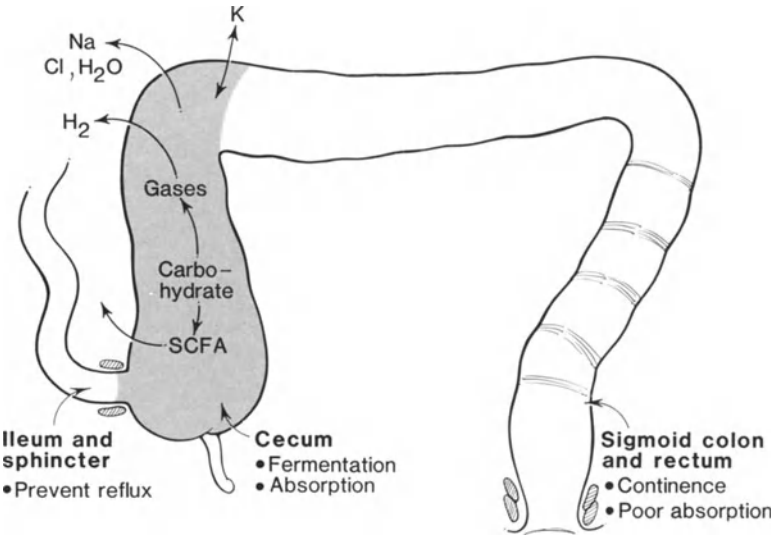

Figure 5. Schematic summary of colonic function. The proximal colon is directed more toward temporary stasis of contents, allowing the processes of bacterial fermentation and absorption to proceed. The distal colon absorbs less, but is directed primarily to continence of formed stool, with convenient evacuation to follow.

into the cecum was followed by a prompt rise of $^{14}CO_2$ excretion in breath.[110] The mechanisms by which carbohydrate is conserved were investigated in normal and germ-free rats.[110] Whereas acetate and lactate, the fermentation products of glucose, were rapidly coverted to $^{14}CO_2$ in germ-free animals, minimal excretion of $^{14}CO_2$ occurred after glucose. These experiments imply that colonic bacteria metabolize glucose to SCFA's, which are then absorbed and oxidized to CO_2 by the host. Other products of carbohydrate fermentation are the gases H_2 and methane. All humans produce H_2 by this mechanism, which is the only endogenous source of this gas; only one-third of humans have flora able to generate methane.[111] Gases can be quantified in expired air and can be used as an index of carbohydrate malabsorption.[112]

B. Ammonia Generation and Absorption

Further symbiosis between host and fecal flora is exemplified by the intestinal recycling of nitrogen. Thus, it has been estimated that about 7 g of urea, or 20% of that which is synthesized daily, is catabolized by the colonic flora.[113] And, since only very limited quantities (approximately 0.4 g) enter the colon from the ileum each day,[114] most of the urea metabolized must arrive at the colon through other routes. Wolpert et al[115] suggested that urea is hydrolyzed at a "juxtamucosal site"; urea administered intravenously was metabolized faster than was urea perfused through the lumen.

Since minimal quantities of ammonia and urea are found in feces,[98] ammonia is thought to be well absorbed by the colon. However, the mechanism of ammonia absorption is unclear. Down and colleagues[116] presented evidence favoring nonionic diffusion of NH_3, a process presumed to dominate the transport mechanisms for weak acids and bases. This would be favored by a high luminal pH, as dictated by the pK_a of ammonia. On the other hand, other mechanisms of transport have been proposed. Bicarbonate ions (HCO_3^-) and ammonium (NH_4^+) may interact in the lumen, thus facilitating absorption of the nonionic species,[11] and Wolpert et al.[117] even found evidence favoring ionic diffusion of NH_4^+.

Limited absorption of the ionized species (NH_4^+) would provide rationale for the treatment of portosystemic encephalopathy with lactulose. Bacterial metabolism of the disaccharide lactulose is rapid *in vivo,*[118] the SCFA's so produced will establish an acid environment that should retard absorption of NH_4^-. However, two studies[119,120] using a dialysis technique show that lactulose does not increase the fecal content of ammonia. Of considerable interest are studies by Vince et al.[112] who found that lactulose stimulated the incorporation of ammonia into bacterial proteins. Further, ^{15}N-labeled amino acids, which have been incorporated into protein by bacteria, have been identified in the portal blood of animals.[122] These observations raise interesting possibilities for new insights into relationships among hosts, their colons, and the fecal flora.[123]

VII. FACTORS MODIFYING COLONIC ABSORPTION: SECRETION IN VIVO

A. Hormones

The colon is a source of several gastrointestinal hormones, including serotonin, vasoactive intestinal polypeptide (VIP), glucagon, and somatostatin.[124–128] The reasons for this localization and any role of colonic hormones in modifying local functions

are unclear; certainly, no obvious "humoroprival diseases" are recognized after colectomy. Moxey[129] discussed the localization of endocrine cells in the large intestine; she suggested that some hormones may serve a local regulatory or paracrine function. R. Jian and colleagues have shown (see: *Dig Dis Sci* 26:195–201, 1981) that perfusion of the human colon with hypertonic solutions reduced gastric secretion of acid.

The effect of exogenous hormones on colonic absorption and secretion of electrolytes is poorly defined. Moreover, interpretations are complicated by concomitant changes in blood flow and motility, by amounts of hormones administered, and by unknown interactions among hormones. Aldosterone administered exogenously, or secreted endogenously in response to sodium depletion or potassium loading, stimulates sodium absorption and potassium secretion and increases transepithelial PD.[100,130–135] Mineralocorticoids do not have similar effects in the small bowel.[136] The mechanism of action has been explored in the rabbit colon *in vitro*. Aldosterone increases the permeability of luminal membranes to sodium,[37] an effect that would enhance Na absorption. However, other mechanisms have also been proposed.[136] In renal tissues, mineralocorticoids are thought to increase sodium transport by augmenting the activity of Na^+, K^+–ATPase,[137] and comparable effects have been proposed in the colon. In acute (3-hour) experiments using rabbit colon, Na^+, K^+–ATPase activity was unchanged,[37] but a later effect on this enzyme cannot be excluded.[136]

The action of glucocorticoids on the colon is important clinically, as these agents are used in an uncontrolled manner, but with occasional success, in many forms of diarrhea. Binder[138] reported that dexamethasone was a potent stimulus of sodium absorption and potassium secretion and that it also raised the PD of rat colon. In addition, when fluid secretion was induced by bile acids, large doses of glucocorticoids inhibited the secretory response.[138] As with mineralocorticoids, the PD increased before levels of Na^+ K^+–ATPase were raised, suggesting a primary effect upon luminal permeability to sodium. Studies on man are not available; Field has summarized this subject recently.[136]

Antidiuretic hormone decreases sodium, chloride, and water absorption in the human colon[139] as it does in the small bowel.[140] The mechanism is unknown, and the effect appears to be opposite to that seen in other species.[141] Other humoral substances have been implicated in the genesis of diarrheal disease. VIP reverses net absorption to net secretion in rat[76] and human[78] colon *in vivo,* and addition of VIP to serosal fluids of the rat colon *in vitro* causes choride secretion.[77] Although VIP has been incriminated in the diarrhea of the Verner–Morrison syndrome,[142] its predominant site of action is the small bowel, since net absorption of fluid was observed in the colon of one such patient.[143] Intravenous prostaglandins ($F_{2\alpha}$ and E_2) increased rates of ileal flow in man but did not influence colonic absorption.[91]. On the other hand, *in vitro* studies[79] show that exogenous prostaglandins change I_{sc} and PD in a manner that suggests an active secretion of Cl^-; moreover, some evidence exists that secretory laxatives might act via endogenous prostaglandins.[85,86]

B. Laxatives

Previous classifications of laxatives are now thought to be inaccurate.[144] For example, dioctyl sodium sulfosuccinate (DSS) is designated as a "stool softener." How-

ever, it evokes net accumulation of fluid and electrolytes in the rat colon.[71,72] Other laxatives also stimulate fluid secretion in the colon; these include (1) the diphenolic laxatives, bisacodyl and oxphenisatin;[145,146] (2) phenolphthalein;[147] and (3) ricinoleic acid (castor oil).[69]

The mechanisms which mediate fluid secretion are unknown, though much of what is discussed below for bile acids and fatty acids may well apply to the laxatives. Morphological damage and changes in permeability have been described after laxatives,[148,149] some cathartics elevate mucosal levels of prostaglandins,[85,86,150] and their secretory effects can be modified by indomethacin.[85,150]

C. Bile Acids and Fatty Acids

Diarrhea often accompanies bile acid and fat malabsorption, and it is well established that bile acids and fatty acids reduce colonic absorption and even evoke a net secretion of fluid.[67,68,73,75,151,152] The essential features of this phenomenon *in vivo* are that it is (1) dose-related, (2) rapidly reversible, and (3) highly stereospecific. Two separate studies have examined the structure–function relationships of secretion induced by bile acids.[153,154] Bile acids with two α-hydroxyl groups in the steroid nucleus, in the positions 3,7; 3,12; or 7,12 are most potent.

Similar comments apply to long-chain fatty acids. Ricinoleic acid, the active principle of castor oil, is a C18, monohydroxy fatty acid that inhibits net absorption of water and electrolytes by the colon and that induces net fluid secretion at higher doses.[67,68,149] Dietary fatty acids have similar effects, and their hydroxylation by intestinal bacteria[155] enhances the secretory effect.[67] Thus, oleic acid is a less potent secretagogue than is its bacterial hydration product, 10-OH stearic acid.[67] Nonhydroxylated fatty acids are effective secretagogues in other systems, though still of lesser potency than their hydroxylated congeners.[156] These differences may be explained in part by the fact that hydroxylated fatty acids are absorbed more slowly than their more hydrophobic, nonhydroxylated congeners.[157]

The mechanism for bile acids and fatty acid secretion is uncertain and probably multifactorial. At a cellular level, these agents reduce the available level of metabolic energy.[158] They also stimulate adenylate cyclase activity, with a resultant increase in mucosal cAMP.[159–161] Active secretion of Cl, an established property of cAMP, has been proposed as a mechanism for the action of bile acids and fatty acids on rat,[160] rabbit,[151,160]; in one report, however, beta blockade failed clinically to ameliorate bile acid diarrhea.[162]

Bile acid diarrhea is accompanied by increased fluxes of urea, creatinine, and inulin from plasma into the lumen of the colon; bile acids also augment fluxes from colonic lumen to plasma of small-molecular-weight polyethylene glycols. These changes reflect a generalized increase in mucosal permeability.[68,155] However, the role of altered permeability in the phenomenon of secretion is doubtful, since pretreatment with propranolol prevents fluid secretion but does not block the increase in permeability.[163] Finally, these bile acids and fatty acids are potent cytotoxins,[158] and their presence in the colon is accompanied by mucosal damage.[148,149] For more details of the effects of bile acids and fatty acids on the colon, readers should consult specific reviews of this subject.[164,165]

VIII. DIVALENT CATIONS: CALCIUM, MAGNESIUM, AND ZINC

Limited information is available on the role of the colon in the homeostasis of divalent cations. In fact, the small intestine is assumed to be the preferential site of absorption of both calcium (Ca) and magnesium (Mg).[166] However, in rat colon, Ca absorption increases when dietary calcium is restricted,[167] and colonic absorption is considered to be active and dependent on vitamin D.[168] Fragmentary evidence in man is somewhat at variance, and secretion of Ca was noted in one perfusion study of the human colon.[88] Magnesium absorption was reported in the same study,[88] and hypermagnesemia has been described after administration of enemas that contain magnesium sulfate.[169] To our knowledge, no information is available on zinc absorption by the human colon, but negligible absorption occurs from the rat cecum and colon *in vitro*.[170]

IX. COLONIC ABSORPTION IN DISEASE

A. Colonic Overload

The absorptive capacity of the colon should be overwhelmed when the volume of small-bowel contents delivered to it exceeds 5 liters daily.[90] In a patient with pancreatic chlorea and severe diarrhea of small bowel origin, the colon absorbed 1000 meq Na and 6 liters of water daily.[143] However, the load presented to the colon was 10 liters daily, an amount in excess of the functional reserve. In another example, an ileal flow of 12 liters per day was measured in a patient with Asiatic cholera, who passed 5 liters of stool.[171] Still unstudied are the circumstances in steatorrhea, when unabsorbed fat or bile acids might limit the reserve capacity of the colon.

B. Diarrhea after Ileal Resection

Hofmann and Poley[172] reported that the diarrhea of small ileal resections (less than 100 cm) is due to secretory bile acids, and that their sequestration with cholestyramine should be effective treatment. Conversely, "fatty acid diarrhea" should occur after resections of greater than 100 cm; in this instance, reduction of dietary fat should be effective. These important studies focused on the influence of unabsorbed solute on colonic function but did not consider concurrent resection of the colon. It is not surprising that the severity of diarrhea following ileal resection also depends on the amount of colon removed.[26,173,174] The observations of Cummings[173] are most cogent; not only was the loss of colonic absorbing surface incriminated, but reduced production of SCFA's after major resections could be a factor in reducing sodium absorption.

C. Congenital Chloride Diarrhea

This rare, autosomal recessive disease presents at birth with watery diarrhea and metabolic alkalosis. Stools have a low pH and contain high concentrations of Cl^-, and little HCO_3^-. The defect resides in the exchange of Cl–HCO_3^- in the colon and ileum. Colonic perfusion studies by Holmberg et al[89] revealed no absorption of

Cl^- and no secretion of HCO_3^- despite normal absorption of Na^+. Low fecal excretion of Na^+ and a normal rectal PD support the presence of normal Na absorption. Turnberg[175] demonstrated also that ileal exchange of $Cl^-HCO_3^-$ was also absent and that Cl^- accumulated in the ileum. Most untreated patients with chloride diarrhea die in the first weeks of life, from hypokalemia and dehydration. Treatment is directed at lifelong replacement of electrolytes and water, utilizing the passive permeability of the proximal bowel to Cl^-.[95]

D. Mucosal Disease

1. Inflammatory Bowel Disease

Absorption of water and electrolytes is impaired in ulcerative colitis and Crohn's disease.[176–180] However, study of individual patients has not elucidated the mechanisms appreciably. Rask-Madsen et al[178] attributed the defect to increased serosal-to-mucosal back flow of Na. However, support for a "leak" of Na^+ is not strong, and measurements of the permeability of inflamed rectal mucosa have given contradictory results.[178–179] The reduced PD in colitis[22,23] has been interpreted as evidence of a primary disorder of Na^+ transport; however, a direct effect of mucosal ulceration on transmucosal PD cannot be excluded. Sharon and colleagues[181] demonstrated elevated levels of prostaglandins in the rectal mucosa of patients with active colitis. Another study has suggested that levels of prostaglandins in colitic mucosa may be correlated directly to the abnormalities in electrolyte transport.[182]

2. Invasive Organisms and Their Toxins

The initial diarrhea of shigellosis is thought to result from jejunal secretion of fluids, as a response to shigella enterotoxins.[183,184] Later a cytotoxic invasion of colonic mucosa results in an inflammatory colitis. Thus, although fluid and electrolyte transport in the colon is not thought to be affected by the enterotoxin,[79] diarrhea is thought to be aggravated by damage to the colonic mucosa.

Salmonellosis also involved the colon. Rabbit intestine can be infected with different strains of *Salmonella typhimurium,* some of which invade tissues. Fluid secretion is produced only by strains that increase mucosal levels of AMP.[185] Fluid secretion is abolished by pretreatment of rabbits with indomethacin, suggesting a role for prostaglandins.[185] The inflammatory reaction of mucosa may also be important, since pretreatment with nitrogen mustard prevented both the mucosal accumulation of an exudate and secretion of fluid.[186] As for shigellosis, the current hypothesis[187] states that mild salmonellosis produces symptoms by impaired ileal and jejunal absorption, but more severe examples are compounded by colitis and colonic secretion.

E. Villous Adenoma

A specific syndrome of K loss and hypokalemia has been attributed to villous adenoma of the large bowel. Although these lesions can cause diarrhea and major losses of electrolytes, Shields[188] found that losses of K were not disproportionate to losses of Na and water. He concluded that fecal losses of K were explicable by increased fecal

volumes, a view shared by others.[189] However, it is well established that colonic mucus, in health[94] and disease,[99] contains unusually high concentrations of K. Since these tumors elaborate large quantities of mucus, unusual losses of K should still be anticipated.

F. Constipation

Devroede and Soffie used intestinal perfusion[190] to investigate whether patients with idiopathic constipation exhibited "hyperabsorption." When uncorrected for other factors, fluid absorption was greater in constipated individuals. However, intracolonic volumes were increased in constipated subjects and transit of perfusates through the colon was slower. Thus, augmented absorption was explicable entirely by these factors and an intrinsic abnormality of mucosal function could not be implicated.

G. Enteric Hyperoxaluria

Though the genesis of hyperoxaluria after ileal resection has been subject to several different theories in the past decade,[191] it is now generally accepted that increased absorption of dietary oxalate plays, at least, a contributing role. Moreover, the major site of increased absorption of oxalate is thought to be the colon.[192] Thus, this condition is an example of hyperabsorption by the colon. Key to the colon's role is an increase in mucosal permeability to oxalate, thought to be produced by the action of bile acids or steatorrhea on the epithelium. This increased ability to absorb oxalate can be aggravated by high dietary intakes of oxalate and factors which increase the bioavailability of oxalate.[191]

H. The Role of Fecal Electrolyte Measurements in Clinical Medicine

A final practical question is, what role, if any, does fecal electrolyte measurement have in clinical practice? There are few instances in which such analyses are key to diagnosis or therapy. When watery diarrhea is severe, measurements of stool osmolality and concentrations of Na^+, and Cl^-can help distinguish secretory from osmotic diarrhea.[193] In other instances, the finding of large amounts of magnesium, sulfate, or phosphate might suggest laxative abuse. However, despite suggestions of more precise diagnostic benefit,[174] other practical advantages are most unusual.

X. SUMMARY AND FUTURE DIRECTIONS

There is abundant evidence that the human colon has diverse potentials for the handling of electrolytes and water. These functions have relevance to normal physiology and to a number of important processes of disease. Future advances can be anticipated in the areas of microbial digestion of unabsorbed dietary substrates and the subsequent absorption of fermentative end products. In addition, the colon may well emerge as an important "endocrine organ," with peptides of colonic origin having the potential to modify absorption or secretion in the colon or in other segments of the bowel.

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3

The Structure of Colonic Mucus

Adrian Allen

1. INTRODUCTION

Mucus is secreted as a viscoelastic gel that adheres to and covers the gastrointestinal mucosal surface. An accepted function of mucus throughout the gut is protection of the mucosal surface from mechanical damage by the passage of food or fecal material.[1,2] In the stomach and the duodenum mucus forms a continuous gelatinous cover (average thickness about 50–200 μm) over the mucosa[3] and is thought to provide the first line in mucosal defense against corrosive gastric secretions.[4,5] In the colon the enteric microflora produce enzymes that can degrade both the carbohydrate and the protein components of mucous glycoprotein secretions[6–9]. In this way mucous secretions, including those from higher up the gut, can be digested and provide at the same time a nutrient source for the endogenous microflora. In other circumstances mucus may act as an antibacterial or antiviral agent.[2,10] Various other functions have been suggested for gastrointestinal mucous secretions, including the binding of ions, for example, the selective binding of Na^+ by colonic mucus. [11] In general, however, the physiology of gastrointestinal mucus is poorly defined, owing in part to a lack of knowledge of the structure of these secretions.

The viscous and gel-forming properties of mucous secretions are the result of interactions between the constituent glycoproteins. These isolated mucous glycoproteins are large molecules with molecular weights from about 2×10^5 to as high as 15×10^6 (Table 1). The major portion of the glycoprotein is carbohydrate, which is attach to a central protein core as hundreds of side chains per molecule, as many as 800 per molecule in some submaxillary mucous glycoproteins.[12] Up to five different sugars can be present in these carbohydrate units, and the average length of the chains in a given glycoprotein secretion can vary from 2 sugars to 22 sugars per chain. The central protein core, buried within the carbohydrate, is resistant to attack by proteolytic en-

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Table 1. Some Structural Features of Mucous Glycoproteins[a]

1. Large size, molecular weight 2×10^5–15×10^6.
2. Carbohydrate major component, 60–90% by weight.
3. Carbohydrate chains contain *N*-acetylgalactosamine, *N*-acetylglucosamine, galactose, fucose, and sialic acids.[b]
4. Carbohydrate chains often branched and an average of between 2 and 22 sugars per chain for a given glycoprotein secretion.
5. Negatively charged, primarily owing to ester sulfate and sialic acid residues.
6. Protein consists of two parts:
   a. Glycosylated peptide, with the carbohydrate chains attached, which is resistant to proteolysis.
   b. Naked peptide, which is free of carbohydrate and is susceptible to proteolysis.
7. Several mucous glycoproteins shown to be a polymeric structure of glycoprotein subunits joined covalently by interchain disulfide bridges located in the naked peptide.
8. Proteolysis and reduction dissociate polymeric glycoprotein into subunits with loss of viscous and gel-forming properties.

[a]More details of glycoprotein structure see specialized books and reviews.[12–16]
[b]Sialic acid is a generic name for the various neuraminic acids that differ from each other in whether they are substituted with an acetyl or a glycolyl residue on the amino group and in the number of *O*-acetyl substitutions on the hydroxyl groups.

zymes, but over a third of this protein core exists as "naked peptide," which is essentially free of carbohydrate and is accessible to proteolysis. In several mucous glycoproteins it is now clear that the naked peptide contains disulfide bridges that join glycoprotein subunits together to form the larger covalent, polymeric structure of the native glycoprotein,[16,17]

The constitutent glycoproteins of mucous secretions from all regions of the gastrointestinal tract have been isolated and studied. In some respects those from colonic mucous secretions (Table 2) have received rather less attention than those from other secretions of the gut, namely salivary mucus,[18,19] gastric mucus, [20,21] and small intestinal mucus.[22] This applies particularly to rheological properties, and subsequent sections in this chapter will include some discussion of mucous secretions from other regions of the gastrointestinal tract as well as those from the colon.

2. ISOLATION AND PURIFICATION OF MUCOUS GLYCOPROTEINS

To understand mucus structure it is necessary to isolate the soluble glycoprotein, undegraded and with full rheological properties, yet free of the contaminating protein and nucleic acid that are present in mucus scraped from the mucosal surface. This has proved difficult and inevitably involves complicated procedures in which the amount of chemical and physical manipulation is directly related to the potential loss of the viscous and gel-forming properties of the glycoprotein.[16,32] Mucus is present as a gel that does not readily dissolve in aqueous solution and it is therefore necessary to solubilize the glycoprotein constitutents. The methods for dissolving mucus can be broadly divided into two groups (Table 3). The first group are the degradative methods of proteolysis or disulfide bond reduction, which break the covalent structure of the

Table 2. Methods for the Preparation of Colonic Mucous Glycoproteins

Species	Purification method	Analysis of glycoprotein (percent dry weight)				Ref.
		Carbohydrate	Protein	Sulfate	Molecular size	
Human.	Homogenization; extraction with phenol at 65°C;	50.5[a]	39.7	1.6	15×10^6	11,22
a.	ethanol precipitation; gel filtration					
b.	Homogenization; gel filtration; reduction with dithiothreitol	52.6	—	—	—	23
Sheep fraction H	Papain digestion; ethanol precipitation; preparative ultracentrifugation	73.3	25	4.6	2.12×10^5	24,25
Pig	Homogenization; ethanol extraction; trypsin and	8.0–8.7	61.6–70.8	3.2–2.8	$2.32.8 \times 10^5$	26
a.	pronase digestion; ethanol precipitation; DEAE exchange chromatography					
b.	Dialysis; nuclease digestion; equilibrium centrifugation in CsCl gradient; gel filtration	74	13.3	3.0	15×10^6	27
Rat	Homogenization; extraction with phenol at 65°C;	64.7	25	—	—	28
a.	ethanol precipitation; gel filtration					
b.	Homogenization; boil; sonicate; treatment with lithium diiodosalicylate; extraction with phenol; treatment with hydroxylapatite	87.5	13.7	5.4	9×10^5	29,30
c. Fraction IV[b]	Homogenization; gel filtration;	59	23.7	2.6	—	31
	DEAE ion exchange chromatography	51.4	31.2	1.8	—	31

[a]Individual preparations vaaried between 50.1–71.8% and 13.8–36.7% for carbohydrate and protein, respectively.
[b]Different fractions from DEAE ion exchange chromatography.

Table 3. Methods for Solubilization of Mucous Cells

Method	Nature of soluble glycoprotein
1. Degradative methods: destruction of covalent structure of glycoprotein	
Proteolysis (e.g., pepsin, pronase)	Protein component of glycoprotein degraded with formation of lower-molecular-weight glycoproteins, which have lost viscous and gel-forming properties.
Reduction (e.g., mercaptoethanol or dithiothreitol)	
2. Nondegradative methods: dissociation of noncovalent bonds between glycoprotein molecules in gel matrix	
Strong shear force (e.g., homogenization in isotonic saline or stirring in denaturants, like guanidinium chloride)	Primary structure of glycoprotein remains intact. Retains viscous and gel-forming properties of secretion, but these are reduced with excessive manipulation or by denaturants.

constituent glycoproteins. Proteolytic enzymes hydrolyze the naked peptide and reduction splits the intersubunit disulfide bridges of the glycoproteins. This destroys its covalent polymeric structure to yield lower-molecular-weight glycoproteins that have lost the viscous and gel-forming properties of the mucous secretion.[17,33] Since the glycosylated regions of the glycoproteins remain intact following proteolysis, this method can be used to obtain a readily soluble glycoprotein, free of contaminant protein, in which the structure of the oligosaccharide side chains can be studied. Proteolysis of mucous secretions has been a long-established method for preparing blood group substance glycoproteins.[34]

If the relationship between structure and gel-forming properties of mucus is to be investigated, then the soluble glycoprotein must be obtained in its undegraded, gel-forming, polymeric form. This can be achieved by brief homogenization, e.g., 1 min in a Waring blender in isotonic saline, or by prolonged stirring in a denaturant, e.g., 6 M guanidinium chloride (Table 3). The shear forces generated by homogenization dissociate the noncovalent bonds between the glycoprotein molecules forming the gel matrix. The glycoprotein in the sol phase of the gel can be removed by prolonged dialysis, but a residue of insoluble gel is still left behind.[27] Once the glycoprotein is soluble, several methods have been used to purify it from contaminant proteins, and the variations and combinations of such methods used are almost as numerous as the number of different glycoprotein preparations attempted. For detailed accounts of the purification of mucous glycoproteins more specialized reviews or specific papers should be consulted.[12–14]

Colonic mucous gel obtained by scraping the mucosal surface will inevitably be contaminated with other components arising from gut contents, secretions (e.g., IgA and bile), mucus from further up the tract, bacterial products, and sloughed epithelial cells. It is not surprising, therefore, that unfractionated preparations of colonic mucus contain large amounts of protein and nucleic acid. Human colonic mucus contains RNA and albumin[11] and sheep colonic mucus contains both RNA and DNA,[24] while the composition of soluble pig colonic mucus was by freeze-dried weight, 72% protein, 12% nucleic acid, and about 5% glycoprotein.[27] The difficulty in isolating the colonic mucous glycoproteins free from so much protein and nucleic acid is reflected in the

complexity of the purification procedures used and outlined in Table 2. Purification methods include the removal of contaminant protein by extraction with phenol,[11,24,26] fractionation by size using gel filtration to separate the large-molecular-weight glycoprotein from the lower-molecular-weight proteins, [11,23,27,28,31] fractionation by ion exchange chromatography utilizing the strong negative charge of these mucous glycoproteins,[26,31] fractionation by equilibrium centrifugation in a density gradient of CsCl that separates the glycoprotein from the less-dense protein and more-dense nucleic acid,[27] and preparative ultracentrifugation to sediment selectively the large glycoprotein molecules.[24] Exogenous proteolytic enzymes, e.g., pronase,[26] trypsin,[26] and papain,[24,25] have been used to remove protein, and nucleases[27] have been used to remove nucleic acid from the glycoprotein. However, as previously stated, proteolysis will result in a degraded glycoprotein. Digestion with hyaluronidase has been used to remove hyaluronic acid as part of a purification procedure for a mucous-type glycopeptide from human colonic mucus.[35]

A general problem in mucous glycoprotein purification is the complete removal of protein that combines strongly, although noncovalently, with the glycoprotein and separates with it during the isolation procedure. For example, in Sepharose gel filtration some low-molecular-weight protein, which from its size would normally be expected to be included, remains noncovalently bound to the larger-molecular-weight glycoprotein and is excluded with it from the gel.[36] Co-precipitation of glycoprotein and protein has been shown to account for the variable amounts of protein in submaxillary mucous glycoprotein preparations. [37] The best preparative method developed so far that will satisfactorily remove the noncovalently bound protein from mucous glycoproteins is equilibrium density gradient centrifugation in a gradient of CsCl or a similar salt.[32,36] The true value of methods used to assess the purity of glycoproteins, such as forms of electrophoresis and even immunological methods, will only be realized if at some stage this strongly bound protein is separated from the glycoprotein in a solvent that will break these noncovalent interactions. [32,36,38,39] These interactions can be broken by 3.0 M cesium salts, as described above, or by boiling in 1% sodium dodecyl sulfate, but not necessarily by isotonic salt solutions. Polyacrylamide gel electrophoresis in 1% sodium dodecyl sulfate is a good method for assessing the purity of mucous glycoproteins with respect to contaminant protein. A relatively large amount of glycoprotein (200–500 μg) is loaded onto the gel and, because of its large size, remains at the origin, while any contaminant protein present moves into the gel during electrophoresis.

The content of protein and carbohydrate in the different colonic mucus preparations varies widely (Table 2). This is partly due to differences in the moisture and salt content of the preparations, but the ratio of carbohydrate to protein also varies, even in preparations from the same species. While the wide variation in the relative amounts of carbohydrate and protein may be the result of changes in the form of the newly secreted material *in vivo,* it may also reflect, in part, incomplete purification or degradation of the glycoprotein during isolation. For example, noncovalently bound protein may not have always been completely removed or degradation may have occurred due to the action of bacterial proteases or glycosidases that degrade mucous glycoproteins and are

known to be present in the colon.[7,8] Bacterial contamination during the isolation procedure can be minimized by the inclusion of 0.02% azide. The differences in the methods for preparing colonic mucous glycoproteins make it difficult to assess these procedures further. However, all the colonic mucous glycoprotein preparations listed in Table 2 have the special structural features and physical properties characteristic of glycoproteins from other mucous secretions and can be assumed to represent the gel-forming components of the parent secretion.

3. CARBOHYDRATE CHAIN STRUCTURE IN MUCOUS GLYCOPROTEINS

The sugar constituents of the carbohydrate chains are *N*-acetylgalactosamine, *N*-acetylglucosamine, galactose, fucose, and sialic acids (Table 1). Mannose, which is characteristic of membrane and serum glycoproteins, is not found in more than trace amounts in purified mucous glycoprotein preparations. In addition, protein is the major component by weight in serum and membrane glycoproteins, in contrast to the predominance of carbohydrate in mucous glycoproteins. A mannose-rich glycoprotein has been reported as a minor component of human colonic mucosal biopsy specimens and colonic mucosal scrapings.[23,40] Uronic acid, which is found in the proteoglycans of connective tissue, is completely absent from mucous glycoproteins, and in fact the presence of uronic acid can be taken as an indication of contamination in the mucous glycoprotein.

There are wide differences in the molar ratios of the constituent sugars in mucous glycoproteins from the various regions of the gastrointestinal tract, as well as in mucous glycoproteins from the same region in different species. An example of this is seen in the differences in sugar composition of the various colonic mucous glycoproteins shown in Table 4. The detailed structure of the carbohydrate side chains has so far been determined for only a handful of mucous glycoproteins, but it is clear that the differences in sugar composition of glycoproteins reflect wide differences in the size of their carbohydrate chains and the sequence of sugars within them. The simplest carbohydrate side chains are those of sheep submaxillary mucous glycoproteins, which consist of two sugars, *N*-acetylgalactosamine joined to *N*-acetylneuraminic acid,[18] while the carbohydrate chains of rat colonic mucus and pig gastric mucous glycoprotein are branched, complex, and up to a maximum of 12 and 19 sugars long, respectively,[30,41] In contrast, the carbohydrate chains of pig submaxillary mucous glycoprotein[42] and pig small intestinal mucous glycoprotein[38] are relatively short, up to 5 and an average of 6–8 sugars, respectively. Thus, in one gastrointestinal tract, namely that of the pig, there are clear structural differences between the carbohdyrate chains of the glycoproteins from different regions. Despite these differences in the composition and structure of the carbohydrate chains of glycoproteins from the various mucous secretions analyzed, there are also common features. N-acetylgalactosamaine is always the sugar at the reducing ends of the chain and links the chain to the peptide core.[43] Fucose and sialic acid, when present, are found in terminal nonreducing positions in the chains. Another feature of the carbohydrate side chains in mucous glycoproteins

Table 4. The Carbohydrate Composition of Colonic Mucous Glycoproteins[a]

Species	Sugar composition (percent dry weight)						
					Sialic acid		
	Galactosamine	Glucosamine	Galactose	Fucose	NANA	NGNA	Ref.
Human	10.6	10.5	11.9	4.3	—	13.2	11
a. Colonic mucoprotein antigen[b]	(5.1–14.8)	(11.422.7)	(10.9–25.8)	(2.8–19.5)		3.4–19.5	22
b.	14.0[c]	12.1[c]	10.4	3.5		11.8	23
Sheep, fraction H	13.7[c]	27.4[c]	13.8	4.7	5.1	8.6	25
Pig							
a.	21.3 29.3		20.425.6	9.7–14.0		5.5–8.8	26
b.	9.7	25.3	22.7	6.5		3.2	27
Rat							
a.	13.1	17.3	29.0	2.0		3.3	28
b.	10.4[c]	8.4[c]	37.4	15.3	11.7	3.3	29
c. Fractions IV and V[d]	12.9–15.2	14–14.9	12.3–12.7	3.0–3.6		8.2–13.6	31

[a]For details of preparation and overall composition see Table 2.
[b]Range of values for individual preparations in parentheses.
[c]Expressed as *N*-acetylhexosamine.
[d]Different fractions from DEAE ion exchange chromatography.

from human secretors is that they can carry the antigens for ABH blood group substance activity. In such individuals the chains of the mucous glycoproteins have the same terminal sequence of sugars as the ABH antigens on the surface of the erythrocyte, with the result that the glycoprotein will react with the corresponding antibody and inhibit the agglutination reaction.[44,45] In A and B secretors the carbohydrate chains end in *N*-acetylgalactosamine and galactose, respectively. The glycoproteins from both pig and rat colonic mucus have A and H blood group substance activity.

The sequence, the type of linkage, and the maximum number of sugar residues possible per chain are genetically specified, but the number of sugars per chain together with the number of chains per glycoprotein molecule vary among molecules from the same secretion. In pig submaxillary mucous glycoprotein from A secretors, 5 different sizes of carbohydrate chains are found from a single *N*-acetylgalactosamine residue to completed chains with all 5 residues,[42] While in rat colonic mucous glycoprotein 8 different sizes of carbohydrate chain are present, ranging in size from 2 to 12 sugars per chain.[30] This microheterogeneity in size and also in number of carbohydrate chains per molecule is considered to be the reason for the polydispersity in molecular weight shown by the mucous glycoproteins.[46] Thus the size of a mucous glycoprotein from a single secretion will vary about a mean; this results in a pure glycoprotein preparation eluting as a broad peak when fractionated by size using gel filtration. On analytical ultracentrifugation mucous glycoproteins sediment under a broad envelope instead of the sharp profile obtained by this method with a single species of protein. Considerable polydispersity in size was evident on sedimentation analysis of pig[27] and rat[31] colonic mucous glycoproteins.

The overall sugar composition of the different colonic mucous glycoprotein preparations investigated (Table 4) and the variations in the molar ratios of the sugars among preparations from different species are typical of mucous glycoproteins from other regions of the gastrointestinal tract. There are also substantial variations, however, in the molar ratio of the sugars in colonic glycoproteins prepared from a given species, in some cases even after using the same preparation method. Such variations in the sugar composition could be due to differences in the average length of the carbohydrate side chains or to changes in the amounts of different structured carbohydrate side chains in one or more of the secreted glycoproteins in the preparation. Proteolytic or glycosidic enzymes produced by colonic bacteria[7,8,47] *in vivo* or *in vitro* during the preparation procedures could also contribute to variations in the average carbohydrate chain length of the different preparations. More work has to be done on the structure of the carbohydrate chains in colonic mucous glycoproteins before these variations in the sugar composition can be explained and a meaningful comparison with other mucous glycoproteins can be made. Recently 8 different carbohydrate units, of between 2 and 12 monosaccharides per chain, have been sequenced for rat colonic mucous glycoprotein.[30] The larger chains were branched and consisted of the same oligosaccharide core of four sugars: galactose, (β1–4)*N*-acetylglucosamine, (β1–3)*N*-acetylgalactosamine (6–2α)*N*-acetylneuraminic acid. The chains were joined to the serine or threonine residues of the protein core from the *N*-acetylgalactosamine of the above oligosaccharide. Branches with either fucose or *N*-acetylneuraminic acid were present, but these sugars were not observed together on the same branch chain.

All gastrointestinal mucous secretions carry a net negative charge; this is particularly true for glycoproteins from colonic mucous secretions. These glycoproteins have substantial amounts of negatively charged ester sulfate and sialic acid residues on the carbohydrate side chains[24] (Tables 2 and 4), although a contribution to the net charge of the molecule will also be made by the protein. Sialic acids are a family of sugars based on *N*-acetyl and *N*-glycolylneuraminic acids. Both these neuraminic acids have been found in colonic mucous glycoproteins together with mono-, di-, and tri-*O*-acetyl derivatives of *N*-acetylneuraminic acid.[48,49] Sialic acids are always in terminal positions (nonreducing end) on the carbohydrate chains and can be removed by the exoglycosidase neuraminidase. However, frequently not all of the sialic acid is removed by neuraminidase from colonic mucous glycoproteins, because some *O*-acetylated sialic acids are resistant to this enzyme. Treatment of pig colonic mucous glycoprotein with neuraminidase increased blood group substance A activity,[27] which suggests that sialic acid partly masks the antigenic site in this glycoprotein. The position of the ester sulfate groups in the carbohydrate chains of colonic mucous glycoproteins has not been investigated, but in pig gastric mucus[41] and dog submaxillary mucus[49] these groups are located internally in the chains attached to *N*-acetylglucosamine. The contents of both sialic acid and ester sulfate vary among colonic mucous glycoprotein secretions from different species and among molecules within the same secretion (Tables 2 and 4). This microheterogeneity in the number of charged sialic acid and sulfate residues between glycoprotein molecules from one secretion results in polydispersity of charge in the preparation. For example, pig[26] and rat[31] colonic mucous glycoprotein preparations have been separated by ion exchange chromatography on DEAE columns into fractions of different sialic acid and ester sulfate content. Until there have been more studies on distribution of the charge in mucous glycoproteins, it will not be possible to say if there are distinct classes of sulfated and sialylated glycoproteins.

The sialic acid and ester sulfate content of colonic mucous glycoproteins is the basis for their strong staining with the basophilic dyes Alcian blue and high iron diamine.[48] The distinction between the sialic acids and ester sulfate is achieved by varying the salt concentration or the pH of the staining solutions or by using neuraminidase to remove the sialic acids. Extensive histochemical studies on colonic mucosa have shown that the degree of sulfation and sialylation in the mucous glycoprotein depends on the location of the epithelial cells, whether they are in the crypts or on the surface of epithelium, and in which segment of the colon they are located.[48,51,52] Sulfomucins are characteristic of the crypts while neutral mucins predominate in the upper crypt and surface epithelium. The amount of mucus secreted and the sulfation, sialylation, and sugar composition of its glycoproteins also change in disease states such as carcinoma of the colon and ulcerative colitis.[23,40,48,51,52] For example, selective staining has shown that there is a switch from sulfomucins to sialomucins in the "transitional" mucosa associated with colonic malignancy, accompanied by an increase in the sialic acid content of the isolated glycoproteins.[52,53] The sialomucins of human colonic tumors are less resistant to digestion by neuraminidase owing to their lower amounts of *O*-acetylated sialic acids.[54] A reduction of carbohydrate content and a decrease in the chain length of the carbohydrate units was observed in colonic mucous glycoprotein

fractions from patients with ulcerative colitis and Crohn's disease when compared to material from normal postmortem colons.[23] More than half of the carbohydrate chains from normal material contained eight or more monosaccharide residues while the bulk of chains in material from diseased colons were smaller in size than this.

Antigenic studies on colonic mucous glycoproteins have shown structural similarities with mucous glycoproteins from other gastrointestinal secretions but have also revealed antigenic determinants specific to the colon. The human colonic mucoprotein antigen (Tables 2 and 4) is a mixture of antigens including a colonic-specific antigen but also other antigens that cross-react with mucous secretions from all the way up the gut, although there was most cross-reactivity between these antigens and ileal or cecal tissue.[55] Purified human colonic mucous glycoprotein, homogenized by physical methods, contained at least two components by immunodiffusion.[39] After proteolysis this glycoprotein was separated into two fractions by gel filtration on Sepharose 4B. The excluded fraction (mucin A) contained the colon-specific antigen and was enriched in threonine, proline and sialic acid. The included fraction (mucin B) was immunologically distinct from mucin A, with an antigen present in all mucin-secreting tissues, and was enriched in serine, threonine, and fucose. While these two components may have arisen from the same molecule an alternative explanation is the presence of two different mucins in the same secretion. A mixture of antigens was detected with antisera to rat colonic mucoprotein antigen [Tables 2, 4, and 5 (rat a)], but in neoplasia, in the rat model, the colon-specific determinant was lost and a new determi-

Table 5. The Amino Acid Composition of Some Colonic Mucous Glycoproteins

Amino acid	Amino acid content (μmoles/100 moles protein)				
	Human CMA[a,b]	Rat		Pig b[e]	
		a[c]	b[d]	Undigested	Pronase-digested
Asp[f]	5.8	7.7	10.2	5.5	2.1
Thr	23.6	19.1	18.1	15.1	38.9
Ser	9.8	9.7	11.7	12.6	18.4
Glu[f]	8.9	9.4	15.7	5.5	2.4
Pro	11.9	8.5	9.6	15.4	20.9
Gly	8.1	6.6	8.5	5.7	5.3
Ala	6.2	6.9	6.9	5.4	5.6
Val	5.2	7.4	5.1	3.8	1.9
Ile	4.0	3.0	3.0	2.7	1.7
Leu	5.2	5.4	4.6	4.7	2
Tyr	1.9	1.4	—	1.4	—
Phe	2.1	2.0	1.7	1.9	0.9
His	3.7	4.8	—	4.3	—
Lys	1.7	5.2	2.9	2.7	—
Arg	1.9	3.1	1.6	3.6	—

[a]Colonic mucoprotein antigen.
[b]Reference (22).
[c]Reference (28).
[d]Reference (29).
[e]Reference (27).
[f]Asp and Glu, aspartic/asparagine and glutamic/glutamine, respectively.

nant was detected.[28] An antibody to human small intestinal goblet cell mucous glycoprotein, which was distinct from the ABH determinants, cross-reacted equally well with rectal and colonic mucus but showed some species specificity in that it was present in dog, monkey, and rabbit but not in pig or rat.[55] A highly sulfated glycoprotein antigen isolated from gastric tumors has been shown to be a characteristic antigen of normal human small intestinal and colonic mucosa; it was also found in about 50% of colonic carcinomas and in some gastric tumors.[57] This glycoprotein antigen consisted by weight of 50% carbohydrate and 10% protein and had a high ester sulfate content of 7.5% and, interestingly, a molar content of *N*-acetylgalactosamine higher than that of any of the other sugars present.

4. PROTEIN CORE AND POLYMERIC STRUCTURE IN MUCOUS GLYCOPROTEINS

The protein core of the mucous glycoproteins can be divided into (1) glycosylated peptide, to which the carbohydrate is attached, and (2) the naked peptide, free of carbohydrate and hydrolyzed by proteolytic enzymes. The glycosylated region is the major part of the peptide core of mucous glycoproteins and accounts for the very high serine, threonine, and proline content of these glycoproteins, as typified by the amino acid analyses of the colonic mucous glycoproteins (Table 5). Serine and threonine, the sites of glycosylation, are packed closely together on the peptide core [12–14]; from the analysis of pig gastric mucous glycoprotein, one in every three or four amino acids on the peptide chains must carry carbohydrate units of an average 15 sugars per chain.[20] The high proline content may be associated with the provision of a suitable conformation in the peptide backbone for such close packing of these sugar chains. It is not surprising, with its sheath of carbohydrate, that the peptide core is protected from proteolytic attack and that mucous glycoproteins prepared using proteolysis still have a high molecular weight, e.g., pig gastric mucus,[33] 5×10^5; pig colonic mucus,[27] 7.6×10^5; and sheep colonic mucus,[24] 2.1×10^5.

The presence of nonglycosylated naked protein in the peptide of these glycoproteins has been established for pig and human gastric mucus,[33,58] pig small intestinal mucus,[59] and pig colonic mucus[27] and in the related ovarian cyst[60] and cervical [61] mucous glycoproteins. This naked peptide makes up only about 5% by weight of the native molecule; to demonstrate its presence the mucous glycoprotein must be isolated, free of noncovalently bound protein yet undigested by proteolytic enzymes. Pig colonic mucous glycoprotein prepared, apparently free of protein, by preparative equilibrium density gradient centrifugation in CsCl has been shown to possess regions of naked peptide in its protein core.[27] A comparison of the analysis of the native glycoprotein before and after proteolytic digestion shows that 29% of the protein (3% by weight of the glycoprotein) is removed by proteolysis without loss of carbohydrate. The digested peptide remaining exhibits an increase in the relative percentage of threonine, serine, and proline at the expense of all the other amino acids present in the undigested peptide (Table 5).

The native, undegraded gastric mucous glycoproteins from man and pig (molecular weight 2×10^6) are covalent polymers of glycoprotein subunits joined by disulfide

bridges located in the naked peptide component of the molecule.[17,58] In pig gastric mucous glycoprotein there are, on average, four of these subunits with a molecular weight of 5×10^5 per molecule. Reduction of the gastric glycoprotein with 0.2 M mercaptoethanol cleaves the disulfide bridges to produce the subunits together with a protein of molecular weight 70,000[62]. One protein molecule (molecular weight 70,000), on average, is joined by disulfide bridges to one native glycoprotein molecule (molecular weight 2×10^6), and evidence suggests that this protein may play a central role in the glycoprotein by joining the subunits together. Proteolysis with a variety of enzymes, including pepsin, trypsin, and pronase, splits the gastric mucous glycoprotein into subunits of molecular weight 5×10^5, which are, in fact, the glycosylated regions of the undigested glycoprotein.[33] The 70,000 molecular-weight protein is also released and hydrolyzed on proteolysis indicating that it is the major component of the naked peptide in the glycoprotein.[62] Undegraded pig small intestinal mucous glycoprotein (molecular weight 1.8×10^6) has a defined polymeric structure too, but in this case one consisting of subunits of molecular weight 2.4×10^5 joined by disulfide bridges to a protein of molecular weight 90,000.[59] Again this intestinal mucous glycoprotein is split on proteolysis into subunits, in this case of molecular weight 4.7×10^5. There is evidence for similar polymeric structures based on disulfide bridges in glycoproteins from nongastrointestinal secretions, namely cervical,[61] respiratory,[63] and ovarian cyst mucus.[64] The polymeric structures of these mucous glycoproteins are essential for expression of the gel-forming properties of these molecules,[16,17] and this is discussed in the Section 5.

The polymeric structure has been investigated in the glycoprotein from pig colonic mucus.[27] The native glycoprotein, when extracted, was very large, with a molecular weight of 15×10^6. It would seem unlikely that such a large glycoprotein could be a single covalent entity, although sedimentation velocity data suggest that there is only a limited reduction in size in 6 M guandinium chloride, a strong noncovalent-bond-breaking solvent. A summary of the effects of reduction and proteolysis on the pig colonic mucous glycoprotein is shown in Figure 1. Both reduction with 0.2 M mercaptoethanol and pronase digestion produced smaller glycoprotein components, but it was only after pronase digestion followed by reduction that the smallest glycoprotein unit, with an average molecular weight of 7.6×10^5, was obtained. While the sedimentation patterns of the pig colonic glycoproteins were clearly polydisperse and the molecular sizes of such large molecules from these studies approximate, the importance of disulfide bridges and naked peptide in maintaining the structure of this glycoprotein is evident.

The amino acid contents of the colonic mucous glycoprotein preparations all show a similar pattern with high values for serine, threonine, and proline, but the ratios of the individual amino acids in the preparations are different (Table 5). In human colonic mucoprotein antigen the content of a given amino acid was found to vary widely among individual preparations, e.g., the lowest value for threonine, 16.1%, was less than half the highest value, 34.1%, but preparations from normal and carcinomatous colons differed consistently in their analysis.[22] In particular, the proportion of threonine and the amount of glycosylation were decreased while the proportion of aspartic acid was increased in the glycoprotein from the carcinomatous colon. To explain such fundamen-

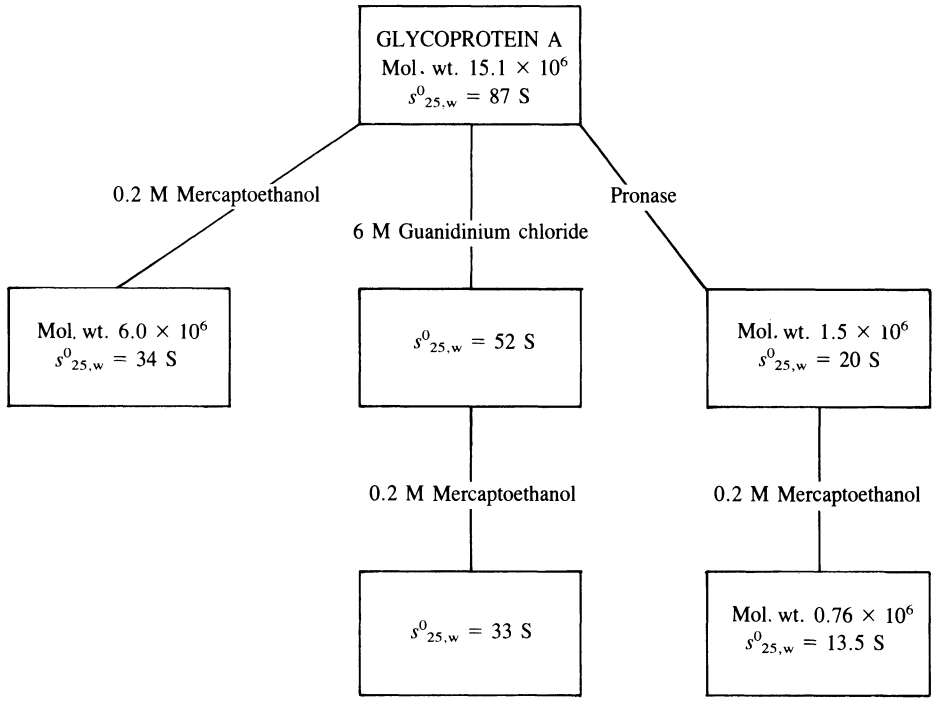

Figure 1. Summary of the physical properties of the high-molecular-weight glycoprotein A from pig colonic mucus. Reproduced from Marshall and Allen.[27]

tal differences in the structure of the glycoprotein, changes at the transcriptional or translational level of the protein core resulting in an altered glycoprotein or changes in the amounts of different glycoproteins secreted were proposed as possibilities.

5. THE RELATIONSHIP BETWEEN GLYCOPROTEIN STRUCTURE AND THE GEL-FORMING PROPERTIES OF MUCOUS SECRETIONS

It is clear that mucous glycoproteins from the various regions of the gastrointestinal tract can differ in (1) the size and composition of their carbohydrate units, (2)the amount of negative charge, and (3) the size and number of glycoprotein units that are polymerized by disulfide bridges to form the native glycoprotein. Yet these different structured glycoproteins all form mucous gels with viscoelastic structures that at the same time have flow properties and will anneal if sectioned. These flow properties, which contrast with the rigid gel structrure of, for example, agar, mean that, if present in sufficient quantity, mucus will form a continuous protective coat over the entire surface of the mucosa. The mucous gel matrix is formed by glycoprotein molecules that interact noncovalently yet can "make and break" in a finite time; this provides an explanation for the flow properties of the gel. In pig gastric[65] and small intestinal[38] mucous glycoproteins, in which gel formation has been investigated, the gel-forming units

are native glycoproteins of molecular weights 2×10^6, and 1.8×10^6 respectively. No gel-forming studies have yet been performed on isolated colonic mucous glycoproteins, and the true size of the covalent gel-forming unit is not certain for any of the preparations in Table 2. The molecular weight values for colonic glycoproteins range from 2.1×10^5[24] up to 15×10^6.[11,27] The lower values refer to the proteolytically degraded glycoprotein while the high values are nearly an order of magnitude greater than those for other native gastrointestinal mucous glycoproteins and may not correspond to the smallest covalent entity present. However, an intrinsic viscosity of 8.9 dl/g for the colonic mucoprotein antigen[11] is high compared with values of 3–5 dl/g for gastric[65] and small intestinal [38,66] glycoproteins (all in 0.1–0.2 M salt solutions), and this would be in keeping with a larger size for the colonic glycoprotein.

Gel formation by glycoproteins in mucous secretions from higher up the tract has been shown to depend on (1) the noncovalent interactions between the glycoprotein molecules and (2) the size and polymeric structure of the glycoprotein itself. For gel formation to take place a certain threshold concentration of glycoprotein has to be exceeded, This concentration is 50 mg/ml in pig gastric mucus,[65] but it is somewhat lower in small intestinal mucus, at about 10 mg/ml.[38] There is no sharp transition in rheological properties on gel formation; as the glycoprotein concentration increases so the intermolecular interactions increase, and the solution becomes progressively more and more viscous until it assumes the viscoelastic properties of the gel.[65] The greater the concentration of glycoprotein within the gel matrix, the more noncovalent intermolecular interactions there will be and the denser, stronger, and less permeable the gel will become. Therefore it is important to consider the concentration of mucous glycoprotein that is secreted when determining the properties of the mucous gels.[5,16]

To form a gel, the mucous glycoprotein must retain its native covalent polymeric structure. This is apparent from the ready solublization of many mucous gels by proteolysis or by reduction of disulfide bridges and the demonstration that, in isolated pig gastric and small intestinal mucous glycoproteins, this was associated with the dissociation of the polymeric structure into glycoprotein subunits.[17,59] The isolated, reduced, or proteolytically digested glycoprotein subunits had a relatively low intrinsic viscosity and, at comparable concentrations, did not show the strong dependence of viscosity on increasing glycoprotein concentrations or the gel formation of the native glycoprotein. An important conclusion from these studies was that proteolytic enzymes, whether of endogenous or bacterial origin, can hydrolyze the mucous glycoproteins and thereby solubilize the surface gel. This finding has important implications higher up the gut, e.g., for peptic erosion of gastric mucus,[5,67] and is an important consideration in determining the factors affecting the degradation of mucus in the colon.[7,8]

The role in gel formation of the carbohydrate units (which constitute the major part by weight of the molecule) and the negatively charged sialic acid and ester sulfate residues is not clear. The carbohydrate units will confer on the glycoprotein its strongly hydrophilic properties, maintaining it in the solution. Since much of the surface of the glycoprotein molecule must be carbohydrate, the noncovalent interactions that form the gel matrix, whatever they may be, are likely to be between the carbohydrate units. However, differences in the precise chemical structure of the carbohydrate units can

occur without apparently affecting the ability of the glycoprotein to form a viable gel. This can be seen from the variation in carbohydrate units among glycoproteins from mucous secretions from different sources. In man the terminal sugars of the carbohydrate units of a mucous secretion from the same tissue will vary according to the secretor status of the individual and glycoproteins from nonsecretors can contain three fewer sugars per chain than A or B secretors.[44,45] The amount of negative charge on the glycoprotein molecule can influence its rheological properties, but this is true at very low ionic strengths, when the viscosity of mucous glycoproteins rises dramatically and there is a corresponding lowering of the threshold for gel formation.[66,68] This effect, which occurs below about 5–10 mM salt, is explained by the lack of a sufficient number of counterions to shield from each other the negatively charged groups within the glycoprotein molecule. The result is that repulsion between these negatively charged groups expands the size of the glycoprotein molecule so that it occupies a larger volume of solution and therefore will interact intermolecularly at lower concentrations. Increasing ion concentrations about 5–10 mM does not effect the viscosity of isolated mucous glycoproteins,[66,68] or presumably their gel-forming ability, and it would follow that changes in ionic strength at the relatively higher values found *in vivo* would not affect gel structure.

The effectiveness of the mucous gel in performing its functions *in vivo* will depend not only on its structure but also on the amount of secreted gel that is adherent to the mucosal surface. Throughout the gut, including the colon, the depth of the surface mucous gel layer will be governed by a balance between its rate of secretion and its rate of erosion by enzymes and mechanical shear. Factors that either slow down mucus secretion by the mucosal cells or increase mucus erosion will cause a weakening and eventual destruction of the surface mucous gel; this could be important in understanding the etiology of colonic disease. The potential for mucus erosion in the colon is likely to be considerable, owing to (1) mechanical shear from the passage of fecal material and (2) the degradation of mucus by bacterial enzymes. Extensive mucus degradation can occur in the colon from extracellular enzymes produced by the enteric flora.[8,9,69] Further up the gastrointestinal tract, proteolytic enzyme secretions will degrade the native mucous glycoproteins and thereby solubilize the adherent gel to produce degraded subunits.[16,20] These subunits are still large (molecular weights $> 200{,}000$) but are not further degraded because of the absence of glycosidase enzymes that break down the carbohydrate chains. In the colon, however, there is further breakdown of the mucous glycoproteins by a variety of glycosidases that are produced extracellularly by distinct subpopulations of anaerobic bacteria.[7,9,69] Fecal extracts and cultures of anaerobic bacteria from human stools will extensively degrade the carbohydrate component of pig gastric mucous glycoprotein and various responsible glycosidase activities have been demonstrated.[8,69] Proteolytic degradation of pig gastric mucus was also observed in this system, although it was not as extensive as the carbohydrate degradation. Since the pig gastric mucus substrate used had been isolated initially by proteolysis, this finding suggests that, once the carbohydrate chains are removed, the protein core is accessible to further degradation.

Several studies have demonstrated that a variety of agents can alter the pattern of colonic mucous glycoprotein biosynthesis in isolated tissue,[70,71] in organ cul-

ture,[72,73] and *in vivo*.[74,75] In such studies radioactive precursors, e.g., glucose, threonine, glucosamine, and sulfate, are readily incorporated into mucous glycoproteins by the colonic tissue, which has an active metabolism and continually produces mucus. The biosynthesis of colonic mucous glycoprotein has been shown to be inhibited by antiinflammatory compounds, e.g., salicylates,[71] and inhibitors of protein biosynthesis, e.g., puromycin[76] and cyclohexamide.[72] There is also evidence that mucus output in the colon is under physiological control, since it is stimulated in organ culture by acetylcholine,[72,77] and in the perfused rat colon by 5-hydroxytryptamine and carbachol.[78] The effect of carbachol, but not that of 5-hydroxytryptamine, is inhibited by atropine, a finding that suggests two distinct mechanisms of stimulation.

While sufficient and satisfactory gel formation is a prerequisite for mucus to perform its functions, the detailed structure of the gel is probably important in such roles as interaction with the endogenous gut flora, protection against pathogenic organisms, and selective permeability to ions and other molecules. It is likely that the detailed differences in structure of the glycoproteins from the various gastrointestinal mucous secretions, as discussed in this chapter, are a reflection of such functions of the mucus gel in the changing physiological environments of the gut.

Over the past few years progress has been made toward solving the problems of isolation and characterization of the undegraded gastrointestinal mucous glycoproteins and determining how their structure is related to the gel-forming properties of the native secretion. In the future, with such knowledge, we can look forward to a better understanding of how the structure of mucus is related to the physiology and pathology of the gastrointestinal tract.

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4

The Physiology of Colonic Mucus Secretion

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I. INTRODUCTION

Physiological studies of the colon have focused basically on motility, water, and electrolyte absorption and secretion, but only occasionally on mucus secretion. Knowledge of the secretion of mucosubstances is scanty probably because of the enormous technical complexity required for its research. As with other organs, the quantity and quality of mucus secretion in the colon may be of major importance in pathology.

The present chapter is concerned with the stimulants of mucus secretion and the mechanisms controlling it. The possible functions of mucus are also briefly considered.

II. SECRETORY STIMULANTS

Extensive research has been carried out in the past few years to determine the secretion-inducing ability of stimulants present in the colon lumen. Some of these substances are physiological, such as short-chain fatty acids; others, like microorganisms and microbial toxins, are pathological; and certain drugs are laxative.

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III. ORGANIC ANION

A. Short-Chain Fatty Acids

In patients with disaccharide deficiency, diarrhea and acid stools are commonly observed. A study published by us[1] showed a highly significant relation between the amount of organic anion in the stools and fecal bulk.

On the basis of the above, we undertook a number of experiments in rats,[2] designed to assess the colonic response to acid stimulation. Our experimental design (Fig. 1) consisted in infusing an organic anion solution (OA) into rat colon for 1 hr. After this period, the infusate was recovered and its volume, electrolyte content, and secreted mucus were then measured.

We have recently studied the colonic response to organic anion as affected by different stimulation conditions, i.e., pH of the solution, amount of organic anion absorption, and length of time under stimulation, and found the following:

1. A mucus secretory response occurs at at pH under 5. At pH 2.9 the response is not significantly higher, and above the 4.5 threshold no response is detected (Fig. 2).

2. With a higher concentration of organic anion at pH 2.9, an increase in the absorption of the anion was observed (Fig. 3), which in turn induced a greater secretory response of mucus, that is, the greater the amount of organic anion absorbed, the greater secretory response (Fig. 4).

3. At 120 min there is a rise in mucus secretion, compared with the level reached after 1 hr stimulation (Fig. 5). After longer periods no enhancement of the response was noted. These stimuli are not physiological, since intraluminal pH is approximately 7 in the large bowel. Nevertheless, the secretion-inducing ability of this anion at low-pH levels may be used as a model for stimulation of colonic mucus secretion.[2]

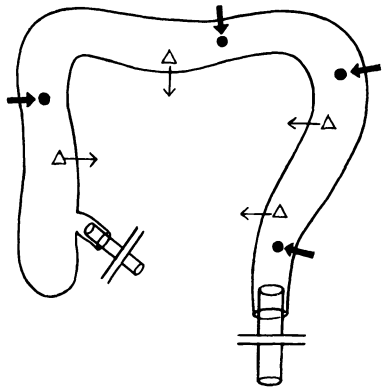

Figure 1. Design for a study of the secretory function of the colon by intracolonic infusion of OA: Secretory stimulant solution pH 2.9; CH_3COOH 100 meq/liter; NaCl 100 meq/liter; polyethylene glycol (PEG) 4000, 0.4%. Absorptive stimulant solution pH 6.9; $NaCH_3COO$ 100 meq/liter; NaCl 50 meq/liter; PEG 4000, 0.4%.

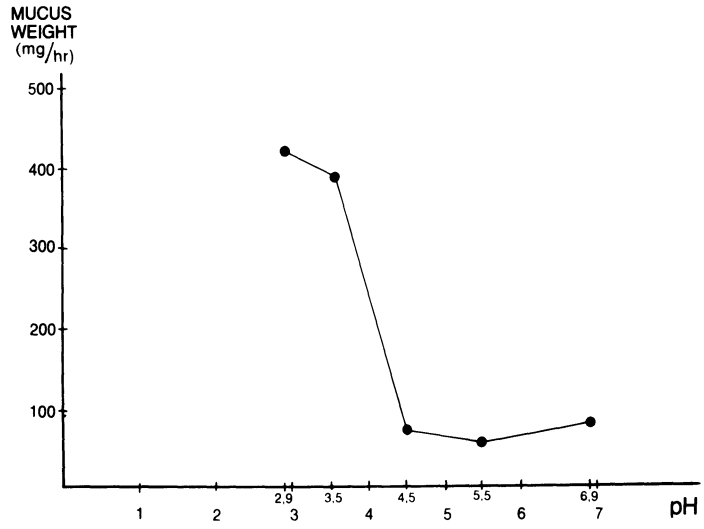

Figure 2. Effect of pH of the OA solution infused into the colon (resulting from different ratios between the acetic acid and Na acetate) on the mucus secretory response.

Independently of the studies on the mucus secretory response in the lumen, additional investigations were made in our laboratory under similar stimulation conditions, in order to determine ^{35}S uptake by the rat colon goblet cells after intravenous injection of the isotope in a single dose of 2.5 μCi/g body weight.

Using fractionation of the labeled mucus macromolecules on Sepharose C1-4B (Fig. 6) from colonic rats, which had been subjected to intraluminal stimulation with OA (100 meq/liter), at different times varying between 15 and 120 min, we found that the labeling was considerable after 15 min in the peak of low-molecular-weight proteins (peak B), but not so in the high-molecular-weight peak (peak A) corresponding to the goblet cell mucus. A faster labeling seems possible, but labeling has not been studied at shorter times. After 30 min there is marked labeling both in the goblet cell mucus (GCM) and in the low-molecular-weight peak (not shown). It does not follow from the above results that those two fractions have a different biosynthetic behavior; however, independent isotopic labeling kinetics for each fraction seems possible. Experiments using isolated mucus from the right and left colon reveal a higher uptake of ^{35}S in the left colon, which may be correlated with histological studies showing a higher sulfomucin concentration in the left colon (unpublished observations).

B. Long-Chain Fatty Acids

It was formerly assumed that only hydroxylated long-chain fatty acids could elicit secretion, on the basis of the similarity of their structure to that of ricinoleic acid. Subsequent studies demonstrated that oleic acid and triglycerides also act as secretory stimulants in the digestive tract.[3]

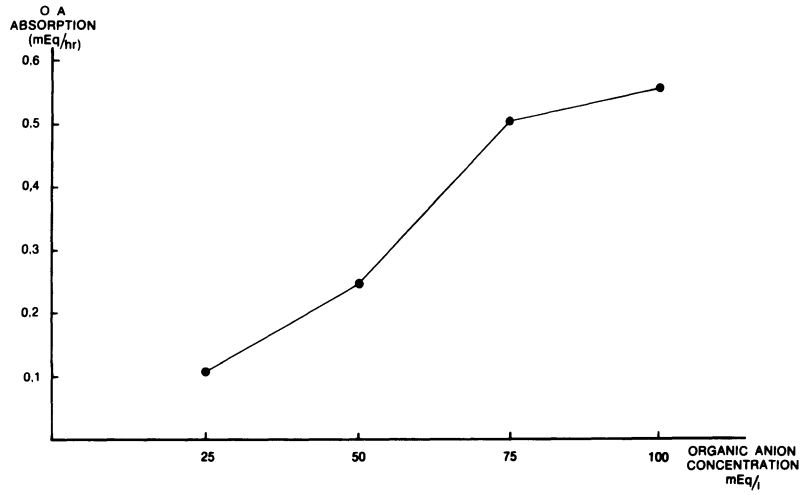

Figure 3. Comparison between concentration and absorption of OA at pH 2.9 (NaCl was used to ensure isosmolality).

Using triglycerides as stimulants of intestinal mucus secretion Freeman[4] was able to obtain a complete exhaustion of secretion from the goblet cells and a continuous synthesis of mucus.

C. Bile Acids

Using 10 mM of deoxycholic acid or cholic acid to perfuse canine colons, Mekhjian and Phillips[5] found that their microscopic appearance was normal and reported no abnormal changes in the epithelial cells. However, the goblet cells showed a diminished amount of periodic acid-Schiff, positive material and the depletion of goblet cells mucus was confirmed by electron microscopy.[5] Other studies of the rat colon[6] and of the canine colon[7] indicate the ability of bile acids to elicit mucus secretion.

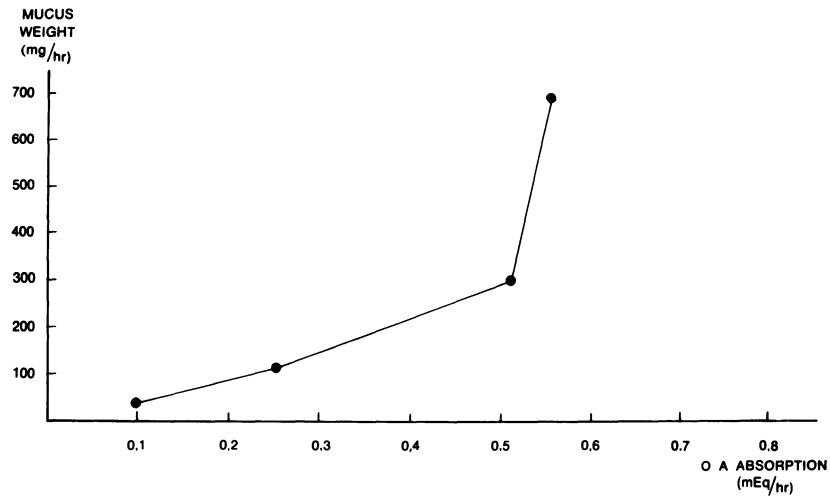

Figure 4. Comparison of OA absorption with mucus secretion.

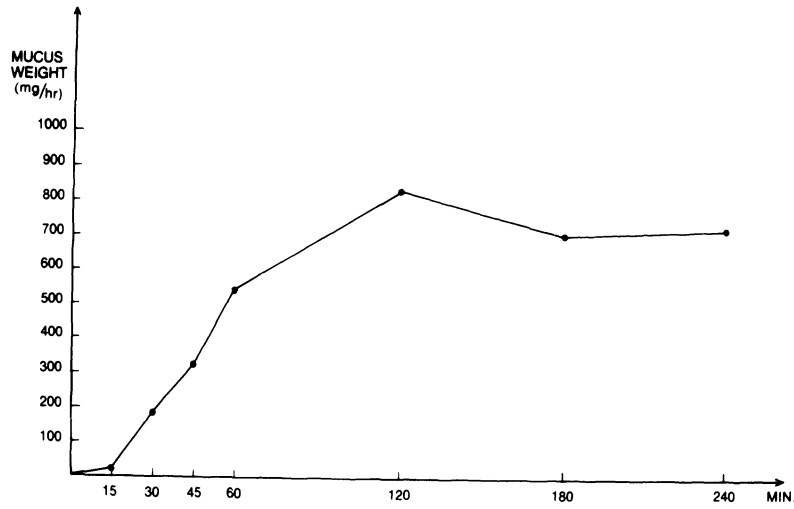

Figure 5. Comparison of length of stimulation period (OA at initial pH 2.9) with mucus secretory response.

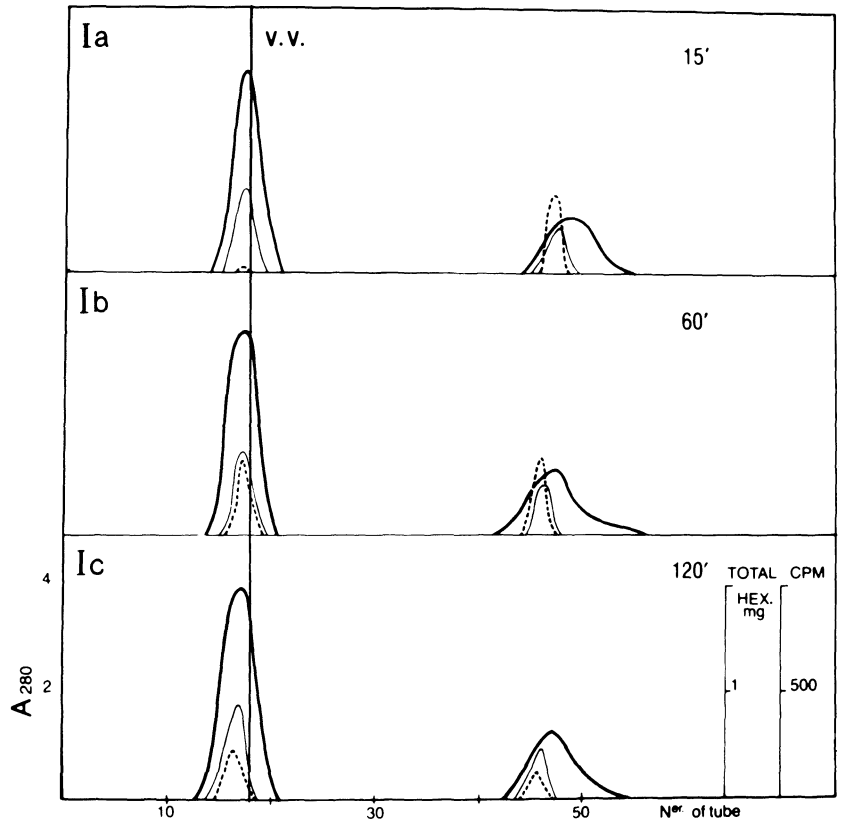

Figure 6. Gel filtration chromatography on Sepharose Cl-4B (1.8 × 80 cm) eluted with phosphate buffer pH 7.2 of colonic mucus isolated at different times. The wide full line represents protein content; the narrow full line, hexosamine content; the dotted line, 35 S activity. Ia at 15′, Ib at 60′, Ic at 120′.

IV. MICROORGANISMS

Germs may induce bowel secretion by a double mechanism: noninvasive toxigenic enteropathy and invasive enteropathy.

A. Noninvasive Toxigenic Enteropathy

Vibrio cholerae and some coli-pathogens multiply and act in the small bowel, causing severe diarrhea. Yardley et al[8] have detected mucus secretion from the small-bowel goblet cells due to cholera toxin action. In recent experiments Donowitz and Binder[9] showed that the purified cholera toxin is capable of eliciting secretion in the large bowel, but found no evidence of mucus secretion.

B. Invasive Enteropathy

Shigella and salmonella multiply in the colon wall itself, causing a sharp inflammatory response. The secretory response seems related to a process similar to that occurring in diarrhea induced by cholera toxin, with a rise in cyclic AMP (cAMP) in the epithelial cells (toxigenic enteropathy). In invasive enteropathy the rise in cAMP is possibly mediated by the release of prostaglandins: Giannella and co-workers[10] found that indomethacin could be used to reduce colonic secretion in such patients.

V. IMMUNE COMPLEXES

Walker et al[11] found that certain immune complexes instilled in the duodenum induced a release of mucus from the small-bowel goblet cells. Subsequent studies by those researchers indicated that intestinal perfusion with the same antigen could elicit mucus secretion only if the rat had been previously immunized orally with that antigen, but failed to do so if parenteral vaccination was used instead[12]. It remains to be elucidated whether such a mucus secretion mechanism also occurs in the colonic mucosa.

VI. LAXATIVES

Several substances traditionally have been used as laxatives. Recent research has shown that in addition to their propulsive motor activity they may facilitate laxation by enhancing colonic secretion.

A. Ricinoleic Acid

An active component of castor oil, ricinoleic acid inhibits colonic absorption of water and electrolytes and induces their blood-to-lumen movement. The effect of ricinoleic acid is similar to that of long-chain fatty acids, such as oleic acid.[13–15]

Although the mechanisms leading to a secretory condition are not yet fully understood, their stimulating effect might be related to the well-established surfactant properties of the molecule. In a study of the morphology of the rabbit colon, Gaginella et al[15] commonly detected a clear secretion of mucus after perfusion of the colon with sodium ricinoleate at a concentration above 5 mM.

B. Dioctyl Sodium Sulfosuccinate

Dioctyl Sodium Sulfosuccinate (DSS) has been used as a laxative on the basis of a supposed softening effect on the stools. Like ricinoleic and bile acids, DSS belongs to the group of surfactant agents.

Donowitz and Binder[16] attribute inhibition by DSS of water and electrolyte absorption to a rise in cAMP in the epithelial cells. Histological studies of the colon stained with hematoxylin eosin, Alcian Blue, and PAS, 2-, 4-, and 6-mM concentration of infused DSS for 3 hr, indicated normal histological sections when compared with the colons of control rats infused with 154-mM NaCl, no depletion in goblet cell mucus was reported. Such findings are apparently in contrast with the observation of LaMont and Ventola[17] that the rise in cAMP elicited mucus secretion.

C. Polyphenolics: Bisacodyl

Bisacodyl action is more effective on the colon than on the small bowel.[18] Saunders *et al.*[18,19] suggest that the laxative effect of bisacodyl in the colon, as observed in *in vivo* experiments during single-pass infusion of rodent and human intestinal segments, might result from an alteration of the membrane with simultaneous stimulation of cAMP. A dose of 2 mg/100 ml bisacodyl infused in the rat colon reduces goblet cell mucus.

VII. CHARACTERISTICS OF SECRETED MUCUS

As early as 1933 Florey[20] carried out a number of brilliant studies of mucus secretion in the colon. The colonic mucus is a viscous mucosubstance formed by the secretion from the goblet cells intimately combined with substances from the lamina propria (immunoglobulins IgA,IgG, and IgM; lymphoid cells) and the bloodstream (albumin, transferrin, etc.). Colonic mucus expressed as dry weight contains 72% proteins, 12.4% nucleic acid from shedding and bacterial cells, and only 6% glycoprotein. The colonic mucus glycoproteins purified by equilibrium density gradient centrifugation in CsCl and gel filtration (Sepharose 4B) contains approximately 30% hexose; 10% sialic acid; 3% sulfate; and 13% protein with a high threonine, serine, and proline content.[21]

One of the most important physical properties of mucus is its gel-forming ability due to its high hydrophilia. Changes in the protein and carbohydrate content result in modifications of the macromolecular aggregates and, consequently, of the viscoelastic and gel-forming properties of mucus.

In a study of mucus secretion in the rat colon, performed in our laboratory Fig. 1 with secretory stimulant, (organic anion at pH 2.9) the insoluble phase was fractionated on a Sepharose Cl-4B column. Two peaks were obtained: peak A in the excluded volume, corresponding to high-molecular-weight glycoprotein known to be GCM; and peak B in the included volume.

The following studies were designed to identify the components of the peak B. Using a specific antiserum (antipeak B), three precipitin bands were observed by immunoelectrophoresis (Fig. 7). In order to identify those arcs of precipitation, differ-

ent antisera were used. One of the arcs was found to be albumin (Fig. 8), while the other two bands remained unidentified. In view of their electrophoretic characteristics, however, it may be assumed that one is IgG and the other is IgA or transferrin. In a similar study of the soluble phase of the perfusate, the presence of albumin, IgG, and a third still unidentified band was confirmed (unpublished observations).

Forstner et al [22] has recently stated that the albumin secreted might enhance mucus viscosity as a result of albumin–mucose complexes being formed. In addition, Ca^{++} reduces mucus viscosity and solubility.

Biosynthesis of Mucus

Mucus, which forms a protective barrier throughout the digestive tract,[23] is made up of glycoproteins synthesized and stored in the goblet cells and subsequently secreted into the lumen of the gastrointestinal tract. The processes of synthesis and secretion seem closely related.

Mucus synthesis in the goblet cells has been studied mainly in rodent species, using radioautography and electron microscopy.[24] By using precursor sugars and amino acids labeled with 3H, the pattern of mucus biosynthesis has been found to follow the same general model as that for secreted glycoproteins in other organs.[25,26]

The peptide core of the mucus is assembled on the ribosomes of the rough endoplasmatic reticulum, travels through the smooth reticulum, and is glycosylated mainly in the Golgi apparatus. In a pattern similar to that for all the glycoproteins studied, glycosylation proceeds via sequential enzymatic addition of sugars from nucleotide-sugar donors to specific acceptors coupled to the peptide core. The sugars are linked via O-glycosidic bonds between serine or threonine and *N*-acetylgalactosamine, *N*-acetylglucosamine, or galactose. Neither uronic acid nor mannose were present. Sulfation, the last stage in the biosynthesis of mucus, occurs in the Golgi apparatus.

The most abundant amino acids found in the mucus of the gastrointestinal tract are threonine, proline, serine, asparagine, glycine, alanine, and valine. The sugar chains of these molecules (with approximately 600 carbohydrate side chains linked to the protein backbone) vary from 2 to 22 sugar residues in length and in some cases are

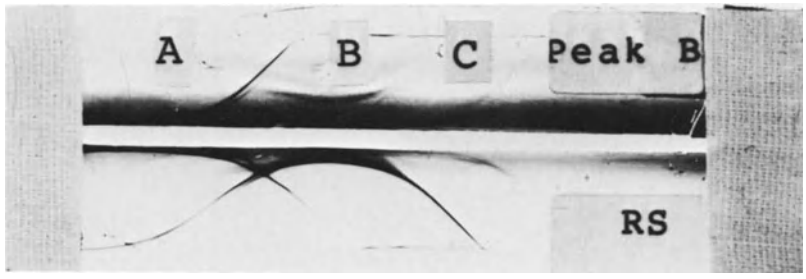

Figure 7. Immunoelectrophoresis of peak B, obtained by gel filtration chromatography of colonic mucus on Sepharose Cl-4B with specific antisera. Three precipitin bands were observed: A, albumin; B IgA or transferrin; C, IgG. Superior hole: peak B; inferior hole: rat serum.

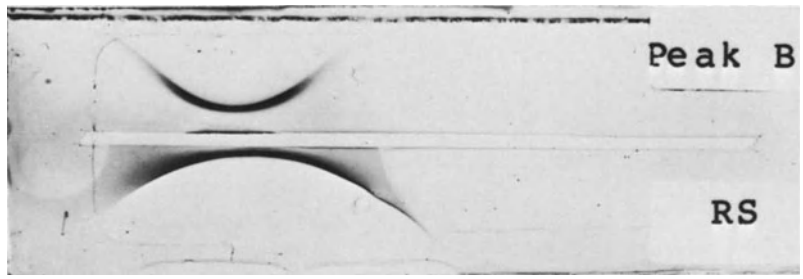

Figure 8. Immunoelectrophoresis of peak B with antialbumin shows the presence of albumin. Superior hole: peak B; inferior hole: rat serum.

branched. Another feature of these glycoproteins is their polydispersity in relation to molecular weight, sugar composition, chain length, and sequence. The above findings reflect the absence of close genetic control in the glycosylation process, which explains why the mucus is not homogeneous, even when isolated from a given tissue or organ. These heterogeneous macromolecules have a molecular weight of about 1.5×10^6, but they are able to form large "aggregates" of very high molecular weight (up to 15×10^6).

Various models for the mucus tertiary structure have been proposed; among them, mention may be made of the "windmill" model of Allen et al[27] suggested for the porcine gastric mucin. In this model, the basic unit is formed by four different subunits not necessarily identical in sugar residues (about 27,000 mol. wt.) held together centrally by disulfide bonds to the "naked" area (nonglycosylated area of the protein core). Several of the above windmill structures polymerize to form aggregates of 2×10^6 mol. wt. Treatment with proteolytic agents is able to produce subunits of about 5×10^5 mol. wt.

Another interesting model is that proposed by Forstner et al [28] for the rat soluble goblet cell mucin, consisting of a "flexible thread" configuration stabilized by noncovalent and disulfide bonds. This molecule has a molecular weight of about 2×10^6, contains 34 disulfide bonds per molecule, and is not reduced to subunits by treatment with sulfydryl agents.

VIII. MECHANISMS OF THE SECRETORY RESPONSE

Secretory proteins are generally secreted by a process of exocytosis, during which the membrane of the secretory granule fuses with the plasmatic membrane. Using electron microscopy, Kramer et al[29] found that the fusion could be divided into several stages:

1. Contact between the secretory granule and plasma membrane forming intramembrane particles.
2. Disappearance of the particles.
3. Fusion of the two membranes into five layers.

4. Transformation of the five layers into three.
5. Conversion of the layers into a diaphragm.
6. Disruption of the diaphragm, allowing the secretory product to be discharged into the lumen.

These researchers also state that the membrane of the granule needs to reach certain maturity before being able to fuse with the plasma membrane.

Although Kramer and Poort[30] have shown that the fusion is possible even in the case of unstimulated cells, the frequency of the fusion between the secretory granule and the plasma membrane increases under the influence of secretory stimuli (hormones, neurotransmitters, or drugs). These investigators from the University of Utrecht suggest that some of the mechanisms possibly implicated in the secretory response are (1) activation of purinylcyclases in the plasma membrane; (2) an increase in cAMP and cyclic GMP; and (3) increased activity of the microtubules and microfilaments, allowing the transport of granules toward the membrane.[30]

The suggestion has been put forward that the fusion is facilitated by the release of calcium or the translocation of the membrane proteins.

It may also noted that when mucus is discharged, various organelles, such as mitochondria, ribosomes, and ground substances from the cytoplasm, are released into the lumen together with the mucus. Both small-bowel and colon epithelia have been shown to be capable of eliciting a secretory response when cAMP is added to the environment or when adenylate cyclase[31] is stimulated by certain agents, such as the cholera toxin, prostaglandins, VIP, bile acids, and acetylcholine. LaMont and Ventola[17] demonstrated that the presence of cAMP dibutyril enhances the synthesis of colonic glycoproteins. MacDermott and co-workers[32] found that acetylcholine induced an increase in glycoprotein release but not in glycoprotein synthesis. Lewis[33] drew attention to the fact that a relation existed between mucus and its globular membrane as a determinant factor of its viscosity. He advanced the suggestion that because of its insolubility the globular membrane influenced the basic pattern for the formation of the mucin network through its union with the glycoproteins. Accordingly, the relative quantity of the globular membrane, as compared with the glycoprotein, determines the network cross-linking. On the basis of the above, the smaller the globules, the greater the amount of membrane and, hence, the viscosity of the mucus. Elstein[34] has speculated about the possible role for soluble serum proteins as agents of mucus cross-linking.

IX. NEUROENDOCRINE AND CHEMICAL MEDIATORS IN CONTROL OF COLONIC MUCUS SECRETION

A. Cholinergic Influence

In an experimental study of the rat colon, we found that methacholine injected i.v. (0.1 μg/kg body weight) induced colonic mucus secretion. Simultaneously, a higher (^{35}S) uptake (as sulfomucin marker) by the colonic mucosa was observed. On the basis of those findings, it may be assumed that there is a greater synthesis of sulfomucin due to the influence of methacholine. Our results with atropine (1 mg/kg) studied in the rat

colon and under organic anion stimulation (pH 2.9) showed a marked decrease in the mucus secretory response. These results seem to suggest that in the colonic secretory response, mucus is stimulated by cholinergic innervation,[32] which is blocked by atropine.

B. Adrenergic Influence

The lower ^{35}S uptake detected by us with noradrenaline (0.3 μg/kg) indicates that a decrease in mucus secretion occurs when the drug is intravenously injected with simultaneous intraluminal stimulation. This finding might be attributed to the inhibitory action of the β-adrenergics on colonic cholinergic innervation in the gut wall ganglia.[35]

C. VIP Influence

It seems to have been proved that VIP plays a role in small-bowel secretion. Recently, evidence has been found of its effects on colonic secretion.

Waldman and co-workers[36] carried out a comparative study of the action of the hormone VIP, secretin, GIP, and glucagon on colonic transport and activation of adenylate cyclase in everted sacs of rat colon. These researchers concluded that the enzyme is activated only by VIP and secretin. Since the activity is not additive, VIP and secretin are likely to share the same receptors. In view of these findings it may be hypothesized that these two hormones promote mucus secretion through their action on adenylate cyclase.

Since the publication of the studies by Florey[20] and, later, those by Hultén,[37] the pelvic nerve has been found to induce mucus secretion and an enhancement of vascularization and of propulsive motor contraction of the colon. Recently, Fahrenkrug and co-researchers[38] have demonstrated that simultaneously with electrical stimulation of the pelvic nerve there is an increase in plasma VIP. It may be assumed that the pelvic nerve, through its peptidergic terminals (VIP) enhances vascularization of the colon and, hence, the amount of nutrients reaching the secretory cells; the nerve might ensure the release of mucus into the lumen through its cholinergic terminals.

D. Prostaglandin Influence

Since indomethacin was known to inhibit prostaglandin synthesis, the following investigation was undertaken in our laboratory. We studied the secretory response and the histological characteristics of the colonic mucosa. We studied rats under organic anion (pH 2.9) stimulation and also in similar experimental conditions with the addition of indomethacin injected i.v. (10 mg/kg body weight). Indomethacin caused a decrease in the secretion of mucus, water, and chloride. The mitotic index was higher in the tissue of the animals treated with indomethacin alone. The relationship that exists between the kinetics of cellular reproduction and mucus secretion may be inferred from the above.

X. FUNCTION OF THE MUCUS

In the foreword of *Mucus in Health and Disease* (1977)[39], Avery Jones states that "mucus is one of Nature's perfections in protection, but unhappily even Nature has not anticipated all the noxious influences of Western civilization, which can reduce its protective powers." It is generally accepted nowadays that in the bowel and, particularly, in the colon, mucus acts as a barrier to prevent contact with potential aggressors present in the lumen.

Of the two layers of the colonic epithelium apparently (designed to control the lumen to blood entry of substances) one is a lipid layer corresponding to the epithelial cell membrane and the other, known as the "unstirred water layer," is identical with hydrated goblet cell mucin. Nimmerfall et al[40] showed a direct relation between absorption and diffusion of substances. Retention depends on the following parameters: molecular weight, pH, ionic strength, and hydrogen bond.

Apart from that protective role, studies performed in our laboratory seem to suggest that the high hydrophilia of mucus secretion contributes largely to fecal weight. We found that the weight of stools in rats fed on a fiber-rich diet was 8.85 times that of rats on a fiber-free diet. Concurrently, a significantly similar difference was observed in total hexosamine excretion, as indicative of mucus secretion in the stools. The hexosamine content was 8.5 times higher for the high-fiber diet than for the low-fiber diet.

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5

Immunology of the Colon

D. P. Jewell and W. S. Selby

I. INTRODUCTION

The colon provides an enormous reservoir of antigenic material—bacterial antigens, dietary proteins and polypeptides, and self-antigens from shed epithelial cells. The majority of immunological studies, however, have concentrated on the small intestine as this is thought to be the major site of immune responsiveness to exogenous antigens. Nevertheless, there is much evidence to suggest that the colon is able to mount local immune responses to the normal gut flora. The following examples illustrate this point. Normal subjects are able to synthesize secretory IgA antibodies to a wide variety of commensal intestinal bacteria.[1,2] In children with double-barreled colostomies, the introduction of polio virus into the isolated distal colon stimulates local specific IgA antibody production in addition to a systemic IgM and IgG response.[3] Patients with shigella dysentery were shown by Davies[4] to produce antibody to the infecting organism in their stool before an antibody response in serum could be detected. Finally, the colonic immune system may play an important role in the inhibition of pathogenic organisms in certain carrier states.[5] The ability of the colon to respond to its contents is also shown by the fact that the colon of a fetal animal is virtually devoid of immune cells. The full complement of lymphoid and plasma cells only develops after birth in response to the colonization of the lumen by bacteria.

This evidence suggests a role for the colon in humoral immune responses to commensal and pathogenic organisms. There is no direct evidence, however, for the role of cell-mediated immune responses in the colon. Nevertheless, the lamina propria contains macrophages and T lymphocytes. The presence of killer cells and natural killer cells is more controversial.

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The immunology of the colon will be discussed by considering the nature of the immune cell population, the origin of these cells, and their possible functions. The way in which differing types of immunological reaction may affect the colon will also be discussed.

II. THE NATURE OF THE IMMUNE-CELL POPULATION

A. Immunoglobulin-Containing Cells

1. Number and Distribution

In 1965, Tomasi et al[6] described the presence of 11 S secretory IgA in human saliva and colostrum and demonstrated the presence of IgA-producing plasma cells in the normal salivary gland. They postulated the existence of a specific mucosal defense system. In the same year, Crabbe et al,[7] using immunofluorescence with antisera against the immunoglobulin subclasses, found that the majority of plasma cells in the human duodenum and jejunum contained IgA. This work was extended throughout the entire gastrointestinal tract, including the colon and rectum, with similar results.[8] IgM- and IgG-containing cells were also found, but in much smaller numbers, and only occasional IgD-containing cells were seen. These studies initiated extensive research into the nature of the immune-cell population of the intestine. Table 1 summarizes the results and compares the distribution of cells in the large and small intestine. The figures represent the percentage of the cells containing the major immunoglobulin classes. In the normal intestine, it is clear that IgA-containing cells predominate (80–90%) and that this is true for the small bowel, colon, and rectum (Fig. 1). The proportion of IgM-containing cells tends to be higher in the small intestine than in the colon or rectum. The percentage of IgG-containing cells is lower than IgA or IgM cells, and the

Table 1. The Proportion of Immunoglobulin-Containing Cells in the Small and Large Intestine

	IgA (%)	IgM (%)	IgG (%)	Reference
Small intestine	83.8	12.4	3.8	8
	66.5	22.9	10.6	9
	58.9	23.4	17.1	10
Proximal	80.8	16.5	2.6	
Ileum	83.1	11.4	5.4	11
	66.1	18.0	5.9	12
Colon	91.5	4.5	4.0	8
	90.8	5.6	3.6	11
	91.1	3.5	4.6	13
Rectum	85.2	9.3	5.5	8
	90.3	6.7	3.0	14
	65.7	17.5	16.8	15
	92.6	2.9	4.5	16
	89.3	5.3	5.5	11

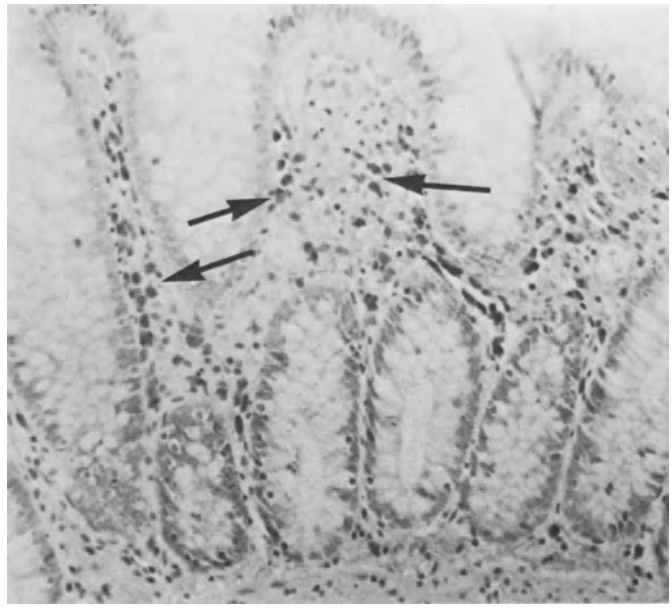

Figure 1. A section of normal rectum showing the distribution of IgA-containing plasma cells (arrows). They are concentrated in the luminal portion of the lamina propria. (Immunoperoxidase technique, × 250)

data of Brandtzaeg and Baklien[11] suggest that IgG cells are more numerous in the ileum and colon than in the jejunum.

There are many difficulties in determining the absolute numbers of plasma cells in the lamina propria because of the problems of tissue shrinkage during fixation, the thickness of the section, orientation, and magnification.[133] Nevertheless, Crabbe and Heremans[8] found mean numbers of IgA-containing cells to be similar in small bowel and colon but with lower values in the rectum (Table 2). IgM cells were less frequent in the colon and rectum than in the small intestine. IgG cells were less frequent in all sites.

In disease states, there is a considerable rise in the number of plasma cells in the colonic lamina propria, and the proportion of cells secreting individual immunoglobulins varies. Table 3 gives the percentages of immunoglobulin-containing cells in the colon in patients suffering from ulcerative colitis or Crohn's disease. IgA cells are still predominant, but there is a relatively greater rise in the proportion of IgM and IgG cells. In patients with IgA deficiency, the majority of plasma cells along the whole of the gastrointestinal tract, including the colon, are IgM cells, with very few IgA-containing cells.[8,11,17]

The distribution of the immunoglobulin-containing cells within the colonic mucosa is not uniform. Approximately 60% of the IgA- and IgM-containing cells are found within 200 μm of the surface epithelium with decreasing numbers toward the muscularis mucosae (Fig. 1). The number of IgG cells tends to be slightly higher in the basal than in the luminal zone.[11]

Table 2. Absolute Numbers of Immunoglobulin-Containing Cells in the Lamina Propria of the Intestine

	IgA[a]	IgM[a]	IgG[a]
Small intestine	352,000	52,000	16,000
Colon	346,000	17,000	15,000
Rectum	156,000	17,000	10,000

[a]Values represent mean counts expressed as cells per cubic millimeter. From Crabbe and Heremans, 1966.[8]

Immunoglobulins can also be detected extracellularly in the lamina propria of the gut using immunofluorescent techniques. The major immunoglobulin class in this site is IgG. This is thought to be the result of diffusion of IgG from the serum, where it is the major immunoglobulin class, rather than the result of local synthesis.[11] IgA is also found in the lamina propria, but fluorescence is often faint despite the large proportion of IgA-producing cells present. The existence of an epithelial transport system for IgA, leading to the rapid removal of locally formed immunoglobulin, is thought to account for this phenomenon. IgA can also be detected on the surface epithelium and the epithelium of the colonic mucosal glands. In this situation it is found within the epithelial cell cytoplasm, on the epithelial cell membrane, and intercellularly. A similar distribution is seen in the small bowel, although the fluorescence is less intense.[18] IgM is found extracellularly in the lamina propria, but the amount varies considerably. Its distribution in the epithelium, both intra- and extracellularly, is similar to that of IgA.[11]

Few IgD-containing cells have been detected in either normal or diseased colonic mucosa.[8,11,19] The one exception is the study of Søltoft et al,[15] who found that 6.5% of immunoglobulin-containing cells were of the IgD class. IgE cells are found in very low proportions in colonic mucosa. The exact proportions vary in different studies, often being negligible,[11,15,16] but found by some to comprise up to 2% of colonic immunocytes.[19–21] Tada and Ishizaka[20] are the only workers to have found the proportion of IgE-containing cells to be greater than that of IgG-containing cells in the human large bowel. The variability in these results may be due to the presence of heterophile antibodies or due to lack of specificity of antisera. Neither IgD nor IgE can be detected in the extracellular spaces of the lamina propria.[11] The reported variation in the proportions of IgE-containing cells is even greater in the diseased colon. No increase of IgE-containing cells has been found by Skinner and Whitehead[16] or

Table 3. The Proportion of Immunoglobulin-Containing Cells in the Colon in Patients with Ulcerative Colitis or Crohn's Disease

	IgA (%)	IgM (%)	IgG (%)	Reference
Ulcerative colitis	83.4	13.3	3.3	14
	52.8	11.8	35.4	15
	79.2	7.5	13.3	16
	52.1	4.4	43.3	11
	50.7	45.2	4.1	13
Crohn's disease	87.0	4.5	8.5	16
	62.3	10.4	27.2	11

Baklien and Brandtzaeg[22] in ulcerative colitis and Crohn's disease, but increased numbers have been reported by others.[21,23] The presence of IgD-containing cells in the lamina propria of the diseased colon has been reported in one case.[8] No information is available concerning IgE-producing cells in the mucosa of patients with helminthic, protozoan, or parasitic infestations of the colon.

2. Mechanisms of Immunoglobulin Secretion

The major immunoglobulin class in most human secretions is IgA.[6,24] The precise concentrations of each immunoglobulin class, however, are difficult to measure because of the presence of proteolytic enzymes and the tendency of IgA to polymerize. Table 4 indicates that, in intestinal secretions, in contrast with serum, IgA forms a much greater proportion of the total immunoglobulin. It is also apparent that colonic juice contains more IgA and IgG than jejunal juice, but much of this may be derived from the ileum, and for the reasons outlined above the jejunal juice concentrations may not be accurate. In contrast to the 7 S monomeric IgA of serum, the IgA found in intestinal fluid is predominantly dimeric 11 S secretory IgA. Dimeric IgA consists of two monomers linked by an additional polypeptide of about 15,000 mol. wt. This is the J piece, which is synthesized within the plasma cell[11,25] and appears to be necessary for IgA to combine with the secretory component (S.C.). This latter glycoprotein with a 60,000 mol. wt. is synthesized within the endoplasmic reticulum of the epithelial cell and is transported to the Golgi complex, where it is found in the free, unbound form.[26–28] Studies with isolated epithelial cells suggest that the primary function of the S.C. is to act as a receptor on the cell surface for the dimeric J-chain-containing IgA. Once S.C.–IgA complexes are formed they are taken into the epithelial cells by pinocytosis and pass through the cytoplasm, to be finally discharged into the lumen.[28,29] Secretory IgA does not cross the intercellular tight junctions. The presence of S.C. within the IgA dimer appears to render the immunoglobulin molecule relatively resistant to proteolysis.[30]

IgM is also secreted by plasma cells as a J-chain-containing polymer.[31] Its transport pathway, by binding to S.C., is similar to that of IgA.[28,32] In intestinal secretions, 70% of the IgM is in the secretory form.[33]

The source of IgG that enters the gut lumen is thought to be serum. The immunoglobulin is probably transported by passive diffusion through intercellular spaces, and this process does not require secretory component.[28] IgE is likewise not a secretory immunoglobulin and probably follows a similar route to that of IgG.[11,28] It is synthesized locally by mucosal plasma cells.

Table 4. Immunoglobulin Concentrations in Intestinal Secretions (mg/100 ml)

	IgM	IgA	IgG	IgG/IgA
Serum	1 32	328	1230	3.8
Saliva	0.55	30.4	4.9	0.2
Jejunum	—	27.6	34.0	1.2
Colon	—	82.7	86.0	1.0

B. T Lymphocytes

Much less information is available concerning the numbers and distribution of T lymphocytes in the gastrointestinal mucosa compared with immunoglobulin-producing cells. Studies both in animals and in man have demonstrated the presence of T cells within the small- and large-intestinal mucosa. T cells are found in two locations: (1) within the epithelial cell layer, lying between the epithelial cells (intraepithelial lymphocytes, interepithelial lymphocytes); and (2) in the lamina propria.

1. Intraepithelial Lymphocytes

Lying between the epithelial cells of the gastrointestinal tract of man and animals there are many mononuclear, nonepithelial cells, mainly lymphocytes. They were first described in the small intestine, but they have since been identified in colonic epithelium by light microscopy[34] and electron microscopy.[35] The majority lie immediately above the basement membrane, but they do not form specialized attachments to either the epithelial cells or the basement membrane. They are larger than peripheral blood lymphocytes, although there is variation in size. A characteristic feature is the presence of metachromatic granules in many of these intraepithelial cells.[36–38]

Initial evidence that intraepithelial lymphocytes were T cells, both in small bowel and colon, was mainly indirect, based on T-cell depletion following thymectomy in animals. This resulted in a considerable reduction in the number of intraepithelial lymphocytes.[38–40] In a series of experiments in which T and B blasts were injected into syngeneic mice and rats, Guy-Grand et al[41] demonstrated that lymphoblasts from mesenteric lymph nodes or from thoracic duct lymph migrated preferentially to the small and large intestine. Only T blasts, however, and not B blasts, migrated to the epithelium. The same authors found that immunofluorescence of the intraepithelial cells in mouse intestine, using an antiserum to murine T lymphocytes, provided direct evidence that they were T cells.[42] Finally, intraepithelial cells have been isolated from the small bowel of mice and guinea pigs, and their T cell nature has been confirmed.[42,43] Colonic intraepithelial cells have not yet been reilably isolated, perhaps due to the presence of excessive mucus.

The surface markers on intraepithelial cells within the human gut have only recently been studied. Strickland et al,[44] using immunofluorescence, examined the cellular infiltrate in colonic biopsies of one patient with Crohn's disease and two healthy control subjects. They found small numbers of both T and B cells in the epithelial layer. Only a limited number of sections were examined, so that the proportions of each cell type could not be determined. In a larger study, Meuwissen et al[45] found that the majority of intraepithelial lymphocytes in ileum and colon were T lymphocytes in control subjects and in patients with ulcerative colitis or Crohn's disease. Neither study was primarily directed toward the intraepithelial lymphocyte population, and the findings received only brief mention. The nature of the intraepithelial lymphocytes along the normal human gastrointestinal tract has subsequently been studied in more detail using an immunofluorescent technique on tissue sections.[46] Between 82% and 98% of intraepithelial lymphocytes in the colon and rectum were T cells (Fig. 2) as defined by an antiserum to human T lymphocytes (Table 5). The proportion was slightly higher in the small intestine (89–100%). Using similar methods, it was found that 72% to 87% of

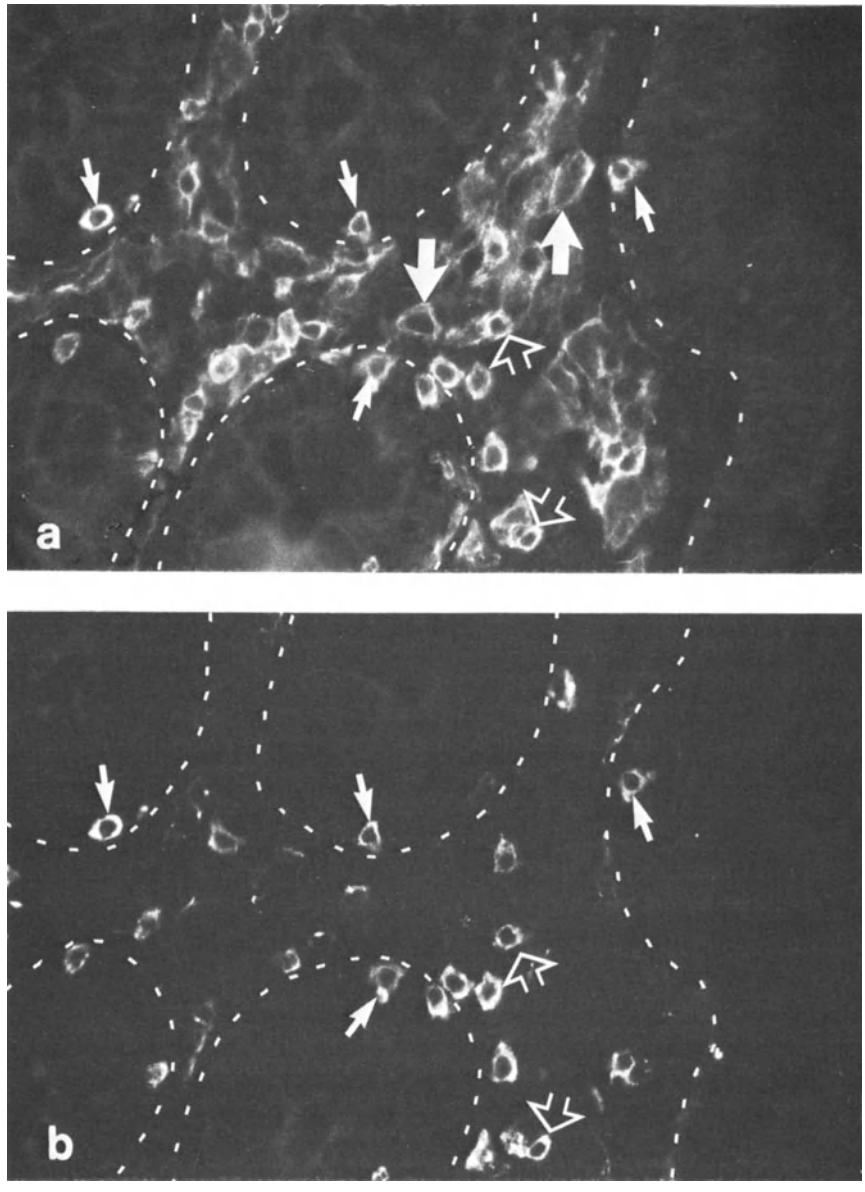

Figure 2. A biopsy of normal ascending colon labeled by indirect immunofluorescence, using different fluorochromes, with (a) HLe-1, monoclonal antibody reacting with all lymphocytes, and a proportions of tissue macrophages; and (b) antiserum to human T lymphocytes. Intraepithelial lymphocytes are present both in the surface and the glandular epithelium (small arrows). Within the lamina propria, T lymphocytes (open arrows) and macrophages (closed arrows) can be seen. The basement membrane is indicated by the broken line.[46] From Selby et al.[46] (×400) Reproduced with permission of editor of *Gut*.

Table 5. Immunological Characterization of Normal Human Intraepithelial Lymphocytes

Tissue	Ratio of T to total intraepithelial lymphocytes (%)[a]	Intraepithelial lymphocytes: ratio of OKT8^{+}[b]T cells to total T lymphocytes (%)[a]	Lamina propria lymphocytes: ratio of OKT8^{+}[b]T cells to total T lymphocytes (%)[a]
Small intestine	97 (89–100)	77 (67–90)	36 (27–47)
Colon	92 (82–98)	85 (84–87)	48 (39–56)
Rectum	95 (85–98)	79 (72–86)	35 (30–39)

[a]Results expressed as mean with range in parentheses. From Selby et al., 1981.[46,47]
[b]a mouse monoclonal antibody specific for the suppressor-cytotoxic T-cell subset (see text).

colonic or rectal intraepithelial T lymphocytes reacted with a monoclonal antibody, OKT8 (Fig. 3), which is specific for the suppressor-cytotoxic subset of T cells as defined by *in vitro* testing of peripheral blood lymphocytes.[47] Similar proportions were found in the small intestine. The remaining intraepithelial T lymphocytes had the phenotype of helper T cells (OKT4^{+}) (Selby et al, in preparation). Intraepithelial T cells rarely expressed Ia-like, p28,33 antigens (thought to be governed by the *HLA-DR* locus of the major histocompatibility complex) (Fig. 4).[46] The presence of these antigens on the surface of T cells from peripheral blood has been associated with activation *in vitro*.

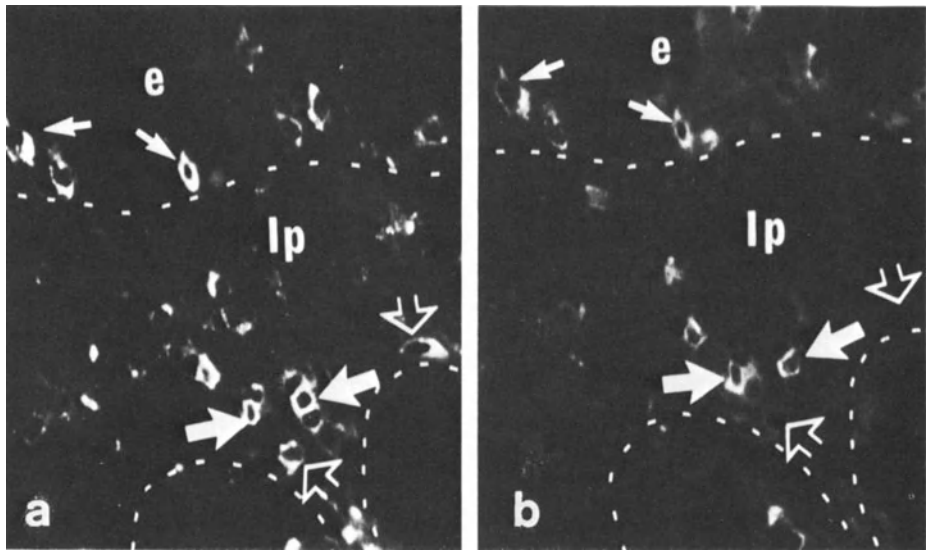

Figure 3. A section of normal human colon labeled by indirect immunofluorescence with (a) antibody to human lymphocytes and (b) OKT8, a monoclonal antibody specific for human T cells of suppressor-cytotoxic phenotype. Within the epithelium (e) intraepithelial T cells are found, as indicated by the small arrows. These are mostly OKT8-positive, suppressor-cytotoxic cells. Within the lamina propria (lp) 2 OKT8-positive T cells (closed arrows) and 2 OKT8-negative T cells (open arrows) are demonstrated. The basement membrane is shown by the broken line. From Selby et al.[47] (×400) Reproduced with permission of editor of *Clin Exp Immunol*.

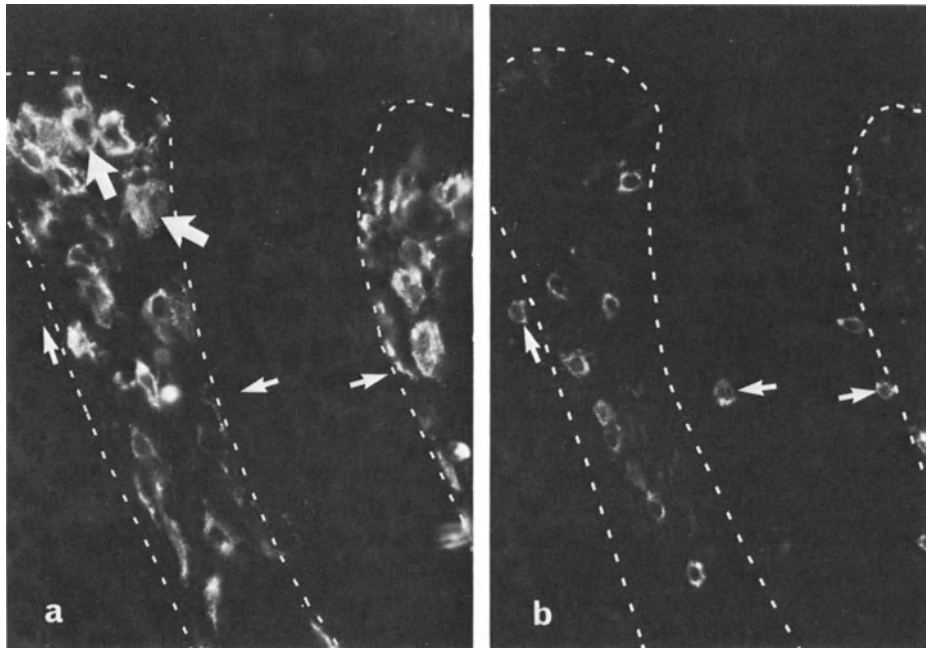

Figure 4. A normal rectal biopsy labeled for (a) Ia-like antigens and (b) T lymphocytes. The lamina propria contains numerous Ia-rich macrophages (large arrows), concentrated toward the intestinal lumen. Above the basement membrane (broken line) lie the intraepithelial T cells (small arrows). These are Ia-negative. From Selby et al.[46] (×400) Reproduced with permission of editor of *Gut*.

Whether the absence of these antigens on intraepithelial lymphocytes reflects a lack of activation remains to be determined. Neither surface nor cytoplasmic immunoglobulin was detected on colonic intraepithelial lymphocytes.[46]

The functions of intraepithelial lymphocytes remain unknown but must be assessed in the light of the findings discussed above. Lying in the epithelial layer, they may be the first immune cells to come into contact with luminal antigen. Antigen may then be taken up by the cell or carried on its surface. Intraepithelial lymphocytes are capable of crossing the basement membrane to enter the lamina propria,[48,49] and they may then modulate the immune response to the antigen by interacting with the macrophage and lymphocyte populations. The demonstration that processes from intraepithelial lymphocytes cross the basement membrane to lie in contact with macrophages[48] and that macrophage processes project into the epithelium[36] supports the possibility of lymphocyte–macrophage interactions. Alternatively, intraepithelial T lymphocytes may have a predominantly cytotoxic function. Study of isolated guinea pig intraepithelial lymphocytes demonstrated their capacity to generate spontaneous and antibody-dependent cytotoxicity.[43] The possession of this property by intraepithelial lymphocytes in man could confer upon them a role in the control of viral infection, tumor development, or autoimmunity. The contribution of this cell population to the pathogenesis of gastrointestinal disease remains unknown.

2. Lamina Propria T Lymphocytes

T lymphocytes are found also in the lamina propria of the colon. Evidence for this is threefold. First of all, the homing of thoracic duct T blasts to the lamina propria has been demonstrated.[41,50] Secondly, the immunohistochemical studies of Strickland et al[44] and Meuwissen et al[45] demonstrated the presence of T cells throughout the lamina propria of the normal colon. T lymphocytes in fact constituted the majority of lymphocytes in this site, and they were found both in a nonaggregated distribution and in lymphoid aggregates, where they formed a corona around non-T lymphocytes. These findings have recently been confirmed. Employing immunohistochemical techniques, Selby et al[47] have demonstrated the presence of T lymphocytes in the lamina propria of normal colon, and found that only 30–56% of lamina propria T cells are of suppressor-cytotoxic phenotype as defined above. The majority of lamina propria T cells are of helper phenotype. This is in contrast to the intraepithelial T lymphocytes (Table 5). In Crohn's disease and ulcerative colitis the proportion of $OKT4^+$ T cells in the lamina propria does not differ from normal, although absolute T-cell numbers appear to be increased (WS Selby et al, in preparation). Finally, cell isolation studies have shown that about 50% of isolated lamina propria lymphocytes are able to form E rosettes (Table 6).

Cell isolation techniques have provided a new tool for the investigation of intestinal cell populations. Two methods are used—mechanical and enzymatic. The mechanical method involves passing homogenates of colonic mucosa through a series of graded steel meshes and finally over siliconized glass beads. The enzymatic procedure employs dithiothreitol to disrupt mucus, EDTA to remove the epithelial cells, and collagenase to release cells from the connective tissue. Studies comparing the yields of the two methods favor the enzymatic method.[53,54] Collagenase does not appear to stimulate lymphocytes or to impair their response to nonspecific mitogens.[54,57] Table 6 lists the findings of the reported studies isolating colonic mucosal cells and demonstrates that the major cell population in the colonic lamina propria consists of lymphocytes, both large and small. The proportion of T cells, as determined by sheep red cell rosettes, varies between individual studies but is usually greater than that of B cells. Non-T, non-B lymphocytes (null cells) were not found by Bull and Bookman[51] or

Table 6. Cell Populations in Normal Colonic Mucosa as Determined by Isolation Techniques

Method	Total lymphocytes[a]	T lymphocytes (E rosettes)	Ig-positive cells	Non-T non-B (null) lymphocytes	Macrophages	Reference
Enzymatic	90[a]	58[a]	37[a]	0[a]	10	51
Enzymatic	81[a]	64[b]	36[b]	0[b]	8	52
Enzymatic	68–82[a]	50[b]	ND	ND	5–11	53,134
Enzymatic	86[a]	44[b]	ND	ND	ND	54,135
Enzymatic	ND	55[b]	23[b]	22[b]	ND	55
Enzymatic	ND	43[b]	ND[a]	ND	ND	136
Mechanical	89[a]	34–54[a]	22	ND	6	56,137

[a]Expressed as a mean percentage of the total isolated cell population.
[b]Expressed as a mean percentage of the isolated lymphocytes.
ND, No data.

Bookman and Bull[52] but were detected by Chiba et al[55]. This discrepancy may be due to technical differences, and further studies using other techniques for identifying T cells, such as the use of anti-T-cell antisera, may be necessary to resolve the issue. In ulcerative colitis and Crohn's disease, the absolute numbers of lymphocytes increases, but there is a decrease in the proportion of T cells with a corresponding rise in the proportion of B cells.[52]

Several functions of mucosal T lymphocytes have been studied. They respond to nonspecific mitogens, and are capable of mitogen-induced cellular cytotoxicity.[57,134,136,138] They can act as responding cells in mixed lymphocyte culture, but are incapable of effecting cytotoxicity of the stimulating cells.[139] In the peripheral blood this latter function is a property of the $OKT8^+$ T cell populations, and suggests that although cells of this type are present within the colonic lamina propria, their functions may be different from the corresponding cells in the circulation.[140] Mucosal T cells are also capable of suppressor activity.[54,137] Alterations in several of these functions have been described in patients with colonic carcinoma, ulcerative colitis or Crohn's disease.[134–137]

C. Killer Cells and Natural Killer Cells

Killer (K) cells are a subpopulation of lymphoid cells, bearing Fc receptors, which are able to lyse target cells coated with antibody (antibody-dependent cellular cytotoxicity, ADCC). Studies of patients with ulcerative colitis or Crohn's disease have demonstrated that peripheral blood lymphocytes are cytotoxic to colonic epithelial cells *in vitro*.[58–60] The immunological mechanisms have not been elucidated, but it has been suggested that K cells may be responsible even though antibody is not added to the system.[61] K cells have also been looked for in lymphocyte populations from the colonic mucosa. Most studies have been unable to demonstrate K-cell activity in this population isolated either by mechanical or by enzymatic means, using Chang cells or human lymphoblastoid cell lines as target cells.[52,54,62,134,138] In several of these studies the presence of null cells or Fc-receptor-bearing lymphocytes could not be demonstrated in the isolated cell population. In contrast, Chiba et al[55] initially reported K-cell activity using a similar system, but were unable to confirm this.[138] Bland et al[53,134] found that K-cell activity in mechanically isolated cells was inhibited by prostaglandin E_2 released during the isolation procedure, and also postulated the presence of a prostaglandin-producing suppressor cell within the mucosa, inhibiting K cell activity. The choice of target cell may be one factor which explains these differences, as MacDermott et al[57] and Chiba et al[138] have shown that isolated cells will mediate ADCC when chicken erythrocytes are used as target cells but not when Chang cells are used. Further studies using a variety of target cells, including colonic epithelial cells, are currently in progress.

Natural killing, or spontaneous cytotoxicity, involves the destruction of malignant or nonmalignant target cells by nonimmune effector lymphocytes in the absence of immunoglobulin. It is thought to play an important role in tumor immunity. The cells responsible for natural killing are heterogeneous. They are nonphagocytic, nonadherent and possess Fc receptors.[63,64] Natural-killer (NK) cells are found both among the T-cell population, where they have a low affinity for sheep red cells, and

among the null cell population.[65–67] There is increasing evidence that natural killing and ADCC are mediated by the same lymphocyte population.[68,69] Lamina propria lymphocytes isolated from normal, neoplastic or inflamed colon produce little natural killing against chicken erythrocytes, Chang cells or a lymphoblastoid cell line.[52,55,57,62,134,136] Further work is required to determine whether NK cells are absent from the intestinal mucosa or whether their function is suppressed.

D. Macrophages

The role of macrophages in the normal or diseased colon has received little attention. Macrophages are found throughout the connective tissue of the gastrointestinal tract[47,70] and are commonly found in rectal biopsies.[71] They can be identified readily by electron microscopy, and in the lamina propria they often lie in close proximity to plasma cells and eosinophils.[72] This close relationship may be important in the local immune response. In the colonic mucosa, macrophages are found mainly in the upper 100–150 μm of the mucosa.[35,47,70] They are very rich in Ia-like antigens[47] (Fig. 4). Recent evidence from combined immunofluorescence and histochemical studies on tissue sections has indicated that at least two distinct populations of HLA-DR-positive, macrophage-like cells are present within the human colonic mucosa.[141] It has been suggested that this heterogeneity may indicate different functions within the lamina propria for each of these cell types. Cell isolation studies have shown that macrophages constitute 5–15% of the population (Table VI) although obviously this does not necessarily represent the *in vivo* proportion. Bull and Bookman[51] have studied isolated intestinal macrophages and compared them with peripheral blood monocytes. In contrast to monocytes, some of the macrophages were large (14–25 μm), contained cytoplasmic granules, were more active in spreading, and had a greater phagocytic capacity. The number of macrophages in isolates from patients with ulcerative colitis and Crohn's disease is increased.[52]

E. Other Cells

Neutrophil and eosinophil polymorphs are found in the normal colonic mucosa by light and electron microscopy and are present in isolated cell populations even though the techniques deplete polymorphs. Increased numbers of neutrophils are a histological feature of ulcerative colitis, where they invade the glands to form crypt abscesses. A similar picture is seen in the colonic mucosa of patients with infective forms of colitis, e.g., amoebiasis, shigellosis, and *Campylobacter* enteritis. Eosinophils are found in the lamina propria and, occasionally, within the epithelium.[48] Numbers may be increased in ulcerative colitis.[73–75] Mast cells are also present in the lamina propria and between epithelial cells in the healthy colon.[35,76] Their numbers increase in response to parasitic infestations in rats[77] and in human ulcerative colitis.[78,79] In Crohn's disease, although information is available only for the ileum, where normal mast cell numbers have been found,[79,80] mast cell hyperplasia has been reported by one group.[81] A major difficulty arises in assessing mast cell numbers, however, in that degranulated cells are not detected by standard staining techniques, and large numbers of degranulated cells are a feature of Crohn's disease.[81]

III. THE ORIGIN OF THE IMMUNE-CELL POPULATION

Most studies examining the circulation of intestinal lymphocytes have involved the small intestine. It seems likely that the findings apply equally to the colon. In 1964, Gowans and Knight[82] showed that the large lymphocytes in thoracic duct lymph, when injected into syngeneic rats, went mainly to the gastrointestinal tract, in particular the lamina propria of the small bowel. This was not seen when small lymphocytes were used. Further studies, using lymphoblasts from mesenteric lymph nodes as well as from thoracic duct lymph, have confirmed that these cells migrate preferentially into the small bowel and the colon.[41,83–86] In contrast, cells from peripheral nodes return either to peripheral lymph nodes or to the spleen and do not migrate into the intestinal lamina propria. The migration of large lymphocytes to the gut involves both T and B blasts. B lymphoblasts migrate to the lamina propria, whereas T blasts move into the lamina propria and into the epithelial cell layer.[41]

The origin of the lymphoblasts that home to the intestine is thought to be the Peyer's patches. These are lymphoid aggregrates found along the intestinal tract, being maximal in number in the ileum.[87] They contain both T-cell and B-cell areas[85,88] and are covered by a specialized epithelium which allows passage of luminal antigens.[89] Craig and Cebra[90] have shown that Peyer's patches contain the precursors for the IgA-producing cells in the lamina propria. The B cells within the Peyer's patch are probably committed to IgA synthesis, since a high proportion of them have IgA on their surface although they lack cytoplasmic immunoglobulin.[41] These cells respond to ingested antigens and become sensitized.[91] They then leave the Peyer's patch and migrate in the lymphatics to the thoracic duct via the mesenteric lymph nodes. During this migration, the cells mature and develop intracellular IgA. They then enter the blood stream and home into the small and large intestine, where they differentiate into IgA-secreting plasma cells.[41,92] A similar pathway probably exists for T cells.[42]

The signal for the homing of lymphoblasts into the lamina propria is not known. Although both T and B cells in Peyer's patches proliferate in response to antigen,[91,93,94] migration still occurs in germ-free mice[83] and in antigen-free small and large-intestinal grafts.[95,96] Secretory component is unlikely to be the signal, since free secretory component is not found in the lamina propria[97] and secretory component does not show detectable affinity for the surfaces of circulating B cells.[29] Furthermore, homing cannot be inhibited by antiserum to secretory component.[86] It is possible that the homing property is partly conferred on the cells while still in the Peyer's patch or it may partly depend on mucosal stimuli.[42] Another possibility is that migration may be nonselective but that local factors, such as antigen, may result in selective proliferation of immunocytes.[11] Finally, antigen-induced differentiation of lymphoblasts may lead to nonmigratory forms.

The differential distribution of T cells of suppressor-cytotoxic phenotype between epithelium and lamina propria may be related to the pattern of Ia-like and HLA-ABC antigens found on the colonic mucosa.[47] Ia-like antigens are expressed strongly on the macrophages, but not on the epithelium. Inducer T cells recognize antigen in the context of Ia,[98] and it is therefore possible that they seek the Ia-rich lamina propria environment. HLA-ABC antigens are found both on epithelial and on lamina propria cells, including the macrophages. Since suppressor-cytotoxic T cells function in the context of HLA-ABC antigens,[98,99] this may explain their presence in the epithelial layer.

IV. SYNTHESIS OF IMMUNOGLOBULIN AND COMPLEMENT

A. Immunoglobulin Synthesis

Quantitative studies of immunoglobulin synthesis in human colon have been performed by several workers. Bull et al[100] demonstrated the synthesis of IgA by the isolated, perfused human colon. Lai A Fat et al,[101] using cultures of biopsies taken along the normal gastrointestinal tract, showed that IgA was the predominant immunoglobulin synthesized in both the small and the large bowel. Secretory component production increased along the gut, being maximal in the colon and rectum. IgG and IgM synthesis were not consistently detected in colonic biopsies, but IgG production appeared to be slightly greater than that of IgM. Oldham et al,[102] also using cultured rectal biopsies, confirmed these findings. Synthesis of IgA varied from 30 to 2675 ng/mg per day, that of IgG from 0–83 ng/mg per day, and of IgM from 0–18 ng/mg per day. In all tissues, the synthesis of IgA was greater than that of IgG, which was in turn greater than that of IgM.

The predominant IgA synthesis is consistent with the large proportion of IgA-containing cells seen in the colon by immunohistochemical studies. However, the same studies show that IgM cells are more frequent than IgG cells. It is possible, therefore, that IgG-producing cells may secrete immunoglobulin more actively than IgM cells or that counts of IgG-containing cells by immunofluorescence are falsely low due to extracellular IgG obscuring the IgG-containing cells.[11]

IgM production by rectal biopsies is increased in patients with selective IgA deficiency,[102] which correlates well with the increased numbers of IgM-containing cells seen in tissue sections from patients with this disorder.[8,11,17] Immunoglobulin production, particularly of IgG, is increased in biopsies from patients with ulcerative colitis or Crohn's disease.[103] This is reflected in the immunofluorescence and immunoperoxidase studies (Table 3).

Immunoglobulin synthesis by the colon has also been studied in isolated lamina propria cell populations. Mucosal cells appear to produce more immunoglobulin during a period in culture than do peripheral blood lymphocytes.[51,54] Bookman and Bull[52] subsequently showed that IgA production by isolated lamina propria cells from the normal colon was greater than that of IgG and this was confirmed by MacDermott et al.[142] In patients with ulcerative colitis, mucosal cells showed a considerable increase in IgG production, whereas production of IgA remained unchanged.[52] However, this was not found by MacDermott et al,[142] who demonstrated a reduction in the synthesis of all classes of immunoglobulin by the intestinal mucosal cells from patients with inflammatory bowel disease.

The relationship between immunoglobulin production and the colonic lymphocyte subpopulations, especially the role of the mucosal T cells, has not yet been examined. The ability to isolate mucosal lymphoid cells should make this study possible.

B. Complement Synthesis

The human colon is capable of synthesizing complement *in vitro*. Colten et al[104] showed the production of C1, and subsequently Lai A Fat[101] demonstrated the synthesis of C3 in cultured colonic biopsies and occasionally in rectal biopsies. Clq synthesis

occurred in colonic biopsies but not in rectal tissue. The techniques used, however, were relatively insensitive. The exact site of synthesis of these complement components is not known. Tissue macrophages are known to synthesize complement components,[105–107] and peripheral blood monocytes synthesize all the components of the alternative pathway, as well as C2, C4, C3, and C5, together with the modulators C3bINA and β1H.[108] It is a reasonable hypothesis therefore that the intestinal mucosal macrophages also synthesize these components and that they may contribute to the raised levels in the serum of patients with active ulcerative colitis or Crohn's disease.[109,110]

V. RESPONSE OF THE COLON TO IMMUNOLOGICAL REACTIONS

As described above, the colonic mucosa contains many of the cellular and humoral components necessary for the immune response. Moreover, since the mucosa is in constant contact with a heavy antigenic load, it is likely that immune reactions may occur within the colon. Many models have been reported to show that the colonic mucosa can be involved in each of the four types of immune reaction.[111]

A. Type I Hypersensitivity

Type I or reaginic hypersensitivity is mediated by IgE antibody bound to the surface of mast cells. Antigen binding to specific IgE antibody causes the release of histamine and other vasoactive amines from the mast cells with the subsequent development of inflammation. Whether this type of immune response has a role in the pathogenesis of human colonic disease is unclear. Nevertheless, the colon has been shown to react, like many other tissues, in type I reactions. Gray and Walzer[112] passively sensitized the skin and rectal mucosa of normal subjects by the injection of serum containing a high titre of reagins from a patient allergic to peanuts. One or 2 hr after sensitization the subjects were given a peanut meal, and within 15–25 min the sites of injection became hyperemic and edematous. The reaction at the rectal site preceded the skin reaction and was associated with increased mucus production. Subjectively, the patients complained of pruritus, rectal burning, and a desire to defecate. A similar response in the rectum was obtained when a peanut suspension was placed in the rectum. Further studies in patients with an exposed ileum or colon (ileocolostomy or colostomy), which was again passively sensitized by an intramucosal injection of reaginic serum, resulted in similar findings.[113] Local passive anaphylaxis has also been demonstrated in the small and large intestine of Rhesus monkeys using serum from patients allergic to a variety of antigens.[114]

B. Type II Hypersensitivity

This form of reaction involves circulating antibody directed toward antigens on cell surfaces. Many groups have induced anticolon antibodies in a variety of animals by the injection of colonic or bacterial antigens but have not always shown any histological change in the colon. Passive injection into dogs of rabbit serum containing antibodies to dog colon has been reported to cause a colitis,[115] but in some cases this

appears to have been part of a terminal event.[116] A colitis has also been described in mice by sensitizing them to mouse colon[117] and in dogs by passive sensitization,[118] but both of these models involved local rectal irritation that may have caused an Auer-type phenomenon by localizing the response to the colon. Perlmann et al[119] noted severe histological changes in the colonic mucosa while raising antibodies to colonic antigens, but similar changes were seen in some animals that had received only adjuvant. Other reports of colitis induced by anticolon antibodies have been less convincing, since contamination with salmonellae was present.[120] No histological changes, however, have been seen in rabbits with circulating anticolon antibodies.[121–123]

C. Type III Hypersensitivity

Arthus-type reactions are caused by the formation and deposition of antigen–antibody complexes. Goldgraber and Kirsner[124] injected egg albumin intramucosally into sensitized rabbits and produced a hemorrhagic, necrotizing response. The response developed after about 6 hr and began with edema, increased vascularity, and polymorph infiltration. Necrosis developed at 3 days, and at the end of the week granulomata were noted. The colon also responds to the deposition of antigen–antibody complexes as demonstrated by the Auer reaction. In this case, the rectum is mildly irritated (e.g., by formalin), which allows circulating complexes to be preferentially deposited within the mildy inflamed mucosa. Kirsner et al[125] showed that intravenous or intraperitoneal injections of egg albumin into sensitized rabbits, which had been given intrarectal formalin, caused superficial ulceration of the rectal mucosa with infiltration of polymorphs, plasma cells, and lymphocytes. This model has been extended by Hodgson et al,[126] who injected preformed immune complexes intravenously into rabbits pretreated with intrarectal formalin. An acute inflammatory reaction developed within 24 hr and was maximal at 5 days. Subsequently, a similar experiment[127] was performed in rabbits who had been immunized to the Kunin antigen (the common enterobacterial antigen). The rabbits again produced a mild acute colitis, but the lesion became chronic and was still present 6 months after the injection of the complexes.

These animal models show that antigen–antibody reactions occurring within the mucosa may cause an acute inflammation which histologically resembles human ulcerative colitis. However, in the case of the models based on the Auer phenomenon, it has not been demonstrated that the circulating complexes actually deposit in the colon.

D. Type IV Hypersensitivity

This type of immune response is cell-mediated and has been induced in colonic mucosa by sensitization with dinitrochlorobenzene (DNCB), by allograft rejection, and by graft-versus-host disease. Contact sensitivity to DNCB has been induced[128] in guinea pigs by applying DNCB to the neck, and the animals were challenged by instilling DNCB into the rectum. Within 24 hr the area of challenge became inflamed and histologically showed edema, perivascular cuffing, and glandular crypt destruction. The reaction could be transferred to other guinea pigs or to swine by lymphocytes but not by serum.[129] In another study, Rabin and Rogers[130] have shown similar findings

but also showed that repeat instillations of DNCB caused edema of the ileal mucosa and infiltration of the portal tracts of the liver with lymphocytes and plasma cells.

Allograft rejection has been studied[131] in mice by inserting fetal mouse colon from one strain under the renal capsule of another strain. After a time lapse of 24–48 hr, the grafts became infiltrated with lymphocytes and the surface epithelium was shed. By 5–7 days there was gross mucosal damage and the graft was then completely destroyed. The appearances, however, did not closely resemble any human disease.

Finally, graft-versus-host disease has been induced in neonatal rats by the injection of spleen cells from a different strain. Of those rats who developed a graft-versus-host reaction, 25% of them had diarrhea and showed some histological changes in the colon—some epithelial cell atrophy and an inflammatory infiltration of the mucosa and submucosa with lymphocytes, plasma cells, and eosinophils.[132]

VI. SUMMARY AND CONCLUSIONS

The colon as an immune organ is an unusual concept. It is nevertheless exposed to a heavy antigenic load, and its role in immunological defense may be as important as that of the small intestine. Like the small intestine, the colon possesses the mechanism for the synthesis of secretory immunoglobulin (IgA, IgM) and IgA is the major immunoglobulin produced. T lymphocytes are present in both the lamina propria and the epithelium. Macrophages staining strongly for Ia-like antigens are found in the lamina propria in association with lymphocytes, and these macrophages are concentrated near the lumen. It seems highly probable, therefore, that this anatomical distribution of cells represents the active processing of antigens that may cross the epithelial cell layer. The recently developed techniques to isolate mucosal cells should extend our knowledge of the immunoregulatory control mechanisms operating within the intestinal mucosa.

Experimentally, the colon can respond to all forms of immune reaction with the production of inflammation. This is relevant for the development of human disease. For example, the granuloma formation in response to the presence of schistosomes represents a cell-mediated, type IV response. It is to be hoped that studies on the immunological effector mechanisms operating in the inflamed mucosae of patients with ulcerative colitis or Crohn's disease may lead to some understanding of the etiology of these diseases.

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6

Bacteriology of the Colon

Gary L. Simon and Sherwood L. Gorbach

I. THE NORMAL MICROFLORA

A. Description of the Microflora

The microflora of the colon is an extraordinarly complex ecologic niche, consisting of both aerobic and anaerobic bacteria. Extensive microbiologic studies have revealed that a single individual harbors more than 400 different bacterial species in the colonic flora.[1] Characterization of such a heterogenous mixture by classic bacteriologic techniques is a monumental task; it has been estimated that a complete microbiologic analysis of a single fecal specimen requires up to 1 year to complete. Fortunately, a reasonable description of the colonic microflora is possible to construct by considering only the most representative microorganisms (Table 1).

The concentration of bacteria within the colon is 10^{11} to 10^{12} colony-forming units per ml (cfu/ml), approaching the theoretical limit that can be accommodated in this mass.[2,3] Nearly one-third of the dry weight of feces consists of bacteria. The luxurient bacterial flora of the colon is predominantly anaerobic, and these oxygen-sensitive forms outnumber aerobic species by a factor of 10^2 to 10^4.[3-6] The most frequent anaerobic isolates are *Bacteroides, Bifidobacterium,* and *Eubacterium;* other common anaerobic bacteria include *Peptococcus, Peptostreptococcus, Ruminococcus, Propionibacterium, Veillonella,* and *Clostridium.*[3-8] *Escherichia coli,* enterococci, and *Lactobacillus* are the most prevalent aerobic or facultative bacteria of the colon. Other gram-negative organisms, such as *Klebsiella, Proteus,* and *Pseudomonas,* are found in about 50% of fecal specimens.[6]

Colonization of the colon of newborn infants occurs within a few days of birth.[9] However, the pattern of colonization may be influenced by such exogenous factors as type of delivery, diet, and gestational age. Long and Swenson[10] found that virtually all

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Table 1. The Gastrointestinal Flora of Man

	Stomach	Jejunum	Ileum	Feces
Total bacterial count	$0–10^3$	$0–10^5$	$10^3–10^9$	$10^{10}–10^{12}$
Aerobic or facultative anaerobic bacteria				
Enterobacteria	$0–10^2$	$0–10^3$	$10^2–10^7$	$10^4–10^{10}$
Streptococci	$0–10^3$	$0–10^4$	$10^2–10^6$	$10^5–10^{10}$
Staphylococci	$0–10^2$	$0–10^3$	$10^2–10^5$	$10^4–10^9$
Lactobacilli	$0–10^3$	$0–10^4$	$10^2–10^5$	$10^6–10^{10}$
Fungi	$0–10^2$	$0–10^2$	$10^2–10^4$	$10^4–10^6$
Anaerobic bacteria				
Bacteroides	rare	$0–10^3$	$10^3–10^7$	$10^{10}–10^{12}$
Bifidobacteria	rare	$0–10^4$	$10^3–10^9$	$10^8–10^{11}$
Streptococci	rare	$0–10^3$	$10^2–10^6$	$10^{10}–10^{12}$
Clostridia	rare	rare	$10^2–10^4$	$10^6–10^{11}$
Eubacteria	rare	rare	rare	$10^9–10^{12}$

full-term, formula-fed, vaginally delivered infants were colonized by anaerobic bacteria within 4–6 days of birth; 61% of these infants harbored *Bacteroides fragilis* in their fecal flora. In contrast, anaerobes were found altogether in only 59% and *B. fragilis* in 9% of infants delivered by cesarean section. Prematurity and breast-feeding were associated with fewer anaerobes in general; *B. fragilis,* for example, was less likely to be found in breast-fed infants than in their formula-fed counterparts. Colonization of the bowel in newborns appear to occur perorally. In infants with congenital small-bowel obstruction, a fecal-type flora is found immediately proximal to the site of obstruction, while the distal bowel remains sterile.[11]

B. Methods of Studying the Microflora

The relative inaccessibility of all but the most distal segments of the colon has prompted most investigators to study the fecal flora as a close approximation of the intracolonic bacterial composition. The validity of this approach has been confirmed by a variety of specialized techniques. [7,12,13] A long tube with a distally attached mercury bag was employed by Gorbach and co-workers[7] to aspirate luminal fluid for bacteriologic study. They found that in general the fecal microflora was quantitatively similar to that found in stool samples. The concentration of microorganisms in the cecum was approximately 100-fold less than that in feces. Bentley et al[12] obtained samples of intracolonic fluid by needle aspiration of the bowel in patients undergoing elective cholecystectomy. They found similar microbial counts in the cecum and transverse colon, but noted that the concentrations of both facultative bacteria and obligate anaerobes were two to four logs greater in feces than in the intraabdominal colon.

Bacteriologic analysis of the colonic microflora requires meticulous anaerobic techniques. The roll tube method, an anaerobic jar, and the glove box have been used to maintain an anaerobic environment for growing these fastidious bacteria. [14–16] Isolation of extremely oxygen-sensitive organisms is more successful with the roll tube. On the other hand, the glove box has greater application in the clinical microbiology

laboratory. The yield of culturing anaerobic organisms by the glove box technique has been improved by the use of prereduced oxygen-free media and inorganic oxygen scavengers.[14,16]

Microbiologic analysis of the colonic bacteria utilizes selective bacteriologic media, which permits the growth of certain organisms while inhibiting others. Inherent difficulties are associated with the use of a selective medium. Besides preventing the growth of unwanted species, it may also inhibit the desired species, thus altering the quantitative data. In an extreme case certain fastidious microorganisms may not grow at all on selective media. Furthermore, a selective medium is only selective for those species which are known and being sought. On the other hand, bacteria present in low concentrations are not apparent on an all-purpose medium; they cannot be identified if the selective medium does not favor their growth.

Classification of anaerobic bacteria has been aided by gas–liquid chromatography (GLC), which identifies bacterial fermentation products.[17] Short-chain fatty acids produced by anaerobic microorganisms are easily recognized by standard GLC techniques. The individual GLC pattern may be quite distinct and can be used for classifying various bacterial species in the laboratory. In addition, Gorbach and co-workers[18] have shown that the presence of these short-chain fatty acids in clinical material such as pus is a marker for infection by pathogenic anaerobic organisms.

C. The Influence of Host Factors on the Microflora

Under optimal growth conditions in the laboratory, coliform organisms can divide every 20 min. Such rapid growth *in vivo* would rapidly overwhelm the host if allowed to go unchecked. In the intestinal tract of animals the rate of bacterial multiplication is substantially reduced, on the order of one to four divisions per day.[19] This is not due solely to a limited supply of nutrients, since the resident flora undergoes rapid growth throughout the bowel shortly after death of the host. Several host and microbial factors act to limit intestinal bacterial populations during life.

In the small bowel, normal peristalsis is the major host defense against bacterial overgrowth.[7,20–22] Reduced peristaltic activity in the large bowel contributes to the luxuriant flora of the colon. Clinical situations in which enteric peristalsis is decreased or ineffective, as in the stagnant loop syndrome, are associated with a dense, colonlike bacterial flora in the small intestine.[6,23–28]

Microbial interactions are of major import in regulating the indigenous microflora of the densely populated colon.[22,29–31] Bacteria may interact to promote or prevent the growth of other bacterial species by several mechanisms: Facultative bacteria (i.e., those which grow either aerobically or anaerobically) help maintain the low oxidation–reduction potential of the colon by utilizing oxygen which diffuses into the colonic lumen.[22] In the absence of such organisms many of the more extremely-oxygen sensitive anaerobic bacteria would not survive. Certain species of bacteria produce substances that inhibit the growth of other species or even control their own growth in an autoregulatory manner. The best-known substances are colicines, bactericidal chemicals produced by strains of *E. coli*. Short-chain fatty acids such as acetic,

propionic, and butyric acid, which are produced by anaerobic and facultative bacteria, are inhibitory to bacterial growth.[31,32] The suppressive activity of these acids is maximal at the pH and Eh levels of the large bowel, thereby serving to limit bacterial populations at this site.

Alterations in the colonic microflora are caused by virtually every antibiotic, depending on the concentration of drug in the luminal contents and its antimicrobial spectrum.[2, 33] A dramatic reduction in the concentration of colonic bacteria can be accomplished by orally administered antibiotics, but usually on a short-term basis. Nonetheless, in certain circumstances even brief suppression can be beneficial. In patients undergoing elective colon surgery, prophylactic administration of oral antibiotics decreases the incidence of postoperative wound infections.[14,34,35] Nichols et al[36] noted that prophylactic administration of neomycin and erythromycin effectively reduced the concentration of aerobic and anaerobic bacteria in the ileum, intraabdominal colon, and feces. In patients receiving this regimen the incidence of postoperative wound infections was 9% compared with 35% in placebo controls.[14]

Pathogenic microorganisms can multiply within the colon to fill the ecologic vacuum created by the administration of broad-spectrum antibiotics. A dramatic example of such on overgrowth syndrome is pseudomembranous colitis in which a toxin-producing anaerobic bacterium, *Clostridium difficile,* proliferates in the large bowel during antibiotic treatment especially with clindamycin and ampicillin.[34,37] The organism elaborates a protein toxin that causes necrosis and ulceration of the colonic mucosa. Similarly, staphylococcal enterocolitis is caused by overgrowth of *Staphylococcus aureus,* a condition usually associated with use of broad-spectrum antibiotics.

Variations in diet have surprisingly minor effects on the fecal flora. When the fecal flora of individuals maintained on a standardized institutional diet was compared with that of subjects eating random diets, few differences were noted except for lower concentrations of total anaerobes and fungi in the former group.[7] Several investigators have failed to find substantial changes in the fecal flora of volunteers fed elemental liquid diets, although a significant reduction in both stool weight and frequency was noted.[38,39] Attebury et al[40] fed an elemental diet, Vivonex, for 8–10 days to three healthy people and found no major change in the total counts of viable fecal bacteria from before the experiment, when the diet was conventional. They did note changes in certain constituents of the flora, most notably that extremely oxygen-sensitive bacteria were virtually eliminated from the fecal flora while receiving the elemental diet. Gorbach[41] studied two volunteers who were kept in a sterile germ-free plastic isolation unit for 3 weeks in order to reduce exogenous contamination. During the 1st week the volunteers ate presterilized canned foods; for the last 2 weeks they consumed only Vivonex and sterile water. Compared with the control period, during which subjects ate a conventional diet, the isolation period produced no significant alterations in the major constituents of the aerobic or anaerobic fecal flora.

Maier et al,[42] and later Hentges,[43] reported on the results of a 4-month diet study in which 10 volunteers consumed a control diet, a meatless diet, a high-beef diet, and finally the same control diet, each for a 1-month period. In all diets the fat content was held constant. No significant differences in the fecal flora were found between the

high-beef and meatless diets, although there was a slight increase in the concentration of anaerobes during the high-beef diet and a mild increase in coliforms during the meatless diet.

These diet studies were performed with classic bacteriologic techniques of counting and identifying microorganisms in a measured aliquot of fresh feces. It must be emphasized that such techniques are imprecise at best; the results are highly liable to error in this complex system in which 10^{11} bacteria and at least 400 different anaerobic bacterial species are encountered in a single gram of feces! As discussed in the latter part of this chapter, the metabolic activity of the flora is quite labile, and measurements of bacterial enzymes in fact have revealed marked changes in the colonic flora as a function of diet.

Alterations in the bacterial populations of the gastrointestinal tract frequently occur during the course of acute diarrheal illness. Under certain conditions the resident microflora may be eclipsed by an identifiable pathogen.[44–47] In patients with cholera, the mean concentration of *Vibrio cholerae* in the characteristic rice water stools is 3×10^7 organisms/ml.[45] Gorbach et al[48] found a predominant flora of enterotoxigenic *E. coli* in the stool and/or small-bowel fluid of patients with acute diarrhea.

Toxigenic *E. coli* has been implicated as a major cause of diarrheal illness in young children in developing nations and in travelers.[49–53] The role of other coliform organisms, some of which produce entertotoxins, remains to be defined. For example, patients with "nonspecific" or viral diarrhea have large numbers of coliforms in the small intestine; cultures of their feces often reveal high concentrations of *Klebsiella, Proteus,* and *Pseudomonas* spp., although there is little evidence that these organisms are pathogenic in this situation.[19,44,48]

The rapid transit of diarrheal stool is associated with a marked reduction in the anaerobic population of the large bowel. In patients with cholera the concentration of *Bacteroides* in the feces may decrease to 10^5 organisms/ml, a reduction of five to six logs below the normal state.[45] These changes are secondary to the underlying acute process, and resolution of diarrhea is accompanied by rapid restitution of the normal flora.

D. Stability of the Microflora

Ecologic forces are extremely important in defining intestinal bacterial populations. Several investigators have attempted to define an "autochthonous" or indigenous intestinal flora in order to distinguish those organisms which are "normal" inhabitants of the gastrointestinal tract and those which are transient colonizers.[54–56] Given the multitude of microorganisms that inhabit the bowel, such a definition is useful only in the abstract, particularly since this work was done in rodents, which have a unique flora.

Although there is considerable variation in the composition of the flora among individuals, studies in a single subject have shown that in general, the composition is quite stable over prolonged periods of time.[7] Under certain circumstances, however,

fluctuatious may be observed.[57] Hospitalized patients are colonized rapidly by specific serogroups of *E. coli* associated with the particular institution. Cooke and her colleagues[58] found clustering of *E. coli* serotypes in patients on individual hospital wards. Attempts to artificially implant *E. coli* into the gastrointestinal tract of volunteers have revealed that colonization is strain dependent. Both Sears and co-workers[59,60] and later Smith[61] noted rapid clearance of ingested *E. coli* strains from the gastrointestinal tract. On the other hand, Formal and Hornick[62] were able to isolate the HS strain of *E. coli* for as long as 3 months following a single feeding to volunteers. Similarly, Cooke et al[58] found persistent *E. coli* colonization after feeding large numbers of a specific organism to volunteers. These studies notwithstanding, the relative stability of the flora is rather remarkable considering its complexity and the constant barrage of ingested microorganisms from the environment.

Ecologic principles assign each species a niche or habitat unique to that microorganism. At equilibrium all niches are occupied. For transient colonization to occur the system must be perturbed, its equilibrium displaced. In the gut, each niche is defined by host physiology, microbial interaction, and environmental pressures. The combination of these effects provides an extraordinarily complex ecosystem. Yet its very complexity is what makes it most resistant to change, and the flora rapidly resumes its original composition after a variety of environmental insults.

E. The Effects of the Microflora on Intestinal Structure and Function

The intestinal bacteria are not merely passive colonizers of the gut lumen. Many of the so-called normal morphologic characteristics of the intestinal epithelium are seen only in animals associated with a resident bacterial flora.[63–66] In the germ-free state the intestinal wall is considerably thinner and less cellular. The most notable changes can be seen in the lamina propria, which in the absence of a microflora has a sparse stroma infiltrated with a few lymphocytes and macrophages. There is little immunologic stimulation of the bowel in the germ-free state, as evidenced by the absence of plasma cells in the lamina propria and by the histologic appearance of Peyer's patches, which are smaller and have fewer germinal centers. In addition, the intestinal villi are thinner and more pointed at the tip, crypts are shallower, and the total mucosal surface area is substantially reduced.[67] In germ-free animals cellular turnover is slower. The mitotic count is decreased, and the time for [^{3}H]-thymidine-labeled mucosal cells to migrate from crypt to villus tip is twice that of conventional animals.[63, 68] There appears to be a greater proportion of mature mucosal cells in the germ-free intestine; they are cuboidal rather than columnar, and are quite uniform in both size and shape.

After exposure to enteric bacteria the bowel of a germ-free animal rapidly assumes a conventional appearance.[64,69,70] There is an increase in the cellularity of the lamina propria, with infiltration of lymphocytes, histiocytes, macrophages, and plasma cells. This picture, called "normal" morphology, is in fact characteristic of a chronic inflammatory response. Further evidence that the microflora induces an inflammatory response in the gastrointestinal mucosa is provided by histologic studies of animals

with surgically created blind loops. In these animals bacterial overgrowth is accompanied by blunting of villi, crypt hypertrophy, and a pronounced interstitial cellular infiltration. [63,71,72]

One of the more impressive structural alterations in the bowel of germ-free rodents is a striking enlargement of the cecum. In the absence of intestinal bacteria this organ may be 10 times its normal size and account for nearly 30% of the animal's total weight.[63,65,73] Water absorption is impaired in the colon of germ-free animals, and the fluid within the dilated cecum is hypotonic and low in chloride and bicarbonate.[74,66] One possible explanation for this cecal enlargement is the presence of osmotically active compounds that are normally degraded by bacteria.[71] It has also been suggested that a small, kininlike peptide or an inhibitor of adrenalin may mediate this process.[65,71] In any event cecal enlargement is rapidly reversed by bacterial colonization of the large bowel, especially with *Clostridium* or *Bacteroides* spp.[65,66,75]

II. INTRA-ABDOMINAL SEPSIS

The pathogenic potential of the colonic microflora is most apparent when the integrity of the large bowel is breached. The resultant leakage of intraluminal contents into the peritoneal cavity produces a characteristic clinical and pathological scenario. There is an initial phase of generalized peritonitis, followed by localized abscess formation.

The hallmark of intra-abdominal sepsis is a polymicrobial flora involving both aerobic and anaerobic bacteria. Among 67 patients with peritonitis and/or intra-abdominal abscesses an average of five different types of bacteria were isolated from the infected site, including two aerobes and three anaerobes.[76] The most common anaerobic isolate was *B. fragilis,* followed by *Clostridium* spp. and anaerobic gram-positive cocci (*Peptostreptococcus* and *Peptococcus*). Among the aerobic microorganisms, *E. coli,* other coliforms, and enterococci were cultured most frequently.

The pathogenesis of intra-abdominal infection has been studied in a rat model.[77] After contaminating the peritoneal cavity by inoculation of cecal contents, these animals developed a two-stage illness. The initial phase was generalized peritonitis and septicemia with a 40% mortality rate. All survivors of the peritonitis phase progressed to the second stage of intra-abdominal abscess formation. Bacteriologic studies revealed that the peritonitis stage was due to coliforms.[78] The second stage of abscess formation was dependent upon anaerobic bacteria, most notably *B. fragilis*. By using antimicrobial probes, the two phases of the disease could be separated.[79] Rats treated with gentamicin, a drug active against coliforms but not anaerobes, survived the initial insult of peritonitis and septicemia. However, all of these animals developed abscesses. Animals treated with clindamycin, which is active against anaerobes but not coliforms, developed peritonitis and septicemia with an initial mortality similar to that of untreated controls, but survivors were spared subsequent abscess formation. Rats treated with the combination of clindamycin and gentamicin developed neither peritonitis nor abscesses.

Despite the polymicrobial nature of infections associated with intraperitoneal leakage of colonic contents, the bacteriology does not compare in complexity with that of the colonic flora in which several hundred species of bacteria may be found. The complex inoculum of fecal material is consistently simplified to a few bacterial species which survive to cause infection. This suggests that the pathogenic species possess certain virulence characteristics not uniformly present among the majority of colonic bacteria.[80] These virulence properties are important in both invasion and proliferation of the bacteria outside their usual habitat in the gut. For example, oxygen sensitivity of a particular anaerobe is related to its virulence.[81] Many extremely oxygen-sensitive anaerobic bacteria exist in the colonic lumen, but these organisms are not isolated from infected sites. Pathogenic anaerobes such as *B. fragilis* are usually aerotolerant and can survive for several hours in oxygenated tissues. It could be argued teleologically that survival by a microorganism in the oxygenated tissues of the host is a prerequisite to its invasiveness. Oxygen tolerance in an anaerobe roughly correlates with its concentration of the enzyme, superoxide dismutase.[82]

Recently, Kasper[83] has shown that *B. fragilis* but not other *Bacteroides* spp. possess a polysaccharide capsule on the outer surface of the cell wall. This capsule is an important virulence factor. Well-encapsulated strains of *B. fragilis* are more resistant to neutrophil phagocytosis and killing than are unencapsulated *Bacteroides* spp.[84] In animal studies, inoculation with encapsulated *B. fragilis* results in abscess formation while unencapsulated strains of Bacteroides do not produce an abscess unless a facultative anaerobe is also present in the inoculum.[85,86] The virulence of encapsulated *B. fragilis* strains is reflected in the fact that they are isolated three times more frequently than unencapsulated strains from clinical specimens, although the concentration of encapsulated strains in the fecal flora is two logs less than that of unencapsulated species such as *Bacteroides vulgatis* and *Bacteroides thetaiotaomicron*.[3,87–89]

III. THE RELATIONSHIP BETWEEN COLON CANCER AND THE INTESTINAL MICROFLORA

It has been recognized for some time that the incidence of colon cancer is higher among North Americans and Western Europeans than among residents of Africa, Asia, and South America.[90–94] Epidemiologic investigations have indicated that these observed differences are not due to genetic factors, but rather appear to be based on environmental considerations. The critical element contributing to the unequal geographic distribution of colon cancer may be the characteristic Western diet, which is high in beef, fat, and protein. Several lines of evidence support this hypothesis. Several investigators have noted a correlation between the adoption of a Western diet and a rising incidence of colon cancer in presumably low-risk population groups. In Japan the overall incidence of colon cancer is low, but those Japanese who develop this malignancy tend to be in a higher socioeconomic class and eat a more Westernized diet than the general populace.[95] Similar findings were noted by Crowther and co-workers,[96] who studied Chinese people in Hong Kong. Haenszel et al[97] found a greater incidence of large-bowel cancer among Japanese immigrants in Hawaii who adopted a Western-

style diet than among those immigrants who maintained a more traditional Japanese diet. Multinational studies by several investigators have revealed a direct correlation between the per capita beef consumption and the incidence of colon cancer.[90,93]

Classical microbiological techniques of identifying and counting bacteria have been utilized to study the relationship between fecal microflora, diet, and the incidence of colon cancer. An early study by Hill and colleagues[98] revealed higher counts of enterobacteria and enterococci and lower counts of *Bacteroides* spp. in fecal specimens from Ugandans, Indians, and Japanese than from Western Europeans and North Americans. Recent investigations done in an attempt to further characterize the fecal flora of groups at different risk for colon cancer have failed to confirm these findings. Finegold et al[87] studied the fecal flora of Japanese-Hawaiians consuming different diets. In general, the differences in flora were minor. Increased numbers of enterococci were noted among subjects who ate a traditional Japanese diet. Higher counts of *Bacteroides* were found among those who consumed a more Western diet, but the differences were not statistically significant. A study of the fecal flora of vegetarian and nonvegetarian Seventh-Day Adventists also revealed few significant differences between the two groups.[5,99] A similar investigation by Moore and Holdeman[3] failed to confirm the existence of a specific type of microflora associated with a "high risk" Western diet. The results of these studies suggest that the bacteriologic composition of the fecal flora is influenced to only a minor extent by diet.

The causal link between intestinal microflora and colon cancer may exist in bacterial enzyme metabolism, a factor not considered in classical bacteriology studies. Many chemical compounds exist as procarcinogens, substances that must be chemically modified before they exhibit mutagenic activity.[100,101] Some of these procarcinogens occur naturally within our environment, while others are ingested as food preservatives, dyes, additives, or pollutants. Colonic bacteria produce a variety of enzymes that mediate reactions resulting in the production of carcinogens.[102,103] These include β-glucuronidase, β-glucosidase, nitroreductase, azoreductase, 7α-dehydroxylase, and cholesterol dehydrogenase. It may be more than coincidence that the most common site for cancer development in Western populations, namely, the large bowel, also harbors an extensive and highly metabolic microflora. Since the metabolic activity of the flora is dependent upon the composition of specific microorganisms, it is reasonable to suspect that any factor that might change the flora, such as diet, could induce profound alterations in the ability to metabolize carcinogens.

The role of bacterial enzymes in the production of carcinogens is illustrated by studies of experimental colon cancer induced by cyacasin. This substance is the β-glucoside of methylazoxymethanol, and is found naturally in the nut of the cycad plant, a tropical fern. When cycasin was fed to normal rats, they developed colon cancer.[104] The carcinogenic activity of cycasin was dependent upon the intestinal flora, since no effect was noted when germ-free animals were fed the same dose of cycasin.[105] Additional studies demonstrated that the route of administration and age of the animal were critical.[106] In newborn rats, tumors could be produced by oral, subcutaneous, or intraperitoneal administration, while subcutaneous or intraperitoneal injection had no effect in older, conventional animals. Similar observations were also noted for germ-free rats. On the other hand, methylazoxymethanol, the parent aglycone,

caused tumors in both conventional and germ-free rats regardless of their age or the route of administration.[107] The explanation for these findings is that cycasin has no intrinsic carcinogenic activity but becomes active when hydrolyzed to methylazoxymethanol. In adult rats the bacterial enzyme β-glucosidase is necessary to effect this hydrolysis. Newborn rats, but not adults, have high levels of tissue β-glucosidase that can hydrolyze cycasin and produce the carcinogenic moiety.

The bacterial enzyme nitroreductase may also be important in the production of carcinogens within the colon. Aromatic nitro compounds are widely distributed in our environment. Nitroreductase acts to reduce these compounds to aromatic amines. This reaction involves the formation of short-lived intermediates such as nitrosoamines and N-hydroxy compounds, which are suspected carcinogens.[108,109] Another bacterial enzyme, azoreductase, causes the reduction of azo food dyes, yielding substituted phenyl and naphthyl amines; these groups include potent chemical carcinogens.[31]

Although variations in diet appear to exert little effect on the specific bacterial composition of feces, studies of the metabolic activity of the microflora have shown it to be quite labile and greatly influenced by dietary factors. Many bacterial enzyme systems are inducible, so that continued exposure of colonic bacteria to substrate results in increased enzyme activity. If the substrate is a procarcinogen, continued exposure will result in increased carcinogen synthesis.

These properties are illustrated by the metabolism of fecal bile acids. These compounds have been studied extensively as candidate carcinogens because of their structural similarity to the carcinogenic polycyclic aromatic hydrocarbons.[8,100,110–113] The ingestion of animal fat promotes augmented bile flow and greater production of conjugated bile acids. Consequently, the concentration of bile acids in the colon is increased in people eating a high-fat diet, and this induces colonic bacteria to produce increased amounts of 7α-dehydroxylase, an enzyme involved in the degradation of bile acids.[110] Increased 7α-dehydroxylase actively results in higher concentrations of secondary bile acids.

Studies of population groups have suggested that such diet-induced alterations in fecal bacterial enzyme activity do occur. High concentrations of secondary bile acids in the feces correlate with a Western style, high-beef diet. Hill and co-workers[98] noted that the fecal microflora of North Americans and Western Europeans contained more bacterial strains capable of 7α-dehydroxylation than did the flora of Ugandans or Indians. Furthermore, they found higher concentrations of coprostanol and coprostanone, the degradation products of cholesterol, in fecal samples from Westerners. Similarly, Reddy and Wynder[114] reported that Americans on a Western diet had higher levels of total bile acids and of deoxycholic and lithocholic acid, but not of cholic acid, than American vegetarians, American Seventh-Day Adventists, or Japanese or Chinese immigrants. They also noted high fecal levels of coprostanol and coprostanone and low fecal cholesterol in Americans on a high-meat diet. These data suggest that the flora of the people eating a meat diet is more active metabolically than that of population who eat lesser amounts of animal fat and protein. Mower et al[115] found that Japanese in Hawaii had higher concentrations of deoxycholic acid in fecal specimens than did Japanese living in Akita, Japan. In a similar vein Mastromarino and co-workers[111] re-

ported elevated levels of 7α-dehydroxylase and cholesterol dehydrogenase in the fecal flora of patients with colon cancer.

Reddy et al[116] examined fecal bacterial β-glucuronidase activity in volunteers on mixed Western high-meat diets and nonmeat diets. Higher fecal enzyme activity was noted in subjects eating the meat diet. An associated decrease in fecal β-glucuronidase was noted in subjects who initially ate a high-beef diet and subsequently shifted to a nonmeat diet. β-glucuronidase is capable of retoxifying compounds which have been glucuronidated in the liver. For example, carcinogens such as *N*-hydroxy-*N*-2-fluorenylacetamide and diethylstilbesterol are activated by hydrolysis of the glucuronide bond in the bowel.[117,118]

The relationship between fecal bacterial enzymes and colon cancer is also evident in experimentally induced malignancies. In rats, Goldin and Gorbach[102] found that the fecal bacterial enzymes β-glucuronidase, azoreductase, and nitroreductase could be altered by diet. Rats maintained on a grain diet had low levels of enzyme activity; shifting from a grain diet to a high-beef diet resulted in significant increases in the activity of all three bacterial enzymes.[119] Similar increases were also noted when the grain diet was supplemented with 30% beef fat without the addition of other meat products.[120] Furthermore, rats on high-beef diets appear to be more susceptible to carcinogens. Dimethylhydrazine (DMH) is an experimental carcinogen metabolized by the liver to methylazoxymethanol-β-glucuronide.[121] Like cycasin, DMH is deconjugated by the colonic flora to form the active moiety. When DMH was fed to rats on high-beef and high-grain diets, the rates of tumor production were 83% and 31%, respectively.[122].

Goldin and co-workers[123] have studied fecal bacterial enzymes in humans and noted similar findings to those found in rats. Enzyme levels were measured in three groups of people: omnivores who ate a mixed Western diet, lactovegetarians who consumed a diet which excluded all animal foods except milk products, and a group of strict vegetarians. Omnivores had considerably higher levels of fecal β-glucuronidase, nitroreductase, and 7α-dehydroxylase than did lacto-vegetarians or strict vegetarians. Azoreductase was not significantly different between omnivores and lacto-vegetarians, but was significantly lower among the strict vegetarians than among the omnivores. In the same study, the effect of eliminating red meat from the diet of omnivores and the effect of fiber supplementation on a mixed Western diet were also measured. The researchers found no significant change in any enzyme activity except that of 7α-dehydroxylase. In both cases this enzyme fell significantly during the period of diet adjustment.

Feeding lactobacilli in an attempt to alter the characteristics of the intestinal flora has been suggested since the turn of the century as a cure for many different maladies. Metchnikoff[124] attributed the longevity and good health of Bulgarian peasants to their penchant for consuming yogurt, a cultured dairy product produced by fermentation with lactobacilli. Subsequent studies revealed that the lactobacilli in conventional yogurt, *Lactobacillus bulgaricus,* was a dairy microbe that could not be implanted in humans. Since then, several investigators have studied *Lactobacillus acidophilus,* a species of lactobacillus found in the human gut.[125,126] Previous attempts to implant this

microbe in the human intestinal tract have met with little success; for example, Paul and Hoskins[127] fed enormous numbers of *L. acidophilus* to humans without evidence of implantation.

Recent observations have stimulated interest in the relationship between lactobacilli and colon cancer. A study of colon cancer in Danes and Finns revealed that the low-risk Finnish group had a higher intake of dairy products in their diet and significantly higher counts of lactobacilli in their feces than did the high-risk Danish group.[128] It was suggested that the higher intake of dairy products might provide some protective effect. Whether lactobacilli are involved in this protection remains speculative.

The role of exogenous lactobacilli in the metabolic activity in the intestinal microflora was examined by Goldin and Gorbach,[129] who fed *L. acidophilus* to rats that were consuming either grain or beef diets. Little change was noted in the activity of the fecal bacterial enzymes of grain-fed animals. However, in the rats consuming a beef diet, a significant reduction in β-glucuronidase, azoreductase, and nitroreductase activity was noted after feeding them *L. acidophilus*. Furthermore, when beef-fed rats were challenged with dimethylhydrazine, there was a significant delay in the time required for experimental tumors to develop, suggesting that lactobacilli might have some protective effect. These studies provide further evidence that experimental colon cancer is influenced by the metabolic activity of the intestinal flora.

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7

Storage and Propulsion along the Large Intestine

Ghislain Devroede

The main function of the large bowel is to store and absorb water, electrolytes, volatile fatty acids, nitrogenous substances, and some vitamins, but at the same time it must be able to propel its contents when they have reached the proper consistency and to interact with the central nervous system in order to provide continence and a socially acceptable pattern of defecation. The complex nature of the motility of the colon, rectum, and anus is not surprising in view of the need for the combination of retention and excretion properties over barely 100 cm of bowel.

At this stage in our understanding of large-bowel physiology it is convenient to discuss separately the factors that facilitate retention of bowel content, i.e., the storage function, and the factors that promote excretion, i.e. propulsion.

I. STORAGE IN THE LARGE BOWEL

A. Storage in the Ascending Colon

There is ample evidence that the main role of the ascending colon is storage. Radiological observations of the abdomen suggest that stools are retained there for long periods,[1,2] and fluoroscopic evaluation of the movements of the large bowel indicates that the transverse colon is a transit structure: prolonged retention occurs either proximally or distally.[3–5] In the ascending colon, most of the movements are of the segmentation type to promote absorption and free mixing.[2] Its contents, under resting conditions, move at only 1 cm/h.[4] Retropulsion occurs often from the transverse colon

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toward the cecum, and is often seen as the dominant movement in the right half of the colon.[4,6] This can be reproduced in the artificial conditions of colonic perfusion. When the cecum is constantly filled with fluid, pressure studies performed simultaneously in the ascending, transverse, and descendign colon indicate that it first accumulates in the right portion of the large bowel. A consciously perceived contraction occurs when a pressure threshold has been reached. The fluid is then transported very quickly to the distal colon and rectum, before much absorption can take place.[7,8] This explains why, in the course of right colonic perfusion, appearance of fluid in the rectum is intermittent rather than continuous.

The control mechanism of storage in the ascending colon is not well understood. There is a high-pressure zone at the ileocecal junction,[9] and distension of the colon increases the level of this pressure barrier, thus preventing reflux of colonic contents into the ileum.[10,11] This probably also explains why the ecology of the fecal flora is very different on either side of the ileocecal sphincter, both quantitatively and qualitatively.[12] The mechanism by which this barrier is regulated is neurological.[13–15] Vagal stimulation reduces transphincteric flow at least partly through a direct effect on the sphincteric muscle. Both guanethidine and atropine block this effect. Stimulation of the splanchnic and lumbar colonic nerves also elicit a contraction of the sphincter, but this sympathetic effect is accompanied by an inhibition of the adjacent parts of the small and large intestine, in contrast to vagal stimulation, which increases motor activity in the ileum and occasionally in the proximal colon.[14,15]

At the level of the transverse colon, both in humans[16] and in animals,[17,18] the basal electrical rhythm (BER) appears to be faster than it is in the right colon. In addition, *in vivo* animal experiments suggest the presence just above the ileocecal junction of a dominant pacemaker[19,20] that forces material back toward the cecum. In some species, forward movement of contents is then seen when a cecal pacemaker becomes intermittently dominant.[18] The human colon can be divided into three segments according to its electrical control activity.[21] The middle segment has the overall dominant frequency in the higher range averaging 11 cycles/min, and control waves are temporarily phase locked. This segment begins in the proximal transverse colon and may end, depending on the individual, in either the distal transverse colon, the descending, or the sigmoid colon. Proximal and distal to this segment, control waves are not phase locked and are of variable frequency. As a corollary, the muscle of the transverse colon generates pressure waves at a greater frequency[3,7,22] and more contractions can be observed there, radiologically,[3] than in the right colon. This pattern is consistent with the view of the transverse colon as a transit segment with storage occurring both proximally and distally according to needs.

B. Storage in the Descending Colon

After defecation, some normal subjects retain feces in the left colon, even while the rectum is being emptied.[23] The pattern is variable: in other subjects defecation empties the entire left half of the colon and rectum. The major difficulty in deciding which pattern is normal is due to the selection of subjects under study: the rectosigmoid junction responds to anxiety induced by painful stimuli or unpleasant memories,[24–27] and a large-scale study came to the surprising conclusion that one-third of ''normal''

subjects complain of the irritable bowel syndrome,[28] a disorder believed typical of neurotic subjects.[29–31]

The angulation between the sigmoid colon and the rectum probably provides a physical barrier which prevents propulsion of stools. The smaller caliber of the left colon and the different consistency of its contents probably also play a role in the mechanism of storage on the left side. Stools do not behave like fluids.[32] Whereas feces are stored in the distal colon, fluids are not, as demonstrated by perfusion studies [17,18]

Pressure studies reveal a higher frequency of contractions at the rectosigmoid junction than is found in the sigmoid colon.[33] However, a physiological sphincter, as suggested long ago by O'Beirne, does not exist at the rectosigmoid junction.[34] The BER also increases in frequency as one approaches the rectum.[16] In almost all patients with the irritable bowel syndrome or colonic diverticulosis who also complain of constipation, a 1.5- to 2-cm hyperactive segment of bowel, characterized by wave activity of a continuous complex type or superimposed repetitive waves, is found 9-15 cm above the anal margin. Unlike a true sphincter, there is no significant elevation of the base-line pressure nor is there a pressure gradient proximal or distal to this segment. Neither secretin nor cholecystokinin influence the wave activity of the hyperactive segment, but atropine and glucagon produce a marked inhibition of its motility, suggesting overactivity of the muscarinic effector cells in these subjects. Interestingly, some healthy controls and patients with the same diagnoses but normal bowel habits were also found to have such a hyperactive segment but on an intermittent rather than persistent basis.[35] This again raises the question of the normality of "normal" subjects. Colonic contractions at a frequency of 2-3 cycles/min are considered characteristic of patients with the irritable bowel syndrome or idiopathic constipation.[36–39] These contractions are nonpropulsive and thought to be the result of segmental contractions that impede aboral fecal movements. [38,40] There is a difference in myoelectrical activity in the distal colon between controls and patients who complain of irritable bowel. The latter have an increased 3 cycle/min slow wave activity in the basal state, and this is even more pronounced after administration of cholecystokinin or pentagastrin. On the contrary, spike potential and motor activity do not appear to differ in patients. [41,42]

It is thus not clear at this stage what sort of barrier exists at the rectosigmoid junction in the normal state.

C. Storage in the Rectum

Stools can be stored in the rectum until the time of defecation becomes socially convenient.[43] Storage in the rectum results from several complex mechanisms that are responsible for the unique capacity of the anorectal area to maintain continence for stools or permit defecation at will.

In clinical practice, the rectum is normally empty. It is easy to perform a proctoscopy after defecation; if stools are encountered they are present only at the rectosigmoid junction. Thus the rectum plays a crucial role in the maintenance of a will-dependent defecation pattern, but it is not essential for continence: colonic continence does exist, and patients with a sigmoid colostomy may not need to wear an appliance if they do not have an irritable bowel syndrome. The principle of colonic irriga-

tion in such patients is based on that property. This does not occur with a transverse colostomy and therefore depends upon properties of the descending colon.

When stools enter the rectum, they can be stored, because the rectal wall accommodates well to distension and has viscoelastic properties resembling those of the urinary bladder.[44] The viscous properties are responsible for rapid accommodation. One may speculate that this is a most useful mechanism in coping with sudden distension such as may occur with gas rather than solids. This rapid accommodation of the rectal wall is accompanied by a rectoanal inhibitory reflex i.e., the short-lived relaxation of the internal sphincter that is induced by rectal distension and recorded in the upper anal canal. This reflex is synchronous with the accommodation of the rectum, and repetitive distensions show that when their frequency is too high, the reflex is abolished.[45] The elastic properties of the rectum are responsible for residual wall tension during sustained distension. This elasticity is increased in ulcerative colitis,[46] and volumes tolerated in the rectum are smaller in active phases of the disease.[47] Patients with Hirschsprung's disease also have a high coefficient of elasticity and thus a poorly distensible rectum.[48] In this condition, it has been possible to demonstrate that patients who not only suffer from constipation but have serious complications such as obstruction and enterocolitis and therefore need a colostomy early in life, develop a much greater tension in the rectal wall during distension than control subjects. From this disease, we thus not only learn that the absence of neurones explains the lack of propulsion of feces in the rectum but also that there is a second variable, an element of spasticity of varying severity that is related to another anatomical or functional abnormality. In this regard, histological studies have demonstrated a direct relationship between the abundance of nervous bundles and the severity of disease in the Zuelzer-Wilson syndrome, a total aganglionosis of the large bowel.[49] That the tonus of the large bowel may be related to parasympathetic innervation is also suggested by the observation that transection of the nervi erigentes produces an atonic colon.[50,51]

Rectal valves are so placed as to prevent linear progression of feces in the rectum. This can be easily appreciated when performing a proctosigmoidoscopy. They create resistance to the forward progress of material in the distal bowel. This resistance simply is related to the rectal morphology, since pressure changes are recorded by large balloons, but not by open-tipped catheters, as they are pulled caudally past the bends and folds of the distal rectum.[34]

The angle between the rectum and the anal canal contributes to fecal continence. Also, the puborectalis muscle which provides a sling around the anorectal junction either prevents defecation by angulating the junction anteriorly during voluntary contraction of the pelvic floor or relaxes as the individual strains to defecate (Fig. 1). The external sphincter is another striated muscle; it surrounds the internal sphincter and extends beyond it, so that at the anal margin its superficial portion provides a closing mechanism of the anal canal. A triple loop system has been described anatomically in the puborectalis and external sphincter.[52] The top loop is composed of the puborectalis, which blends into the deep portion of the external sphincter. Its direction of pull is upward and forward toward the pubis. The middle loop is the distinct midportion of the external sphincter, which is fixed behind on the tip of the coccyx and pulls posteriorly. Finally the bottom loop is made of the most superficial portion of the

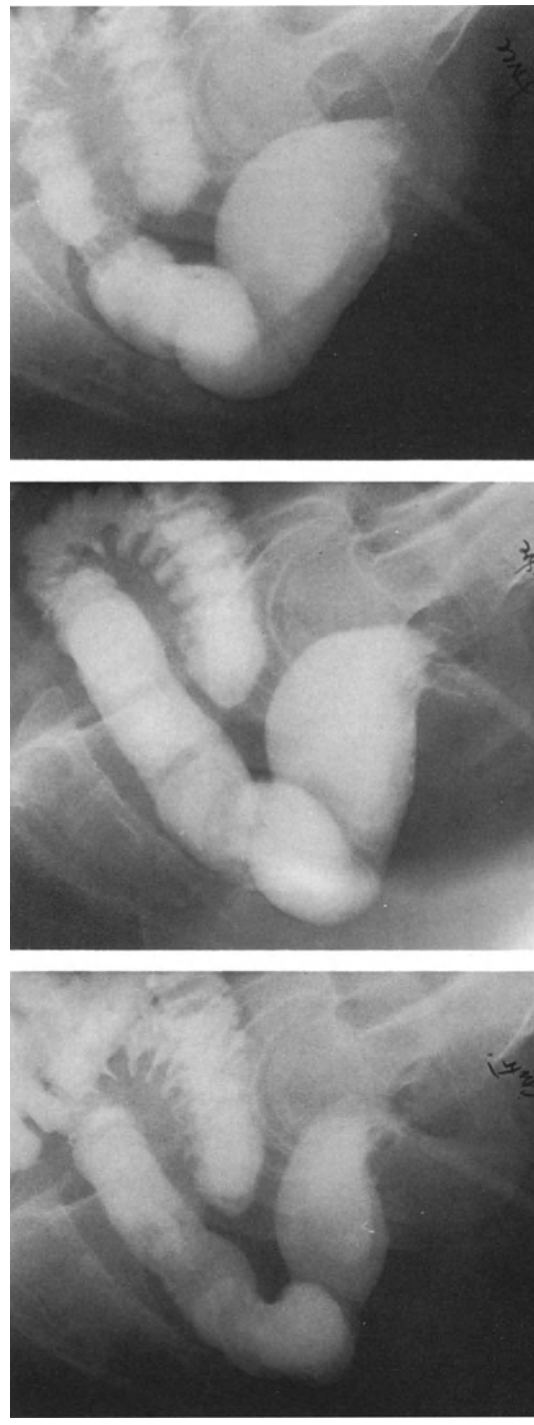

Figure 1. Anorectal angle during maximum anal contraction (left), at rest (middle), and during defecation (right).

external sphincter. Its pull is directed downward and forward. It is the only loop which contains concentric circular muscle bundles. This simple anatomical disposition provides a tight closure with minimal fatigue because anal occlusion derives not only from direct compression of the canal but also from kinking. During defecation, all parts would relax, but in its final phase vermicular contractions of the three loops would occur one after another, in a pseudo-peristaltic fashion, thereby expelling the rear portion of the fecal mass. However, this interesting concept is based solely on anatomical considerations and its validity needs to be confirmed by functional studies.

The internal sphincter is a condensation of the circular muscle layer of the rectum. It is thus made of involuntary smooth muscle. It is not permanently contracted but resists stretch and thus provides a strong pressure barrier between the rectal lumen and the atmosphere.[53–56] This barrier is not circular but flattened from side to side (Fig. 2). This mechanism constitutes a "flutter valve" which transmits sudden increases in intraabdominal pressure to the anal canal and so augments continence mechanically.[56] Longitudinal mucosal folds similar to those seen in the esophagus are seen on plastic molds obtained by rectal instillation of hardening resins.[57] They possibly contribute to mechanical closure of the anus.

Fluctuations in anal canal pressure[58,59] are known to appear shortly after birth,[60] with a higher frequency distally[58,61] than proximally, thus creating a sweeping retrograde movement.

As already stated, the anal canal relaxes in response to rectal distension.[56,62–65] The residual pressure in the anal canal during the rectoanal inhibitory reflex is the combined result of the antagonistic actions of the external and internal sphincters. Pudendal block abolishes external sphincter activity, but the reflex persists, albeit modified, thereby showing that it depends on relaxation of the internal sphincter.[66] Balloon distension above a low rectal anastomosis does not elicit a rectoanal inhibitory reflex in the majority of patients, shortly after surgery. However, it may reappear at a later time, even when the ascending colon has been anastomosed to the anal canal.[67] It is absent in Hirschsprung's disease where the intrinsic plexus of the rectum is abnormal.[68] These observations suggest that receptors for the rectoanal inhibitory reflex are not in extrarectal tissue, but may be present elsewhere in the colon. Also, their action on internal sphincter tone is either via an extrinsic reflex pathway or directly across the anastomosis. The former is unlikely when the anastomosis is done with a segment of colon proximal to the splenic flexure. Furthermore, the reflex persists in the absence of parasympathetic innervation,[62,63,65,69–72] but parasympathetic stimulation induces a rectoanal inhibitory reflex.[73] No study has been done of combined bowel transsection and extrinsic denervation. Perhaps there are two pathways, extrinsic or local in the gut wall, and the reflex may involve purinergic nerves. It may be learned through operant conditioning.[74,75] Although the reflex is classically present at birth,[76] diminished rectal activity may be present[58] and appearance of the reflex may be delayed for 2 weeks after birth.[60] Neuronal immaturity has been proposed as the histological counterpart to the delay in maturity of this reflex.[77,78] This could explain the fact that premature infants defecate their first stool slightly later than full-term or postterm infants, although the difference is only measured in terms of hours.[79]

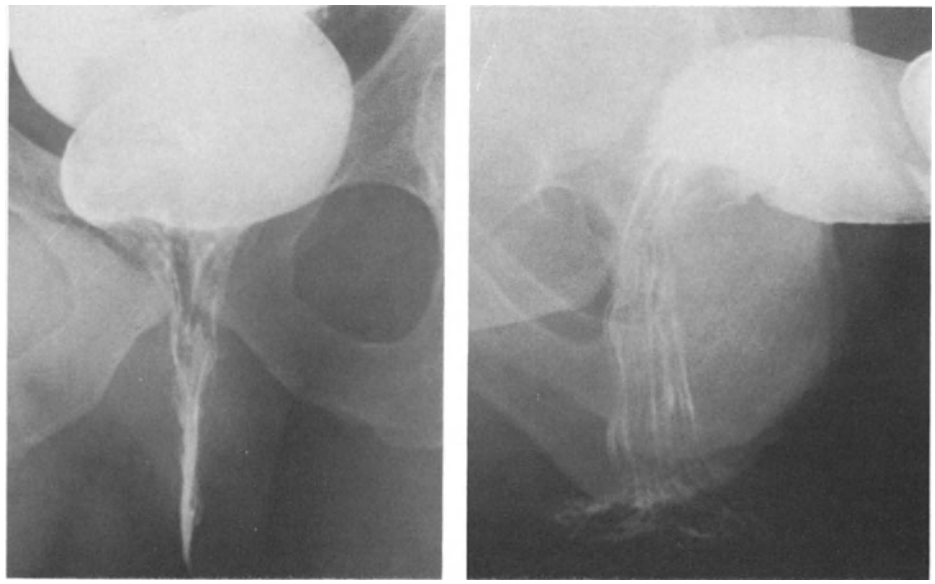

Figure 2. The anal canal is flattened from side to side. Left: antero-posterior view. Right: lateral view.

The rectoanal inhibitory reflex is contined to the upper part of the anal canal.[80] Simultaneously, the external sphincter contracts. This is known as the rectoanal contractile reflex and is best recorded in the lower portion of the anal canal, where there is no internal sphincter.[64,65,81,82] Cauda equina lesions abolish this external sphincter reflex, which is mediated through the spinal cord.[83,84] The contractile reflex persists in the majority of patients who underwent a low rectal anastomosis when stimulation of the bowel is done above the anastomosis. At large volumes, total inhibition occurs and marked excitation is then seen when the distending balloon is deflated. A minority of patients, however, have a poorly distensible rectum and cannot tolerate volumes exceeding 50 ml.[67] This suggests that the receptors for the reflex must be outside of the rectum, possibly in the levator ani muscles. The combination of the rectoanal inhibitory and contractile reflexes results in a change of configuration of the anorectal area which takes a funnel shape, closed distally by the external sphincter contraction. As a result (Fig. 3), pressure in the cloacogenic zone, slightly above the dentate line, is lowered to that of the rectal lumen. Fecal contents can thus descend into an area which is abundantly innervated.[85] Without losing continence, it thus becomes possible to analyze rectal contents (gas, liquid, solid) and consciously make appropriate decisions.

Voluntary contraction of the pelvic floor and external sphincter is the last barrier of defense against incontinence. This final protective mechanism does not last beyond 1 min.[56] Incontinence then occurs if the other factors promoting continence have all been overcome.

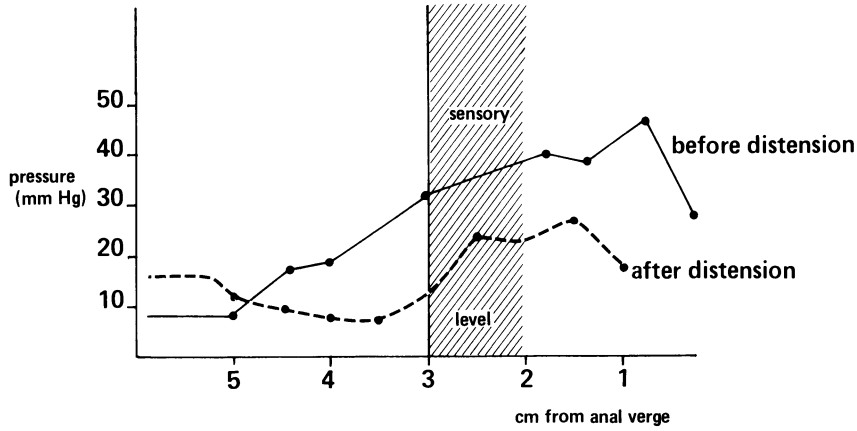

Figure 3. Pressure profile of the rectoanal area before and after rectal distension. At rest (before distension), the pressure in the area of conscious sensation (sensory level) is greater than in the rectum and fecal content of the rectum cannot reach this zone. As a result of rectal distension, a rectoanal inhibitory reflex occurs but its magnitude varies along the anal canal. This time, the pressure in the area of conscious sensation is the same as in the rectum and allows descent of fecal contents, which can be consciously analyzed while continence persists through a distal pressure barrier. From Duthie.[80]

The mechanisms for these continence factors are of course very complex. The pelvic floor is in a perpetual state of electrical activity even in the resting state and during sleep.[84,86–88] This is unique among striated muscles. This activity encompasses both the levator ani muscle and the external sphincter.[86,89] It is involuntary and persists in spinal man. The origin of the activity is reflex from the muscle itself.[84] Furthermore, unlike other striated muscles, even when separated from its nerve supply the external sphincter does not degenerate, it retains its responsiveness to externally applied faradic stimulation, and with continued stimulation it will regain much of its tone.[90,91]

The BER of the internal sphincter is faster than that seen in any other part of the bowel. It is abolished during the rectoanal inhibitory reflex.[92–94] It is likely that this electrical activity is myogenic rather than neurogenic in origin. Two patterns of slow waves are recorded: a constant sinusoidal pattern and a spindle-shaped or waxing-and-waning pattern. It appears that the constant pattern originates from oscillators generating electrical activity at frequencies that, while they may be out of phase, are not canceling each other. The spindle-shaped pattern probably derives from generators with frequencies out of phase in such a manner that they alternately augment and cancel each other. The sphincter relaxation that accompanies inhibition of electrical activity supports the concept that the sphincter is normally in a state of tonic contraction governed by the electrical slow wave and that relaxation results from inhibition of this electrical activity.[94]

II. PROPULSION IN THE COLON

Propulsion through the large bowel is slow. Thus, although the transit time of food through the small bowel is measured in hours,[64] it is measured in days in the

large bowel.[64,95,96] Neither electromyograms of the colon in man[21,41,97] nor measurements of intraluminal pressures have yet described the propulsive mechanisms. Both approaches are difficult to apply to physiological conditions because of the slowness of colonic events. Stool consistency is another factor which prevents an easy way to investigate colonic propulsion.

Colonic volume and transit have been measured during perfusion.[98] The average volume of fluid is relatively small at 300 ml, but conditions during perfusion are quite artificial and this volume possibly only represents volumes in the ascending colon. Transit of perfusates from right to left colon is almost instantaneous.[7] Transit from cecum to rectum is fast, occurring in minutes. The greater the rate of perfusion, the faster the transit and the closer the composition of rectal effluent to the original perfusate. Thus rapid transit appears to decrease absorption.[98]

Under physiological conditions, noraml subjects who change from a mixed diet, containing unabsorbable residue, to one containing fluids only have a decrease in stool frequency. At the same time, however, the weight of each stool does not change.[99] This finding supports the concept that critical volumes of distension are needed for propulsion to occur. Fiber content of the diet is thus crucial in terms of colonic propulsion. Bran accelerates slow intestinal transit and slows down fast transit.[100,101] Although mixing is seen in different segments of the colon,[2] progression of markers swallowed by normal subjects is remarkably smooth.[64,102]

Visualization of a human colon full of barium permits a reasonable classification of movements.[4], [5] Propulsion occurs when the contents of a single segment of haustrum are displaced by systolic contraction into an adjacent segment, and thence to another. Multihaustral propulsion affects three or more segments together as a coordinated unit. Annular constriction may originate as an interhaustral fold and advance more or less steadily in either direction along the bowel. Mass propulsion starts at the transverse colon and occurs mainly after food intake.[4,5,103] Peristalsis can occasionally be recorded in man as a wave of pressure at rest,[104] but it is best elicited by distension of the bowel[105] or laxatives.[106] In the guinea pig colon, propulsive motor activity is initiated when basal intraluminal pressures exceed 5–10 cm of water, and consists in a peristaltic contraction. At a lower basal luminal pressure, motor activity is nonpropulsive and consists of slow, irregular, segmental pressure waves that do not empty fluid from the colonic segments.[107]

Parasympathetic innervation plays a crucial role in the control of propulsion. The supply ascends from the nervi erigentes of S2-S3-S4 to innervate the left part of the transverse and the rest of the distal colon. Bilateral resection of these nerves induces obstipation.[69,108] Trauma to the cauda equina results in the same problem.[70] Rectal pressure is reduced compared with controls, probably because of inelasticity of the rectal wall. The anal canal becomes hypertonic. Minimal distension of the rectum induces maximal relaxation of the internal sphincter, accompanied by a rectoanal contractile reflex of less than normal amplitude and of shorter duration. This is conducive to constipation with incontinence to liquids. In some animals, electrical stimulation of pelvic nerve afferents arising in the colon or distension of the colon or rectum evokes reflex increases in efferent firing, and sustained propulsive contractions that are associated with defecation. Both responses are abolished by transsection of the pelvic nerves or

sacral dorsal roots but not after transsection of the spinal cord. Thus, the sacral parasympathetic reflexes to the large intestine are mediated via a spinal pathway and have an essential role in the initiation of propulsive activity during defecation.[109]

Colonic motility is also regulated in part by postganglionic sympathetic neurons, whose cell bodies, in animals, are located in the inferior mesenteric ganglion. Several studies have demonstrated that the neural activity of these postganglionic neurons results from the synaptic integration of input received from the central nervous system via the inferior splanchnic nerves and from sensory receptors located in the periphery and received via the lumbar colonic, intermesenteric, and hypogastic nerves. Gross afferent input from colonic mechanoreceptors to inferior mesenteric ganglion neurons does not vary with changes in intraluminal pressure until basal pressure exceeds 5 cm water, and increases above that level up to a maximum which corresponds to physiological levels of intraluminal pressure. A major component of this afferent input results from the activity of slowly adapting mechanoreceptors. When propulsive contractions occur, there is a clear correlation between afferent input and intraluminal pressure. Only then does a peristaltic contraction develop. This suggests that colonic intraluminal pressure modulates the genesis of peristalsis and fecal progression, thus at least partly through the sympathetic nervous system. Both peripheral and central preganglionic inputs to the inferior mesenteric ganglion reduce or abolish colonic motility but with varying degrees of effectiveness, [107,110–112] and supraspinal mechanisms are not essential for action.[113]

Application of local anesthesia to the colonic mucosa prevents the occurrence of peristalsis but only when anesthesia is induced before the onset of the reflex.[104] This suggests a reflex which is triggered at the mucosal level but which also involves a deeper plexus. Removal of the mucosal and submucosal layers results in a lack of peristalsis.[114] *In vitro* studies confirm that tetrodotoxin and ganglion blocking agents abolish the peristaltic reflex elicited by an intraluminal stimulus. Sympathetic denervation does not affect propulsive activity, but stimulation of the pelvic nerves facilitates propulsion activity.[15] Local reflexes of ascending excitation and descending inhibition contribute to propulsion.[16]

Food[22,117–119] can affect intraluminal pressure recording. The mechanism for this is at least in part neural.[21,118–121] A rapid increase in rectosigmoidal spike activity occurs after ingesting a standard or fat meal. There is a return to fasting levels, 1h later, and a secondary increase later again only after fat ingestion. Anticholinergics inhibit the early increase in spike activity but not the secondary rise. Introduction of bile acids into the proximal or distal colon elicits a marked motor response in the sigmoid colon.[122] In the rabbit, maximal response occurs within 45 min after bile-acid infusion into the cecum. Infusion into the sigmoid also increases sigmoid motility according to a dose-response curve, and defecation occurs when concentrations exceed 15 mM. The response is much faster than with cecal infusion and begins within 10 min of infusion. Secondary bile acids, normally found in the distal colon exert little effect on motility at that site. Finally, in man, patients with an abnormal primary bile acid excretion have greater motility than those who excrete only secondary bile acids. These studies suggest a major role of bile acids in controlling colonic motility. Since they are closely related to fat absorption, they may be implicated in the late increase in rectosigmoidal spike activity that occurs after a fat meal. Alternatively, hormonal

mechanisms may be responsible for it. Finally, whole proteins and amino acids, but not carbohydrates, modulate the biphasic response of the colon to fat.[123] How these intricate relationships influence not simply changes in bowel tone but propulsion remains unclear. Several drugs[117,124–126] and hormones[35,127,128] can affect intraluminal pressure recordings, and these effects may differ from one part of the bowel to another. Secretin depresses motor activity of the sigmoid colon[35] as does glucagon.[129] Conversely, gastrin and cholecystokinin stimulate the sigmoid colon.[35],[118] Neither hormone affects the rectum. Atropine sulfate and glucagon almost entirely abolish the motility of the colon and rectum.[35] Dopamine stimulates motor function of the sigmoid colon possibly through specific receptors.[130]

Ingested radiopaque markers progress smoothly from one segment of the large bowel of man to the next and are easily visible on plain films of the abdomen. This provides a simple way of assessing clinically the transit through the large bowel. If small bowel motility is adequate all markers are within the confines of the large bowel on the day after ingestion. Bony landmarks can be used to distinguish between right colon, left colon, and rectosigmoid area, although occasionally a large cecum may overlap the pelvic area and the transverse colon may also do so on rare occasions. Data have been obtained in healthy subjects eating a standard high-residue die (0.2 g/kg per day of crude fiber). Mean transit time of the markers can be calculated from the following formula:

$$\overline{\Delta t} = \frac{T}{N} \sum_{i=1}^{j} n_i$$

where $\overline{\Delta t}$ = mean transit time of one marker; T = time interval between films (usually 24 h and must be constant) N = number of markers ingested; n_i = number of markers present in any given site (right colon, left colon, rectosigmoid area); and i to j = days, or successive days, until all markers have gone through the given site.

With this formula, one can calculate a transit time in a segment of large bowel which is site-independent (Table 1). Although total transit through the large intestine is not statistically larger in adults, it appears from the comparison of adults and children that there are segmental differences between the two groups of subjects. Stools of adult volunteers are retained proportionally longer in the colon, while stagnation in children occurs in the rectum. This has bearing on the understanding of the irritable bowel syndrome so prevalent in adult subjects otherwise in good health.[28] Despite the rationalization that psychogenic constipation occurs mainly at the rectal level, where sensation supposedly is abolished because of chronic distension and refusal to defecate, data obtained with markers suggest that storage occurs mainly in the colon. Thus the role of the rectosigmoid barrier is crucial in this regard.

III. DEFECATION

The act of defecation is a complex process that involves conscious and involuntary elements. Normal bowel habits are not known, because establishing them would re-

Table 1. Transit of Radiopaque Markers through the Human Large Bowel

Site	Days after ingestion (n markers) 1	2	3	4	5	6	7	Mean transit time (hr)
				Adults				
Right colon	20	10	4	1	0	0	0	38
Left colon	15	11	8	4	3	1	0	37
Rectosigmoid	9	12	10	7	3	1	0	34
Large bowel	20	20	18	10	5	3	0	93
				Children				
Right colon	14	1	0	0	0	0	0	18
Left colon	12	4	0	0	0	0	0	20
Rectosigmoid	20	12	7	5	0	0	0	34
Large bowel	20	13	7	4	0	0	0	62

quire putting a random sample of a healthy, nonneurotic population on a standard diet for a prolonged period of time during which defecation frequency would be recorded. This has not been done and is difficult to do. Population surveys of bowel habits based solely on questionnaires are of doubtful value, because, although there is a close correlation between data coming from questionnaires and those from diaries, subjects who defecate less than once a day tend to underestimate bowel frequency at interview.[131] Finally, the act of defecation involves elements of sensation which are not taken into account when simply counting stools, weighing stools, or investigating bowel function: the call to defecate, like other physiological "tumescent" feelings, includes some anticipation of pleasant detumescency. Emptying should not be accompanied by straining or pain; finally, following defecation, a pleasant feeling of emptiness and a good mood appears.[132,133] There may be cultural differences, not only related to dietary content in fiber but also in psychosomatic factors.

The limited information available indicates that a normal person defecates at least five times per week and up to twice daily.[64,134,135] Most people defecate once a day. Few normal people skip defecation for two consecutive days[64,131] and none does it three days in a row.[64] Similarly, few people have two or more bowel movements between meals.[131]

Only one study has been performed on normal stool weight.[136] It was done on prison inmates, a sample hardly representative of the normal population. Weight ranged from 35 to 225 g per stool. Healthy volunteers in Japan produce stools weighing on the average 200 g, while similar volunteers in Hawaii, either Caucasian or Japanese, produce stools of around 120 g. This difference is not related to transit time through the bowel, because both Japanese groups have a much shorter bowel transit time than the Caucasian group. These data have been used to suggest that increased stool weight rather than shorter transit time is related to a decreased cancer risk in the large bowel.[137,138] Output is thus probably of key importance in this regard. Stool output (weight x frequency) is increased by the addition of fiber.[99,100] Digestibility of fiber influences its mechanism of action in the human colon: cabbage fiber, which is extensively broken down, provides a readily usable substrate for the stimulation of microbial growth, largely responsible for stool weight,[139] whereas wheat fiber remains

largely undigested and retains water in the gut lumen and thus stool wet weight.[140] Finally, stool production is also influenced by nonfiber elements as demonstrated by a comparison of three defined formula diets.[141] This is possibly related to influence on bile-acid output, which stimulates colonic motility[122] as well as absorption.[142,143]

Stool consistency is highly variable and difficult to appreciate objectively. The composition of feces also varies. Surprisingly, more than half of total solids in stools are composed of bacteria, fiber representing less than 20% of the weight.[139] Finally, in healthy subjects, there are striking variations in wet and dry weight, size, volume, and frequency of stools and in colonic transit time. Fecal water content, however, remains relatively constant.[136,144] Thus, individual values have limited use in colonic function studies unless they lie outside of an established normal range. Of note is the fact that no difference has ever been shown between healthy males and females,[144] although conflicting data have been offered on the influence of phases of the menstrual cycle.[144,145]

The urge to defecate is elicited when a threshold volume is reached in the rectum.[47,81,146] Distension of the descending colon is felt predominantly as gas, while stretching of the rectum induces a sensation of urgency.[147,148] Distension above a low rectal anastomosis still elicits a normal feeling of perineal fullness coupled with a sense of impending evacuation, except when it is done 15 cm from the anal margin, where a sensation of gas or pain is triggered.[67] This suggests that the receptors responsible for the appreciation of impending evacuation must lie outside of the bowel. It is probable that the receptors described in pelvic floor muscles play a major part in the sensory pathway.[149,150] Rectal sensation normally reaches the conscious level through parasympathetic innervation. But when it is deficient, as in spinal man with a sacral lesion,[71] not all sensation is abolished and rectal filling is perceived as a vague abdominal discomfort. The message is then carried through sympathetic innervation and is known to disappear when a high spinal transsection is present.[71] When rectal distension is maintained, all electrical activity from the striated muscles stops.[71] This reflex is mediated through the pelvic autonomic nerves. During voluntary defecation, intraabdominal pressure increases, resulting in a mechanical change at the anorectum. The anorectal angle decreases during straining,[151] and the rectum takes a funnel shape, with shortening of the anal canal.[56,152] Straining to defecate produces a much greater pressure in the abdomen than in the chest.[153] One may thus theorize that the higher incidence of hiatus hernia in Western countries may be related in part to prolonged periods of straining on hard stools.[154] Straining causes a profound inhibition in muscle activity of the pelvic floor following a transient increase. Flexion of the hips, as in the squatting position, straightens the anorectal angle further [151] and facilitates defecation. After the stool is expelled, the pelvic floor ascends to its normal position and its myoelectrical activity resumes. This reflex persists in spinal man.[84] When the autonomic innervation is absent, defecation is autonomous.[72]

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8

Bulk Agents in the Colon

M. A. Eastwood and J. A. Robertson

Food can have many effects along the gastrointestinal tract, one of which is the distension caused by the bulk of the food. In the colon, residual food, yet to be digested, will produce a bulking effect believed to be involved in the physiological activity of the colon and the production of fecal material.

1. FIBER AND FECES

Feces are a complex mixture of microorganisms, undigested food residues, soluble ions and organic compounds, and water. Studies on microbial flora and their metabolism are numerous[1-4] and almost without exception have considered the fecal mass as a homogeneous mixture from which the various components can be isolated for study.[5] Microscopy shows that this is not in fact the case.[6] The matrix of feces contains a large number of bacteria, intermingled with smaller or amorphous particles of food residue. Some of the bacteria are grouped in colonies. Within the fecal mass, fragments of plant cell residues are embedded. Bacteria can be observed within areas of damage on the outer seed coat of fiber residues, suggesting that extracellular bacterial enzymes may be responsible for the production of these changes in the fiber.

It is not known what makes for the stool bulk. Clinicians and physiologists agree, however, that as far as diet is concerned, fiber has a greater effect on stool volume than protein, fat, and carbohydrates[7,8].

The effect of fiber on stool weight varies with the fiber source and from person to person[7,9] Originally the fermentation of carbohydrates in the cecum, with the release of short-chain fatty acids, was believed responsible for stool bulk. Slowly this concept changed to one in which it was believed that the water-holding capacity (WHC) of the fiber was all-important. This view has been reinforced by the observation that bran

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preparations of different WHC had effects on colon function that paralleled the WHC of that bran. Fine bran with a low WHC had less effect on colon function than coarse bran, which had a higher WHC.[10,11] Williams and Olmsted suggested many years ago that the fermentation products of polysaccharides had a laxative effect.[12] These fermentation products are likely to be gases and fatty acids. Williams and Olmsted suggested that these provided a chemical stimulus to the production of stool, aided by the hygroscopic quality of the residue escaping degradation. The agent responsible for stool weight is less likely to be volatile fatty acid, since it has been shown that there is rapid absorption of short-chain fatty acids from the colon.[13] However, studies which show absorption of fatty acids from dialysis bags may not be the same as the absorption of fatty acids from the dense heterogeneous system that constitutes the colonic contents.

It seems likely that the ability of a particular fiber to resist fermentation is related to its final fecal bulking effect. Cummings et al[9] have suggested that it is the pentosan-containing fractions of the polysaccharides in dietary fiber which are most resistant to bacterial hydrolysis. This suggestion is based on experiments with dietary fiber concentrates. In a further study Stephens and Cummings[14] have shown that the bacterial content of the stool may be an important factor in dictating stool weight. They suggest that the fecal bulking effect of bran is due to its WHC, whereas fiber from fruit and vegetables may influence stool weight by increasing the fecal flora mass. Bornside[15] concluded that diet had no effect on the major groups of bacteria and their numbers per gram of feces. However, the fecal mass increases with dietary supplements of fiber; therefore the total number of bacteria excreted daily is directly related to dietary fiber.

Hellendoorn[16] is not convinced of the importance of the relationship between fiber WHC and feces production and suggests that the fiber percentage diminishes during transit through the bowel. He believes that volatile fatty acids, lactic acid, and bacterial mass largely account for the fecal mass and that the bulking capacity of fiber in feces is not caused by the WHC of the pentosan fraction. The bulking action of lactulose results from fermentation and proliferation of bacteria.

The effect of the products of bacterial metabolism on the production of stool mass is likely to be complex. Stephen and Cummings[5] have commented on the high nitrogen content of feces (6% of the dry weight), which suggested to them that the bacterial component of fecal matter may be larger than previously thought. Previous estimates of the bacterial components of the wet fecal mass were 30–40%[17]. These estimates were based on direct microscopic counts, which were then converted to a weight, assuming an average size of the bacteria. Stephen and Cummings[5] developed a method that fractionates feces into three main components, believed to be bacteria, undigested fiber, and insoluble substances. Such a method has the advantage of not needing to calculate the weight of the bacteria. Separation techniques, however, have the great problem of removing bacteria from other materials. Bacteria adhere to fiber, which is broken down to small particles that may be indistinguishable from bacteria. There is also the possibility that any method may be diet specific, working in a particular environment but not in others. This is certainly the finding of the ruminant physiologists.[18] However, Stephen and Cummings' results suggest that in the feces of nine healthy subjects on a metabolically controlled British-type diet, bacteria formed 55% of the total solid, fiber

17%, and soluble materials 24%. These findings are in keeping with an important, consistent factor in stool production: the stool weight is always about 75% water. Bacteria are 80% water, which suggests that the constancy of fecal water is dictated by bacteria.

II. FECAL BULKING AGENTS

Fecal bulking agents are generally of plant origin and are generally categorized as dietary fiber. The main sources of fiber are cereal bran, fruit, and vegetables. Less important nutritionally but important in the catering and pharmaceutical industries are gums, mucilages, and polysaccharide isolates, e.g., pectin (Table 1). The manner in which these complexes increase stool weight has yet to be defined but is probably related in some way to the hydration properties of each preparation.[19,20] The difficulty in understanding the function of fiber is due in part to diverse physical properties of the bulking agents. For example, cellulose is insoluble in water and will have properties which are those of surfaces, but carboxymethylcellulose is water soluble and will have concentration-related properties in solution. These colligative properties include osmotic pressure. A slight chemical change therefore can have consequences for the properties of bulking agents. Dietary fiber, a mixture of water-soluble and water-insoluble material, will also have a combination of surface and colligative properties; these may change with temperature, with pH, with osmolality, or after partial degradation by bacteria.

III. DIETARY FIBER AND THE PLANT CELL WALL STRUCTURE

Dietary fiber is mainly plant cell wall material, defined as a plant polysaccharide and lignin that are resistant to hydrolysis by the digestive enzymes of man.[21] Although many polysaccharide isolates and gums are not of plant cell wall origin they are nevertheless considered by some to be dietary fiber.

Table 1. Bulk-Producing Polysaccharides

Fiber type	Example	Source
Cereal	Grain	Bran
		Bread
Fruit	Apple	—
	Orange	—
Vegetable	Carrot	Root
	Cabbage	Bud/leaf
	Potato	Stem
	Beans	Seed
Gum	Gum arabic	Plant exudate
	Guar	Seed endosperm
Mucilage	Ispaghula	Seed coat
Isolate	Pectin	Plant cell walls
	Cellulose	Plant cell walls
	Carrageenan	Algal cell walls

The major polysaccharides in fiber are cellulose, hemicellulose, and pectins, while the major nonpolysaccharide materials are lignin, waxes, cutin, inorganic ash, and protein. Starch may also contribute to the dietary fiber, depending on the degree of breakdown of the starch granules during cooking.[22] The components of fiber each have their own specific function in the cell wall and are intermeshed for anatomical and physiological function in the plant. The physical properties important in the plant structure are retained when eaten and will produce the physiological effects of fiber in nutrition.[20] The cell wall is a rigid structure that encloses the cell.[23] It consists of a middle lamella, an inner primary cell wall, and an innermost secondary cell wall. Each is produced in an ordered sequence beginning with the middle lamella. The middle lamella is particularly rich in pectins and is the point of origin of lignification; the primary cell wall contains pectins and hemicelluloses enmeshed in a randomly arranged network of cellulose; microfibrils and the secondary cell wall are mainly an ordered arrangement of cellulose microfibrils in a matrix of hemicelluloses. Lignification of the cell wall progresses from the middle lamella toward the secondary cell wall, reduces the plasticity of the wall, and is usually associated with cell death. Dietary fiber consists almost exclusively of secondary wall material. Other suggested components of dietary fiber include gums produced as a plant wound response; mucilages that help prevent desiccation of seeds; and polysaccharide isolates, resulting from the chemical modification of what originally may have been cell wall material.

IV. CHEMISTRY OF FIBER

The chemistry of gums, mucilages, and polysaccharide isolates has been extensively studied because each is commercially important in the food and pharmaceutical industry.[24,25] The complex structure of plant cell walls make their chemistry more difficult to define, but cell walls can be considered to consist of the polysaccharides cellulose, hemicellulose, and pectins, and the phenyl propane polymer lignin.[26] Cellulose is a β-1-4-linked glucan of 3000 or more residues and is arranged in microfibrils. Hemicelluloses are a mixture of linear and branched polysaccharides each with 150–200 sugar residues. The sugar residues may be either pentoses or hexoses, and uronic acids may also be present. Pectins are similar to hemicelluloses but consist mainly of glucuronic and galacturonic acids. Lignin is a complex cross-linked polymer formed by the condensation of phenyl propane monomers.[27] The three main monomers present are sinapyl, coniferyl, and coumaryl alcohol. Condensation of these monomers forms a rigid three-dimensional network through the cell wall. Only a trace of lignin is present in most fiber sources, since lignification is associated with plant maturity, and most fiber sources are used when the plant is succulent. Cereal grains, the major exception, are also the most lignified sources of dietary fiber.

Chemical changes in cell wall composition, such as lignification and the change from a primary cell wall to a secondary cell wall, occur not only with increasing developmental age but also between species.[28,29] Thus carrot and cereal bran have a very distinct chemical composition. Differences in chemical composition between varieties have not been found, although recently introduced improved varieties of vegetables may often appear morphologically different from the original cultivar.[29,30,31]

V. THE MORPHOLOGY OF FIBER

Differences in chemical composition can also result in differences in cell wall anatomy,[32] as shown in Fig. 1. In all examples the cell structure is at least partially retained. In potato the cells are relatively large and the fiber appears succulent, but in bran the cells are smaller and the fiber appears more coarse. Bran also shows evidence of lignification of the outer cell layers, but lignified tissue is rarely found in the vegetable organ of the potato. Bagasse, which appears to be very fibrous, is highly vascularized and shows little evidence of cell structure. Gums, mucilages, and polysaccharide isolates will have no distinctive structure except that conferred by processing to produce flakes or granules.

VI. THE PHYSICAL PROPERTIES OF FIBER

Differences in cell wall structure and chemical composition will result in differences in the physical properties of the cell walls,[33] which in turn will be different from those of polysaccharide isolates, gums, and mucilages. The physical properties of fiber

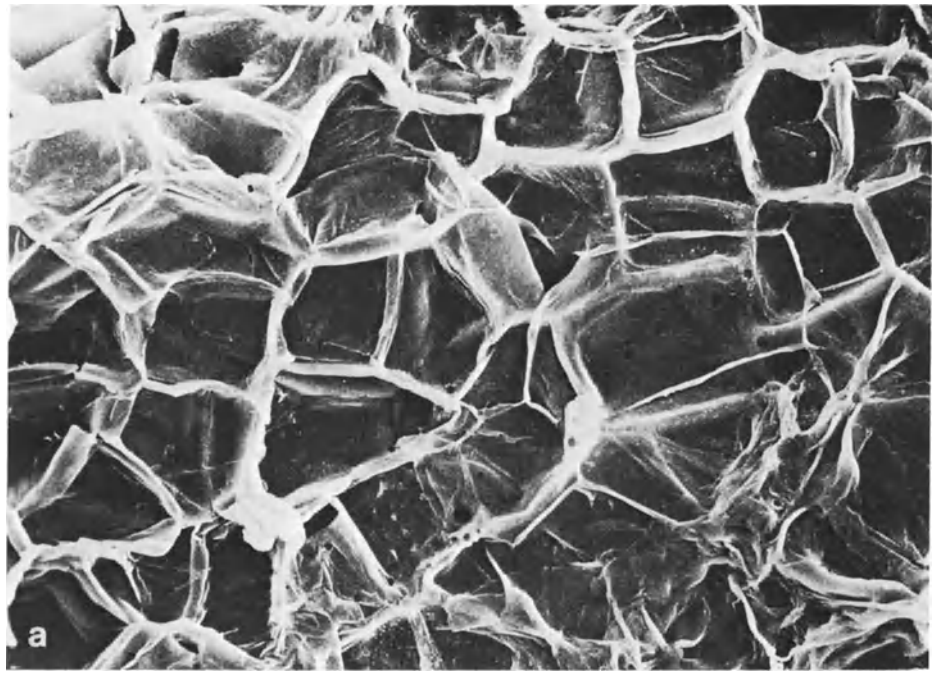

Figure 1. The structure of dietary fiber under scanning electron microscope. (a) Potato. The cell wall material still has intact cell walls, although much cellular disruption has occurred during preparation. (b) Bran. Starch granules have been removed from the preparation with neutral detergent solution to make the structure of the bran more evident. (c) Bagasse. No cell structure can be seen. The bagasse fiber appears as highly vascularized plant cell wall preparation composed of xylem vessels with secondary thickening (×200). 35 mm is calculated at 100 μ.

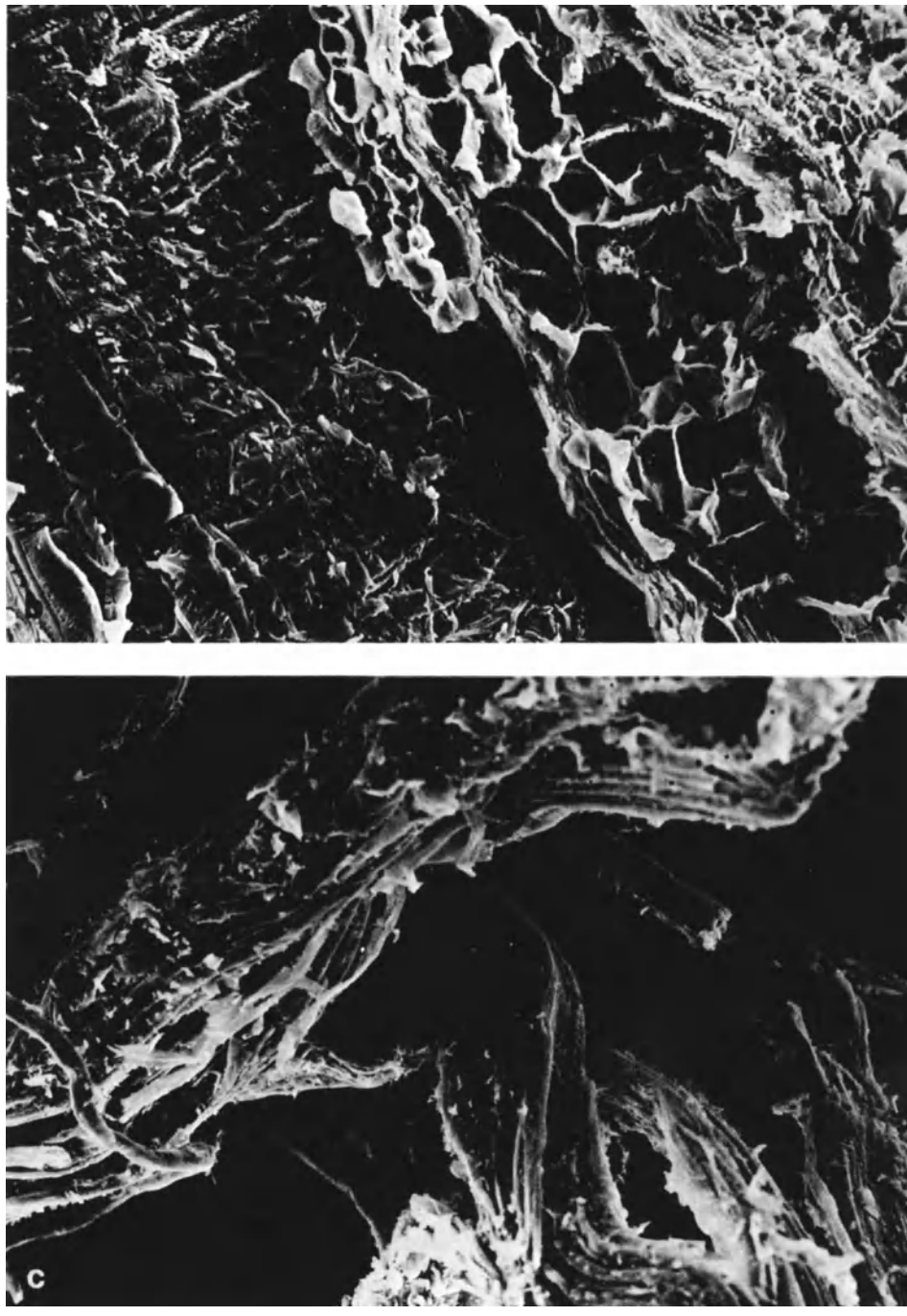

Figure 1. (*cont.*)

will be a combination of the colligative properties of the water-soluble fiber components and the surface properties of the water-insoluble fiber components. These properties include WHC, cation exchange capacity, organic adsorptive ability, gel filtration phenomena, and particle size distribution.[34] Each property is related to the other properties. For example, it has been suggested that dietary fiber can act as an ion exchange column and as a sieve chromatography column in the colon.[35] The cell wall provides a solid matrix through which the soluble contents of the gut can flow; and soluble fiber sources will provide a fluid matrix. The gut flora and the fiber itself can modify the gut contents, as occurs in the bacterial transformation of glycocholate to deoxycholate by colonic bacteria. Such modified substances can be toxic, as has been shown for cyclamate metabolites,[36] but the presence of fiber can reduce their toxicity.[36–38] This can be effected either by adsorption of the toxin to the fiber, or by dilution of the toxin concentration due to the increased bulk in the colon. The increased bulk may also help to reduce intestinal transit time and thereby also reduce the time of contact between toxin and gut mucosa. The bulking effect of the fiber may, however, be altered by the fermentation of the fiber by colonic bacteria.

A. Cation Exchange

Fiber can protect against the effects of certain cations, such as strontium,[39] probably because of the cation exchange capacity of the fiber. The cation exchange capacity of dietary fiber is a property of the cell wall, and the type of exchange varies from strong and monofunctional to weak and polyfunctional depending on the fiber source.[33] The cation exchange capacity of vegetable fiber has been shown to increase with developmental age[40] and depends on the species of cation exchange.[41] The cation exchange capacity of fiber is similar to that of commercially available weak ion exchange resins, i.e., of the order of 1–2.4 meq/g fiber.[40] Gums, mucilages, and polysaccharide extracts also have a cation exchange capacity, but their solubility often makes measurements difficult.

The variability of the cation exchange capacity and the absence of an anionic exchange capacity suggests that the exchange is due to groups, such as carboxyl groups, though it is not clear which cell component is responsible for the cation exchange capacity.[42] Presumably, different cell preparations with different composition owe their exchange properties to the proportion and conformation of ionic groups in the fiber source. Fiber samples with a high cation exchange capacity, such as carrot,[33] tend also to have a high WHC due to water associated with the anionic groups and hence may also be good bulking agents. The cation exchange capacity of vegetable fiber does not alter electrolyte metabolism,[43] which suggests the property is lost in the gut as a result of fermentation by bacteria. This could also affect the bulking ability of these preparations.

1. Organic Adsorption

Fermentation by bacteria may also affect the organic adsorption ability of fiber preparations. Adsorption ability has been investigated mainly using bile acids,[44–47] since bile acids are involved in cholesterol metabolism and the bacterial metabolites of

bile acids may be important in the colon.[48] Many fiber preparations have been shown to have an adsorption ability,[44,47,49,50] the adsorption being pH dependent and greater for more hydrophobic materials. Adsorption is a surface phenomenon and therefore may be affected by particle size and fermentation by bacteria. Pectin, however, which is water soluble, can affect cholesterol metabolism.[51,52] This suggests that other factors besides adsorption may figure prominantly in the interaction between fiber and the other colonic contents. One possibility is that apparently, adsorbed substances are actually bound to the fiber; but since almost complete recovery of bile salts from fiber can be achieved by solvent extraction, this seems unlikely.[53]

Compounds apparently adsorbed, however, may just be held in solution by the hydrated fiber, forming a gel. The stronger the gel, the slower the rate of diffusion from the gel and the greater the apparent adsorption to the fiber. It is not known if true adsorption or gel filtration is most important in the colon. Adsorption to fiber will remove potential toxins from the soluble phase of the colonic contents, and gel filtration will reduce the rate of diffusion of potential toxins toward the gut mucosa. Both mechanisms will reduce the amount of contact between toxin and gut mucosa. Since it is the water-soluble fiber preparations that can form gels, gel filtration is likely to be associated with increased bulk in the colon. This assumes that the gel is not destroyed by fermentation.

a. Water-Holding Capacity. The ability of a fiber source to form a gel may be considered to be the ability of the fiber to retain water. This can be measured as the WHC of the fiber. Various methods have been used to measure WHC,[33,40,54–57] but few have considered how the water is actually held by the fiber. Centrifugation has shown that fiber from different plant species has a distinct WHC and that vegetable fiber has a high WHC and cereal fiber a lower WHC.[33] The WHC of a fiber source is not affected by age or plant variety,[40] but fiber particle size does affect the results[10] of adding fiber to the diet. Similar results for WHC are found after filtration of corresponding fiber preparations, which supports the hypothesis that the bulking effects of dietary fiber are due to their ability to retain water in the colon.[19,20,58] However, an inverse relationship has been shown between the measured WHC of fiber and the ability of fiber to increase stool weight.[59] One possible explanation for this is that in fiber preparations with a high WHC the water is only loosely associated with the fiber and is effectively free water, whereas in fiber preparations with a low WHC the water is very strongly held. Measurements of WHC against a known suction potential can show whether this is the case.[60] When applied to dietary fiber the results obtained differ from the WHC determined by other techniques, as shown in Table 2. A small change in suction potential can cause a large change in the WHC of a fiber preparation. At a suction potential of only 0.04 atm (1 atm = 1.013×10^5 Nm^{-2}), the WHC is similar to the WHC measured by filtration. The WHC of water-soluble fiber sources can also be measured by this method, and these appear to have a WHC similar to that of vegetable fiber. A pressure difference of 1 atm has an effect on the WHC of fiber, especially vegetable fiber. Most of the water in vegetable fiber must therefore be only loosely associated with the fiber. A suction potential difference of 1 atm does not have as great

an effect on the gel-forming polysaccharides, and even a difference of 10 atm results in the WHC of the gel-forming polysaccharides still being twice the WHC of the other fiber sources. At a suction potential difference of 10 atm the WHCs of cereal fiber and of vegetable fiber are very similar.

In the colon, therefore, all dietary fiber preparations could have the same effective WHC, depending on the magnitude of the forces involved in water uptake from the colon, and hence could have the same bulking effect. The differences in bulking effects found for different fiber sources could be explained by fermentation and also by the ability of fiber to hold water in the absence of a pressure difference. For example, 100 g of hydrated fiber taken daily could contain 25 g bran or only 4 g carrot fiber. Theoretically, if a 10-atm difference in suction pressure was involved in water uptake from the colon the effecitve WHC of the fiber would then result in a bulking effect of 50 g for bran but possibly only 12 g for carrot.

The water content of feces is usually 75% fresh weight, i.e., WHC = 3; this might argue against a large force being required for water uptake from the colon. Table 2 lists the suction pressure required to change the WHC of a fiber preparation from that measured by filtration to a WHC of three. Very little pressure is required to change the WHC of cereal fiber, but, then, the WHC of cereal fiber is originally almost 3. A pressure difference of approximately 1 atm is required to change the WHC of vegetable fiber, but over 5 atm difference is required to change the WHC of water-soluble fiber preparations. This can also be illustrated by regarding all fiber preparations as water soluble and investigating how storngly each binds water (see Fig. 2).

The more strongly water is held by the fiber, the greater the gradient of the curve describing the water loss.[61] This implies that water-soluble fiber sources such as gum arabic bind water more than other sources of fiber. Potato fiber, for example, is also a fairly avid binder of water. Bran, however, may experience only a small pressure increase to result in water loss. This is consistent with the fact that bran is water insoluble and hence has the properties of a surface whereas other fiber sources are either partially

Table 2. The Water-Holding Capacity of Various Fiber Preparations[a]

Sample	Water-holding capacity					ΔP^b (atm) (WHC =3)
	Centrifugation	Filtration	ΔP^b = 0.04 atm	ΔP^b = 1 atm	ΔP^b = 10 atm	
Pectin	—	—	21.1	11.1	3.2	10.0
Gum arabic	—	—	7.7	5.7	2.6	6.6
Carrot	24.9	22.8	18.0	4.3	1.8	0.9
Potato	23.6	16.5	13.7	3.3	1.7	1.2
Bagasse	12.2	6.0	7.5	2.6	1.5	0.5
French fine bran	6.4	4.1	3.4	1.6	1.2	0.0
French coarse bran	9.1	5.0	3.6	1.6	1.1	0.1
AACC bran	5.8	3.0	4.2	1.4	1.0	0.1

[a]Results are the mean of determinations made in duplicate.
[b]Δ P, suction pressure.

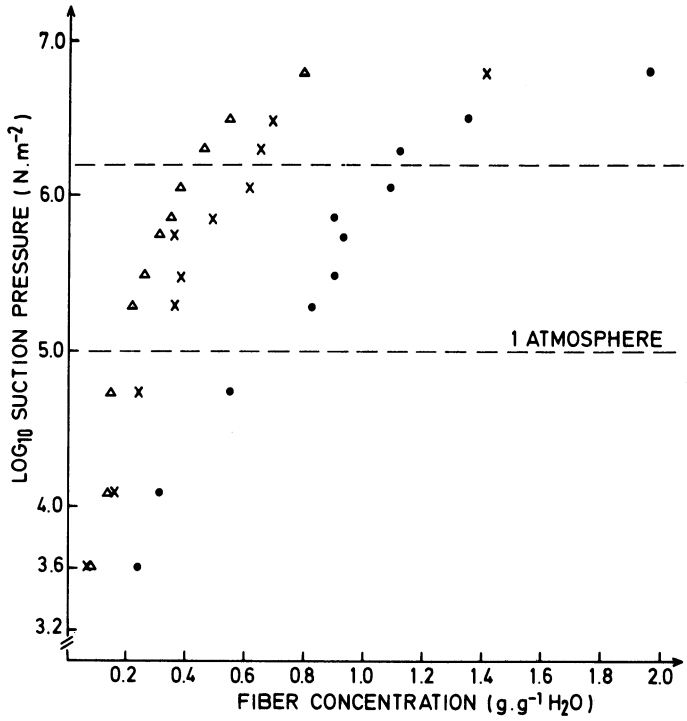

Figure 2. The effect of suction pressure on the hydration of dietary fiber. Δ, Gum arabic; × potato fiber; •, AACC standard bran. Results for bran are significantly different from the results for gum and potato ($p < 0.05$). Suction pressure measurements above log 6.2 Nm^{-2} cannot be determined accurately and are extrapolated from measurements made up to log 6.2 Nm^{-2}.

or completely water soluble and therefore require a reduction in their solubility before water can be removed.

For all fiber sources investigated, fiber concentration is apparently directly proportional to $\log_{10}$ suction pressure up to a suction pressure of 1 atm ($\log_{10}$ suction pressure = 5 Nm^{-2}), but the gradient of water loss depends on the fiber source. Water-soluble fiber preparations lose water at a constant rate up to a suction pressure of 10 atm, but for plant fiber there is a reduced loss of water between 1 and 10 atm suction pressure. This implies that free water is removed from cellular fiber up to a suction pressure of 1 atm. Thereafter only bound water is present, which is more difficult to remove.[62] With water-soluble gel forming fiber material such as gum arabic, the results suggest that water can be steadily removed up to a suction pressure of 10 atm.

Beyond a suction pressure of 10 atm, water can be removed from all fiber sources. This implies that some bound water can also be removed from the fiber and agrees with the concept that water is held by fiber in different phases,[63] namely, water bound by the fiber, water associated with bound water in the fiber matrix, and free water trapped within the fiber matrix.

Water bound to the fiber will be difficult to remove, and the proportions of loosely bound and free water will vary with the fiber source. Bran, which is very insoluble,

will contain little bound water, whereas vegetable fiber, which is partially water soluble, will contain a higher proportion of loosely bound water. Little suction pressure is thus required to produce a highly concentrated bran solution, but a relatively high suction pressure is required to achieve the same result for vegetable fiber. For water-soluble polysaccharides the formation of a gel requires the water to become an integral part of the gel[61]; hence a high suction pressure is required to concentrate the gel.

b. Flow Rate Considerations. A further estimate of the ability of fiber to retain water can be obtained by measuring the flow rate of water through the fiber when it has been packed into a column as in chromatography. The flow rate at a given pressure difference will depend on the mean pore size of the fiber and the affinity of the fiber for water.[64] A fiber source with a high affinity for water will have less free space for water within the fiber matrix, which will affect the pore size distribution and hence the flow rate. Table 3 lists the flow rate of solution through various fiber preparations. Apparently cereal fiber, with the lowest WHC, allows the highest flow rate, which can be 10 times that through the other fiber preparations. Bagasse and cellulose have a similar flow rate, but carrot has a flow rate significantly less than that of cellulose. Potato fiber has a flow rate significantly less than that of the other fiber preparations. The flow rate is not related to packing density.

The high flow rate and low WHC of bran suggest that when bran is present with the liquid phase of the cecal contents the result is a free solution passing through a fiber matrix. There will be no inhibition of either water or nutrient uptake from the colon, and the movement of water may modify fermentation. Bran will act therefore as a bulk-producing support material in the colon and act as a sieve to collect nonabsorbed material and bacteria. This will be concentrated in the colon and contribute to the formation of fecal mass.

The slower flow rate of solution through vegetable fiber will result in a reduction in the absorption rate of nutrients from the cecum and colon. This will increase the concentration of nutrients in the cecum, which will favor increased fermentation. The slow movement of water through the fiber matrix will favor a localized high concentra-

Table 3. Flow Rate of Water Through Various Fiber Preparations[a]

Sample	Packing density (g/ml)	Flow rate (ml/hr)
Cellulose	0.25	60
AACC bran	0.16	550
French fine bran	0.16	460
French coarse bran	0.15	430
Bagasse	0.10	45
Carrot	0.08	34
Potato	0.09	11

[a]Flow rate was measured as milliliters of solution collected per hour, and results are the mean of three determinations. Density was measured as grams of fiber per milliliters of column. Fiber was packed under a weight of a 60-g packing rod. Ascending flow of solution with a pressure difference of 0.1 atm was used.

tion of bacteria and bacterial enzymes and hence bacterial proliferation to utilize the fermentation products. Such a localization of fermentation may also result in the destructive fermentation of the fiber matrix. With gel-forming polysaccharides the situation will be more defined. Absorption will be very slow, and fermentation of the fiber will be effectively complete. Therefore, although vegetable fiber and gel-forming polysaccharides may have a high WHC the gelation properties of the fiber could result in its increased fermentation, which could explain why the measured WHC of fiber may bear an inverse relationship to the bulking effect of the fiber in the colon.[59] Cereal fiber may have a direct bulking effect on the colon, but the bulking effects of other fiber sources may result from other mechanisms.

VII. NORMAL STOOL

What constitutes a normal or even an ideal stool weight has not been defined. Variations in stool weight in different communities may be largely a consequence of diet, though other factors such as an individual response to that diet are also involved. Most data for stool weight are recorded for small groups and vary greatly. The average stool weight for young adults eating an average Western diet was 100–150 g/day,[65–67] for British vegetarians was 225 g/day,[68] and for Dutch students eating a diet rich in fruit and vegetables and with whole-grain bread was 184 g/day but only 69 g/day if fiber-rich foods were excluded.[69]

One hundred white South African students eating considerably more fruit than is customary in the United Kingdom had a daily stool weight of 173 g.[68] One of the most extensive studies recorded was that of Rendtorff and Kashgarian,[70] who recorded stool weight in men interned in a prison in Texas from 1950 to 1953. One hundred fifteen men were studied and 8267 stools collected. Of course, this study may not be typical for all Texans. The average stool weight of these individuals was 124 g $\pm$ 40 g. A study of 80 normal individuals in North Edinburgh, Scotland, showed the great variation in stool weight in a population.[71] The results ranged between an output of 11 and 280 g/day. Age had no effect on stool weight, but the range between individuals was considerable. This had also previously been described in a study[66] in which 20 healthy subjects eating normal diets made repeated 5-day stool collection. The size of the individual stools varied by up to or more than a 10-fold factor.

Clearly the response of the bowel to fiber is variable. If an adult is put on a residue-free diet, then stool weight falls considerably.[69] With a baby, however, as pediatricians suggest, a pint of milk results in a pound of feces. Therefore there may be differences in colonic function in the baby, the child, and the adult. Such differences have not been explored. Williams and Olmsted[7] showed that the high-protein or high-fat diet had little effect on stool weight. A diet containing a considerable amount of sugar increased stool weight, although this was studied in only three subjects. Williams and Olmsted ascribed this to the laxative effect of sugar breakdown products. Overall, however, they pointed out that dietary fiber was the most effective dietary constituent to increase stool weight. They believed that the hemicelluloses were the most efficacious fraction of the diet in influencing stool bulk. A large number of other studies have shown that stool weight is most effectively increased by cereal bran.[72,73] In the North

Edinburgh Study[71] no relationship was found between stool weight and the total calorie, protein, and fat content of the diet. However, the total dietary polysaccharide hexoses were closely related to stool weight. A much poorer relationship existed between total calories and polysaccharide pentoses.

Fig. 3 summarizes a number of experiments in which bran and fruit have been added to the diet. Table 4 summarizes the experimental conditions.

VIII. DILUTION OF FECAL CONTENTS

Stool weight increases in a variable manner in response to adding fiber to the diet. An increase in stool weight does not always result in a corresponding increase of all fecal constituents. The consequence of an enhanced fecal bulk, therefore, is to increase the water content of the stool and to decrease the concentration of other fecal contents. Dietary fiber may have the effect of diluting fecal constitutents.[74] It was observed that feeding 16 g of cereal bran daily for 4 weeks to eight subjects resulted in a considerable decrease in the concentration of fecal constituents (e.g., bile acids). This has since

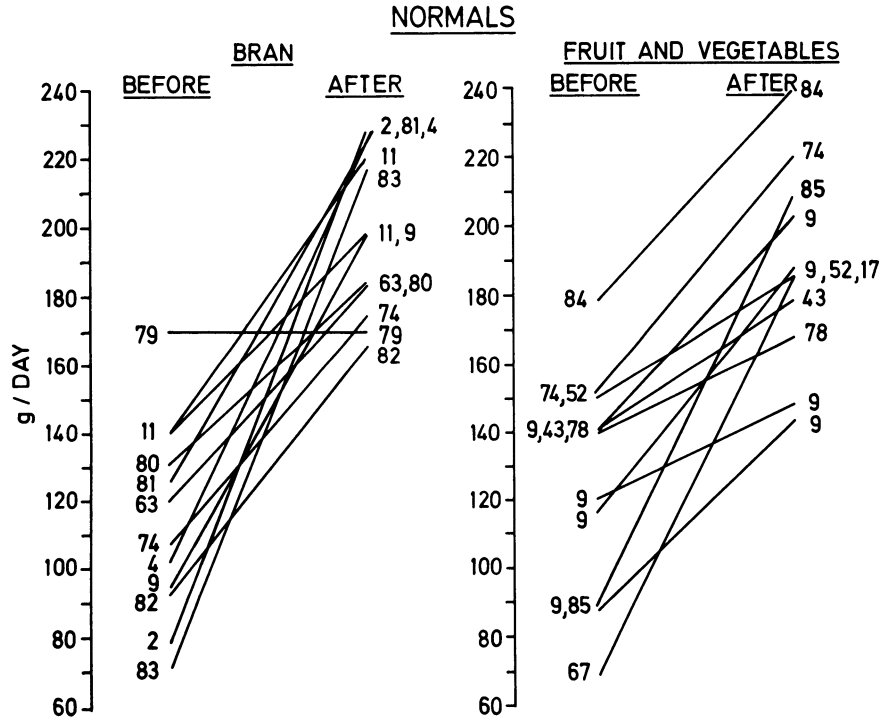

Figure 3. The effect of bran or fruit and vegetables on stool weight before and at the end of a period of ingesting this enhanced fiber intake (mean value). The number refers to the reference from which the information was taken.

Table 4. Effect of Fiber on Stool Weight (in Normals)

Reference	Number of subjects	Fiber weight	Period	Initial fecal weight	Final fecal weight
			Bran		
79	6	2 oz.	30 weeks	170 128–212	171 125–215
74	8	16 g	3 weeks	107±44	174±51
63	6	20 g	4 weeks	120±18	183±22
80	10	Cooked: 12 g	14 days	131±17	12 140±9 20 164±20
		Raw: 20 g			12 183±21 20 159±13
2	6	17–45 g	3 weeks	79±7	228±30
81	6	23–35 g	3 weeks	125 (59–194)	225 (154–290)
82	6	38 g	7 days	93±10	166±15
4	6	3 oz	3 weeks	103±40	226±90
11	28	20 g	3 weeks	140	Coarse: 220 g Fine: 199 g
9	6	20 g	3 weeks	95	197
83	6	36 g	3 weeks	71±6	217±12
		Fruit and vegetable fiber sources			
9	6	Carrot concentrate (20 g)	3 weeks	117	189
		Cabbage concentrate (20 g)	3weeks	88	143
		Apple concentrate (20 g)	3 weeks	141	203
		Guar concentrate (20 g)	3 weeks	120	139
84	6	Plant fiber (60 g)	4 weeks	177±40	240±35
85	12	Fruit and vegetable 20 g N.D.F.	26 days	89±9	208±9
78	9	Citrus pectin (15 g)	3 weeks	140	168
52	12	Pectin	3 weeks	150±10	186±15
43	5	Carrot (6 g)	3 weeks	142±37	177±33
67	43	Fruit and vegetable 50%; bran 50% (20 g)	3 weeks	69±50	184±75
74	8	Cellulose 16 g	3 weeks	152±32	221±58

[a]Weights are given as either mean ± or mean and range.

been confirmed in other studies, with normal subjects[75] and with patients with diverticular disease.[76] A dilution of colonic contents presumably coincides with a diminution of any deleterious effects of a concentrated stool.

IX. FECAL CONSTITUENTS

Fiber affects the stool by increasing its weight, largely by increasing the amount of water in the stool. However, water remains a constant percentage of stool weight.[66]

The fecal excretion of fat, nitrogen, minerals, and bile acids can rise when fiber is added to the diet. The extent of the increase depends on the type of fiber added.[77] Pectin, psyllium seed colloid, large amounts of cellulose, and certain legumes enhance fecal bile-acid loss. Wheat bran has no effect on fecal bile-acid excretion, but in two studies bile-acid excretion increased 3–4 weeks following the period of bran administration.[74,78] This suggests that the adsorption of bile acids to fiber is a less important factor in the fecal excretion of bile acids. Wheat bran reduces the deoxycholic acid content of bile, which suggests that this fiber may reduce deoxycholic acid absorption or formation by colonic bacteria.[76,86–88]

It is possible therefore that the main effect of dietary fiber on the excretion of bile acids is accomplished by the cecum's dictating whether or not bile acids are reabsorbed or excreted in the feces.

X. MICROFLORA OF THE COLON

The total microflora of the colon appears to be relatively stable in both absolute and relative amounts of bacteria.[15] The variation appears to be throughout the colon and also between the lumen of the colon and close to the mucosa.[89] The feces contains bacteria capable of digesting polysaccharides.[90–92] The microflora is apparently stable, and even changes in diet such as dietary supplements of fiber have little effect on the flora. Fiber appears to have no effect on the microflora.[93] Bornside[15] has shown, however, that while increased or decreased dietary fiber has no effect on the numbers and major groups of fecal bacteria, the total output of fecal bacteria is related to dietary fiber. The total number of bacteria per gram of feces therefore remains constant, but the daily fecal mass increases with added dietary fiber and decreases in its absence.[3,94]

XI. THE DIGESTION AND UTILIZATION OF FIBER

Southgate and Durnin[95] showed that a large proportion of dietary fiber was in fact hydrolyzed during passage along the gastrointestinal tract. The hydrolysis of fiber in the cecum was very important in so far that this means some fiber can pass intact along the gastrointestinal tract to the cecum. In the cecum some fiber, e.g., pectin, may be hydrolyzed to unknown moieties of small molecular weight. Other sources of dietary fiber, mainly cereal fiber, appear to pass through the gastrointestinal tract almost unfermented. It is believed that dietary fiber is hydrolyzed to end products such as volatile fatty acids, hydrogen, and methane. Hydrogen is produced by bacteria in the cecum through the metabolism of polysaccharides and oligosaccharides.[96] Breath hydrogen and methane can be measured as an indirect method of investigating metabolism of fiber.[97,98] The source of methane has yet to be elucidated. Hemicellulose, raffinose,

and lactulose increase hydrogen production, while cellulose, pectin, and lignin do not.[97] Hemicellulose-fermenting bacteria are the principle bacteria fermenting fiber in human feces, though there are some cellulolytic bacteria.[90,99] It has also been observed[92] that many species of anaerobic bacteria from the human colon are able to react to constantly changing mixtures of complex carbohydrates entering the colon. Therefore, the same microbial flora can have very different overall metabolic activities, depending on the diet of the host.

XII. STOOL CONSISTENCY

Stool consistency has been regarded as one of the important factors in the symptomatology of constipation. Most studies in which stool consistency has been reported rely on a subjective comment. Such comments, however, have little value in comparing areas of high and low prevalence of colonic disease. It is commonly thought that fiber softens the stool when it is initially hard, but this has yet to be quantified. Often in these studies there is a confusion between consistency, shape, and texture of the stool.

XIII. INTESTINAL TRANSIT TIME

Intestinal transit time is the time taken for the passage of material from the mouth to the anus. It is possible to estimate the emptying time of the stomach,[100] the transit time to the cecum,[101] and also the colonic transit time.[102] The overall transit time varies considerably from person to person, and even in an individual the transit time is not constant.[103] The usual method of measuring transit time is with radioopaque markers.[104] The results from experiments in which Hinton markers were used to measure transit time are not precise.[105] Differences in transit time on the order of 46 hr are necessary to show a significant difference between individuals in the same trial, or 19 hr for comparisons of means of groups of six people or replicates. It has also been shown that the variability of transit time in 10 subjects repeatedly tested was considerable, with a coefficient of variability of 28.[66] The mean transit time in these studies of Wyman et al was 82 $\pm$ 17 hr, and some subjects showed a range of between 47 and 143 hr.

The radiopaque markers are solid substances and behave like solid particles in the stool and not as liquids. Markers of the liquid and solid phase pass along the intestine at different rates.[73] Improved methods to measure transit time give markers over several days.[77] In Western countries the transit time tends to be slow and associated with a small fecal output, whereas in Africa and the East the transit time is much faster and the fecal weight greater.[65] When many ethnic groups in different environmental circumstances are compared,[68] an inverse relationship is found between transit time and stool weight. Fiber also tends to speed a slow transit time and to retard a fast transit time.[106] Fat does not appear to influence the transit time along the gastrointestinal tract when studied under metabolic conditions.[107] Table 5 shows some of the results of administering different sources of fiber and the effect on the gastrointestinal transit time.

Table 5. The Effect of Fiber on Intestinal Transit Time

Reference	Type and weight of fiber used	Initial transit time (hr)[a]	Final transit time (hr)[a]
74	Bran (16 g)	62	45
	Cellulose (16 g)	52	32
63	Bran (20 g)	66±18	50±11
2	Bran (17–45 g)	58±8	40±9
82	Bagasse (10.5 g)	46±6	37±3
9	Bran (20 g)	73±24	43±8
	Cabbage (20 g)	80±26	64±20
	Carrot (20 g)	60±23	50±14
	Apple (20 g)	50±21	43±16
84	Plant fiber (60 g)	60±9	35±8
52	Pectin (12 g)	57±9	44±7
78	Citrus pectin (15 g)	34 (12–60)	37 (14–103)
67	Fruit, vegetables, bran (20 g total)	55±17	37±12
85	Fruit and vegetables (20 g Neutral Detergent Fiber	52±4	38±4

[a]Mean ± standard deviation or range.

XIV. FIBER AND COLONIC MOTILITY

There have been few, if any, studies of fiber and colonic motility in the normal subject. Most studies have been confined to the irritable bowel syndrome and diverticular disease. However, studies of resting pressure in the rectum and upper and lower anal margin are now available.[108] These studies were applied to 114 normal subjects and measured anal pressure at rest, amplitude and duration of the rectoanal inhibitory reflex and inflation reflex, and amplitude of rectal contraction in response to rectal distension. Normal values were obtained of 16.4 ± 1.1 cm water (range 6 – 29) for the rectal ampulla, 54 ± 2.7 cm water (range 32–80) for the upper anal canal, and 35 ± 2.5 cm water (range 2–82) for the lower anal canal. These studies by Martelli and his colleagues are of great importance for studies of constipation and the exclusion of Hirschprung's disease. However, the paucity of data on normal individuals of colonic motility makes the interpretation of motility in disease states even more difficult than it is at the moment.

XV. CONSTIPATION

Constipation can be regarded as a problem in its own right. It is not just a minor personal inconvenience but a problem associated with recumbency and may lead to complications and extended stay of patients in hospital. In the elderly it may make the difference between dependence and independence. With the present interest in dietary fiber the subject has attracted new scientific study. It is probable that dietary fiber is of greatest importance in the treatment of constipation.

Many healthy people do not defecate every day; a few do so only once or twice a week.[108–110] Perhaps a diagnosis of constipation should be reserved for those individuals when the delay in defecation causes discomfort and indigestion. In general it appears that only 1% of the population will have fewer than three bowel motions per week, though it is fair to say that many subjects who complain of symptoms ascribable to constipation pass more stools than this per week. Constipation can be diagnosed by the passage of pelleted stools; the feeling of distension and the pain in the left or right iliac fossa are relieved by the passage of stool. The two common causes of constipation are small dietary fiber intake and persistent neglect of the call to defecate.

Certain diseases are complicated by constipation, while others may present as constipation. Gastrointestinal diseases complicated by constipation are diverticular disease,[111] the irritable bowel syndrome,[112] and, curiously ulcerative colitis, with approximately 14% of patients.[113]

Constipation is treated by increasing the intake of dietary fiber, predominantly in the form of cereal bran, but also as fruit and vegetables. The most important factor is the WHC of the cereal fiber. The addition to the diet of fruit and vegetables such as apples, oranges, and carrots also adds variety to the treatment regime.

XVI. IRRITABLE BOWEL SYNDROME

The irritable bowel syndrome is a common problem in hospital gastrointestinal practice.[114] In a recent survey in Britain, it was suggested that 14% of a normal population had symptoms compatible with the irritable bowel syndrome.[115] Two main groups appear to have symptoms charcteristic of irritable bowel syndrome.[112,116] Group 1 includes those in whom constipation, i.e., small, pelleted stools, is the most common sign; group 2 includes those with watery diarrhea.[112] It is not known if these are variations of the same problem or are of different etiology. The feces of the two groups have chemical differences. Patients with watery diarrhea have a significant increase in the percentage water content of the stool and excrete primary bile acids.[117]

In another study[118] 26 patients with irritable bowel syndrome were given a diet containing either high or low wheat fiber. After 6 weeks on a high-wheat-fiber regime there was significant improvement in symptoms and subjective changes in colonic motor activity. The high-fiber diet was achieved by adding 20 g of bran to the daily diet, either as bran or as whole-grain bread or as a combination of the two. No clinical improvement occurred with a low-fiber diet.

These experiments support the general clinical impression that many patients with irritable bowel syndrome respond to wheat bran.

XVII. DIVERTICULAR DISEASE

A clear relationship between diet and colonic diverticulosis has been suggested. Diverticular disease is considered to result from a diet with low-fiber content.

Forty years may be necessary for the genesis of diverticular disease. Epidemiological evidence for this is small, though Gear[119] has compared vegetarians with individuals in an Oxford general practice eating an orthodox Southern English diet. In the

45–70 age group, 32% of the Oxford patients and only 13% of the vegetarians had diverticular disease. The association between fiber and diverticular disease is strengthened by the fact that cereal bran can alleviate symptoms.

Studies have shown that many patients with diverticular disease have an increased pressure in the rectum.[120] It also appears that with diverticular disease, stool weight, transit time, and colonic pressure cover a 10-fold range. This suggests that 10% of patients who present with symptoms are in fact constipated and that constipation is a complication of diverticular disease that leads to symptoms.[121]

Diverticular disease may be regarded as asymptomatic, symptomatic, and complicated. A large number of older people in this country may have diverticula of the colon. A small proportion will develop symptoms due to constipation. A few will become complicated by bleeding, inflammation, perforation, and fistula formation. This proportion may further dwindle with adequate dietary advice.[122]

Constipation relief by an enhanced-fiber diet is responsible for relief of the problem. Painter et al[123] treated 62 patients with diverticular disease on a low-sugar diet and sufficient additional bran to make sure that their bowels were opened once or twice daily without straining. The patients were followed for a mean of 22 months and found to be taking 3–45 g of bran daily. Fifty-two percent of the symptoms were abolished and a further 36% relieved. It was this report that revolutionized the treatment of diverticular disease. Prior to this, treatment had been with a low-fiber diet; clearly the treatment was unsuccessful, since the number of operations for this complaint was increasing. Many studies suggest that increasing the bran content of the diet relieves symptoms of diverticular disease. The length of follow-up of these studies extends up to a year.[73,124] The first therapeutically valuable double-blind trial of an enhanced dietary fiber intake in diverticular disease was conducted by Brodribb.[125] Significantly greater symptomatic relief was obtained by those on a high-fiber regimen than those in the control group. There was a marked placebo effect initially. The effectiveness of treatment with a high-fiber diet increased with time, suggesting that trials in which bran was given for a shorter period may give misleading results particularly when the placebo effect has been allowed for. Undoubtedly patients feel greatly relieved for being reassured that they do not have carcinoma of the colon, and this must affect trials of this nature.

The rational basis for the treatment of diverticular disease is principally to reduce the raised intraluminal pressure. It seems logical that any agents which can increase stool volume can help to reduce colonic pressure. However, ispaghula hydrophilic colloid, while increasing stool weight consistently, increases the basal intracolonic pressure and is without effect on the food-stimulated pressures.[121] This raises some doubt about the overall importance of features of diverticular disease formerly thought to be hallmarks of this disease. Agents that raise or leave pressure unchanged may in the long run still risk producing further damage to the bowel. This suggests that bran remains the most efficacious agent in treating diverticular disease.[121] In a detailed study, 22 patients with symptomatic diverticular disease of the colon were given either a control or a high-fiber diet.[76] The long-term effects of up to 6 months of administration of bran on serial fecal serum and biliary lipids was studied. Fecal mass was increased by bran, and the change was positively related to the change in dietary fiber. Fecal fat and fecal dry weight were also increased. Fecal bile acids were initially

slightly raised and were positively related with wet weight both with and without bran. With bran there was a significant decrease in the excretion and concentration of bile acids, particularly those with an initially high value.

It appears that symptomatic diverticular disease is a condition favorably influenced by dietary fiber. Once symptoms appear they can be alleviated by enhancing the fiber content of the diet, preferably with cereal fiber.

XVIII. INFLAMMATORY BOWEL DISEASE

It has been suggested that ulcerative colitis and possibly Crohn's disease are due to a fiber deficiency in the diet.[126] There is, however, no evidence of this being the case. The only situation where fiber has been of value in ulcerative colitis is in those patients who are constipated.[127] Conversely, in situations where there are strictures along the intestine and in Crohn's disease a low-fiber or even elemental diet has been prescribed.

In two most interesting papers,[128,129] Heaton and his colleagues have shown that dietary fiber can minimize the complication and prognosis of Crohn's disease. These papers have important implications in the management of this condition, which affects the mid- and hindgut in so many ways.

XIX. CONCLUSIONS

Vegetable dietary fiber plays a role in human nutrition, the extent of which has yet to be determined. The fecal bulking effects of fiber preparations have been fairly well established, but as yet no definite mechanism has been found to explain the diverse effects of fiber preparations as bulk producers. Certainly many clinical conditions can be treated by the addition of fiber to the diet, but whether the relief of symptoms is due only to the bulking effects of fiber or whether some other effects, such as changes in the chemical environment of the lower intestine in the presence of fiber, are involved is still unclear. Attempts have been made to correlate the nutritional and clinical effects of dietary fiber in the colon and cecum with the source of the fiber, using the physicochemical properties of fiber. These properties and possible nutritional effects have been summarized in Table 6 and illustrate how complex the interrelationship of fiber and

Table 6. Physiological Actions of Fiber in the Colon and Cecum

Physicochemical properties	Modifying effect
Water holding capacity	Transit time
	Fecal weight
	Intraluminal pressure
	fecal electrolytes
	bacterial growth
Matrix formation	Cecal bacterial metabolism
Bile-acid adsorption	Fecal steroids
	Cholesterol turnover
Cation exchange	Fecal minerals
Digestibility	Energy availability
	Chemical environment of colon

nutritional effects can be. Clearly no single property will result in a single nutritional effect. Rather each property will be related to each other property and each nutritional effect to the result of the interrelationship of physicochemical properties and also to the other nutritional effects. The net result of this interrelationship is that the addition of fiber to the diet leads to increased fecal bulk, which could be important clinically. However, the extent of fecal bulking depends on the fiber source and hence is difficult to predict: a source can vary between plant species and also with processing and refining.

ACKNOWLEDGMENTS Much of this work was made possible by financial help from the Kellogg Company of Great Britain; Reckitts and Colman Ltd.; and Nordreco/ Nestle, Sweden.

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9

The Regulatory Peptides of the Colon

Julia M. Polak, Anne E. Bishop, and S. R. Bloom

I. INTRODUCTION

During recent years a large number of regulatory peptides, including vasoactive intestinal polypeptide (VIP), substance P, enkephalin, neurotensin, bombesin, enteroglucagon (EG), and somatostatin, have been discovered in a variety of different tissues in man and many other mammalian species.[1] These peptides have a widespread distribution in the brain and/or periphery. They can be found in typical endocrine cells or nerve fibers in, for example, lung, urogenital tract, adrenal and salivary glands, as well as the gut and pancreas.[2] The colon is well provided with the majority of these peptides.

The following is an account of the nature and distribution of colonic regulatory peptides.

II. TECHNOLOGY

The combined use of two immunological techniques, immunocytochemistry and radioimmunoassay, can provide extensive information concerning the localization of peptides in tissue (immunocytochemistry) as well as their precise quantities and molecular forms (radioimmunoassay) in both blood and tissue.

A. Immunocytochemistry

A number of different steps have to be taken, in any immunocytochemical technique, in order to ensure reliability and specificty.[3] Prior to immunostaining, the pep-

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tides in the tissue under examination must be rendered insoluble. This is best done using any of a range of cross-linking agents (e.g., aldehydes) that can insolubilize peptides without altering their antigenicity.

In our laboratory, we prefer to use the cross-linking agent *p*-benzoquinone, either in solution or in vapor form, as a fixative for immunocytochemistry.[4]

For immunostaining, it is important to use a range of well-characterized antisera reacting to the N terminal, mid-portion, or C terminal of the peptide molecule. These antisera should be used at the highest dilution compatible with strong immunostaining, so that unwanted subpopulations of cross-reacting antibodies can be diluted out.

Testing of the specificity of an antiserum can be done by applying a number of different controls, as suggested by Sternberger.[5] The most important control, however, is the quenching of the immunostain by prior absorption of the antibodies with a purified preparation of the corresponding peptide.

Another means of ensuring specificity is through the use of monoclonal antibodies.[6] These antibodies are raised by fusion of antibody-producing lymphocytes from the spleen with myeloma cells. The fused cells are then grown as clones, which produce a continuous supply of antibodies. Only one antibody is produced by each clone, so that optimal specificity is obtained.

B. Radioimmunoassay

Radioimmunoassay can be used to detect the presence of peptides in blood and tissue and also to determine the nature of different molecular forms. This information helps in the interpretation of the mode of action of a peptide and may also reveal abnormalities in disease states. Nowadays, advances in protein chemistry and the use of chromatographic procedures, including ion exchange, gel filtration, and high-performance gas–liquid chromatography, have enabled the thorough investigation of the biochemistry of peptides. This has led to the production of purified natural and synthetic peptides, providing a rich source for use in both immunocytochemistry and radioimmunoassay.

III. REGULATORY PEPTIDES OF THE COLON

Numerous biologically active peptides, with differential distribution patterns, are present in the gut (Table 1).

Four peptides are found in significant quantities in the colon. These are VIP, substance P, enteroglucagon, and somatostatin. Other peptides, including the enkephalins, bombesin, and a cholecystokinin (CCK)/gastrin-like peptide (CCK^{-8}), have also been found in the colon.

VIP and substance P are found principally in the autonomic nerves. They, or closely related peptides, may also be found in mucosal cells in the gut of some mammals other than man.

EG and somatostatin, on the other hand, are present mainly in the mucosal endocrine cells. Somatostatin is also found in autonomic nerves but in lesser quantities.

Thus, due to their prominence in the colon, we shall discuss individually the detailed distribution and pathological involvements of VIP, substance P, EG, and somatostatin.

Table 1. Mode of Action and Distribution of Regulatory Peptides

Function	Peptide	Stomach		Upper gut		Lower gut		Pancreas
		Fundus	Antrum	Duodenum	Jejunum	Ileum	Colon	
Circulating hormone	Gastrin (17)		Yes	Yes				
	Gastrin (34)		Yes	Yes	Yes			
	Secretin			Yes	Yes			
	Motilin			Yes	Yes			
	Cholecystokinin			Yes	Yes			
	Glucose-dependent insulinotropic peptide		Yes	Yes	Yes			
	EG				Yes	Yes	Yes	
	Neurotensin					Yes		
	Pancreatic polypeptide							Yes
Local/paracrine	Somatostatin	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Neurotransmitter	Vasoactive intestinal polypeptide	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Substance P	Yes	Yes	Yes	Yes	Yes	Yes	
	Bombesin	Yes	Yes	Yes	Yes	Yes	Yes	
	Enkephalin (leucine)	Yes	Yes	Yes	Yes	Yes	Yes	
	Enkephalin (methionine)	Yes	Yes	Yes	Yes	Yes	Yes	

IV. PEPTIDES IN THE AUTONOMIC INNERVATION OF THE COLON

A. VIP

VIP is a 28-amino-acid peptide discovered by Said and Mutt[7] while they were searching for a lung vasoactive factor. It was first extracted from the gut and later found in significant quantities in the brain and in almost every peripheral tissue.[8] (Fig. 1). VIP has been shown to have very powerful pharmacological effects on vasodilation, secretion, and muscle relaxation[9] (Table 2). It is found predominantly in autonomic nerves. In the colon, numerous VIP-containing fibers can be seen across the width of the gut wall, innervating the mucosa, submucosa, muscularis mucosae, longitudinal and circular muscle coats, as well as blood vessels.

The VIP-containing nerves of the gut have recently been shown to originate from ganglion cells located mainly in the submucous plexus.[10] This intrinsic origin has been demonstrated in the guinea pig cecum, where extrinsically denervated pieces of taenia coli show no significant changes in either content or immunostaining of VIP. (Fig. 2). In addition, separate cultures of the myenteric and submucous plexuses reveal that VIP fibers are derived from the submucous plexus (Fig. 3) and are not present in cultures of the myenteric plexus. The VIP fibers, demonstrated by immunostaining of sections of colonic tissue, surrounding nonimmunoreactive ganglion cell bodies in the myenteric plexus thus originate from the submucous plexus. These fibers run across the gut wall, to innervate the outer plexus in a newly discovered VIPergic pathway, connecting the two main ganglionated gut plexuses.

The results of a number of neurophysiological experiments provide evidence of a role for VIP as a neurotransmitter. In the gut, for example, increased local concentra-

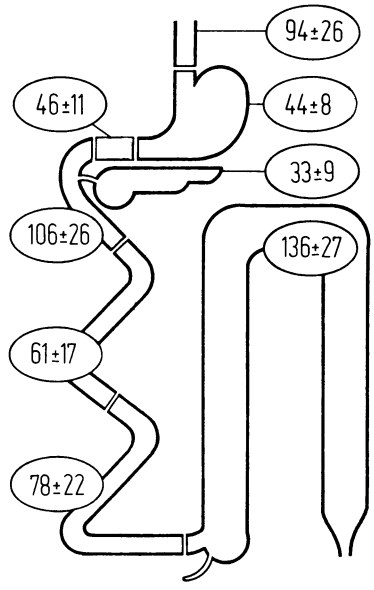

Figure 1. Diagrammatic representation of the various areas of the human gut showing the mean concentration of VIP (pmol/g wet weight of tissue) in each area.

Table 2. Pharmacological Effects of VIP

Vasodilation
Stimulation of water and electrolyte secretion
Slow relaxation of muscle
Stimulation of cyclic AMP
Increased electrical activity in myenteric nerves
Inhibition of gastric acid secretion
Release of insulin, glucagon, and pancreatic polypeptide
Stimulation of pancreatic alkaline juice flow

tions of VIP are found after neurally induced gastric relaxation and intestinal vasodilation.

In the colon, VIP appears to be particularly involved in the control of two major functions: storage of feces and homeostasis. VIP is known to cause long-acting relaxation of gut musculature,[9] and immunocytochemical studies reveal a close relationship between VIP nerves and colonic muscle fibers, suggesting a direct effect of VIP on the muscle. Recent studies show that VIP can also modulate the release of acetylcholine[11] from cholinergic nerves, and may thus have an additional, indirect effect on colonic motility. Thus, VIP may play a prominent role in the control of colonic motor activity and, hence, fecal storage. The fact that VIP has also been shown to be involved in the defecation reflex supports this view.[12]

In the Verner-Morrison, or VIPoma, syndrome, watery diarrhea occurs as a result of high levels of VIP, secreted by a tumor usually of pancreatic or neural origin.[13] VIP can therefore cause secretion of water from the colon and small intestine, in sufficient amounts to cause severe diarrhea. This effect is thought to be mediated via a system of adenylate cyclase in colonic mucosa, which VIP stimulates in a dose-dependent manner.[14] Such is the potency of VIP that a dose of as little as 10^{-10} M will inhibit mucosal-to-serosal water flow.

1. Pathology

Neuropeptides located in autonomic nerves have recently been shown to be involved in a number of different gut diseases. Thus, VIP nerves have been found to be markedly decreased in Hirschsprung's[15] (Fig. 4) and Chagas'[16] (human and experimental) disease, where absence or degeneration of ganglion cells is observed. The decrease in VIP fibers appears to be in proportion to the degree of hypo- or aganglionosis present, with virtually no fibers being found in the mid-region of extensively aganglionic segments of colon.

An opposite situation is observed in Crohn's disease,[17] where ganglion cells are hyperplastic. In this disease VIP fibers are thickened, distorted, and very brightly immunostained (Fig. 5). The VIP content of the diseased colon is significantly increased, sometimes to more than 200% of the normal VIP concentration. These changes, which are particularly consistent in the mucosa and submucosa, are not seen in the other major inflammatory bowel disease, ulcerative colitis.

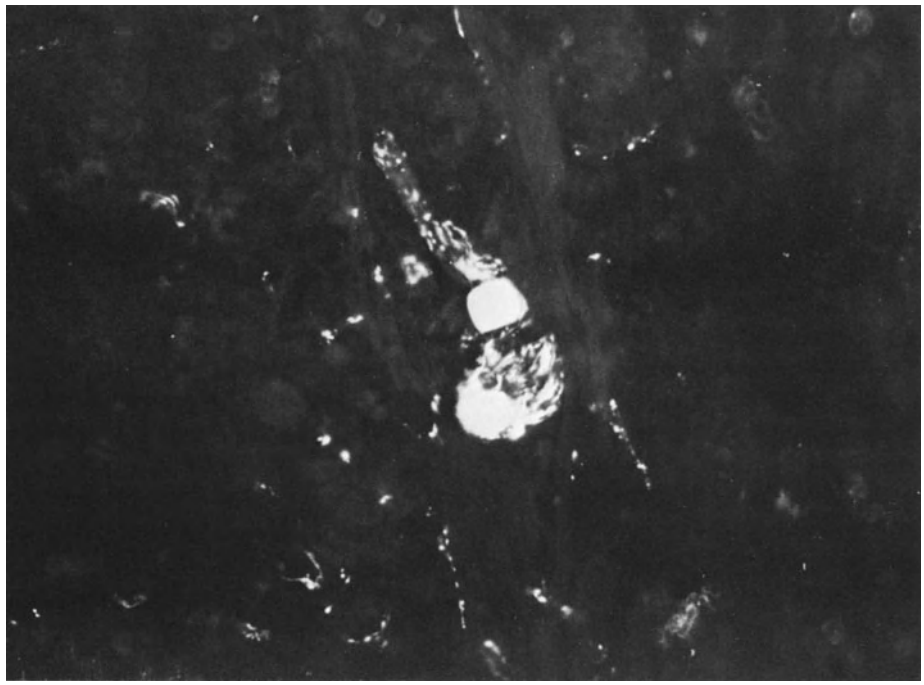

Figure 2. VIP ganglion cells immunostained using the technique of indirect immunofluorescence, in the submucous plexus of extrinsically denervated guinea pig taenia coli (×320), reproduced at 85%).

B. Substance P

Substance P is an 11-amino-acid peptide discovered by von Euler and Gaddum[18] while they were studying the distribution of acetylcholine in the gut. These investigators noted the release, after nerve stimulation, of a substance capable of producing an atropine-resistant contraction of gut muscle. The noncommittal name "substance P" was chosen for the peptide.

Substance P was also extracted from the brain and later found to be identical to the sialogogic factor described by Leeman and Hammerschlag.[19] In addition, its high concentration in the dorsal horn of the spinal cord and its disappearance following dorsal rhizotomy led Otsuka et al[20] to postulate a role for substance P as a sensory neurotransmitter. Various other actions of substance P have been described, including vasodilatory and muscle constrictant.

Immunocytochemistry localizes substance P to the peripheral autonomic innervation of numerous tissues, including lung, urogenital tract, carotid bodies, skin, and heart.[21] In the colon, fibers containing substance P can be found in all layers from the mucosa to the serosa, in relatively smaller numbers than VIP nerves.

Like VIP nerves, substance-P fibers have been shown to have an intrinsic neuronal origin in the gut wall.[10] This was demonstrated using an experimental ap-

proach identical to that described earlier for VIP nerves. Examination of separate cultures of the submucous and myenteric plexuses has shown that, unlike VIP, substance-P nerves originate from the myenteric plexus and also connect the two main plexuses by means of the peptidergic pathway running along the gut wall.

In the gut, substance P exerts a range of varied actions (Table 3). It has recently been reported that substance P is released after a meal and that increased concentrations can be detected in the plasma of experimental dogs following the ingestion of high-protein food.[22] This points to substance P having a role as a circulating hormone as well as a probable neurotransmitter. It is possible that if substance P does indeed circulate it may originate from enterochromaffin cells[23] or the blood content of substance P may represent overspill of neurally released peptide.

1. Pathology

Substance P is also involved in gut diseases where abnormalities of the autonomic innervation exist. Marked decreases of both the content and the number of immunostained substance-P fibers are observed in both Hirschsprung's[15] and Chagas' disease[16] (Fig. 6) in a similar manner to the way VIP fibers are altered.

The presence of substance P has also been detected in some gut carcinoid tumours,[24] although the involvement of the peptide in the resulting syndrome has not been established.

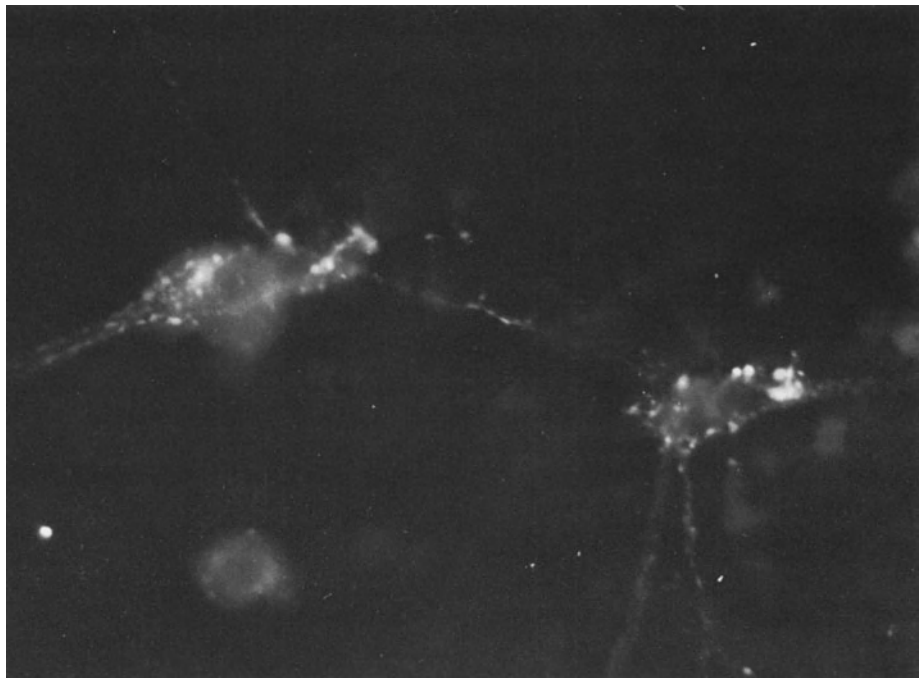

Figure 3. VIP-immunoreactive fibers in cultured submucous plexus of guinea pig colon (× 500 , reproduced at 85%).

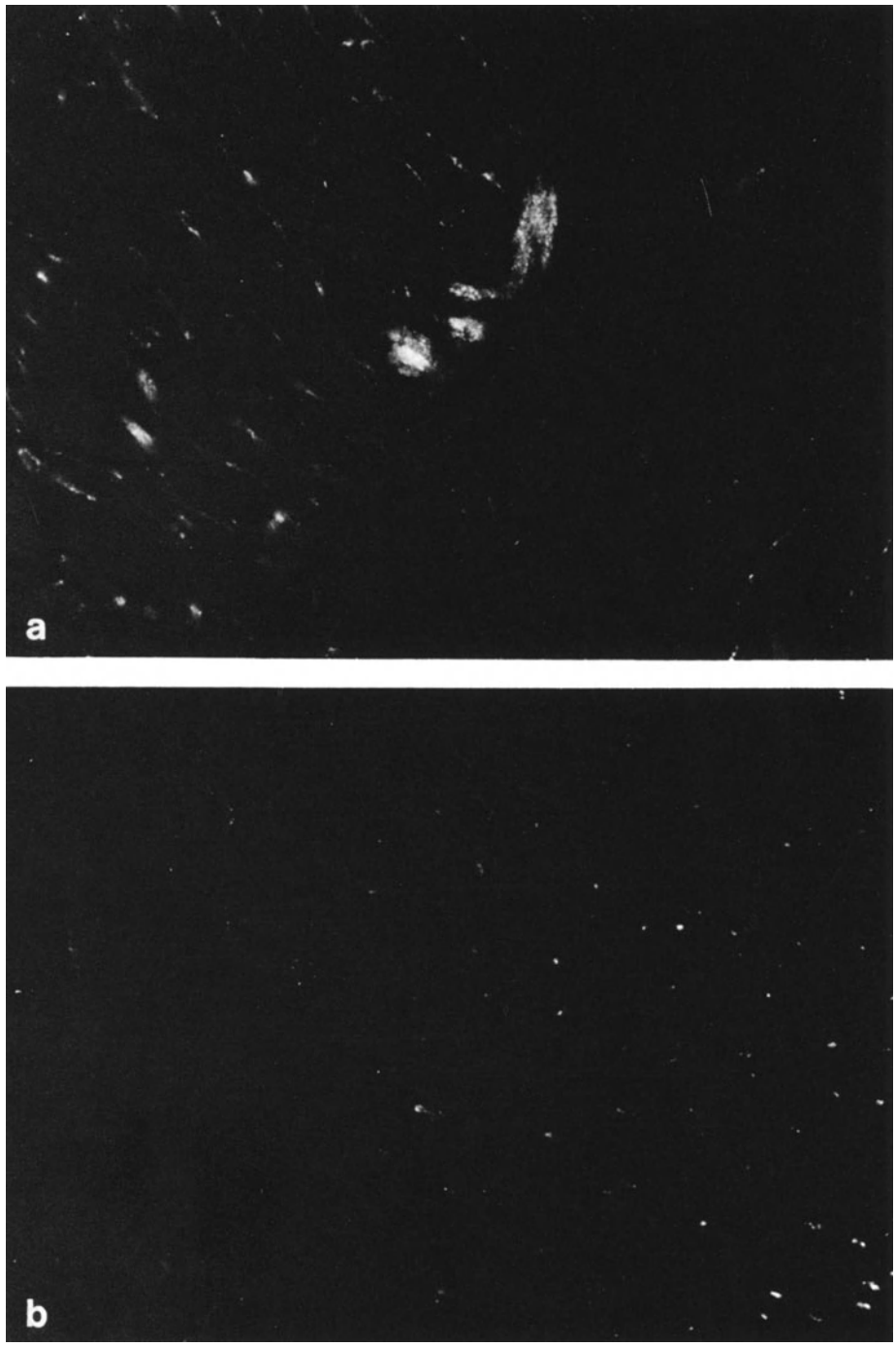

Figure 4. VIP nerves in the region of the myenteric plexus of (a) normal neonatal colon and (b) colon taken from a child with Hirschsprung's disease (×300, reproduced at 85%).

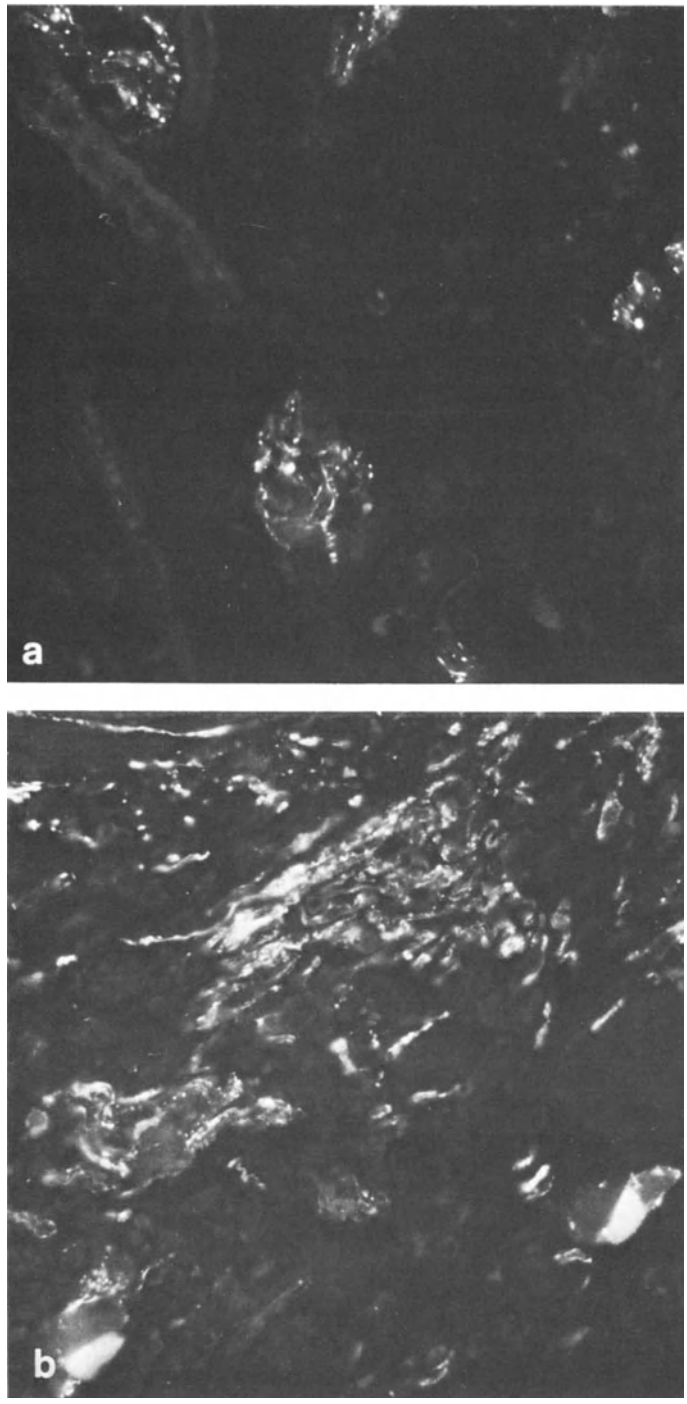

Figure 5. VIP nerves in the submucosa of (a) normal colon (b) colon from a patient with Crohn's disease (×350, reproduced at 85%).

Table 3. Pharmacological Effects of Substance P in the Gut and Pancreas

Vasodilation
Muscle contraction
Stimulation of salivary secretion
Stimulation of pancreatic exocrine secretion
Release of glucagon and glucose

V. PEPTIDES IN ENDOCRINE MUCOSAL CELLS

A. Somatostatin

This peptide consists of 14 amino acids and was originally extracted from the hypothalamus during the search for a factor regulating the release of growth hormone.[25] Somatostatin was subsequently found in the gut, where it also displays powerful inhibitory actions, not only on the release of other peptides but also on many gut functions[26] (Table 4). Considerable quantities of somatostatin are found in the colon of man and other mammals, mainly in classical endocrine cells in the mucosa. A small proportion of somatostatinlike immunoreactive material can also be found in the intestinal wall and is localized to the autonomic nerve supply.

Somatostatin cells are frequently connected to the lumen by means of microvilli. They also often have basal elongations[7] (Fig. 7).

Ultrastructural studies reveal the presence of characteristic electron-dense secretory granules (D type) with tightly fitting membranes, measuring 300 nm on average (Fig. 8).

1. Pathology

Rectal carcinoids of a mixed type frequently contain variable proportions of somatostatin cells.[28] Also, somatostatin cells are reduced in number in both Hirschsprung's and Chagas' disease. The finding of a mucosal cell depletion in areas of gut where autonomic nerves are absent or reduced suggests that the nerves have a trophic effect on mucosal cells. This is in agreement with reports of mucosal atrophy following autonomic denervation[29] and accelerated mucosal mitotic activity during nerve stimulation.[30]

B. Enteroglucagon

EG-like immunoreactivity can be detected in mucosal cells of the gut of various species. In man it is found mainly in the colon and rectum, where it is present in typical endocrine cells, with clear connections to the lumen and frequent basal elongations.

A molecule called glicentin[31] has recently been extracted from the gut and may be the precursor molecule of EG. It is a large peptide consisting of 100 amino acids and containing the entire sequence of pancreatic glucagon, which can be separated out by protease action. Glicentin has been detected by radio-immunoassay in pancreas and gut mucosa using antisera that do not recognize pancreatic glucagon. Possibly the same large peptide is present in both pancreas and gut, but because of differences in enzy-

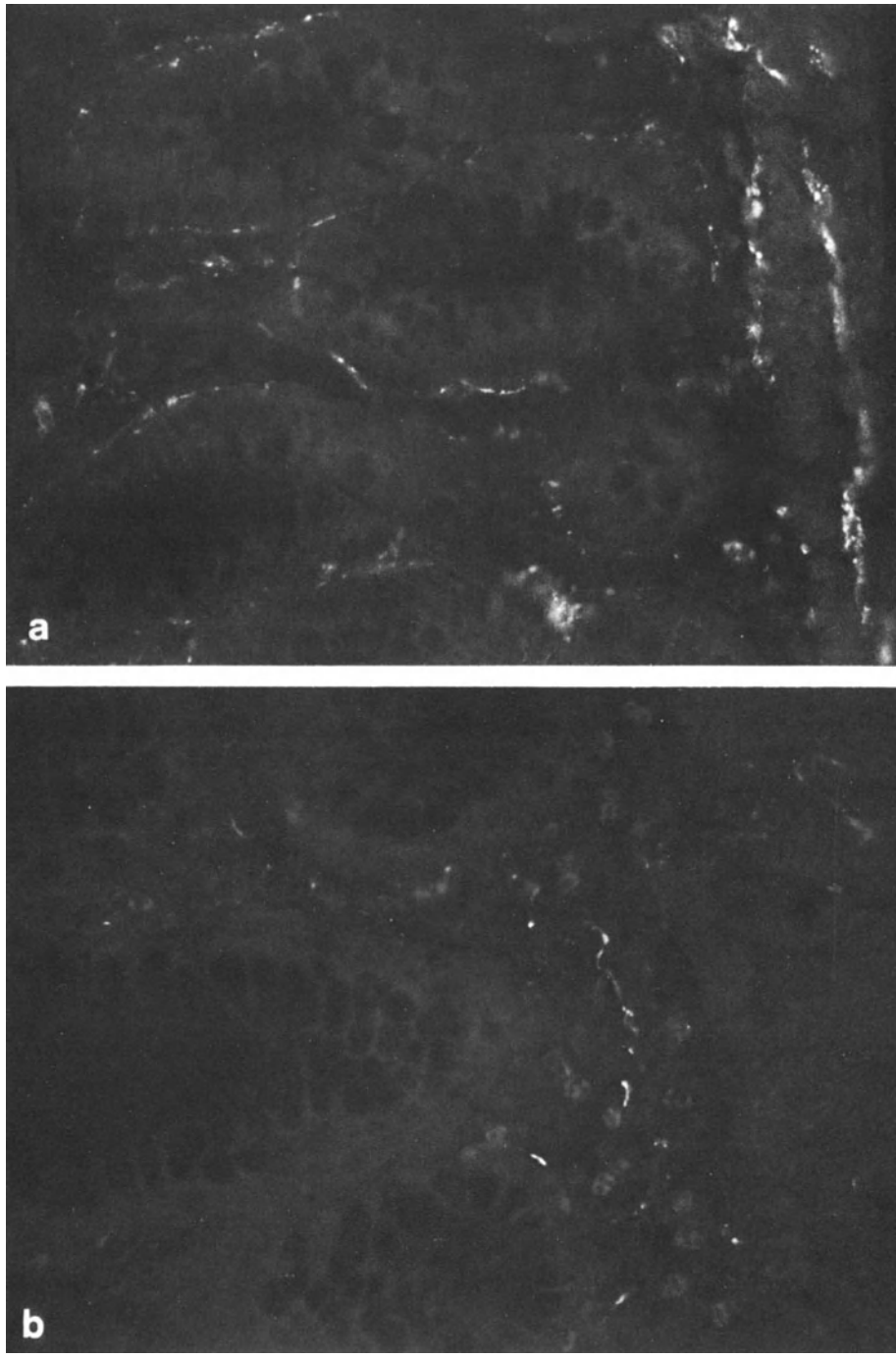

Figure 6. Substance P in the mucosa of (a) normal rectal biopsy and (b) rectal biopsy from a patient with Chagas' disease, (×350, reproduced at 85%).

Table 4. Pharmacological Effects of Somatostatin in the Gut and Pancreas

Inhibition of:	
Insulin	Gastric acid
Glucagon	Pepsin
Pancreatic polypeptide	Gastric emptying
Gastrin	Pancreatic enzyme secretion
CK	Pancreatic bicarbonate
Motilin	Choleresis
EG	Gallbladder contraction
Gastric inhibitory peptide	Motility

matic breakdown, the final hormonal products of pancreatic glucagon and EG are not the same.

Combined immunocytochemistry and electron microscopy using the semithin–thin technique shows that EG cells contain large electron-dense granules measuring 160 nm on average (Fig. 9).

1. Pathology

Cells containing EG can often be detected in rectal carcinoids.[24] In addition, an EG-producing endocrine tumor (APUDoma) of the kidney has been reported[32] (Fig. 10). The patient presented with severe constipation and marked intestinal hyperplasia. Both features disappeared after removal of the tumor. Extrapolation from the clinical picture observed during overproduction of EG suggests that it may act in the control of fecal passage, altering either motility or fluid secretion, and may also contribute to the trophic maintenance of the gut mucosa. Plasma EG levels are, in addition, elevated in situations of intestinal mucosal damage[33] or in massive intestinal resection[34] or bypass for obesity.[35]

In cases of intramural denervation such as Hirschsprung's and Chagas' disease, EG cells, like somatostatin cells, are significantly decreased in number.

VI. OTHER REGULATORY PEPTIDES

Other regulatory peptides present in the colon include bombesin, the enkephalins, and CCK/gastrin-like peptide.[21]

A. Bombesin

Bombesin is one of a number of peptides originally extracted from amphibian skin and later found in several mammalian species, including man. It is a 14-amino-acid peptide found in the discoglossid frog *Bombina bombina,* hence its name.[36]

Bombesin is a potent releaser of gastrin and many other gastrointestinal hormones, including neurotensin, EG, motilin, and pancreatic polypeptide[37] (Table 5).

In the human colon, bombesinlike immunoreactive material can be detected principally in the autonomic innervation, albeit in lesser quantities than either VIP or substance P.

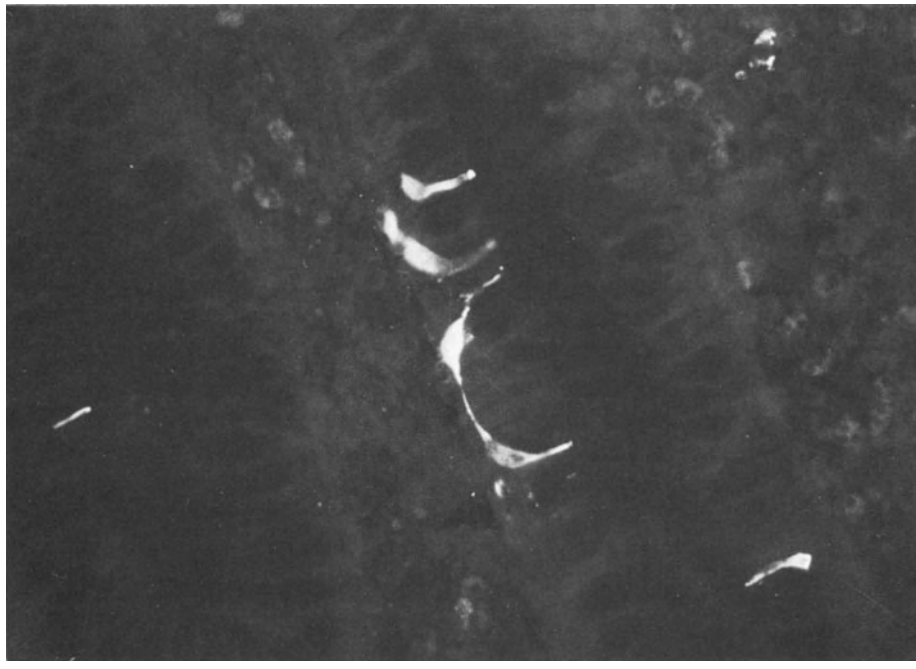

Figure 7. Somatostain cells in human colonic mucosa showing basal and luminal elongations (×400, reproduced at 85%).

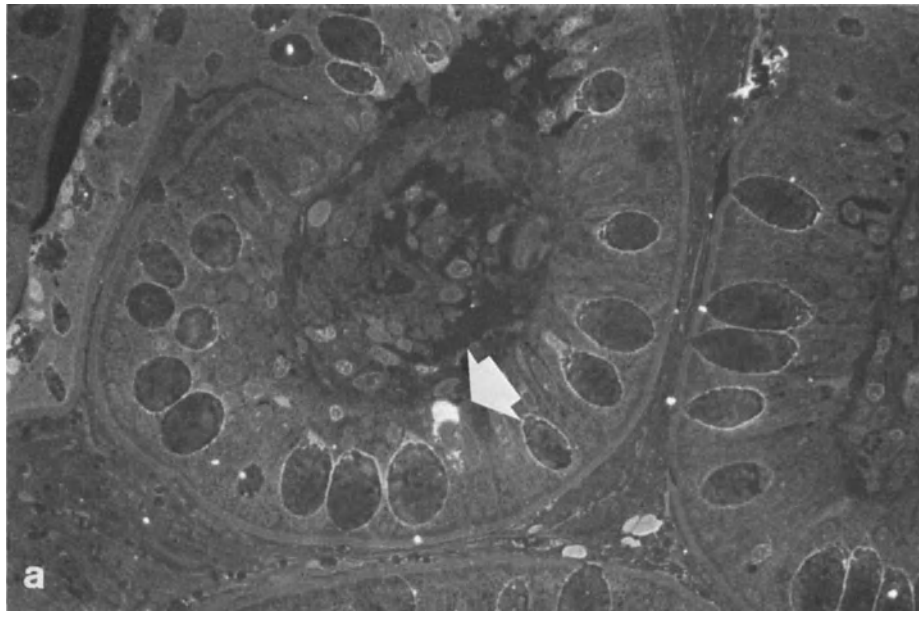

Figure 8. (a) Somatostatin cell (arrowed) in a semithin (800 nm) section (×500). (b) Serial thin (60 nm) section showing secretory, granules (×4800). (c)High magnification of secretory granules (×27,000) (all reproduced at 85%).

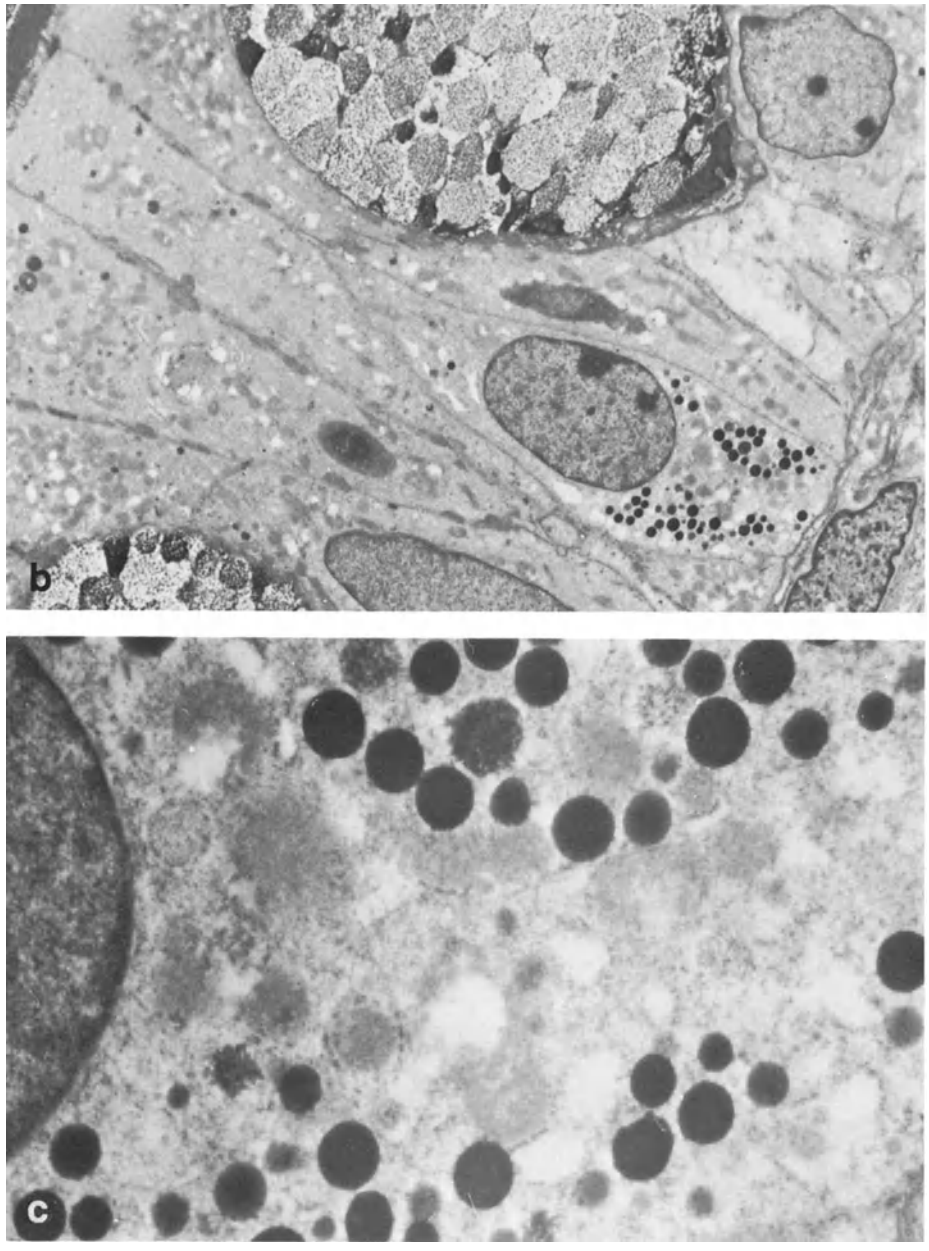

Figure 8. (*cont.*)

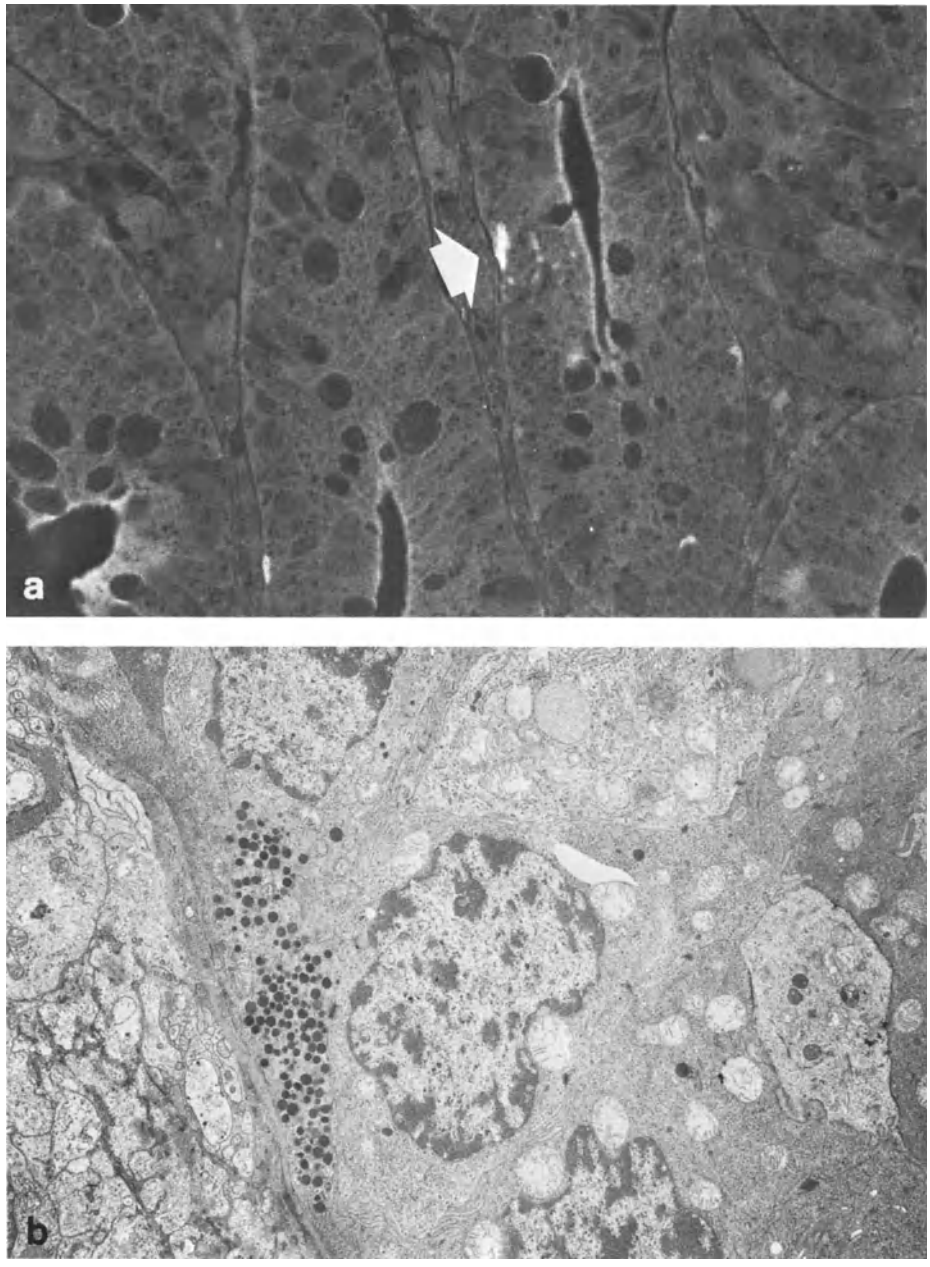

Figure 9. (a) EG cell (arrowed) in a semithin (800 nm) section (×500). (b) Serial thin (60 nm) section showing secretory granules (×6000). (c) High magnification of secretory granules (×30,000) (all reproduced at 85%).

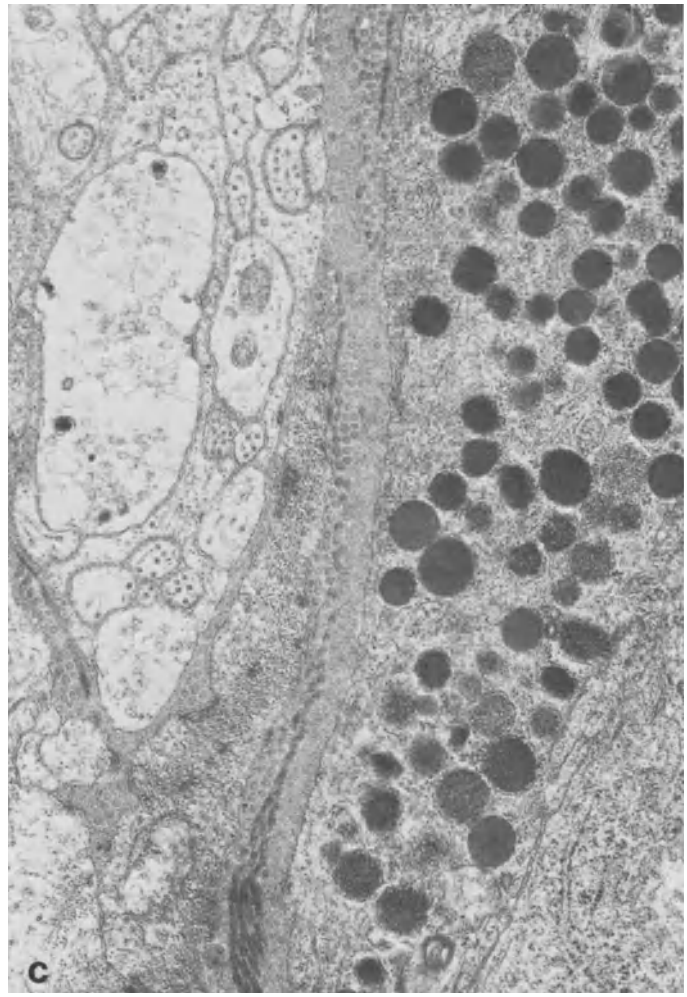

Figure 9. (*cont.*)

B. The Enkephalins

This term refers to a pair of pentapeptides that differ by only one amino acid situated at the C terminal of the molecule. The enkephalins belong to a group of related peptides classed together as endorphins (*end*ogenous m*orphin*es).

The enkephalins, like opiates such as morphine, modulate a number of gut functions (Table 6). They affect motility and secretion so as to cause constipation if administered in sufficient amounts.[38]

In the colon, enkephalin nerves also have an intrinsic origin.[10] In man the fibers mainly innervate the myenteric plexus and circular muscle coat, with occasional fibers being found in the muscularis mucosae. This distribution fits well with the reported

Table 5. Pharmacological Effects of Bombesin in the Gut and Pancreas

Stimulation of:	
pH-independent gastrin release	Glucagon
Gastric acid secretion	Motilin
Pancreatic enzyme secretion	Neurotensin
Gallbladder contraction	EG
Pancreatic polypeptide	Insulin

inhibitory action of the enkephalins on the firing of neurons in the myenteric but not submucous plexus.[39] The enkephalins also appear to have no direct actions on the longitudinal muscle coat,[40] which could explain why no fibers were found in this location.

C. CCK-8 (Cholecystokinin Octapeptide)

Although CCK was first characterised as a 33-amino acid peptide, it has subsequently been shown to exist, in blood and tissues, in several molecular forms. A small form of CCK, consisting of the 8 amino acids at the C-terminal of CCK-33, was originally isolated from brain but has recently been reported to be present in both endocrine

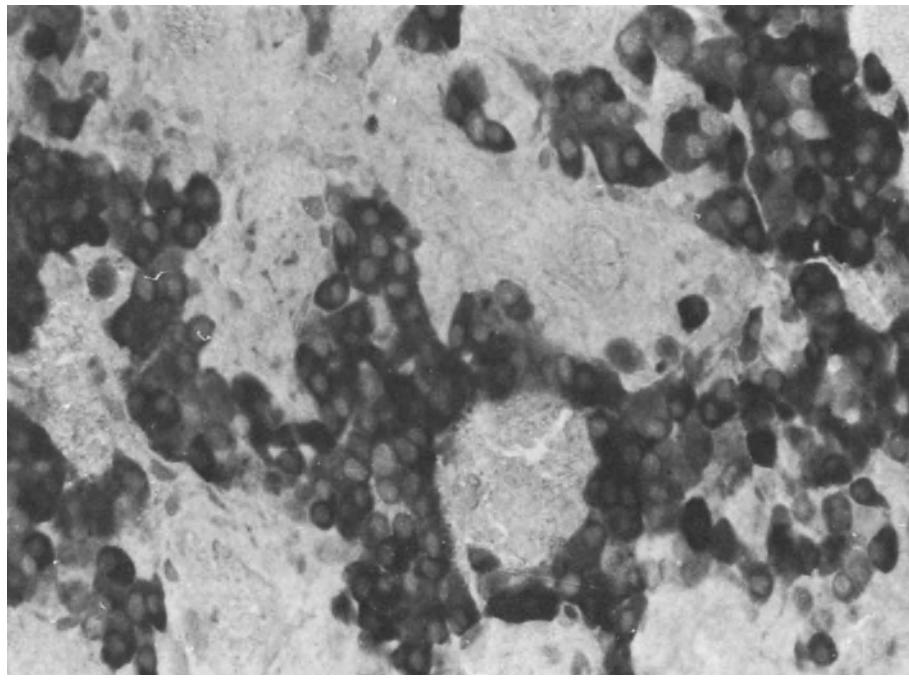

Figure 10. EG cells immunostained using the unlabeled antibody enzyme method, in an enteroglucagonoma (×300, reproduced at 85%).

Table 6. Pharmacological Effects of the Enkephalins in the Gut and Pancreas

Secretory	Decreased	— Gastric secretion
		— Pancreatic secretion
		— Biliary secretion
Motor		— Decreased peristalsis
		— Increased tone
		— Increased segmentation

cells and nerve fibers in the gut.[41] CCK-8 containing nerve fibres have been found in small numbers in the muscle of guinea pig and rat gut.

The main actions of CCK include stimulation of enzyme secretion from the pancreas and contraction of gall bladder. In addition, CCK causes contraction of the longitudinal muscle of guinea pig ileum. This is thought to be an indirect effect involving the myenteric plexus and the release of acetylcholine. It is thus possible that the CCK-8 containing nerves found in the rodent gut may act as excitatory interneurons.

An even smaller portion of the CCK-33 molecule has been isolated. This is the tetrin molecule which consists of the 4 amino acids at the C-terminal of all forms of CCK.[42] Although there is a report of its presence in colonic nerves, there is a certain amount of controversy as to whether or not tetrin exists as a separate entity.

VII. CONCLUSION

Numerous regulatory peptides are known to be present in the colon of man and many other mammals.

These peptides all have a variety of biological actions influencing colonic secretion, motility, and blood flow. It is therefore highly probable that they constitute important factors in the control of large-bowel function.

Many of the peptides may potentiate one another's action (e.g., bombesin stimulates the release of EG). There also appear to be antagonistic relationships between peptides (e.g., the actions of bombesin are predominantly stimulatory, while those of somatostatin are inhibitory).[37]

Immunocytochemistry has localized these peptides to autonomic nerves or to typical endocrine cells in the gut mucosa. The peptide-containing nerves appear to form a division of the autonomic nervous system additional to the two classical adrenergic and cholinergic parts.

The presence of peptides in nervous and endocrine elements, previously considered to belong to totally separate control systems, has provided evidence for the existence of a unified diffuse neuroendocrine system.

At present a number of colonic functions are little understood. For example, the mechanisms controlling the secretion of electrolytes and water are still uncertain, as are those governing colonic motility. The discovery of a system of regulatory peptides, incorporating both the neural and endocrine components of the colon and involved in diseases affecting key colonic functions, is beginning to provide some explanations.

Further investigations of this extensive control system will undoubtedly yield new understanding of its responses in disease.

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10

Nerves of the Colon

Mats Jodal and Ove Lundgren

The colon contains a nervous system of its own, consisting of the different nerve plexuses in the wall. Langley[1] once proposed that the plexuses throughout the gastrointestinal tract should be considered a special part of the autonomic system, the "enteric nervous system." This abandoned view was in part based on the incorrect notion that there are no nervous connections between the plexuses and the central nervous system. However, overwhelming experimental support now exists for the view that the intestinal plexuses can induce complex, specialized physiological adjustments, such as peristaltic movements, in the absence of any central connections. Furthermore, recent histochemical investigations have produced evidence for the possible involvement of a large number of neurotransmitters, mainly peptides, in the neurons of the intestinal wall, adding to the complexity. Thus, the time may be ripe to revive Langley's concept of an enteric nervous system.

This chapter reviews mainly the anatomy and histology of the colonic nerves. The main emphasis is on the more recent developments in this field; for the older work the reader is referred to earlier reviews.[2-4] In some instances conclusions have been drawn from work performed on the small intestine, since in some respects that organ has been investigated more thoroughly than the colon.

I. THE EXTRINSIC INNERVATION OF THE COLON

The autonomic innervation of the colon is complex. No more than four major nerves are involved in regulating colonic function, i.e., nn vagi, nn splanchnici, nn pelvici (erigentes), and the nerves from the inferior mesenteric ganglia, sometimes called the lumbar colonic nerves. See Gabella[3] for a detailed review of the structure of these nerves and their plexuses.

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A. The Sympathetic Nerves

1. The Lumbar Supply

The lumbar sympathetic supply to the colon arises from the second, third, fourth, and sometimes fifth lumbar roots. Most of the preganglionic fibers to the colon pass uninterrupted to the inferior mesenteric plexus, usually containing four ganglia located in a ring around the inferior mesenteric artery close to its origin from the aorta.[3,5] The main sympathetic supply to the colon, the lumbar colonic nerves, arises from these ganglia. The nerves accompany the inferior mesenteric artery to the colonic wall. Preganglionic neurons are many times less numerous than the postganglionic ones, indicating a divergence of efferent impulses.[3]

Histological and physiological studies have clearly revealed the presence of norepinephrine-containing nerve cell bodies in the ganglia and adrenergic neurons in the lumbar colonic nerves.[3,6] However, in recent years immunohistochemical methods have demonstrated the presence of different so-called peptidergic neurons containing polypeptides of varying length, which have been implicated as putative neurotransmitters (for a more detailed discussion, see Section IV.E). Fig. 1 summarizes the results obtained by Hökfelt and co-workers with regard to the inferior mesenteric ganglia.[6,7–10] It is clear from this figure that peptide-containing neurons run in both afferent and efferent directions between the spinal cord, the inferior mesenteric ganglia, and the colon. Histological evidence also exists for the coexistence of two neurotransmitters (norepinephrine and somatostatin) in the same neuron.

The functional importance of these peptidergic nerves is unknown, but Szurszewski and collaborators[11] have provided experimental evidence for the view that afferents from mechanoreceptors in the colonic wall may influence the firing of efferent fibers at the level of the prevertebral ganglia. To what extent such afferent fibers are peptidergic is not known.

2. The Thoracic Supply

The proximal part of the colon is innervated sympathetically via the splanchnic nerves, which follow the superior mesenteric artery and its branches.[5] The splanchnic nerves arise from the 5th to the 12th thoracic spinal segments. The innervation of the colon probably emanates from the lower thoracic segments. The synapse between the pre- and postganglionic neuron is located in the celiac ganglion.

The distribution of norepinephrine and peptides in the neurons of the splanchnic nerves and in the celiac ganglia resembles, generally speaking, that illustrated in Fig. 1.[11] Little is known about the functional significance of the splanchnic nerves containing putative peptide neurotransmitters, but a functional arrangement similar to that described for the inferior mesenteric ganglia seems likely.

B. The Parasympathetic Nerves

1. The Sacral Supply

The main parasympathetic supply to the colon arises from the second and third sacral roots, reaching the colon as the pelvic nerves (nn erigentes). The extent of distri-

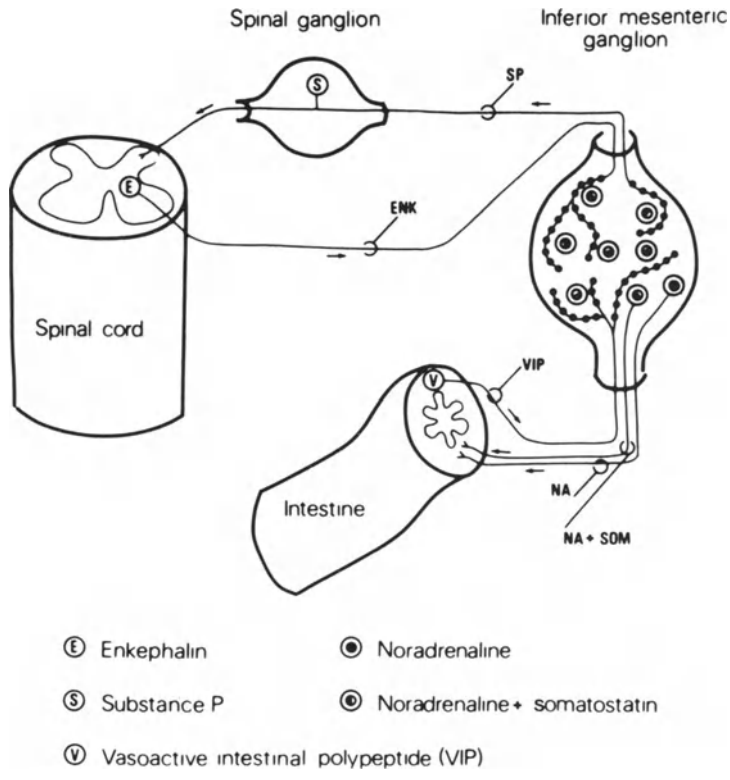

Figure 1. Schematic illustration of the peptidergic connections between the inferior mesenteric ganglion, the spinal cord and the intestine, as revealed by Hökfelt and co-workers. From Hökfelt et al[6] by persmission.

bution of the pelvic nerves within the colon varies among species, but to judge from experiments on cats, approximately the distal two-thirds are supplied by nerve fibers from the pelvic nerves.[5]

Physiological studies clearly indicate the presence of cholinergic neurons in the pelvic nerves.[5] They probably also contain peptidergic neurons (see Section IV.E), but studies have yet to be published on this subject.

2. The Cranial Supply

The cranial parasympathetic fibers to the colon reach this organ via the vagi, the peripheral autonomic nerves that have been most thoroughly investigated. The vagi probably are representative of many autonomic nerves in the body.

The vagal nerves comprise both myelinated and unmyelinated fibers, which convey action potentials in afferent and efferent directions.[3] Afferent fibers are more numerous than efferent ones. In fact, in the cat the abdominal vagi contain 10 times more afferent than efferent neurons.[3]

At the cervical level the vagi contain cholinergic fibers, also axons exhibiting immunoactivity toward substance P, vasoactive intestinal polypeptide (VIP), and

enkephalins, as recently demonstrated in rats[12] and man.[13] Experiments were also performed on rats to demonstrate the presence of fibers immunoreactive against somatostatin and gastrin/cholecystokinin (CCK). In both man and rats, substance-P fibers are the most abundant in the vagi. On the basis of animal studies Lundberg et al[12] concluded that substance P, somatostatin, gastrin/CCK, and possibly VIP are present in afferent fibers, while enkephalin neurons are present in efferent fibers. The functional significance of these fibers is not known. The precise distribution of these peptidergic neurons to the colon has not been investigated.

The vagal fibers are distributed to all gastrointestinal organs, including the colon. However, only the proximal part of the colon is apparently innervated by the vagi, to judge from both anatomical and physiological observations (for a review, see Hultén[5]).

II. THE INTRINSIC INNERVATION OF THE COLON

A. The Nerve Plexuses

The nerve plexuses contained in the colonic wall are netlike structures that represent a continuation of similar nervous structures found in the rest of the gastrointestinal tube.[2,3,4] Fig. 2 illustrates schematically the different plexuses in the wall as described by Furness and Costa.[14] The nervous meshworks described below contain afferent and efferent nerves as well as nerve fibers contained entirely within the colon wall. The sympathetic and parasympathetic extrinsic innervation intermingle quickly with the plexuses and can only be followed by such a special histochemical method that is available for the adrenergic fibers (see Section IV. A).

The histological appearance of all the ''intrinsic'' neurons seems principally similar. Thus, the nerve fibers seem to attain a beaded appearance as they approach the effector cell (another neuron, a smooth-muscle cell, an epithelial cell, etc). The ''beads'' contain varicosities in which the neurotransmitter is located and from which it is released (see Fig. 3).

1. The Subserous Plexus

The subserous plexus is located in the mesentery and on the outside of the colonic muscle layer particularly around the attachment of the mesentery. This plexus considered transitional between the paravascular extrinsic nerves and the myenteric plexus, consists of a net of fine nerve branches, However, it also contains a few cell bodies of its own.

2. The Myenteric Plexus (Auerbach's Plexus)

This nerve plexus is localized in the interspace between the circular and longitudinal muscle layers. It contains clusters of cell bodies (ganglia) in a netlike arrangement of nerve fibers. The average number of neurons per ganglia is 43 (range: 10–100[14]) in the myenteric plexus of rat small intestine. When the wall of the colon is stretched, the myenteric and submucous plexuses become two dimensional, the neurons of the ganglia being located in virtually one single layer. The number of neurons, expressed per

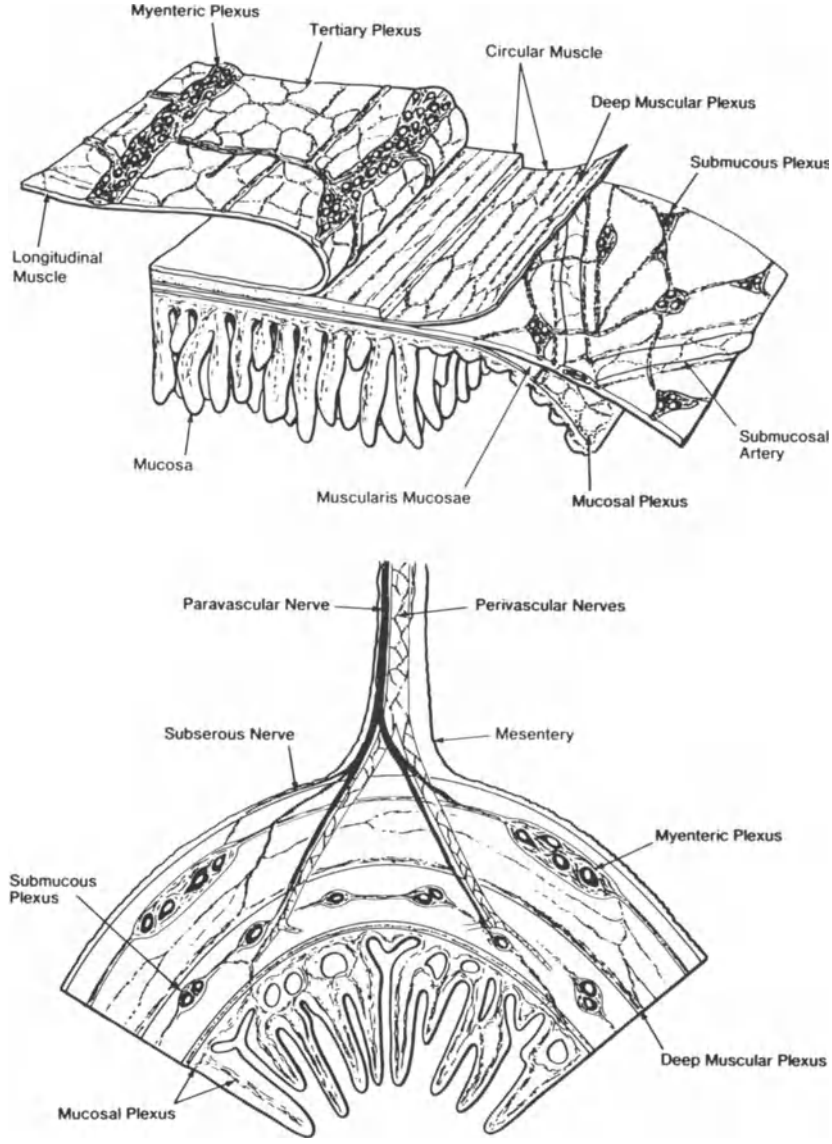

Figure 2. The anatomical arrangement of the nerves of the intestines. Modified from Furness and Costa[14] by permission.

square centimeter of serosal surface, in the colonic myenteric plexus in different species is given in Table 1. To judge from the observations made on the guinea pig the myenteric plexus contains considerably more neurons than the submucous one.

Like the submucous plexus, the myenteric one is often described as composed of three plexiform meshworks.[4] The primary plexus is composed of the bundles of nerve fibers between ganglia of variable size and shape. The secondary (interfascicular)

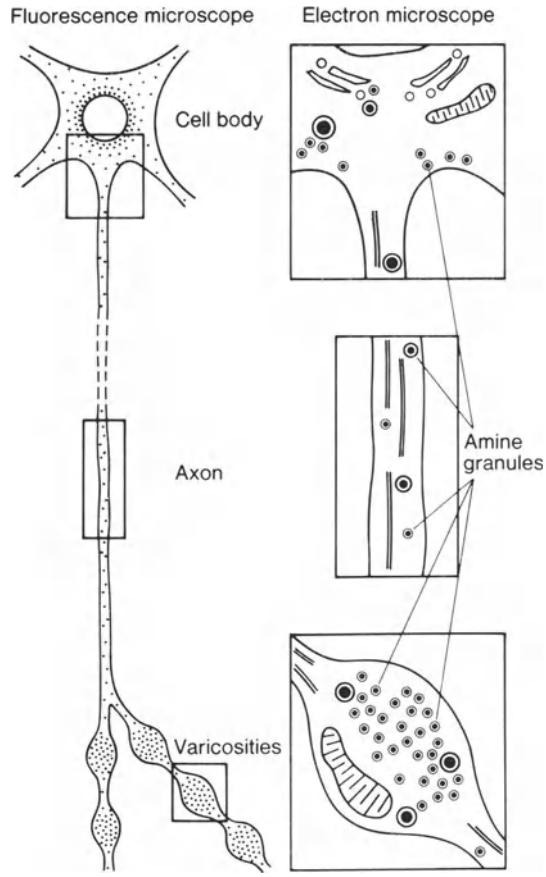

Figure 3. Schematic illustration of a monoamine-containing neuron as revealed by the Falck–Hillarp fluorescence method. The variocisities, containing very high concentrations of norepinephrine, are particularly frequent in the terminal varicosities. The general appearance of any neuron (i.e., also those containing putative peptide transmitters) is the same as that shown in this figure. From Fuve and Hökfelt[92] by permission.

plexus, situated within the primary networks, consists of smaller bundles of unmyelinated fibers in which a few neurons are apparent. The secondary plexus is continuous with a tertiary one consisting of fine nerve fibers that ramify within the longitudinal and circular muscle coat as an autonomic ground plexus. In species with taeniae coli the myenteric plexus contains more neurons subjacent to the taenia than elsewhere in the myenteric plexus.

Numerous so-called glial cells are found in the colonic plexuses, including the myenteric one. In the myenteric plexus of the rat small intestine the glial cells are three times more frequent than the ganglion cells.[3] The shape of the glial cells is very irregular. They cover part of the surface of the ganglion cells, dendrites, and axons, sometimes even completely surrounding bundles of axons. However, it should be pointed out that some workers argue that intestinal glial cells are not true glial cells.[15] What-

ever the cells' proper name, the functions of these cells in the colon are largely unknown.

A *deep myenteric plexus* is situated deep within the circular muscle and composed of numerous small anastomosing nerve bundles (compare the tertiary plexus of the myenteric plexus) running parallel to the muscle cells. This plexus has close connections with the myenteric plexus and also some links with the subserosal one.

3. The Submucous Plexus (Meissner's Plexus)

This plexus in the connective tissue of the submucosa is in principle organized in the same way as the myenteric plexus. Gunn[16] has described an external and an internal portion of the submucous plexus in cat and pig.[16] The external (Henle's) plexus is situated adjacent to the inner layers of the circular musculature, while the internal one lies close to the mucosa. For the number of neurons, see Table 1. The submucous ganglia in rat small intestine contain an average of eight cell bodies.[14]

B. The Innervation of Colonic Muscle Layers

The neuromuscular junction in skeletal muscles is a well-defined histological structure. However, this junction in intestinal smooth muscle is less precisely defined. Thus, there is no one-to-one relationship between neuron and muscle. Instead, bundles of smooth-muscle cells are innervated via the low-resistance pathways (nexuses) between muscles.[17] The transmitter substance is released from the varicosities of the autonomic ground plexus. If the muscle cells then become depolarized, an all-or-none action potential may be initiated that propagates through the tissue via the nexuses. Thus, muscle cells exist that are not innervated, yet are under strong nervous control. This arrangement is illustrated schematically in Fig. 4.

C. The Innervation of Colonic Vasculature

The nerves accompanying the vasculature, particularly the arteries, can be divided into two components, i.e., paravascular and perivascular. The paravascular nerves fol-

Table 1. Number of Neurons in the Myenteric and Submucous Plexuses of the Colon in Different Species

	Number of neurons per cm^2 serosal surface area		
Species	Myenteric plexus	Submucous plexus	Reference
Monkey	1,400	ND[a]	85
Guinea pig	15,000, 19,000	ND	86
	14,800	ND	87
	12,500	ND	88
	35,570	ND	89
	ND	4500	90
Mouse	90,800	ND	91

[a]Not determined.

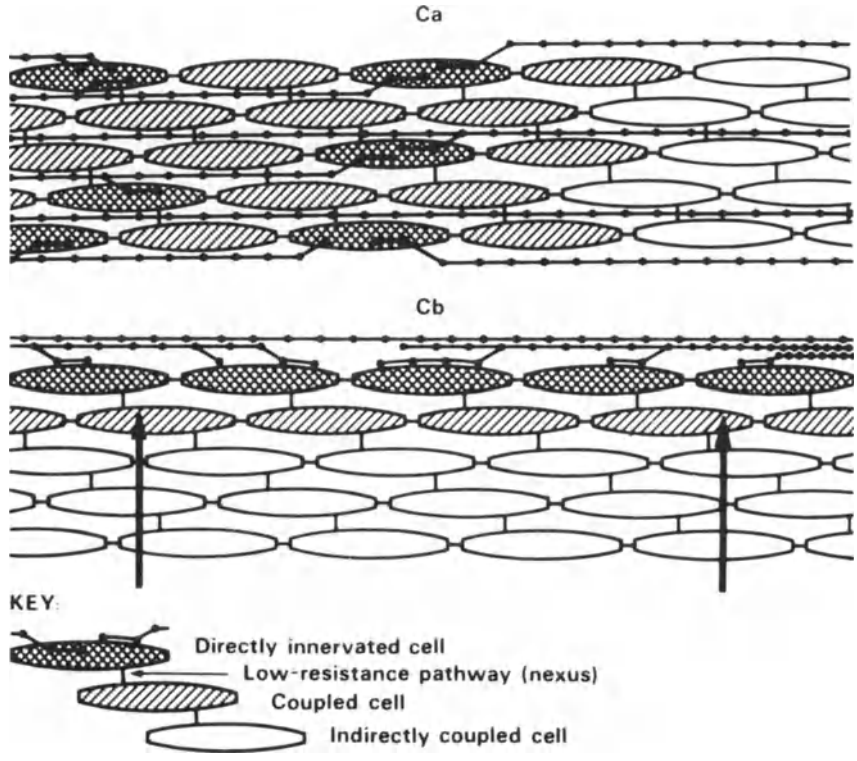

Figure 4. Schematic illustration of two models of the autonomic neuromuscular junction. The upper part (Ca) depicts the arrangement seen in the muscularis propria of the colon, while the lower part (Cb) represents the control of colonic vascular smooth-muscle cells. The arrows indicate the muscle control exerted by substances entering the muscle layer from the vascular lumen. From Burnstock[17] by permission.

low the blood vessels in the mesentery and in the colonic wall but do not innervate the vascular smooth muscles (Fig. 2).

The perivascular nerves, on the other hand, exert the nervous control of the vasculature. The following description of the anatomical organization of this innervation is based on the findings made using the Falck–Hillarp fluorescence technique for adrenergic fibers[18] but has also recently been shown to apply to the peptidergic innervation (F. Sundler, personal communication). The perivascular nerves form a plexus of fine anastomosing nerve fibers (ground plexus) located on the outer surface of the media (see Fig. 4). The release of transmitter from these fibers at the outer circumference of the media controls mainly the adjacent smooth-muscle cells. Indirectly, however, the transmitter exerts an influence also on the more remotely and deeply situated vascular smooth-muscle cells via the nexuses (see Section II.B). Hence, the transmitter released from the perivascular nerves can quickly and efficiently control the tone of the vascular smooth-muscle cells, the wave of muscular contraction moving from the outer to the inner layers of the media.

III. THE MORPHOLOGY OF COLONIC NEURONS

The morphological classification by light microscopy represents the earliest and probably the least successful way of describing different types of enteric neurons. The lack of success depends both on methodological problems and on difficulties to correlate morphology with function.

The first and best-known morphological classification on the light-microscopic level was that proposed by Dogiel,[19,20] who described three different types of neurons: type I: motor neurons with numerous short dendrites and one long axon; type II: sensory neurons with fewer and longer dendrites probably reaching the mucosa; type III: sensory neurons with intermediate-length dendrites surrounding other ganglion cells. The division into sensory and motor neurons was only based on morphological criterions. Dogiel's classification is too simplistic, and different authors have therefore tried other classifications according to criteria such as staining characteristics, cell size, or number of processes.[2,4,15] It is evident, however, that none of these attempts fulfill the requirements of a useful classification system.

Several attempts have been made to base a classification system on the ultrastructural appearance of the cell bodies and their axons. At the magnification of the electron microscope the heterogeneity between the neurons is, however, very large, depending partly on different histological techniques used and partly on problems inherited in trying to achieve a three-dimensional picture of a tissue. The following discussion of the main types of neurons described, and the attempt to correlate these morphological results with histochemical observations, is based mainly on the reports by Baumgarten, et al,[21] Cook and Burnstock,[15] and Gabella.[2]

The classification of the neurons in the colon wall is based on the differences found in size and appearance of the axonal varicosities (Fig. 3). The sympathetic nerve fiber endings are generally considered dominated by small granulated vesicles, mostly somewhat flattened, with an approximate diameter of 30–60 nm. The varicosities also contain some small, clear, ungranulated vesicles together with a few larger, granulated ones, diameter 60–130 nm. The criteria to be fulfilled to accept axons as sympathetic is that they should be of extrinsic origin, i.e., degenerate upon extrinsic denervation, and that they show the ability to take up and store the false transmitters 5- and 6-hydroxydopamine. Such neurons are present in the enteric nervous system (type 3 according to Furness and Costa[14]).

The neurons considered cholinergic contain small clear vesicles and a few large granulated ones. This opinion is based on the similar vesicle appearance at the skeletal muscle motor end plates. There are, however, doubts that this is a homogeneous group of axons.[2] Furthermore, Palay and Chan-Palay[22] have proposed that vesicles of similar appearance in the central nervous system release 5-hydroxytryptamine (5-HT).

Another very common group of axons in the colon was originally described by Baumgarten et al.[21] These axons are richly supplied by large granulated vesicles of variable density and mostly provided with a clear zone at the vesicle membrane. Intermingled with these large vesicles are seen a varying number of small clear vesicles. As this appearance was strikingly similar to the ultrastructural morphology of neurosecretory granules in the hypothalamus, the authors named these axons "p type"

neurons (polypeptide neurons). Similar axon profiles were described in guinea pig ileum by Gabella,[2] and throughout the gastrointestinal tract by Cook and Burnstock.[15] The latter authors, however, divided these axons in three subgroups, implying that functionally they probably represent several different types of neurons. The axons of the "p fibers" has been proposed to represent the so-called purinergic fibers (large, opaque vesicles[23]), fibers containing VIP[24] and perhaps also the proposed 5-HT fibers.[25,26]

Besides the three fairly well defined types of axons described above, Cook and Burnstock[15] could distinguish three further types. One of them was found only in cecum and contained numerous elongated structures similar to microtubules and a few large granulated vesicles. Nothing is known of either the biochemical content or the function of these axons.

The ultrastructural appearance of the cell bodies in the intramural plexuses has received much less attention.Most investigators have found a large variation in size and fine structure of perikaryon of the intestinal neurons. In a careful study, Cook and Burnstock[15] attempted to classify the cell bodies according to size, distribution of organelles, and to their location and relationship to surrounding so-called satellite cells. Nine different types of cell bodies were described, but little correlation was observed between the type of cell body and the type of axonal profile. Furthermore, an equally low correlation existed between type of axon profile making synaptic contact with a certain type of cell body. Cook and Burnstock made an important observation, however, that most types of axons make synaptic contact with several, sometimes all, of the different types of cell bodies. Furthermore, the majority of cell bodies have synaptic contact with several different types of axons. Thus, the enteric nervous system shows the morphological organization necessary for the important functional principles of the autonomic nervous sytem known as divergence and convergence.

The enteric nervous system contains a large number of afferent fibers (see Gabella[2,3]). However, no specialized nerve structures have been identified that can be regarded as a receptor, either mechanical or chemical. If the receptor function is performed by free nerve endings these cannot be identified by the electron microscope.

In summary, the attempts to use a morphological classification, preferentially based on vesicle morphology, to describe functional characteristics of the enteric nerves has not been very successful due to the great variability in neuron appearance.

IV. THE HISTOCHEMISTRY OF COLONIC NEURONS

The attempts to classify the enteric nervous system by means of electron microscopy have proved difficult (see Section III), and little consensus has been reached with regard to the chemical nature of the neurotransmitter involved in the different types of neurons. The histochemical approach, exemplified, e.g., by fluorescence microscopy or immunohistochemistry, has been shown to be much more successful and has yielded an increasing amount of information that presently is difficult to analyze in functional terms. The histochemical "revolution" started 15–20 years ago with the development of the fluorescence method by Falck and Hillarp for demonstrating the presence of norepinephrine, serotonin, and dopamine. In the 1970s it was realized that most of the

nerve cells and nerve fibers in the gut, which were noncholinergic and nonadrenergic, contained peptides. Examples of such peptides are substance P and VIP. Hence it was proposed that these peptides were neurotransmitters (neuromodulators?); in a few cases physiological experiments have also confirmed the neurotransmitter hypothesis (see Section IV.E). Following is an account of the distribution of the different histochemical types of fibers, together with a brief survey of the biochemical events in connection with the release of the transmitter, when these are known.

A. Norepinephrine

The development of the Falck–Hillarp fluorescence method for mapping norepinephrine in tissues has made possible a detailed study of the adrenergic innervation in the gastrointestinal tract, as reviewed by Furness and Costa.[27] Studies in which the extrinsic nerves have been allowed to degenerate demonstrate clearly that most adrenergic fibers are extrinsic, with one exception: in the proximal part of guinea pig colon adrenergic fluorescence is seen in the myenteric plexus also after severing all the extrinsic nerves.[28]

The adrenergic fibers enter the colon as paravascular nerves. When reaching the plexuses, adrenergic filaments make contact with the neurons in the plexuses. However, the adrenergic fibers form no pericellular nerve endings around the neurons in the plexuses, and hence no one-to-one relationship exists between the adrenergic fiber and the ganglion cell. On the contrary, one and the same fiber runs for a long distance (up to 1cm) close to many ganglion cells (divergence).[27] All the ganglion cells in the nodes of the myenteric plexus are surrounded by adrenergic fibers, while the number of adrenergic fibers in the submucous plexus is somewhat smaller than in the myenteric plexus.

According to classical concepts the muscle layer of the intestines is directly innervated by sympathetic and parasympathetic fibers. However, the Falck–Hillarp method has shown that virtually no adrenergic fibers are present in the longitudinal layer except where it is gathered into taeniae. There is some innervation of the circular layer. Experimental work indicates that most of the adrenergic influence on colonic motility is mediated via a presynaptic inhibition of excitatory parasympathetic influence at a ganglionic level, probably in the myenteric plexus.

The muscularis mucosae receives a sparse adrenergic innervation. Adrenergic fluorescence is found in the mucosal portion, particularly around the basal parts of the crypts.

The colonic arteries are provided with a dense adrenergic innervation located at the border between media and adventitia. The veins located in the colonic wall have a scarce innervation, while the larger vessels in the mesentery are provided with a comparatively dense nervous supply. Direct electrical or reflex-elicited activation of these fibers produces a vasoconstriction of resistance as well as capacitance vessels.

The biochemistry of adrenergic transmitter release has been the subject of intensive experimental study (see Burnstock and Costa[29] for a review). A summary of the events taking place at the neuroeffector junction is shown in Fig. 5. It is apparent from this picture that the inactivation of the released norepinephrine is accomplished in three ways: (1) a reuptake into the axon, (2) an extracellular breakdown via monoamine oxi-

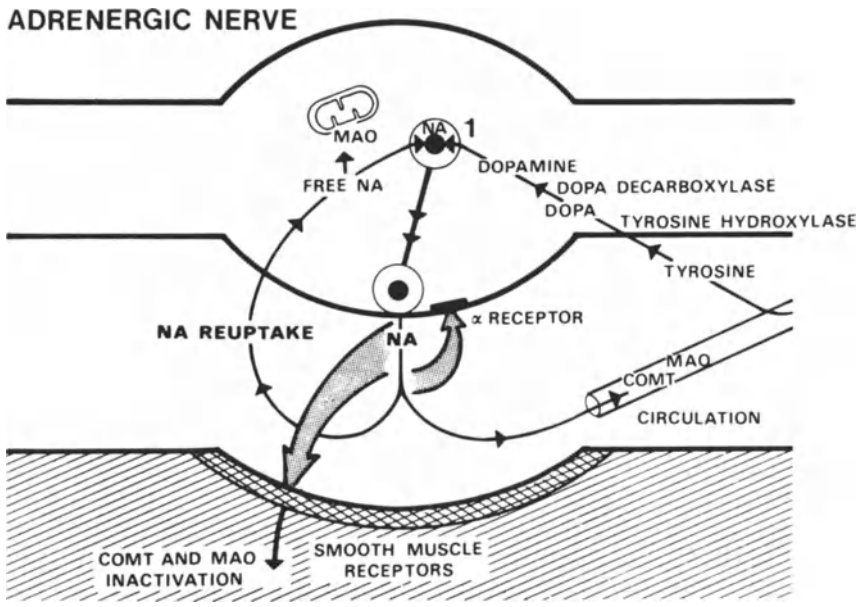

Figure 5. Schematic illustration of synthesis, storage, release, and inactivation of norepinephrine (NA) at adrenergic neuromuscular junctions. MAO, monoamine oxidase; COMT, catecho-O-methyltransterase. From Burnstock[23] by permission.

dase or catecho-*O*-methyltransferase, (3) a diffusion of norepinephrine into the blood circulation.

B. Acetylcholine

The most commonly used histochemical method for the study of cholinergic neurons is the technique demonstrating the presence of acetylcholinesterase (AChE). The specificity of this method in demonstrating the presence of cholinergic neurons has been questioned.[14] Electron microscopy seems a more reliable test in the study of cholinergic nerve fibers in the intestines (see Section III).

Biochemical methods have clearly demonstrated the release of acetylcholine (ACh) from, e.g., the myenteric plexus *in vitro* upon transmural electrical field stimulation (see Kosterlitz et al[33]), also after degeneration of the extrinsic nerves.[34] Similar observations have been made on the small intestine with regard to AChE.[35] In fact, the transferase content was barely affected by extrinsic innervation, suggesting that the parasympathetic cholinergic supply constitutes only a small part of the cholinergic neurons, at least in the small intestine.

The biochemistry of the formation, release, and inactivation of ACh is summarized in Fig. 6. The released transmitter is degraded by means of AChE in the synaptic cleft. ACh may also diffuse into the circulation.

C. 5-HT, Serotonin

The histochemical fluorescence method of Hillarp and Falck makes it possible to study three biologically important amines in tissues, i.e., noradrenaline, serotonin, and dopamine. The fluorescence of noradrenaline and dopamine is greenish with a peak wavelength around 490 nm, while the 5-HT neurons appear more yellow with a peak around 540 nm. One can therefore identify these two groups of transmitters from the color of the fluorescence. This method has been used in attempts to demonstrate the presence of 5-HT-containing neurons in the colon and the small intestine. Most studies report a failure to find any yellow fluorescence 30, 31, 36, 37 even when depleting the tissue of norepinephrine by α-methyl paratyrosine. However, the presence of serotoninergic neurons ins the central nervous system has also been technically difficult to demonstrate (compare Cooper et al[38]).

Gershon and co-workers have provided several indirect experimental observations supporting the presence of 5-HT-containing neurons in the gut.[11,39] To summarize Gershon's findings briefly, the experiments, performed mainly on the mammalian small intestine but also in some instance on the colon, have shown that nerves exist in the intestines, particularly in the myenteric plexus, that can take up and retain 5-HT or 5-hydroxytryptophan, a precursor to serotonin. Furthermore, the presence of the ap-

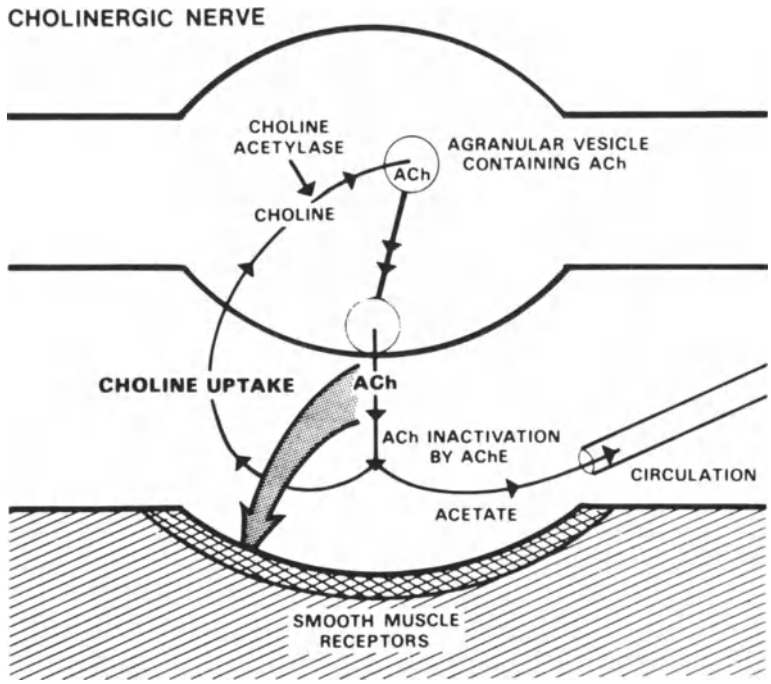

Figure 6. Schematic illustration of synthesis, storage, release, and inactivation of (ACh) at cholinergic neuromuscular junctions. From Burnstock[23] by permission.

propriate enzymes has been demonstrated in intramural nerves. The pros and cons on this question are discussed in detail in a recent review by Costa and Furness[40] that the interested reader should consult for lengthy discussions. In conclusion, the question remains open whether 5-HT is a transmitter in the enteric nervous system.

D. Adenosine 5'-triphosphate

Burnstock and co-workers have claimed that adenosine 5'-triphosphate (ATP) and/or related adenosine dervates are neurotransmitters in the autonomic nervous system (see Burnstock[23] for review). Burnstock has coined the name "purinergic" for these nerve fibers. One of the most thoroughly investigated tissues with presumed purinergic innervation is the taeniae coli. Electrical stimulation of the purinergic fibers in these structures induces a nonadrenergic, noncholinergic inhibition of the smooth-muscle cells. This is demonstrated in electrophysiological studies as an inhibitor junction potential.

In a series of reports, Ålund and collaborators[41,42] (see Ålund[43] for a review) provide indirect evidence for the proposal that the quinacrine histofluorescence method makes it possible to study the distribution of purinergic neurons in tissues. Ålund finds fluorescent beaded fibers in the myenteric plexus in the colon when incubating whole-mounts of sheets of the colonic smooth-muscle layer with quinacrine. Some cell bodies were also stained. The claim that these fibers are in fact Burnstock's purinergic nerves remains, however, to be established.

E. Peptidergic Neurons

The study of peptidergic nerves is a rapidly advancing field of research. So far most of the studies are morphological, attempting to use immunohistochemical methods to map the neurons containing the different peptides (see Hökfelt et al[6] for a recent review). The results obtained with immunohistochemical methods should be interpreted with caution, however, since these techniques cannot be checked with independent methods. It is therefore not possible to exclude immunological cross-reactions with peptides having similar amino acid sequences.

The biochemistry of the putative peptidergic neurotransmitters is poorly understood. Probably the peptides, in contrast to norepinephrine, for example, are only produced on the ribosomes of the cell soma, possibly as a larger precursor molecule.[6] Hence, no local synthesis of the peptide transmitter takes place in the nerve endings. Furthermore, there appears to be no reuptake mechanism for peptidergic neurotransmitters. Fig. 7 summarizes some of the differences between "classical" (e.g., norepinephrine, ACh) and peptide transmitters with regard to formation, release, and inactivation.

1. Substance P

Substance P is a polypeptide composed of 11 amino acids. Its presence in intestinal nerves has been demonstrated in several species (rat, mouse, guinea pig, pig, dog, and man.[44–48] The cell bodies are primarily located in the colonic myenteric plexus (Table 2). Nerve fibers with a positive immunohistochemical reaction to substance P

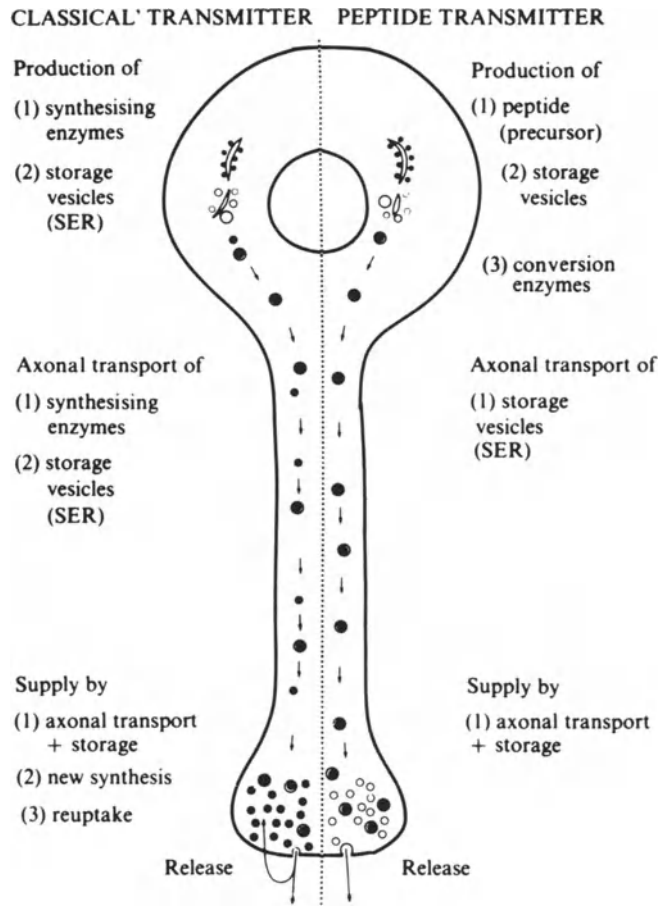

Figure 7. Schematic figure of a neuron illustrating some of the differences between a neuron using a "classical" transmitter, e.g., norepinephrine (left), or a peptide transmitter (right). SER denotes smooth endoplasmatic reticulum. From Hökfelt et al[6] by permission.

are found in all layers of the colon, especially in the myenteric plexus. In this plexus no other type of peptidergic neuron is more frequent than substance P in the species investigated. The longitudinal and circular muscle layers are both innervated rather heavily by substance-P neurons, while the submucosa and the mucosa contain a small or moderate number of fibers, located in the periglandular plexus and also around vessels (Fig. 8). After denervation the fibers surrounding the blood vessels in the small intestine disappear, while there is no appeciable change of the substance-P contents in the rest of the intestine.[44,49,50]

Substance P is often considered to be a transmitter in afferent pain fibers. However, extrinsic denervation barely reduced the colonic contents of this compound, strongly suggesting that intrinsic neurons containing substance P also exist.

Table 2. Proportion of Cell Bodies Exhibiting Immunoreactivity against Putative Peptidergic Neurotransmitters in the Colon Nerve Plexuses of the Rat and the Guinea Pig[a]

	Rat[b]	Guinea pig[b]
Substance P		
Submucous plexus	1.7 (118)	4.3 (1088)
Myenteric plexus	13.0 (300)	12.9 (1241)
VIP		
Submucous plexus	65.9 (992)	27.0 (1525)
Myenteric plexus	5.6 (2670)	2.3 (1631)
Enkephalin		
Submucous plexus	0 (453)	0 (1255)
Myenteric plexus	4.9 (2667)	12.9 (2442)
Somatostatin		
Submucous plexus	3.2 (1160)	20.4 (1621)
Myenteric plexus	14.4 (3332)	2.6 (1793)

[a]From Schultzberg et al.[47]

[b]Extraparenthetical numbers—percentages of total number of cell profiles counted. Parenthetical numbers—number of cells counted.

Substance P, being a potent smooth-muscle stimulator, is a proposed candidate for the nonadrenergic, noncholinergic contraction observed in the gastrointestinal tract, including the colon.[49,50] Substance P also evokes a long-lasting depolarization in membrane potential of certain neurons in the myenteric plexus.[51] Similar changes are seen when eliciting the peristaltic reflex.

2. VIP

VIP is composed of 28 amino acids and was originally considered to be a so-called candidate hormone located in the epithelial cells.[52] The more recent reports, however, have consistently demonstrated that VIP is almost exclusively located in the nervous tissue.[53–56] The cell bodies containing VIP, as demonstrated by immunohistochemical techniques, are located predominantly in the submucous plexus[54] (Table 2). VIPergic axons are the most abundant of the peptidic fibers investigated by Schultzberg et at[47] and are found in all wall layers of the colon. The density of VIP fibers in the myenteric plexus of the guinea pig is so great that Costa et al[54] had difficulties estimating the number of VIP-containing cell bodies. There is an extensive innervation of blood vessels. The mucosal glands in the colon may also be influenced by the VIPergic fibers, to judge by the close contact between the VIP fibers and the epithelium. However, Schultzbeg et al[47] proposed that the large number of VIP fibers in the mucosa may be sensory, at least in part. The longitudinal muscle layer is less heavily innervated by VIP-containing fibers, at least in the rat (see Fig. 8). Most of the VIP fibers in the gastrointestinal tract seem to be intrinsic, since extrinsic denervation had an insignificant influence on the VIP contents in the intestinal wall.[54]

The functional significance of the VIP-containing neurons in the colon is little understood. The electrical or reflex activation ofthe nerves inducing the nonadrenergic, noncholinergic vasodilatation in the colon is accompanied by an increased release of

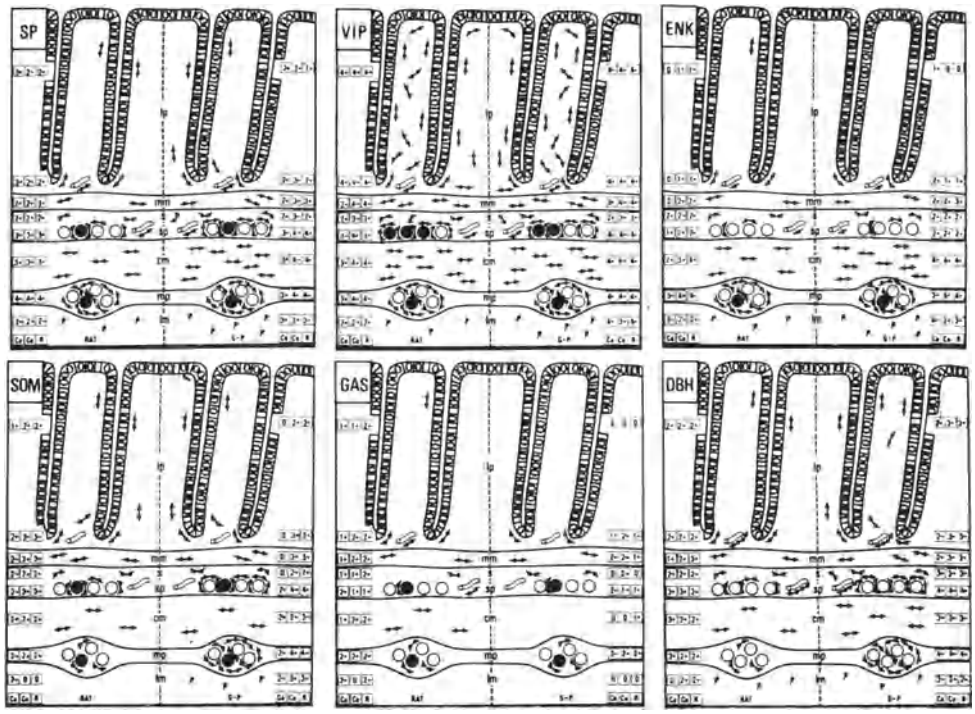

Figure 8. Schematic illustration of the relative distribution of the different peptidergic neurons as demonstrated by immunohistochemical methods. Each panel is divided in two parts showing the results in the rat (left) and the guinea pig (G-P) (right). Each panel illustrates the results of one particular antibody, as indicated by the box in the upper left corner of the panel. The following antibodies were tested: substance P antiserum, SP; VIP antiserum, VIP; met-enkephalin antisera, ENK; somatostatin antiserum, SOM; gastrin/CCK antiserum, GAS; dopamine β -hydroxylase antiserum, DBH. The abbreviations for the various layers are as follows: lp, lamina propria; mm, lamina muscularis mucosae; s, submucosa (includes the submucosal tissue except for the ganglia); sp, submucous plexus; cm, circular muscle layer; mp, myenteric plexus; lm, longitudinal muscle layer. The lamina propria has been divided into a superficial and a basal part. Results of the distribution and density of immunoreactive nerves collected from the cecum (Ca), colon (Co), and rectum (R) of the large intestine are shown in the small boxes on each side of a panel. The number of peptide-containing immunoreactive fibers around clearly identifiable blood vessels in the lamina propria and the submucosa has been indicated (—•—, single fibers; —•—•—, small numbers; —•—•—•—, moderate numbers; —•—•—•—•—, large numbers). The illustration of the ratings of cells and nerve fibers is as follows. *Cell bodies:* ○⊙○○, 1–25; ⊙⊙ ○○, 26–50%; ⊙⊙⊙ ○, 51–75%; ⊙⊙⊙ ⊙, 76–100%. *Fibers:* 1+, single fibers; 2+, small numbers; 3+, moderate numbers; 4+, large numbers. From Schultzberg et al.

the VIP into the venous effluent.[57] It has therefore been proposed that this vasodilatation is mediated by VIP acting as a neurotransmitter.

3. Enkephalins

Met-enkephalin and leu-enkephalin are polypeptides containing five amino acids, four of which are identical in the two compounds. When these substances originally were isolated, the ileum of the rat was used as a bioassay.[58] Immunoreactivity against both substances in the colon is found in the tissue, although met-enkephalin seems more abundant than leu-enkephalin.[59] The cell bodies containing enkephalins are al-

most entirely located in the myenteric plexus[32,47,60–62] (Table 2). It is possible to differentiate between met-enkephalin and leu-enkephalin neurons using immunohistochemical methods,[63] but the overall distribution of the two neuron populations is the same. Fibers are seen in the myenteric plexus and in the deep muscular plexus as well as in the circular layer of the colon. A low density of fibers is apparent in the mucosa–submucosa. Some fibers seem to innervate blood vessels (Fig. 8).

Schulz et al[64] have shown in *in vitro* experiments that electrical stimulation of gut nerves releases enkephalins. Generally speaking, the action of the enkephalins is inhibitory and the substances inhibit both the electrically evoked contractions of smooth muscles from the small intestine[65] and the release of ACh from the myenteric plexus.[66]

4. Somatostatin

Somatostatin-immunoreactive structures in the colon are less numerous than the structures of the previously described peptides.[47,67,68] The cell bodies are localized mainly in the myenteric plexus in the rat and in the submucous plexus in the guinea pig[47] (see Table 2). Most of these nerves are of intrinsic origin. The fibers are found in all layers in the colon wall but seem to end predominantly in the plexuses. The axons in the myenteric plexus project in an anal direction. (Fig. 8).

The functional importance of these neurons is unknown, partly because of the difficulties in *in vivo* experiments to differentiate between the "hormonal" effect of somatostatin and those effects that are conveyed via nerves. In the small intestine somatostatin has been shown to inhibit the firing of myenteric neurons,[69] decrease the release of ACh[70] and at high concentrations to inhibit the peristaltic reflex.[69]

5. Other Peptidergic Neurons

Schultzberg et at[47] reported the presence of gastrin/CCK-immunoreactive cell bodies and fibers in the rat and the guinea pig. A moderate number of immunoreactive cell bodies were found in the colon in the myenteric plexus and a small number in the submucous plexus. Nerve fibers were observed in all parts of the colon except in the longitudinal muscle layer, but in small numbers. Blood vessels in the mucosa and submucosa seemed innervated by gastrin/CCK fibers (Fig. 8).

Schultzberg and co-workers[47] also investigated the possible presence of neurotensin immunoreactivity in the colon. None was found in cell bodies, while some nerve fibers were observed in the circular muscle layer in the cecum.

6. Concluding Remarks

The proportion of cell bodies with immunoreactivity to the various peptides described above has been determined in the colon of the rat and the guinea pig by Schultzberg et al[47]; their results are presented in Table 2. Assuming that only one transmitter is present in each neuron, it is possible to estimate the proportion of cell bodies in the plexuses that contain none of the investigated peptides. In the myenteric plexus about 40% of the neurons are peptidergic, while in the submucous plexus the corresponding figures are about 70% and 50% in the rat and the guinea pig, respectively. It is likely that many of the other cells are cholinergic.

A number of reports suggest that central and peripheral neurons may contain more than one putative transmitter substance (for a recent review, see Hökfelt et al[6]). In the sweat glands[71] and in the submandibular salivary gland,[6] VIP and ACh seem to be present in the same nerves controlling blood flow and secretion. Similar neurons may also be involved in the control of colonic secretion (compare Fahrenkrug et al[57] and Hultén et al[72]).

V. ELECTROPHYSIOLOGICAL CHARACTERISTICS OF ENTERIC NEURONS

Investigations of the electrophysiological characteristics of neurons in the intestinal tract are fairly sparse; in the case of the colon they are almost nonexistent. The obvious reason is the difficulties to control or eliminate the influence of motility on the recordings. By means of extracellular recordings Wood,[73] on the basis of spike characteristics, classified the neurons in the myenteric ganglia of the ileum into mechanosensitive (slow- or fast-adapting and tonic type), single-spike, and burst-type neurons. On the other hand, intracellular registrations from the same organ enable one to distinguish two types of neurons, called S (type 1) and AH (Type 2).[74,75] The S cell shows electrical properties similar to those of autonomic ganglia, while the AH or tonic-type neuron is characterized by a long hyperpolarization following the action potential. The functions of the two types of nerves are largely unknown, but Hirst[76] has considered the S neurons to be interneurons mediating both excitatory and inhibitory responses. Wood and Mayer[77] have proposed that AH neurons are sensory nerves and also interneurons regulating interganglionic impulse traffic.

The electrophysiological techniques have also been used to test, mainly in the small intestine, the nerve membrane effects of different transmitter candidates. Thus substance P has a potent depolarizing action on the membrane of myenteric neurons that is similar to that seen in AH neurons.[51,78] The same depolarization and increased excitability have also been shown in myenteric neurons following microiontophoretic application of 5-HT.[79,80] Furthermore, VIP and neurotensin increase the firing rate in certain neurons in the myenteric plexus,[81] while hyperpolarization of myenteric neurons is observed on application of met- and leu-enkephalin.[82] These results demonstrate that the membrane potentials in enteric neurons are sensitive to the transmitter candidates, but they do not conclusively show that the applied substances are involved in physiological regulation of the activity in these neurons.

VI. CONCLUDING REMARKS

Clearly the extrinsic and intrinsic nervous supplies of the colon are indeed complex. The ultimate goal of research in this field is to describe in detail the circuitry of the extrinsic nervous control and of the intrinsic nervous reflexes, as well as defining the neurotransmitters involved and their action. We are far from this goal. Although the extrinsic nervous control of colonic function has been studied to some extent, our knowledge of the intrinsic nerves and their function is minimal. To judge from the

number of neurons and neurotransmitters, a fair number of intrinsic nervous reflexes probably exist that control all the major physiological functions, i.e., absorption, secretion, and motility. However, only two intrinsic reflexes are known, namely, the peristaltic reflex controlling propulsive motility[76] and the colonic vasodilatation elicited by mechanical stimulation of the colonic mucosa.[83,84] Even with regard to these reflexes our knowledge of the number of neurons involved and their arrangement and transmitter substances is meager, to say the least.

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11

Circulation of the Colon

Ove Lundgren and Mats Jodal

The circulation of blood through the colon has received little attention as compared to that of skeletal muscle or even the small intestine. One obvious reason for this neglect is the relative inaccessibility of the organ, even though qualitative information has been gained from studies of human colostomies. Our ignorance is reflected in the fact that not until a few years ago was any quantitative information available concerning blood flow in the human colon. This review attempts to summarize our knowledge in this field, but in some instances analogous conclusions from observation made on the small intestine have been made, due to the scarcity of data pertaining to the large bowel. Furthermore, the review is almost exclusively based on observations made on carnivorous animals, where the cecum is small or absent.

I. ANATOMICAL AND FUNCTIONAL CHARACTERISTICS OF THE COLONIC VASCULAR BED

In most species the colon is supplied from the superior and inferior mesenteric vessels. Like the rest of the gastrointestinal tract a striking feature is the presence of a large number of vascular arcades. These are macroscopically observable in the mesentery of most species, but intramural arcades also exist at several levels (Fig. 1). This arrangement ensures the necessary blood supply in most situations to tissues constantly changing their position within the closed space of the abdomen. Furthermore, the movements of the muscularis may locally impede the blood supply, as will be discussed below. Despite these precautions taken by nature to ensure a sufficient supply of blood, ischemic lesions are not infrequent in this organ, particularly at the boundary region between the transverse and descending part of the colon.[1] This part represents the area where the vascular supplies from the superior and inferior mesenteric arteries meet, implying that the arterial perfusion pressure is normally comparatively low there.[1]

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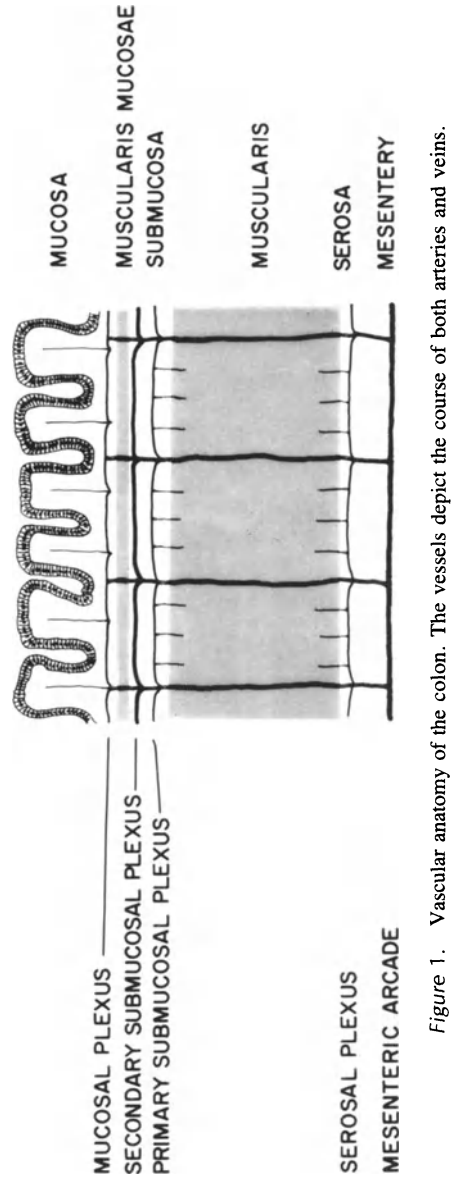

Figure 1. Vascular anatomy of the colon. The vessels depict the course of both arteries and veins.

There has been surprisingly little investigation of the colonic microvasculature, and the description below is to some extent based on anatomic studies of the small intestine. The early literature on the subject is covered by Patzelt.[2] The vessels supplying the intestinal wall (the vasa recta) pierce the intestinal muscularis and during their course through the muscle layer give off a few branches to this layer. Reaching the submucosa they form two vascular networks. One of them, smaller and adjacent to the muscularis, provides the muscle layer with blood. The other plexus is larger and supplies the mucosa via the submucosal vascular plexa the arteriae rectae of opposite sides anastomose at the antimesenteric border. The mucosal arteries pierce the muscularis mucosae, after which they again form a small plexus (Fig. 1). Vessels from this vascular network supply the colonic mucosa. The capillaries form an abundant network around the crypts and drain into veins at the mucosal surface facing the colonic lumen (Fig. 2). The mucosal capillaries are fenestrated,[3] while the muscle capillaries probably are continuous.

From a functional point of view the colonic vascular bed can be looked upon as consisting of a number of parallel-coupled and series-coupled vascular sections (Fig. 3). The parallel-coupled vascular circuits, supplying the different wall layers, were described in detail above. Most studies show that these vascular circuits are parallel, i.e., the nutritional vessels (mainly capillaries) of the mucosa, submucosa, and muscularis are coupled in parallel. Observations using microspheres[4] suggest that in the small intestine the mucosal and submucosal vascular beds are coupled in series. This is, of

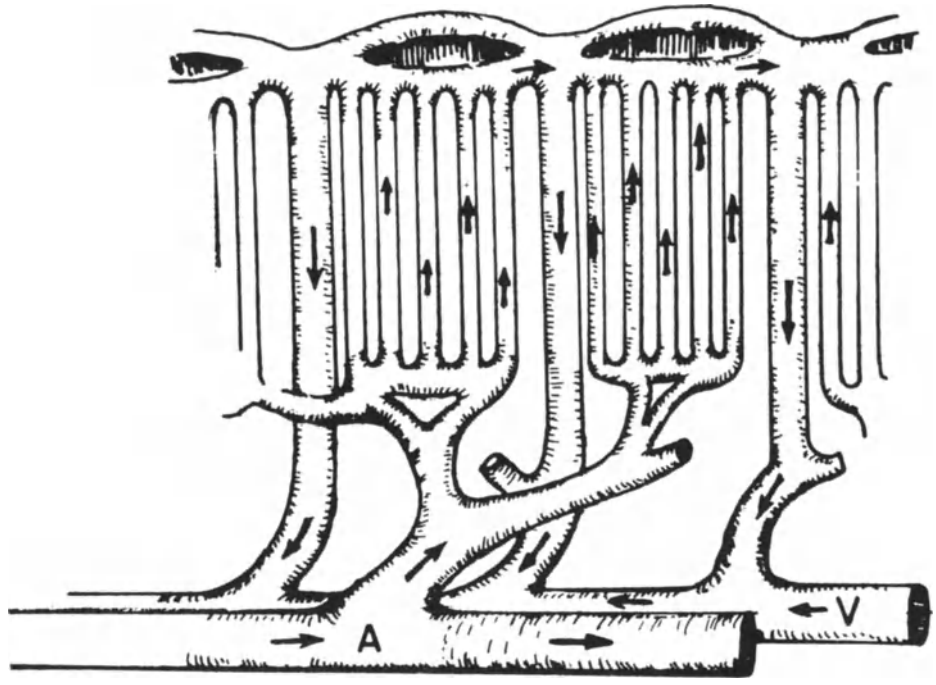

Figure 2. The vascular architecture in the colon mucosa as described by Reynolds.[83]

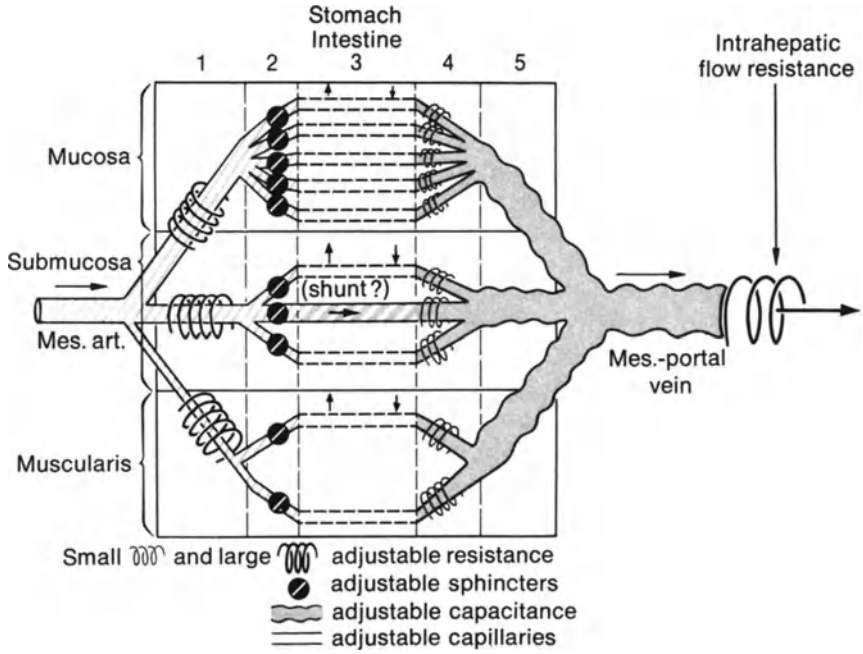

Figure 3. Schematic illustration of the different parallel-coupled vascular circuits of the colon with their consecutive vascular sections: (1) precapillary resistance vessels; (2) precapillary sphincters; (3) exchange vessels; (4) postcapillary resistance vessels; (5) capacitance vessels. From Folkow,[5] by permission.

course, true in the sense that the blood diverted to the mucosa must pass through the submucosal vascular network of fairly large-size vessels. However, no anatomical observations in the colon or in the small intestine indicate that the capillaries of the connective tissue in the submucosa are series-coupled with the mucosal capillaries.

Each parallel-coupled vascular bed is made up of several anatomically and functionally different series-coupled (consecutive) vascular sections (Fig. 3).[5–7] The *precapillary resistance vessels* consist of muscular vessels, usually named arterioles and metarterioles. These vessels are the main determinants of blood flow to a particular region, and the site of the local and the remote control systems which regulate the rate of blood flow from moment to moment by changing vascular smooth-muscle tone. The *precapillary sphincters* are a specialized section of the smallest precapillary resistance vessels. This section usually contributes little to total regional vascular resistance, but it is of paramount importance as regards the control of the numbers of perfused capillaries and, hence, the mean blood–tissue diffusion distance and the time available for transcapillary exchange. It should be stressed, however, that the term *precapillary spincter,* as used by us, is a functional concept corresponding to different anatomical arrangements in different organs. The anatomical counterpart to the precapillary sphincter in the colon is not known.

The capillaries or the *exchange vessels* constitute the key section in any vascular circuit. It is across their thin endothelial lining that the all-important exchange takes place between intra- and extravascular compartments.

Distal to the capillaries are the venules and veins, called *postcapillary resistance vessels* from a flow resistance point of view. The tonus of this vascular section is one of the main determinants of mean hydrostatic capillary pressure and the rate of hydrodynamic fluid exchange across the capillary wall. An isolated constriction of these vessels, for example, increases hydrostatic pressures upflows, thus producing a capillary filtration of fluid into the colonic tissue. The postcapillary resistance section overlaps anatomically with the *capacitance section* composed of the venous compartment as a whole. Changes in the smooth-muscle tone of these vessels may markedly alter regional blood volume without significant changes of the total regional vascular resistance to blood flow.

The presence of arterio-venous shunts in the submucosa of the gastrointestinal tract has been a subject of great controversy (see Grayson[8]). Such shunts have been described in all the consecutive segments of the alimentary tube, including the colon.[9] Their functional significance seems to be small, to judge from experiments on the small intestine showing that only 3% of blood flow passed vessels with a diameter larger than 20 μm.[10] No corresponding experiments have been performed on the colon.

II. METHODOLOGICAL CONSIDERATIONS

Qualitative studies of the colonic circulation in humans have been performed for almost half a century making use of the parts accessible in patients with colostomies. Flow reactions in the colonic mucosa have then been followed by estimating color changes[11–16] or by heat-measuring devices.[17–22] Such studies have provided information particularly on integrated flow reactions elicited by reflexes or induced by emotions. The *quantitative* study of colonic circulation has to a large extent been performed in animals, since such studies were not possible to perform in man until recently (see below). Some earlier studies by Bacaner et al[23–26] are considered unreliable because a water-soluble substance (PO_4) was used as a tracer. The transcapillary exchange of such compounds is not flow limited except at very low flow values.[27,28]

When studying total organ blood flow on anesthetized animals, a number of different techniques are at hand, including electromagnetic flow meters, ultrasonic flow meters, venous drop-counting devices, and microspheres (for a recent review of methodology, see Svanvik and Lundgren[29]). The microsphere technique has also been used to study blood flow distribution within the different wall layers in the colon, although this should be done with caution. Another technique for investigating flow in the different wall layers involves local microinjections of inert substances (krypton, xenon, and radioiodinated antipyrine) into the mucosa and the muscularis. This method is based on the high diffusibility of these lipid-soluble compounds, implying that the elimination of the tracer from the colonic tissue is blood flow limited. Using the equations developed by Kety[27] it is possible to estimate quantitatively local blood flow (compare Fig. 4).

The study of the reactions of the consecutive vascular sections can only be performed in the anesthetized animals. Several methods are available, but so far only the plethysmographic technique has been utilized on the colon.[30] In this method the volume changes of the organ are continuously followed by enclosing the organ in a plethysmograph, usually together with recording total blood flow and pressure in the

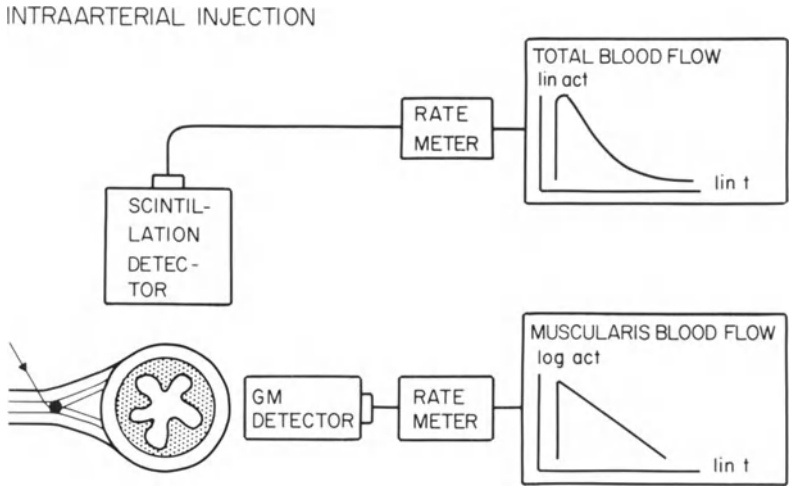

Figure 4. The ^{85}Kr washout method for determining intestinal blood flow distribution in man as described by Hultén et al[33] by permission.

draining vein. Since changes in tissue volume depend on alterations of regional blood content and/or transcapillary fluid exchange, these two quantities must be separated. This is usually possible from the volume recordings alone, since changes in the two parameters often have quite different time courses. Hence, these methods permit the continuous recording of blood flow (resistance vessels), net capillary fluid exchange (exchange vessels), and changes of regional blood content (capacitance vessels). It is also possible to measure changes in capillary surface area available for transcapillary exchange by determining the capillary filtration coefficient (CFC). This parameter is a measure of the outward capillary filtration induced by a sudden increase of mean capillary hydrostatic pressure.

Mucosal blood flow in awake man has been estimated quantitatively by following the tracer elimination after local microinjection of ^{125}I-antipyrine into the mucosa of colostomies.[31,32] This radioactive compound is lipid soluble and highly diffusible, and flow can be calculated as described above for xenon and krypton (see also Fig. 4).

During surgery total colonic blood flow has been estimated in man using the method illustrated in Fig. 4.[33] The elimination of the tracer is followed by a scintillation detector placed above the organ and a Geiger–Müller tube at the antimesenteric border. The scintillation detector records γ radioactivity from the whole organ, and the recorded curve can be analyzed by the height : area formula proposed by Zierler[34] to determine total blood flow. This method also makes it possible to estimate blood flow distribution within the human colon, since the Geiger–Müller tube only records the low-energy β radiation emitted from the tracer located in the muscularis. The registered exponential decay of β radiation thus reflects flow in the muscularis, which can be calculated according to the Kety equations. With the information of the weight relationship between mucosa–submucosa and muscularis (determined from histological

sections) and knowing total and muscularis flow, blood flow in the mucosa–submucosa can be calculated.

III. BASIC HEMODYNAMIC DATA

The "resting" blood flow in the colon in various mammals is given in Table 1. Colonic blood flow in man is about 20 ml/min per 100 g tissue as measured during surgery with the inert-gas washout method described above.[33] Since the colon weighs about 1200 g in man,[33] total colonic blood flow measures about 240 ml/min at "rest," constituting about 5% of cardiac output. The term "resting" in this context usually implies that the subject has been deprived of food for some hours. After a meal, gastrointestinal blood flow increases moderately; colonic blood flow increases too, as demonstrated by qualitative methods.[15]

The potential flow range of the colonic vasculature is high indeed, as illustrated by investigations in the cat showing that blood flow in the colon can be increased approximately 10 times to about 200 ml/min per 100 g tissue by infusions of a potent vasodilator drug.[30] This probably mainly reflects an extraordinary vasodilatation in the mucosa as discussed below. In man a blood flow of a similar magnitude has been measured in fulminant cases of ulcerative colitis, implying that total colonic blood flow is around 1500 ml/min.[35] This represents about 30% of the resting cardiac output. Hence, even if cardiac output is doubled, the fraction diverted to the colon is still quite high in this clinical disorder.

The blood flow rate recorded in the colon is lower than in the small intestine.[33,36] In the human jejunum and ileum, for example, blood flow measures about 50 ml/min and 30 ml/min, per 100 g tissue, respectively. On the other hand, colonic blood flow in man is several orders of magnitude higher than that of resting skeletal muscle. In all probability these differences reflect the different nutritional demands of the organs.

A. Blood Flow Distribution

Most investigators studying the hemodynamics of the colon have recorded total blood flow. When elucidating the relationship between blood flow and function, it is,

Table 1. Blood Flow in the Large Intestine of Various Animals

Species	Colonic blood flow, ml/min per 100 g[a]	Technique	Reference
Man	18 ± 8	Inert gas	33
Rhesus monkey	91 ± 47	Microsphere	84
Dog	82 ± 33	Microspheres	85
	124 ± 57	^{86}Rb clearance	86
	73 ± 9	Flow recorder	74
	35	Flow recorder	43
	40 ± 7	Inert gas	38
Cat	22 ± 7	Drop counter	33
Rat	30 ± 76	^{86}Rb clearance	87

[a]Mean ± standard deviation.

however, most important to obtain detailed information on the regional distribution of flows, since blood flow is not homogeneous throughout the intestinal wall. There are several functional reasons for uneven flow distribution within the colon wall. In part it reflects the varying demands for oxygen and other nutrients of the different colonic functions. Hence, secretion and absorption, two metabolically demanding tasks, are localized to the mucosa, where blood flow is comparatively high. However, as regards the mucosa, functional demands other than nutritional ones must be of importance, since mucosal blood flow provides not only the solutes for cell metabolism but also the fluid necessary for the secretory activity.

A quantitative survey of the intramural blood flow and flow distribution is given in Table 2 based on studies made on the human, canine and feline colon.[32,33,37–39] It is evident from the table that mean mucosal blood flow in the colon is about three times greater than muscularis flow expressed per unit weight. With increasing total blood flow mucosal blood flow is augmented markedly. This naturally implies that an increasing proportion of flow is diverted to the mucosa.

In Table 2 the colonic wall is divided into three anatomical regions. There are reasons to believe that some of them may not be homogeneously perfused. For example, the nervous plexus situated between the circular and longitudinal muscular layers may be perfused at a rate different from that of the muscular coat. Moreover, the cell renewal zone at the base of the crypts may be well perfused.

B. Consecutive Vascular Sections

Apart from the studies of the resistance vessel outlined above, few studies have been devoted to investigations of the series-coupled vascular sections in the colon. Thus any quantitative studies of the length and surface area of capillaries in the different wall layers of the colon have not been performed. The overall hydraulic conductivity in the colonic capillaries has been estimated in the cat by determining the CFC.[30] At resting blood flow levels CFC was about 0.05 ml/min per mm Hg per 100 g tissue and increased to about 0.15 ml/min per mm Hg per 100 g tissue at maximal vasodilatation when blood flow measured approximately 200 ml/min per 100 g. The

Table 2. Blood Flow and Flow Distribution in the Wall Layers of the Large Bowel

	Man	Dog	Cat
Total blood flow, ml/min per 100 g	18	51	22
Regional blood flow, ml/min per 100 g			
Mucosa–submucosa	28	74	31
Muscularis	11	28	10
Blood flow distribution, %			
Mucosa–submucosa	66	73	80
Muscularis	34	27	20
Reference	33	38	33

corresponding values for resting and maximal CFC in the skeletal muscle capillaries are about 0.01 and 0.04 ml/min per mm Hg per 100 g tissue, respectively. The difference in CFC between the two organs in all probability reflects both varying capillary density and capillary "porosity." The so-called fenestrated capillaries in the colonic mucosa[3] are probably more porous compared with skeletal muscle capillaries, which are continuous. The difference in porosity is explained by variations in number and/or size of pores.

In textbooks the capillary pressure in the skeletal muscle vascular bed is considered to center around a mean value of 25 mm Hg, in accordance with the classical studies of Pappenheimer.[28] This figure represents the mean capillary hydrostatic pressure as well as the plasma colloid osmotic pressure. In the mucosal capillaries in the digestive tube it seems reasonable to assume that the Starling equilibrium is set at a lower pressure level, for at least three reasons. First, the mucosal capillaries are fenestrated with a large hydraulic conductivity (see above), implying that the reflection coefficient for the plasma colloids may be below one. Second, the protein content of the lymph, probably reflecting the protein concentration of the interstitial fluid is high, reaching values above 60% of the plasma protein concentration at "physiological" venous outflow pressures (see, e.g., Johnson and Richardson[40]). Hence, the colloid osmotic pressure of the interstitial space is not negligible, as usually assumed. Third, measuring mean hydrostatic capillary pressure in the intestinal villi of the rat, Gore and Bohlen[41] recorded pressures around 14 mm Hg. In the muscular layer of the small intestine, on the other hand, the same authors measured a mean hydrostatic capillary pressure of about 25 mm Hg, implying that this part of the intestinal wall may more resemble skeletal muscle as regards the capillary fluid equilibrium (see above).

The comparatively large hydraulic conductivity of the mucosal capillaries in the colon represents a potential risk, since even a small increase of mean hydrostatic capillary pressure might translocate large intravascular volumes to the extravascular space, an event that may be of great general hemodynamic importance. Mean capillary pressure is particularly sensitive to changes in venous pressure, since the postcapillary flow resistance is much smaller than the precapillary one. Two mechanisms tend to counteract fluid losses induced by increased capillary hydrostatic pressure. First, the precapillary sphincters constrict in response to the augmented transmural pressure, reducing the number of perfused capillaries and the exchange area for fluid.[42] This mechanism has not explicitly been demonstrated in the colonic vasculature. It has been shown, however, that the precapillary resistance vessels constrict in response to an increase of transmural pressure, secondly to a venous pressure rise.[43] A similar chain of events seems probable with the capillary sphincters. Second, if venous outflow pressure remains increased for a long time, a new Starling equilibrium is attained, owing to an increased interstitial hydrostatic pressure and/or a decreased interstitial colloid osmotic pressure.[40,44]

The blood volume, mainly contained in the colonic capacitance vessels, amounts to 7–10 ml/100 g.[30,45] A large portion of this blood volume is localized in the veins of the delicate mesenteric tissue, and the blood volume in the colonic *wall* is about 5 ml/100 g colonic tissue.[46] These regional blood volumes are two to four times larger than that reported for skeletal muscle. This difference is at least in part explained by the

denser vascularization of the colon. In the colonic wall proper the blood content of the submucosa is comparatively great, owing to the presence of a dense vascular plexus in this part. In fact, in the feline colon nearly 15% of the submucosal tissue volume is blood, mainly localized in veins.[46]

IV. LOCAL AND HORMONAL CONTROL

A. Local Chemical Control

The local chemical milieu is believed to be of great importance for the control of flow resistance in many vascular beds. Thus, the cerebral and coronary vascular beds are assumed to be under constant influence of such factors, making it possible to adapt the magnitude of blood flow to the prevailing metabolic demands. Our knowledge of the importance of local chemical environment in the regulation of colon blood flow is scarce. In recent experiments, Gilmour (personal communication) and Gilmour et al[38] have demonstrated a good direct relationship between blood arterial carbon dioxide tension levels and colonic blood flow, implicating a possible role of CO (pH?) in the control of colonic circulation. Furthermore, varying arterial P_{O_2} within wide limits above and below normal suggested that also oxygen may be of importance in the local control of colonic blood flow. However, these experiments were difficult to analyze since the hypoxia evidently also induced a reflex nervous vasoconstriction via the chemoreceptors. It should be emphasized that the local metabolic control of most vascular beds is most probably multifactorial. Hence, the quantitative importance of the compounds discussed above for the control of the colonic circulation is not known.

B. Postprandial Hyperemia

A functional hyperemia in the splanchnic region has been recorded in man and animals following food intake. Most observations suggest that the overall blood flow to the abdominal organ increases approximately 100% above control. Observations on the colonic circulation indicate the presence of postprandial hyperemia also in this organ. Welsh and Wolf[47] recorded an augmented colonic temperature for 60–80 min after a meal, probably implying a mucosal vasodilatation for the same length of time. Furthermore, in a recent experiment Bond et al,[37] using microspheres, demonstrated an increase of mucosal blood flow in the canine colon mucosa 45 min after a meal.

The mechanisms underlying the intestinal functional vasodilatation are not known in detail. In the small intestine three different mechanisms have been proposed i.e., an accumulation of local metabolites, a local nervous reflex mechanism, and a hormonal influence. The question of the local chemical control was reviewed above. Mechanical stimulation of the mucosa in the denervated small and large bowel evokes a marked hyperemia via a local nervous reflex[48] in which 5-HT receptors in some way are involved.[49] It has been proposed that the food present in the intestinal lumen is the natural stimulus for this nervously mediated hyperemia.

The number of potential and established hormones in the gastrointestinal tract has increased greatly during the last decade (see, e.g., Grossman[50]). Several hormones exhibit a marked increase in plasma concentration in connection with a meal. Their

vascular effects in physiological blood concentration are not established, however, but it is quite possible that hormones play a role in inducing the postprandial hyperemia of the colon.

C. Vascular Effects of Hormones and Pharmacological Agents

A comparatively large body of information is available on the effects of exposing the mucosa of human colostomies to various drugs,[13–16, 51] including hormones such as epinephrine and norepinephrine. Such experiments are difficult to evaluate for several reasons. Since drugs have varying intestinal permeabilities, it is difficult to estimate the tissue concentration of the compound from the concentration in the applied solution. Comparisons between drugs are therefore difficult, if not impossible. Furthermore, only a small part of the total vascular bed is exposed to the vasoactive substance. The visual observations in such experiments, though only qualitative, convey the impression that the drug effect evoked by mucosal application is largely the same as that observed in other vascular beds upon intravascular administration. Hence, norepinephrine seems to be a pure vasoconstrictor substance and acetylcholine a vasodilator drug in the colon. For a detailed account of these results, the reader is referred to the original work.

Kerr et al[52] studied in a more conventional way the effect of vasopressin on the colonic circulation of the rhesus monkey. This compound was shown to produce a potent vasoconstriction at infusion rates that did not induce any significant blood pressure increase.

D. Autoregulation of Blood Flow

Many vascular beds in the body exhibit autoregulation of flow, i.e., blood flow tends to stay constant in the face of fairly large variations in perfusion pressure. Hanson and Johnson[43] and Hanson and Moore[53] studied this phenomenon in the colon when lowering arterial blood pressure, some animals exhibiting signs of autoregulation. Increasing venous outflow pressure induced an increase of total blood flow resistance in the colon in all experiments, which is usually taken to indicate a myogenic response of the precapillary vessels to the increase of transmural pressure. The increased transmural pressure induces a contraction of the vascular smooth muscles. Hanson and Johnson[43] concluded that the colonic vascular bed "is capable of autoregulation of blood flow."

In the study by Hanson and Moore[53] the colonic autoregulation of flow was analyzed further. The colonic autoregulation was abolished by the vasodilator drug papaverin given i.a. or by distending the colon with a luminal pressure of 50 mm Hg.

The mechanism underlying autoregulation of flow is much debated. Two main schools of thought exist. According to one school, the constancy of blood flow is secondary to strictly myogenic properties of the intestinal vascular smooth muscles. Such a mechanism is suggested by the experiments cited above that show that increasing venous outflow pressure induces a constriction of the precapillary vessels.[43, 53] It may also be supported by the observation that increasing the pressure in the intestinal lumen

abolished autoregulation, since such a procedure would increase tissue pressure and hence decrease transmural pressure.[53]

According to the other hypothesis, autoregulation of flow reflects a relaxation of vascular smooth muscle secondary to an accumulation of vasodilating metabolites. Probably both mechanisms exist in most tissues. The quantitative importance of transmural pressure versus that of tissue metabolites may, however, vary in between vascular beds, and no studies to elucidate this particular question have been performed on the colon.

The phenomenon of autoregulation is usually discussed in terms of blood flow. However, autoregulation is probably also designed to keep mean capillary hydrostatic pressure constant. Functionally, such a mechanism is of great importance, since the vascular beds with great ability for autoregulation, such as kidney and intestine, also are provided with extensive capillary networks. Hence, small changes in capillary pressure may provoke sudden and large shifts between intra- and extravascular compartments.

V. NERVOUS CONTROL OF COLONIC CIRCULATION

A. The Sympathetic Control

The sympathetic control of the colon is mediated in part via fibers in the splanchnic nerves and in part via fibers in the lumbar colonic nerves. In man the boundary between the areas of distribution of the two nerves is located in the *colon transversus*.

The distribution of adrenergic fibers within the colonic wall has been described in detail in Chapter 10. To recapitulate briefly the adrenergic fluorescence is demonstrated in particular around the nerve cells in the nervous plexa of the colon. Nerve fibers are also seen around blood vessels in all layers of the intestinal wall. In the mucosa sympathetic nervous varicosities are especially frequent in the deeper layers. The sympathetic fibers make contact with the outer vascular smooth-muscle layers of the vessels, indirectly controlling also the rest of the smooth muscle cells via the intercellular nexa.

Activating the sympathetic outflow to the colonic vasculature evokes a characteristic flow pattern that has been investigated most thoroughly in cat[30, 39, 54] and to some extent in man.[45] When stimulating the nervous fibers electrically a fairly intense vasoconstriction is initially observed (Fig. 5), particularly at the higher stimulation frequencies (above 4 hz). However, within 2–4 min blood flow again increases, despite a continued nerve stimulation, reaching a new steady state level only moderately below control. During the steady-state phase of nervous vasoconstriction, the increase of blood flow resistance seldom exceeds 100%, even when the rate of nerve stimulation is high. The secondary increase of flow during nerve stimulation has been called "autoregulatory escape from vasoconstrictor fiber influence" [53] in the small intestine and is also seen in the gastric, the hepatic, and the renal vascular beds. It is not due to fatigue of nervous transmission since the intestinal veins remain constricted throughout the stimulation period. This constriction of the capacitance vessels expells approxi-

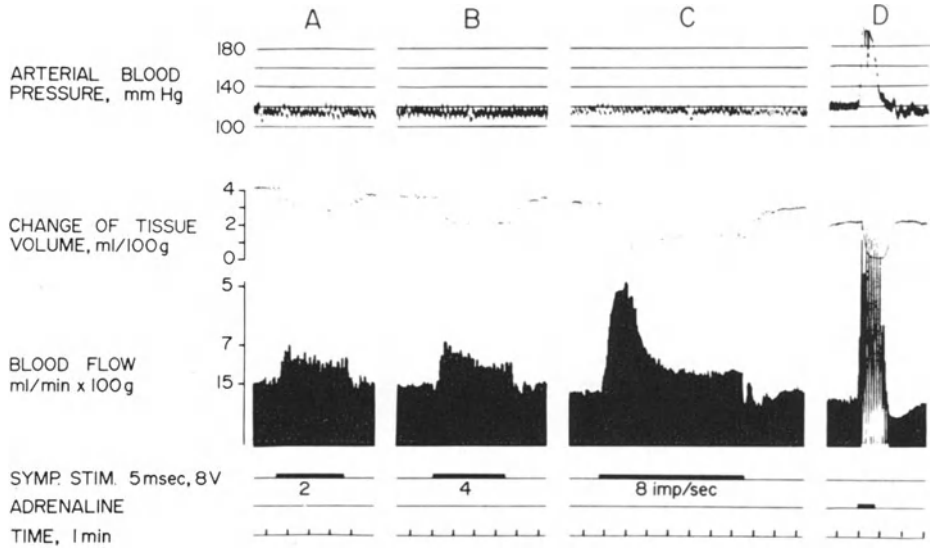

Figure 5. The effect of graded stimulation of the regional vasoconstrictor fibers on arterial blood pressure, blood flow, and tissue volume of the cat large bowel (panels A, B, and C). The changes in tissue volume reflect variations in regional blood volume. As a comparison the effect of a large dose of adrenaline is illustrated in panel D. From Hultén et al[30], by permission.

mately 20% of the regional blood content when the nerves are activated with a stimulation frequence of 2 hz and 30–50% at a stimulation frequencey of 8 hz (Fig. 5). It can be calculated that such a venous vasoconstriction in man would mobilize about 50 ml blood from the intestine.[45]

It has been clearly demonstrated that activation of the sympathetic nervous fibers to a skeletal muscle vascular region increases the pre- to postcapillary resistance ratio to such an extent that mean capillary hydrostatic pressure is lowered and extravascular fluid is absorbed into the vascular compartment.[6] When the blood volume is decreased by bleeding, the activation of the sympathetic nervous system induces in this way an ''autotransfusion'' tending to restore blood volume. Stimulation of the sympathetic nervous fibers to the colon, on the other hand, does not significantly change mean capillary hydrostatic pressure, at least to judge from recordings of total organ volume (Fig. 5).

The sympathetic nerves also control the number of perfused capillaries. The direction and extent of this control have been investigated by following the nervously induced changes in the CFC.[30] The number of perfused capillaries is decreased during the steady-state phase of vasoconstriction. Quantitatively, a nerve stimulation at 4 hz reduces the CFC to about 60% of control. This may be explained by assuming nerv-

ously controlled "precapillary sphincters" exist that constrict upon nervous stimulation.

Blood flow distribution between the different layers of the colon has been investigated during the steady state phase of nervous vasoconstriction with inert gas washout techniques in cat[39, 45] and man.[45] In these two species a nervous vasoconstriction is observed in both the muscle layer and in the mucosa upon electrical activation of the sympathetic fibers. In man the vasoconstriction seems to be most pronounced in the muscularis since a larger portion of total blood flow is distributed to the mucosa-submucosa during nervous activation in the experimental group than in the control, while no clear net redistribution occurs in the feline colon. However, some observations suggest that a redistribution of blood flow may occur *within* the mucosal layer.[39]

The "autoregulatory escape" mechanism (Fig. 5) described above has been investigated in great detail as regards the underlying mechanisms, although all studies have been performed on vessels from the small intestine (for a review, see Svanvik and Lundgren[29]). The phenomenon is most probably of multifactorial origin, and several hypotheses have been presented. According to one school of thought the escape reflects an inherent property of the vascular smooth muscles. This hypothesis is mainly based on results obtained on vascular smooth-muscle strips of large vessels in organ baths. During such *in vitro* conditions vascular smooth muscle tends to escape from adrenergic influence.[55] Somewhat in line with this it has also been suggested that the autoregulatory escape is secondary to an adaptation of the α receptors on the vascular smooth muscle in the gastrointestinal canal.[56]

Another proposal involves the concomitant activation of adrenergic α receptors, causing vasoconstriction, and adrenergic β receptors, inducing vasodilatation.[57] Evidence for this hypothesis has been reported in experiments where the autoregulatory escape was abolished after β blocking. The results obtained in such experiments are not consistent, however, some researchers being unable to demonstrate any effect of β blocking agents.[58] Finally, one obvious explanation is the accumulation of vasodilating metabolites during the initial intense vasoconstriction. The importance of all these possible mechanisms in explaining autoregulatory escape in the gatrointestinal tract remains to be determined.

The pharmacological analysis of autoregulatory escape was performed on the small intestine of experimental animals. In the cat colon it has been demonstrated that the initial vasoconstriction of the resistance vessels is α adrenergic.[54] The presence of α-adrenergic receptors in the human colon has been reported repeatedly in reports describing qualitative observations on colostomies. Local or intravenous administration of norepinephrine induced a vasoconstriction recorded with thermocouple methods or from color changes.[14–16, 20, 22]

B. The Parasympathetic Control

The parasympathetic supply to the colon consists of two main nerves, i.e., the vagal and the pelvic nerves. The distribution areas of the two nerves meet at the transverse portion of the colon. The intramural distribution is largely unknown due to the lack of specific methods for demonstrating the presence of cholinergic nervous pathways.

Electrical stimulation of the vagal fibers to the colon does not elicit any flow changes apart from those secondary to an increase of colonic motility.[54] Stimulating the pelvic nerves, on the other hand, induces marked changes in colonic hemodynamics. A fairly detailed analysis of this has been performed in the cat,[30, 39, 54] and Fig. 6 illustrates an experiment on this animal. This experiment was performed on the colon enclosed in a plethysmograph (middle tracing), recording total venous outflow with a venous drop recorder unit. The animal had received atropine i.v. (1 mg/kg body weight). Upon pelvic nerve stimulation a transient increase of colonic blood flow was observed, the magnitude of which was dependent upon the rate of stimulation. Concomitantly an abrupt increase of tissue volume was recorded reflecting an augmentation of the regional blood volume. In the upper range of physiological discharge rate (8 hz) colonic blood flow values up to 200 ml/min per 100 g tissue were registered in some experiments. The increase of the volume in the capacitance vessels amounted to 2–2.5 ml/100 g tissue at most (Fig. 6), corresponding to a 25% increase of regional blood volume. The latter response is probably in part secondary to the increased transmural pressure in the veins which accompanies the vasodilatation and, in part, due to a direct nervous relaxation of the capacitance vessels.

The intramural flow distribution during the nervous vasodilatation has been studied in the cat using local microinjections of inert tracers.[39] These studies demonstrated that the initial marked vasodilatation is accompanied by an increase of blood flow located exclusively in the colonic mucosa, especially the superficial layers.

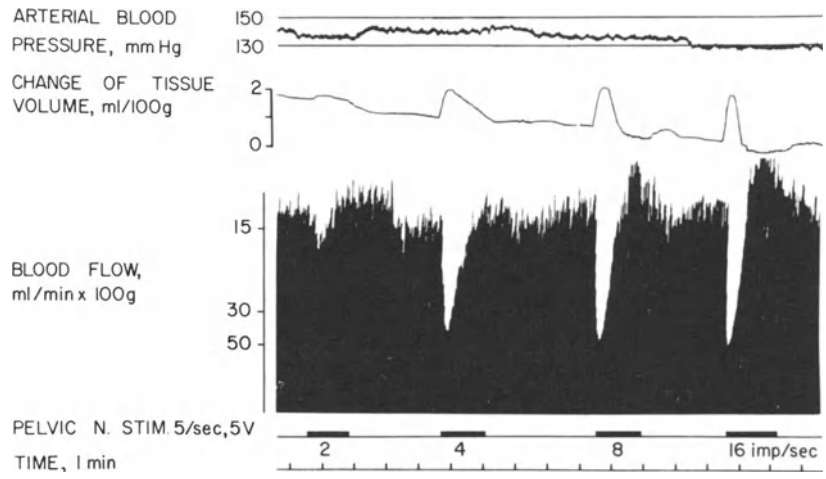

Figure 6. The effect of graded stimulation of the pelvic nerves on blood pressure, blood flow, and tissue volume of the cat colon. The animal had received atropin (1 mg/kg b.w.). From Hultén et al.[39]

Quantitatively, blood flow in the mucosa increases from 25–40 ml/min per 100 g mucosal tissue at rest to 150–200 ml/min per 100 g mucosal tissue at a total colonic blood flow of 60 ml/min per 100 g colonic tissue.

The vascular response upon pelvic nerve stimulation described above and shown in Fig. 6 is also accompanied by a mucus secretion (atropine sensitive) and a marked contraction of the colon musculature (atropine resistant). All these nervous effects are probably involved in the act of defecation.

A pharmacological analysis showed that the parasympathetic vasodilatation in the colon is evoked via receptors that are not blocked by conventional cholinergic or adrenergic blocking agents.[54] Three proposals have been made with regard to the nature of the transmitter involved. Burnstock[59] has proposed that all the autonomic nervous effects that are noncholinergic and nonadrenergic are mediated via the release of ATP. No experimental support for this hypothesis exists with regard to the nervous colonic vasodilatation.

According to the kininhypothesis,[61] the nerves release from the gland cells an enzyme (kallikrein) which acts on a globulin in the interstitial space to split off a peptide (kallidin or bradykinin). These kinins are extremely potent vasodilator agents and also increase capillary permeability. The experimental support for this hypothesis includes the demonstration that close intraarterial infusion of bradykinin mimicked the circulatory effects of pelvic nerve stimulation.[62–64] Moreover, electrical stimulation of the pelvic nerves in the atropinized cat markedly decreased the kallikrein contents in the mucosa.[65,66] Finally, administering aprotinin, a protease inhibitor, reduced in a dose-dependent manner the vascular response upon pelvic nerve stimulation.[67] However, so far there is no study that shows the cellular localization of the kinin enzymes in the colon mucosa.

The hypothesis that vasoactive intestinal polypeptide (VIP) is the neurotransmitter in the vasodilatation induced by pelvic nerve stimulation is based on the demonstration of VIP in nerve fibers in close contact with the colonic vasculature.[68] Furthermore, activating the pelvic nerves by electrical stimulation or reflexly produces the release of VIP into the venous effluent from the colon.[69] Moreover, VIP is a potent vasodilator agent in the colon in atropinized animals. On the other hand, no specific VIP blocking agent exists so far that can be tested on the pelvic-nerve-induced vasodilatation.

C. Integrated Nervous Control

Both branches of the autonomic nervous system are involved in reflex and central nervous control of the colonic circulation, but our understanding of this topic is still very meager. For example, we do not know if nervously mediated flow changes are exclusively the result of variations of the firing rate in the sympathetic vasoconstrictor fibers or if the parasympathetic vasodilator fibers participate in the moment-to-moment control of colonic circulation. To judge from the experimental work performed on the vasodilator fibers to the skeletal muscle, it seems probable that the vasodilator fibers are only involved in special reflexes such as the defecation reflex. Furthermore, only single observations exist on a number of possible nervous control mechanisms, in most cases studied with inadequate techniques.

Only one report has been published on *central nervous control* of colonic blood flow.[70] Stimulation in the posterior part of the hypothalamus increased arterial blood pressure, and concomitantly a vasoconstriction was observed in the colon of largely the same magnitude in all colonic layers. The functional significance of this response is not known, since the exact position of the stimulating electrodes was not determined.

The effects of *changing body temperature* on colonic blood flow has been investigated by several research groups on patients with colostomies.[15,18–20,71] These studies demonstrated an inverse relationship between blood flow in the colon and the skin. Hence, cooling of an extremity caused colonic vasodilatation and skin vasoconstriction, while heating produced the opposite effects. These vascular reactions have been shown to be nervously mediated since they are abolished by infiltrating a local anesthetic around the colonic nerves.[19] Such vascular reactions are probably involved in the control of body temperature. Two early reports are at variance wit these reports on the effect of skin temperature on colonic blood flow. Ruhmann[72] and Kuntz and Haselwood[73] reported that colonic flow was increased upon skin heating and decreased upon skin cooling. The explanation for the observed descrepancies as compared to the more recent reports is not known.

It has been noted on subjects with colostomies that the *sight or smell of appetizing foods* results in a marked reddening and engorgement of the mucosa.[32] In awake animals a similar reaction has been observed recording blood flow in the superior mesenteric artery.[73,74] It seems reasonable to assume that this is predominantly a nervously mediated response. The observations on the colon suggest also that this organ may be involved in the cephalic phase usually considered to be limited to the upper part of gastrointestinal tract.

As was described in some detail above (Section V. B) the electrical stimulation of the pelvic nerves elicited a typical response characterized by a hyperemia, a mucus secretion, and a marked contraction of the colonic muscles. This response pattern can be reflexly elicited via a pelvico-pelvicus reflex arch when mechanically stimulating the rectal or sigmoid mucosa.[61] This complex reflex pattern is probably involved in the *act of defecation,* a proposal that is also supported by observations of a transient colon vasodilatation during defecation in dogs.[75] During the actual emptying of the rectum, on the other hand, colonic blood flow is apparently decreased.[75]

Florey and co-workers[59,76] made some pioneering observations on awake dogs provided with a colonic patch on the abdomen that made it possible to study the color of the mucosa, when, e.g., allowing the dog to run. It was shown that *alarming the animal* by noise produced a swift blanching of the mucosa, suggesting a nervous vasoconstriction. *Physical exercise* also produced an initial marked bleaching of the mucosa. Upon prolonged exercise, however, mucosal color almost returned back to control, indicating only a small remaining vasoconstriction, possibly reflecting an "autoregulatory escape from vasoconstrictor fiber influence."[53]

Bleeding causes a sympathetic vasoconstriction in most vascular beds initially elicited via baroreceptors, but in later stages of bleeding induces hypotension also via central and peripheral chemoreceptors. A colonic vasoconstriction of resistance vessels is also seen upon bleeding.[77,78] Presumably it is mediated via an increased firing rate in the sympathetic fibers. The colonic vasoconstriction helps to restore arterial blood

pressure toward normal values by increasing flow resistance and by augmenting venous return to the heart by expelling blood from the colonic capacitance vessels (see Fig. 5).

The description above has exclusively dealt with fairly well defined experimental situations in man and animals and their effects on colonic blood flow. However, there is a fairly large body of information concerning the reactions of the different functions of the human colon to various types of emotional stimuli. The interest in this field has evolved, since it is a clinical impression that ulcerative colitis is a so-called psychosomatic disease. The works by Wolf et al[13,15] and by Almy and Tulin[11] represent the most thorough studies in this field. Blood flow reactions in these studies were followed by noting changes in the color of the colonic mucosa or by measuring mucosal temperature. Generally speaking, situations colored by anger or hostility are associated with an increased colonic blood flow, while emotionally overwhelming situations or depressing circumstances are accompanied by a decrease of colonic mucosal blood flow. The central nervous control mechanisms that underlie these reactions are not known.

VI. COLONIC BLOOD FLOW AND FUNCTION

Three major functions are represented in the colon: motility, secretion, and absorption. Their relationship to colonic blood flow has been discussed in a limited number of reports.

A. Motility

Motility may affect total blood flow to the colon in two ways. First, it may increase blood flow owing to enhanced metabolism, leading to an accumulation of metabolites in the same way as exercise causes hyperemia in skeletal muscle. Second, the muscular movements may mechanically interfere with blood flow. In animal experiments designed to study the relationship between flow and motility in the small intestine a prolonged increase of total intestinal blood flow has never been demonstrated. This seems to rule out the first possibility described above. However, a slight increase of muscularis blood flow may not be easily discerned by the total blood flow recordings.

The mechanical interference of motility on blood flow has been studied in a few investigations.[75,79] The expected results were obtained; i.e., during strong colonic contractions blood flow transiently decreased, to increase again when the colonic muscles relaxed.[79] Prolonged contractions, as during defecation, reduced colonic blood flow for longer periods of time.[75]

B. Secretion

The current discussion concerning physiological mechanisms underlying secretion across epithelia centers around transcellular "active" transport of solutes via carrier molecules. In the colon a mucus secretion also occurs via exocytosis. Blood flow is considered to be of importance only for delivery of nutrition and raw material. In situations of increased secretion the vessels supplying the gland dilate in response to the

altered metabolic demands, as shown for the colon during pelvic nerve stimulation.[30,39,54]

Colonic secretion of fluid and electrolytes is considered an active transport process, and transcapillary filtration is not believed to contribute to any measurable extent. Lately, however, observations have been reported which strongly suggest that the movement of electrolytes and fluid occurs also through intercellular spaces of certain epithelia. These paracellular routes were earlier assumed to be closed by the tight junctions observed in most epithelia by electron microscopy. The importance of the forces in the Starling equilibrium across the capillary wall for the fluid movement between epithelial cells is not known, however, and this question has never been investigated in the colon.

C. Absorption

According to textbook concepts colonic absorption in carnivorous mammals occurs mainly in the proximal parts of the organ, and sodium chloride and water are the main compounds being absorbed. Blood flow fulfills two functions with regard to intestinal absorption: it represents the major transport vehicle for the absorbed material, and it provides the necessary nutrients for the cellular metabolism underlying active transport across the intestinal epithelium. Again all of our knowledge on the flow–absorption relationship has been gained in studies on the small intestine, most of them being performed by Winne.[79,80] This relationship becomes fairly complicated for an actively absorbed solute, since it may be difficult to decide to what extent an altered absorption rate, induced by a blood flow change, is caused by flow *per se* and/or by an indirect effect on cell metabolism. Passively absorbed solutes are more easily studied, and Winne has explored the effect of variations in total intestinal blood flow on the absorption rate of such solutes. The results demonstrate that the absorption rate of lipophilic compounds increases with blood flow. Water-soluble substances, with the exception of water itself, are on the other hand largely unaffected by the rate of blood flow, probably because the passage across the intestinal epithelium represents the rate-limiting step for the absorption of hydrophilic compounds.

VII. PATHOPHYSIOLOGICAL CONSIDERATIONS

There is an abundance of reports regarding ischemia of the colon and its clinical significance. Three forms of this disease are usually described graded according to severity: (1) gangrene of the colon, (2) ischemic stricture, and (3) transient ischemic colitis. It is beyond the scope of the present review to describe and discuss these clinical entities. The interested reader is referred to the recent reviews by Marston[1] and Saegesser et al.[82]

ACKNOWLEDGMENTS The authors are indebted to Dr. Donald Forrester and Dr. Douglas Gilmour for manuscripts in press. The work described herein as emanating from this laboratory was supported by grants from the Swedish Medical Research Council (14X-2855).

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12

The Enterohepatic Circulation

M. J. Hill

I. INTRODUCTION

The principal components of bile are the conjugated bile acids, cholesterol, and phospholipid, but in addition to these major components there is a vast array of minor components. Some of these are waste products of metabolism excreted by secretion in the bile, for example, bilirubin and the bile pigments. Many are dietary components that have been absorbed from the gut lumen by passive diffusion (because of their lipid solubility) and are detoxified by the liver and secreted in the bile. Some are functioning compounds that are not waste products but that are nevertheless biliary secreted, for example, the bile acids and a wide range of other steroid conjugates.

Following secretion via the bile into the small intestine, many of these compounds are reabsorbed from the gut lumen either by active transport (as with conjugated bile acids) or by passive diffusion following deconjugation (as with unconjugated bile acids) or as part of the micellar phase during lipid absorption (as with cholesterol); such compounds will be returned to the liver via the portal blood, where they are reconjugated (if necessary) and resecreted in the bile. This process is known as enterohepatic circulation and is illustrated diagrammatically in Fig. 1. The proportion of a dose of compound which enters the enterohepatic circulation depends on many variables, including the proportion secreted in the bile, the ease with which conjugates are hydrolyzed by bacterial enzymes, the distribution of deconjugating enzymes along the gut, and the existence of active transport mechanisms, etc.

This chapter will describe the various stages in the enterohepatic circulation of foreign compounds in general terms, and then describe in more detail the circulation of some compounds of major interest (e.g., the bile acids, cholesterol, and polycylic aromatic hydrocarbons). The enterohepatic circulation of urea (which does not involve biliary secretion and intestinal transit) will also be described.

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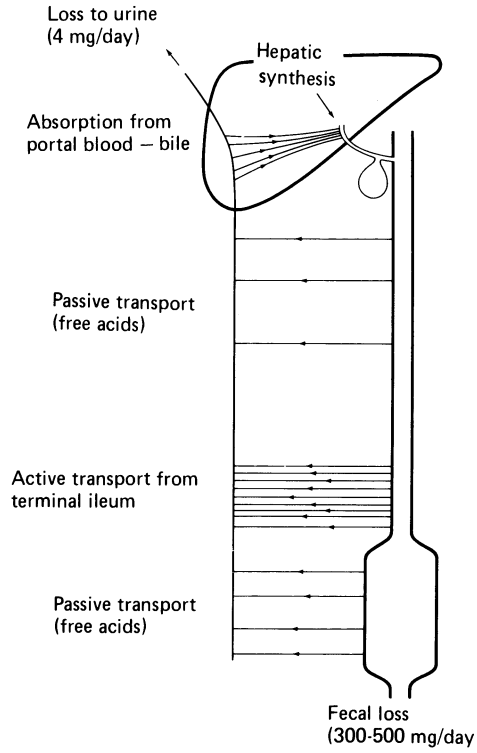

Figure 1. A diagrammatic representation of enterohepatic circulation (illustrated for the bile acids).

The two major stages in the enterohepatic circulation—secetion into bile and absorption from the intestine—will be discussed in turn. The latter is dependent, to some extent, on bacterial deconjugating enzymes, and so a discussion of these will be included.

II. THE HEPATIC SECRETION OF COMPOUNDS IN BILE

Bile is an extremely complex mixture containing seven major and an as yet undetermined number of minor components (Table 1). The major components are bile salts (1%), inorganic salts (0.5%), protein (0.3%), lecithin (0.3%), bilirubin (0.1%), fatty acids (0.1%), and cholesterol (0.1%). Bile is stored in the gallbladder, where it is concentrated by removal of water and electrolytes (through coupled absorption of sodium and chloride ions), so that the organic components of bile are concentrated by a factor of 5 to 10.

The secretion of compounds into bile is determined by two principal mechanisms, one dependent on the secretion of bile acids, the other on sodium excretion. These have been reviewed recently by many experts in the field, including Paumgartner,[1] Erlinger,[2] and Forker,[3] and the reader is referred to these reviews for a detailed treat-

ment of the subject. In essence, the bile-acid-dependent mechanism involves the active transport of bile-acid anions from the parenchymal cells of the liver; this then sets up an osmotic gradient along which water and other solutes flow. Before excretion into bile the bile acids are conjugated with the amino acids glycine and taurine; but, although under normal circumstances this conjugation is complete, this is not a prerequisite for biliary bile-acid secretion. The amount of bile formed is dependent to a large extent on the bile-acid pool size and the hepatic bile-acid concentration, the bile acids being potent choleretic agents. Their excretion appears to be carrier mediated and is saturable.

Bile-acid secretion appears to be the major determinant in the secretion of cholesterol, phospholipid, and other lipophilic agents, although the actual mechanism of this control is unclear. Although Swell et al[4] showed that the secretion of both cholesterol and phospholipid was related to bile salt secretion probably via the formation of mixed micelles, Lindblad et al[5] showed that the effect on the two was quantitatively different. Thus there is a great diurnal variation in the rate of secretion of bile acid during the day which is not accompanied by a large variation in cholesterol secretion, leading to an increased proportion of cholesterol (and an increase in lithogenicity of bile) during the night. This is important in the pathogenesis of gallstones.

Cholesterol is secreted unconjugated in bile, but most other compounds secreted in bile first undergo conjugation either as the glucuronide, the sulfate, or glutathione derivatives, although in addition there are a host of minor conjugation mechanisms. These conjugation mechanisms have been reviewed extensively by Smith[6] and by Williams[7] and are often preceded by metabolic reactions that introduce polar groups and increase the molecular weight of the compound; both increase the efficiency of biliary excretion. For example, biphenyl is first hydroxylated to the 4-hydroxy- or 4,4′-dihydroxy derivative and then conjugated as the mono- and diglucuronides, respectively, both of which are extensively excreted in bile. The metabolism of compounds prior to biliary secretion has been divided by Williams into two stages: in the first (phase 1) the compound undergoes oxidation, reduction, or hydrolysis (Table 2); in the second (phase 2) the product of the phase 1 reaction is conjugated with

Table 1. The Composition of Hepatic and Gallbladder Bile

Substance	Hepatic	Gallbladder
Bile-acid conjugates	1.0%	11.5%
Cholesterol	0.1%	0.6%
Phospholipid (leathin)	0.3%	3.5%
Bilirubin	0.1%	1.0%
Fatty acid	0.1%	1.0%
Protein	0.3%	0.4%
Inorganic salts	0.5%	0.8%
Bicarbonate	40 meq/liter	10 meq/liter
Chloride	100 meq/liter	20 meq/liter
Calcium	3 meq/liter	6 meq/liter
Potassium	6 meq/liter	—
Sodium	150 meq/liter	—
Magnesium	2 meq/liter	0.2 mq/liter

Table 2. Phase 1 Metabolism of Compounds Prior to Conjugation and Biliary Excretion

Class of reaction	Type of reaction	Examples
Oxidation	Hydroxylation	Biphenyl → 4-hydroxy-biphenyl → 4,4-dihydroxy-biphenyl
	Dealhylation	Phenacetin → *N*-acetyl-*p*-aminophenol
	N oxidation	Imipramine → imipramine *N*-oxide
Reduction	Nitro reduction	Nitrobenzene → aniline
	Keto reduction	Keto bile acids → hydroxy bile acids
	Azo reduction	Food colors
Hydrolysis	Ester Hydrolysis	Ethyl *p*-chlorophenoxy isobutyrate
	Amide hydrolyois	Thalidomide

glucuronic acid, sulfate, or glutathione (Fig. 2). This biphasic metabolism is common to most foreign compounds excreted in bile except that, where the compound already has a suitable conjugating group (e.g., hydroxyl or amino group), phase 1 metabolism is unnecessary and conjugation proceeds directly.

The conjugation phase is, for most compounds, an essential step in the efficient biliary secretion for two reasons. First, it increases the molecular weight of the compound; there appears to be a threshold molecular weight of about 300–400, below which little (i.e., less than 10%) of a compound is biliary excreted.[8] This threshold has been determined for the rat, guinea pig, and rabbit and is also thought to be true of the dog, monkey, and human.[6] Second, it increases the polarity of the compound, and this existence of a strongly polar group appears to be a requirement for extensive biliary excretion. In this respect, the term "polar group" implies one such as a carboxyl, sulfonyl, or sulfate (or a cationic group such as a quaternary ammonium group), which will be fully ionized at physiological pH.

By far the most widely used conjugation mechanism in the human and other animals is the glucuronide formation. This does not mean that glucuronide conjugates are specifically favored in biliary secretion but merely reflects the apparently restricted nature of the other conjugation reactions; for example, glycine and taurine conjugation are virtually restricted to the bile acids.

Bile is stored in the gallbladder, which contracts in reaction to the cholecystokinin response to eating, delivering the bile into the duodenum. It may then either be absorbed from the small intestine unchanged by an active transport mechanism (as with the bile acids) or by some passive mechanism (as with cholesterol); alternatively it may undergo deconjugation as a result of microbial enzyme activity followed by passive absorption from the intestine. These various mechanisms of absorption from the intestine will be considered in turn.

III. ABSORPTION FROM THE INTESTINE

A. Active Transport Mechanism

Bile acids are recovered fom the terminal ileum by an active transport mechanism that appears to be carrier mediated, energy linked, and sodium dependent. The charac-

teristics of this mechanism, which is an essential component in the conservation of bile acids, have been well reviewed by Dietschy[9] and by Matern and Gerok.[10]

The mechanism is utilized by both free and conjugated bile acids, and there is some dispute concerning the specificity of the transport system involved. Dietschy[9] reported that the carrier has no greater specificity for conjugated than for free bile acids (Table 3), while Krag and Phillips[11] also reported no differences. Dietschy also reported that the carrier has a greater specificity for conjugated trihydroxy than for dihydroxy bile salts.

In practice this specificity is largely immaterial. Because of the distribution of bacteria along the gastrointestinal tract, only a small proportion of the bile acids entering the terminal ileum are deconjugated. Further, the biliary bile acids are principally primary (Table 4), with on average less than 20% in the form of the bacterial metabolites deoxycholic and lithocholic acids. Thus, in normal persons the overwhelming proportion of the bile acids reaching the relevant region of the small intestine are conjugates of the primary bile acids.

The importance of this mechanism of bile-acid conservation is well illustrated in persons with ileitis, ileal resection, or ileal bypass. In such patients the increased bile-acid loss gives rise to bile-acid-induced (cholerrheic) diarrhea, which responds to bile-acid-sequestering agents such as cholestyramine. If the increased loss is small then hepatic synthesis is increased to maintain the bile-acid pool and so fat digestion is

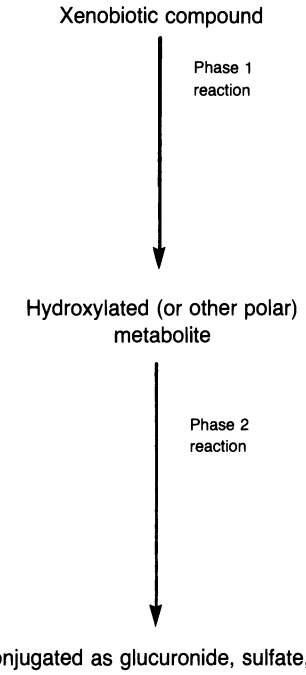

Figure 2. Phase 1 and phase 2 reactions in the detoxification of xenobiotic compounds prior to biliary secretion.

Table 3. The Relative Specificity of the Carrier in the Active Transport of Bile Acids from the Terminal Ileum[a]

Bile acid	Conjugating amino acid	V_{max} pmole min per cm
Cholic acid	None	1906 ± 462
	Glycine	1543 ± 326
	Taurine	1629 ± 236
Deoxycholic acid	None	225 ± 169
	Glycine	114 ± 32
	Taurine	397 ± 27
Chenodeoxycholic acid	None	512 ± 150
	Glycine	173 ± 40
	Taurine	337 ± 13
Lithocholic acid	Glycine	45 ± 8
	Taurine	57 ± 21

[a]Data from Dietschy.[9]

unimpaired; if the increased loss is too great the pool size is diminished until eventually it is insufficient to achieve the critical micellar concentration in the small bowel, fat malabsorption (steatorrhea) ensues, and this exacerbates the cholerrheic diarrhea already present. The consequences of this can be fatal. It has been estimated that the loss of 40 cm of ileum by resection or bypass or as a result of disease is the maximum tolerable before hepatic synthesis is no longer able to replace the losses.[12] If 40—100 cm is resected, severe cholerrheic diarrhea is a direct consequence even before the bile-acid pool has been depleted.

A number of factors are important in determining the efficiency of bile-acid absorption from the terminal ileum in addition to the availability of carrier sites already described. These include bile-acid sequestration, which prevents the bile acids from reaching the carrier sites, and small-intestinal transit, which, if sufficiently rapid, can reduce the opportunity for active transit. These will be discussed later together with other factors influencing the enterohepatic circulation of bile acids.

B. Passive Absorption Mechanism

There are a multiplicity of passive absorption mechanisms by which compounds can be removed from the intestinal tract. For the bile acids these include a nonionic mechanism, an ionic pathway, and at least two other minor pathways; of these the nonionic mechanism is by far the most important, and is at least five to six times as active as the ionic pathway.

The nonionic pathway depends on the lipophilic properties of the compound. Conjugated bile acids or other compounds are ionized at the pH of the normal human intestine and so are very poorly absorbed by this mechanism; consequently deconjugation by bacterial enzymes is a prerequisite for utilization of this pathway.

The ease of passive absorption may also be affected by further metabolism of the compound by bacteria, since the products of such metabolism are normally less polar than the substrates. This is illustrated by the bile acids, where the major reactions are dehydroxylation and hydroxysteriod dehydrogenation (Fig. 3). In contrast, the metabo-

lites of cholesterol, coprostanol, and coprostanone (Fig. 4) are almost totally unabsorbed from the colon. Many compounds secreted in the bile undergo extensive bacterial metabolism, so the nature of these reactions and the site at which they occur is crucial to our understanding of the rate of passive absorption from the intestine.

IV. BACTERIAL METABOLISM IN THE INTESTINE

Two factors need to be taken into account when considering metabolism by the gut bacterial flora, namely, the distribution of bacteria along the gastrointestinal tract and the biochemical activity of the different genera of bacteria. These have been reviewed in depth by Drasar and Hill.[13]

A. Distribution along the Gastrointestinal Tract

In the normal healthy human the small intestine contains very few bacteria, and these are mainly of oral origin. Because of the large volume of fluid secreted into the upper small intestine during the digestion process, the mucosal surfaces and the lumen are continually flushed, which prevents a resident flora from establishing, especially since the rate of flow of the lumenal contents is so high. The stomach contains few organisms because of the bactercidal effect of the gastric acid, so there is little inflow of bacteria into the duodenum. The volume and rate of flow of material down the small intestine makes retrograde colonization unlikely. Thus the human small intestine contains a rich flora only in persons with blind loop syndrome or small-bowel diverticuli, or in persons with small-bowel stasis. The picture is very different in, for example, small rodents.[14] In the rat and mouse the gastric acid barrier is ineffective and a rich flora is resident in the stomach; because these animals are coprophagic the rodent gastric flora is qualitatively similar to that in the colon and is continuously seeded by further fecal ingestion. In these rodents the small-bowel flora is, effectively, a 0.1–1% suspension of feces.

In all animals tested, the large bowel is heavily colonized, with about 10^{11} bacteria per gram of colonic contents. The colonic flora is a rich mixture of strictly anaerobic bacteria (e.g., *Bacteroides,* spp., *Bifidobacterium* spp., *Eubacterium* spp., and *Peptostreptococcus* spp.); CO_2-dependent organisms (e.g., *Lactobacillus* spp., and some *Streptococcus* spp.); facultative aerobes (e.g., *Escherichia* spp., *Klebsiella* spp., *Proteus* spp., and *Streptococcus* spp.); and spore-forming organisms, both anaerobic (*Clostridium* spp.) and aerobic (*Bacillus* spp.). In general the organisms isolated from feces are much more metabolically active against biliary secreted compounds than are the oral bacteria found in transit through the upper small intestine.

Table 4. The Percentage Composition of the Biliary Bile Acids[a]

Acid	American adults	British youths	African youths
Chenodeoxycholic acid	28%	30%	35%
Cholic acid	45%	47%	47%
Deoxycholic acid	23%	23%	18%
Lithocholic acid and others	1%	—	—

[a]Data collected from various sources.

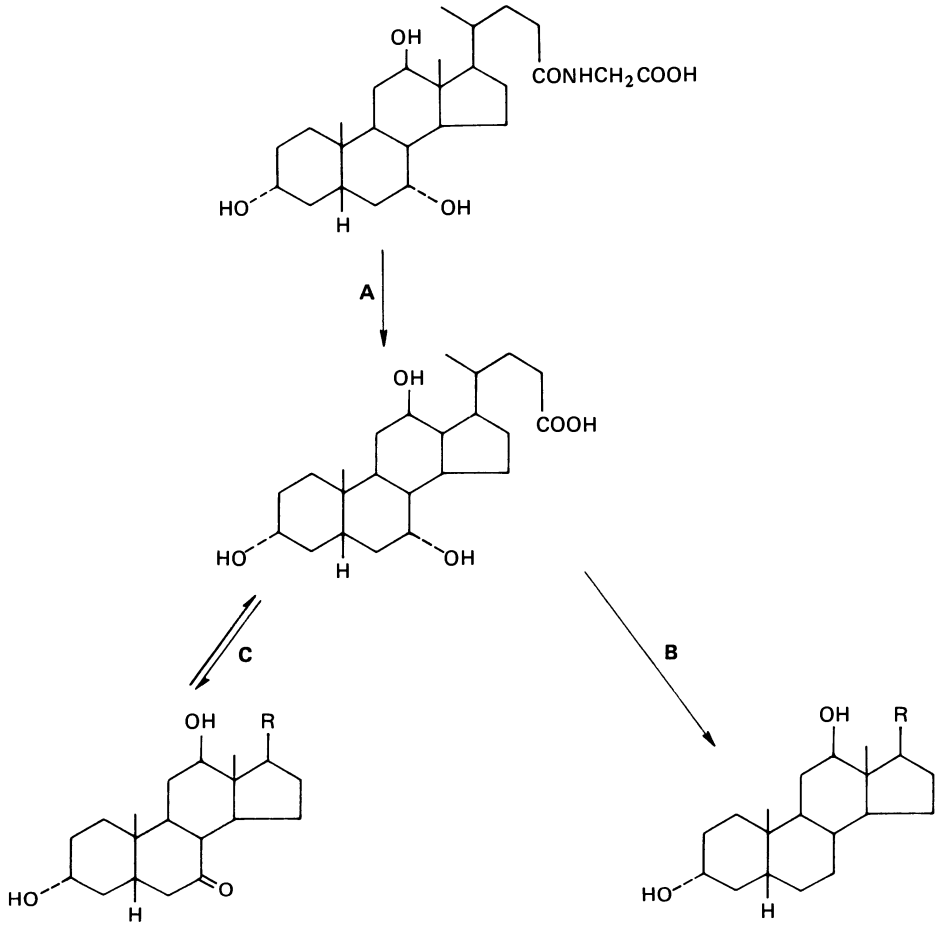

Figure 3. The bacterial metabolism of bile salts. Reaction A—cholylglycine hydrolase; reaction B—cholyl Ta-dehydroxylase; reaction C—T-hydroxysteroid dehydrogenase.

B. Biochemical Activity

The major activities of interest in the context of this chapter are the enzymes deconjugating β-glucuronides, sulfates, bile-acid conjugates with glycine or taurine, glutathione conjugates, and minor metabolites.

The production of β-glucuronidase by gut bacteria was studied by Hawksworth et al.[14] The organisms producing the highest mean activity of this enzyme are *Escherichia coli* and the clostridia, but because of the relative numbers of the various bacterial species at different subsites in the gastrointestinal tract there is nowhere these organisms actually contribute significantly to the total enzyme activity (Table 5). Note that the enzyme activity is very low in the upper small intestine of humans and rabbits compared with the enzyme activity of the small rodents (rat, mouse, guinea pig), while the activity is high in the cecum of all five animal species (Table 6). From these results

we would expect that glucuronides would be excreted much more slowly (in other words, would be more efficiently enterohepatically circulated) by the small rodents than by the rabbit or human; this has been demonstrated with a number of compounds and will be discussed later. The production of bile conjugate hydrolases has been studied extensively by Aries et al,[15,16], Shimada et al,[17] and by many other groups. In general, the hydrolases act on both glycine and taurine conjugates, although hydrolases specific for a particular amino acid have been reported. In general the hydrolases are intracellular, although an extracellular hydrolase produced by *Bifidobacterium* spp. has been described.[15] Production of hydrolases is a common feature of anaerobic species of bacteria (Table 7) but is rare in the facultatively aerobic organisms.[16]

Much less work has been carried out on the enzymes hydrolyzing sulfate and glutathione conjugates by gut bacteria; (however, there has been a lot of work on sulfatases produced by bacteria of nonintestinal origin such as soil organisms and pseudomonads). Lithocholic acid is very poorly enterohepatically circulated because it is converted by the liver to the 3-sulfate, which is excreted in feces. Only a small proportion is thought to be deconjugated by the colonic bacteria, compared with the total hydrolysis of the amide conjugates; the major products of desulfation[18] are lithocholic acid; released by the action of sulfatase; *iso*-lithocholic acid, released by the action of sulfonase; and an unsaturated cholenic acid, produced by removal of the elements of sulfuric acid.

V. ENTEROHEPATIC CIRCULATION OF SOME CLASSES OF COMPOUNDS

A. Compounds Absorbed by Active Transport without Deconjugation

Since the best example of this class of compound is the bile acids, these will be discussed in some detail. Bile acids are the end products of cholesterol metabolism and

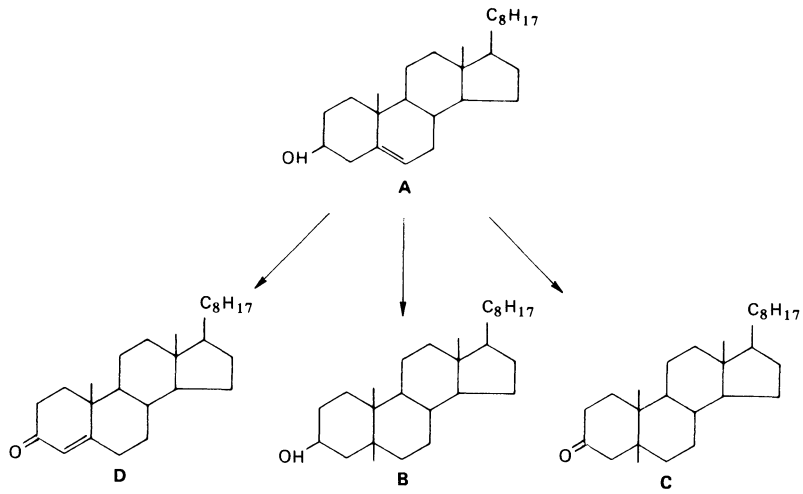

Figure 4. The bacterial metabolism of cholesterol (A) to coprostanol (B), coprostanone (C) or cholesterone (D).

Table 5. β-Glucuronidase Activity at Various Subsites in the Gastrointestinal Tract of the Rat and the Human

		Rat				Human			
		Small intestine		Colon		Small intestine		Colon	
Organism	Activity[a]	Number	% total activity	Number	% total activity	Number[b]	% total activity	Number	% total activity
Bacteroi des spp.	6.0	2×10^6	41%	2×10^8	58%	3×10^2	90%	$10''$	50
Bifidobacterium spp.	1.9	5×10^6	29%	4×10^8	36%	1×10^2	9.2%	$10''$	50
Clostridium spp.	11.3	1×10^3	< 0.1%	1×10^3	< .01%	ND	< .01	10^6	< .01
Lactobacillus spp.	1.6	5×10^6	27%	6×10^7	5%	10	0.8%	10^8	< .1
Streptococcus spp.	2.9	5×10^4	0.5%	4×10^6	0.5%	ND	< .01	10^7	< .01
E. coli	24.9	3×10^4	2%	7×10^5	0.8%	ND	< .01	10^7	< .1

[a]Enzyme activity is expressed as picomoles of substrate hydrolyzed per hour per 10^8 organisms.

[b]ND, not determined.

Table 6. The Calculated Mean Activity of β-Glucuronidase in the Duodenum, Lower Ileum, and Cecum of Various Animal Species[a]

Animal	Duodenum	Lower ileum	Cecum
Mouse	1200[a]	5075	16,400
Rat	300	1340	10,500
Guinea pig	2.7	140	6,300
Rabbit	2.4	45	6,010
Human	0.02	0.9	9,000

[a]Activity is expressed as nanomoles of substrate hydrolyzed per hour per gram of contents.

are excreted in bile as their taurine or glycine amide conjugates. Unlike most compounds excreted in bile, the bile acids play a key role in the digestive process since they are important in fat absorption; consequently their conservation is important to the health of the animal concerned. This discussion will be limited to the human.

The enterohepatic circulation of bile acids is a highly efficient process in humans. The bile-acid pool of 2–5 g is recycled 6–10 times per day (2–3 times with each meal). The pool size is larger in women than in men, depends on diet (being reduced by both a high-fiber and a low-fat diet), and is inversely related to the frequency of recycling and to the rate of gallbladder emptying. Transfer of bile acids from the portal blood to the bile is highly efficient, so that the serum bile-acid concentration, which is a measure of the proportion of the portal bile acids that is not transferred to the bile during the first

Table 7. Production of Cholanorgl Amide Hydrolase by Various Species of Gut Bacteria[a]

Species of bacterium	Number of strains tested	% with hydrolase activity
Aerobic bacteria		
Pseudomonas spp.	58	0
Facultative anaerobic bacteria		
E. coli	75	0
Proteus mirabilis	1	0
Streptococcus fecalis	109	50
Streptococcus salivarius	30	0
Streptococcus viridam	12	0
Microaerophilic bacteria		
Lactobacillus spp.	12	0
Anaerobic bateria		
Bacteroides spp.	237	48
Bifidobacterium spp.	89	38
Clostridium spp.	16	94
Veillonella spp.	47	30
Fungi		
Candida spp.	34	0
Others	11	0

[a]Data from Draser and Hill.[13]

passage through the liver, is normally very low, less than 15 μmole/liter in healthy persons. Consequently the urinary loss of bile acids is normally less than 5 mg/day. Absorption from the small intestine of normal persons is minimal because the bile acids are in the conjugated form. Unconjugated bile acids are present if there is a defect in conjugation due to hepatobiliary disease or if there if profuse bacterial overgrowth associated with diverticular disease or blind loop syndrome.

The major route for bile-acid absorption is the active transport mechanism located in the terminal ileum. This is a carrier-mediated, energy-linked, sodium-dependent system estimated to be 98% efficient. Its importance is demonstrated in persons who have undergone ileal resection, which results in increased fecal loss of bile acids; this increased loss is replaced by increased hepatic synthesis, but if more than 40 cm is resected the liver is unable to make good the loss, resulting in depletion of the bile-acid pool. A similar impairment in active absorption occurs in patients with regional ileitis (Crohn's disease). As well as through loss of carrier sites, the active transport system can be impaired by factors interfering with the access of bile acids to these carrier sites; bile-acid sequestrants, both therapeutic and dietary, interfere with active transport in this way.

Bile acids not absorbed by active transport enter the colon, where they are extensively metabolized by bacterial enzymes. The degradative reactions include deconjugation, which makes the acids available for passive absorption, and dehydroxylation or hydroxyl oxidoreduction (Fig. 3). All of these products are less polar than the substrates, which should facilitate passive absorption from the colon; but in fact, increased bacterial metabolism is associated with *increased* fecal loss. This must presumably be because the decreased polarity of the products also increases their adsorption to colonic particles and because some of the products of bacterial metabolism remain inside the bacterial cell.

The normal fecal loss of bile acids per day is 300–500 mg, and representing the proportion that escapes ileal recovery less that which is passively absorbed from the colon, estimated as about 50 mg/day. Some of the factors affecting fecal loss are listed in Table 8, and the relation between diet and fecal bile-acid loss is described in Table 9. The mechanism of many of these relationships is largely conjecture, but some tenta-

Table 8. Factors Affecting Fecal Loss of Bile Acids

Factor	Mechanism
Increased fecal fat	Sequestration of bile acids, interfering with ileal and colonic transport, increasing fecal loss
Increased cereal fiber	Bile-acid sequestration, increasing fecal loss
Increased dietary mannitol, lactulose, and other "unavailable" sugars	More rapid transit, decreasing absorption and increasing fecal loss
Increased pectin or guar	Trapping of bile acid by gel matrix in the terminal ileum, increasing fecal loss
Increased lignia	Sequestration of bile acids, increasing fecal loss
Residue-free diet	Decreased sequestration, increasing absorption and decreasing fecal loss

Table 9. The Relation between Diet and Fecal Bile-Acid Loss

Dietary change[a]	% change in daily fecal weight (g)	% change in fecal bile-acid concentration	% change in daily fecal bile-acid output
Increased available carbohydrate	< 10%	< 10%	< 10%
Increased protein 60 → 160 g/day	< 10%	+ 10%	+ 10%
Dietary fiber supplements			
Bran (16 g)	+ 40%	–30%	< 10%
(39 g)	+ 69%	–37%	< 10%
(100 g)	+200%	–43%	–70%
Cellulose (20 g)	< 10%	< 10%	< 10%
Bagasse (10 g)	+ 67%	< 10%	+67%
Pectin (36 g)	+ 25%	+ 17%	+ 46%
Guar (36 g)	+ 62%	< 10%	+ 77%
Soluble defined diet	–70%	–50%	–85%
Increased fat 60 → 150 g/day	< 10%	+100%	+100%

[a]All diets were isocaloric.

tive conclusions can be reached based on the results of metabolic ward studies. Available carbohydrate (in the form of sucrose, starch, oligosaccharide) is completely digested and is cleared from the digestive tract before the ileum is reached; consequently it has no effect on fecal bile-acid loss. Similarly, any but extreme changes in the amount of protein have no effect on fecal bile acid loss. Cellulose and bran are also without effect unless, in the case of bran, very large supplements are made when, presumably due to weak sequestration, there is an increased loss of bile acids. In contrast, the gelling polysaccharides pectin and guar both give a big increase in fecal loss of bile acids, presumably because the gels trap the bile acids and prevent their absorption from the terminal ileum. In the colon these polysaccharides are completely hydrolyzed by the colonic bacteria but passive absorption is insufficient to prevent large losses. Bagasse, rich in lignin and not degraded by gut bacteria, sequesters bile acids and so prevents active or passive absorption. Fat presumably traps the bile acids in micelles and thus prevents their access to the carrier sites in the terminal ileum. Although there is no relationship between persons between fecal fat and fecal bile-acid loss, there is a good relationship for individuals.[19]

The ideal diet for bile-acid conservation should therefore be one which has no solid residue to act as a sequestrant and little fat to trap the bile acids; one such diet is the kind of residue-free diet used in preoperative bowel preparation. As expected, these lead to low fecal losses of bile acids.

The relationship between diet and fecal bile-acid loss has been reviewed recently by Hill[20] and Kay.[21]

In any discussion of the enterohepatic circulation of bile acids the great variation in fecal loss of bile acids in persons on an identical diet (Table 10) needs to be ex-

Table 10. The Variation in Fecal Bile-Acid Loss per Day between Persons on Identical Diets

Diet	Number of persons	Range in fecal bile-acid loss per day (mg)	Reference
High fat	6	164–404	19
Low fat	6	68–198	19
High protein (low fat	4	133–222	22
Low protein (low fat)	4	147–165	22
Supplement of 100 g bran	4	208–333	23

plained, and there are a number of possible reasons. One is that there is a range in the density of bile-acid carrier sites between persons; this might be expected, although the size of the range needed to explain the range in fecal loss of bile acids would be surprising. Alternatively we know that there is a considerable range in the numbers of bacteria per gram of terminal ileal contents, and perhaps this is accompanied by a similar range in the extent of bile-acid sequestration by the bacteria.

The enterohepatic circulation of bile acid has been reviewed briefly by Hill[20] and in depth by Hofmann[24] and Paumgartner,[1] to whom the reader is referred for a more detailed treatment of the subject.

B. Enterohepatic Circulation without Deconjugation and Active Transport

The best example of this class of compound is cholesterol, secreted in the bile as the free sterol. There it forms mixed micelles with the bile conjugates and phospholipid; after it has been squirted into the duodenum and mixed with the food, triglyceride, monoglyceride, and fatty acids are added to the mixed micelles. Cholesterol is absorbed from the small intestine as part of this mixed micellar phase. Dietary cholesterol does not enter the micellar phase as completely as does biliary cholesterol and so is relatively poorly absorbed.

Because the bile acids are so well absorbed from the terminal ileum the opportunity for micellar absorption from the colon is greatly reduced, and it has been estimated that cholesterol entering the colon is mainly excreted in feces. This has been confirmed in studies of germ-free rats. In addition, cholesterol is metabolized by the colonic bacteria to coprostanol and coprostanone, both of which are poorly soluble and unavailable for absorption. Numerous studies of ileostomy fluid have shown that cholesterol is virtually undegraded when it enters the colon (see, for example, Kay et al[26]).

The enterohepatic circulation of cholesterol is interfered with by many drugs used to lower serum cholesterol. Some are known to decrease the absorption of cholesterol from the small intestine (for example, probucol, as demonstrated by Mieltinen et al[27]), resulting in increased fecal loss of neutral steroids. Similarly, dietary pectin, guar gum, and other components with gelling properties cause increased fecal loss of neutral steroids. Presumably the gel traps cholesterol in the small intestine and prevents its absorption. Although the pectic substances are completely metabolized by the bacteria and the gel is broken, the cholesterol is not released in this way until it reaches the large intestine and so is not recovered.

The enterohepatic circulation of cholesterol has been reviewed in depth by Dietschy and Wilson[28] and Grundy,[29] to whom the reader is referred for more details.

C. Enterohepatic Circulation Dependent on Deconjugation

Most of the xenobiotics which are biliary secreted have first been metabolized by phase 1 and phase 2 reactions as described earlier. The resultant conjugates are polar compounds and are not passively absorbed from the gut. Following deconjugation, however, the aglycone is sufficiently hydrophobic to be readily absorbed by passive diffusion; the efficiency of the enterohepatic circulation—that is, the proportion of a dose entering the bile which is returned to the liver and secreted in the bile for another cycle—depends on the distribution of bacteria along the gastrointestinal tract and on the ease with which the bacteria deconjugate the compound.

Phenolphthalein is a lipophilic compound with cathartic properties. After stimulating intestinal motility it is absorbed and returned to the liver, where it is conjugated as the glucuronide. This is a good substrate for bacterial β-glucuronidase (indeed it is a standard substrate in the assay of the enzyme), and consequently the conjugate is rapidly hydrolyzed on reaching the bacterially colonized regions of the gut; the aglycone then exerts its cathartic effect before being absorbed and recycled. The compound is excreted principally as a result of the catharsis, which of course impairs its reabsorption from the intestine.

The antibiotic chloramphenicol is the drug of choice in the treatment of the small-bowel pathogen *Salmonella typhi*. It is absorbed from the intestine and returned to the liver, where it is conjugated as the mono- and diglucuronide (Fig. 5), both of which are inactive antibiotically. However, on reaching the infected part of the gut the conjugate is hydrolyzed by the *S. typhi* and the aglycone is then free to exert its antibiotic effect and also to be reabsorbed for another cycle. Thus the enterohepatic circulation of this antibiotic allows a single dose to have more than one attack on the pathogen. However, it also gives the rest of the bacterial flora multiple exposure to the antibiotic, which often results in the flora's adapting to metabolize the antibiotic by reducing the nitro group and hydrolyzing the amide group to yield a thyrotoxic diamine.

The distribution of bacteria along the gastrointestinal tract differs between animals; in particular the small intestine of rats and mice is densely populated when compared with that of, for example, rabbits. Consequently the activity of β-glucuronidase in the small intestine of the mouse or rat is relatively high (Table 6) and compounds secreted in bile as glucuronides will be less effectively excreted in these animals. This is illustrated with stilbestrol; a 50% dose takes 24 hr to be excreted from the rabbit compared with 7 days from the rat. In addition to this species difference in distribution of bacteria along the gastrointestinal tract, there is also a between-persons variation in the population density of bacteria in the lower ileum; those with a densely populated ileum will have a higher ileal β-glucuronidase and therefore more effective enterohepatic circulation than those with only a sparse flora. This is important in morphine treatment, for example, where the drug may accumulate in persons who retain a high proportion of each dose.

Polycyclic aromatic hydrocarbons (PAH's) undergo enterohepatic circulation, which has implicated them in the causation of cancer of the colon and of the biliary

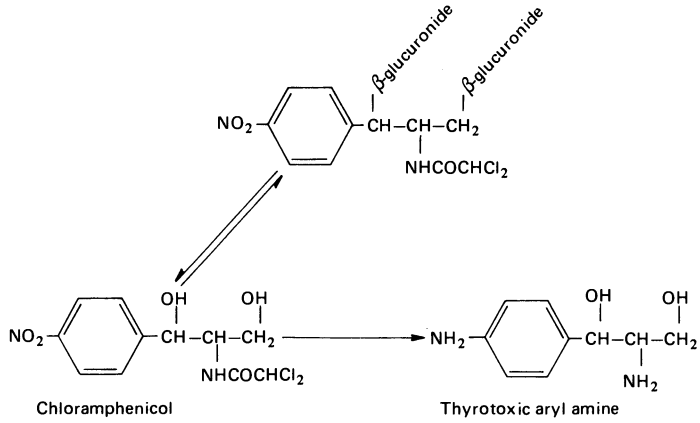

Figure 5. The metabolism of chloramphenicol by the liver to the diglucuronide and by the gut bacteria to a thyrotoxic arylamine.

tract. PAH's, enter the body in food as pyrolysis products formed during cooking (especially grilling) or during preservation by smoking,[30] or are transferred to food from cooking oil during frying. They also enter the body airborne in inhaled smoke from fires or cigarettes or from motor exhaust fumes. Inhaled PAH's are absorbed into the blood and carried to the liver, where they are hydroxylated then conjugated as the glucuronide or glutathione conjugate. Dietary PAH's being very hydrophobic, are absorbed readily from the small intestine, returned to the liver by the portal blood, then hydroxylated and conjugated and secreted in bile. The biliary glucuronides are hydrolyzed by the bacterial β-glucuronidase in the lower intestine, and the released hydroxy PAH's are absorbed and returned to the liver. Hydroxylated PAH compounds are neither carcinogenic nor mutagenic and do not bind to DNA. However, Renwick and Drasar[31] showed that intestinal bacteria, in particular some strains of *Clostridium* spp., are able to dehydroxylate hydroxybenz-(*a*)-pyrene to release the free benzpyrene. These authors then suggested that this might be a mechanism for colon carcinogenesis.

In contrast, Kinoshita and Gelboin[32] showed that during the hydrolysis of hydroxybenz-(*a*)-pyrene glucuronide by bacterial β-glucuronidase a short-lived active intermediate is formed that binds to DNA. They suggested that the carcinogenicity of hydroxylated and conjugated PAH's was worthy of reexamination under conditions in which it might be hydrolyzed. The site where this might occur to yield the greatest concentration of short-lived intermediate would be in the bile itself during biliary tract infection; interestingly it has been noted that biliary infection with *S. typhi* is associated with an increased incidence of biliary tract carcinoma. This is discussed in greater detail by Hill.[33]

D. Enterohepatic Circulation by Extrabiliary Routes

This is best illustrated by the enterohepatic circulation of urea. Small amounts of urea secreted in the bile are degraded to ammonia and carbon dioxide by bacterial urease in the intestine. The ammonia returns to the liver, where it is reconverted to urea. Most of the urea, however, stays in the peripheral blood system, to be excreted into the urine. The urea enters the colon during the process of osmotic equilibration that

accompanies the absorption of water from the large bowel; it is then split by urease, and the ammonia generated is absorbed back into the blood system and returns to the liver, where much of it is reconverted to urea. The rate of entry of urea into the colon depends on the serum concentration, so in uremia, for example, more is enterohepatically cycled. Interestingly, this is not simply because the amount of urea entering the colon is greater but also because activity of bacterial urease in the large intestine is also greater.[34] Thus, in uremia the enterohepatic circulation helps to keep down the serum concentration of urea and also maximizes the availability of ammonia for protein synthesis. In contrast, in cirrhosis the ammonia generated from urea by the colonic flora is not restricted to the portal blood and escapes into the peripheral system, where it exerts a powerful toxic action and is thought to cause the hepatic coma experienced by cirrhotics in response to dietary protein.

The enterohepatic circulation of ammonia has been discussed by Summerfield.[35]

VI. CONCLUSIONS

A wide range of compounds undergo enterohepatic circulation (Table 11), and our knowledge of this range is increasing daily. Nevertheless, much of the process is little understood and the subject has received little notice except for the intensive studies carried out on bile acid and cholesterol. In this brief review I have tried to indicate the principles involved in the process and the sources where more details may be sought. The reader will appreciate, however, all there is to learn in this field and how long the learning process is.

Table 11. Some Examples of Types of Compounds Undergoing Enterohepatic Circulation[a]

Compound	Type of biliary conjugation	Type of transport from small intestine
Bile acids	Glycine and aurine amides	Active
Cholesterol	None	Micellar
Steroid hormones		
Estradiol	Glucuronide/sulfate	?
Testosterone	Glucuronide	?
Progesterone	Glucuronide	?
Stilbestrol	Glucuronide	Passive[b]
Antibiotics		
Benzylpenicillin	None	
Ampicillin	None	
Chloramphenicol	Glucuronide	Passive[b]
Erythromycin	None	
Rifamycin	None	
Others		
Imipramine	Glucuronide	
Morphine	Glucuronide	Passive[b]
Indomethacia	Glucuronide	Passive[b]
Methylamphetamine	Glucuronide	Passive[b]

[a]Data from Smith.[6]

[b]Passive absorption of the aglycone after deconjugation.

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13

Colonic Adaptation

H. Menge and J. W. L. Robinson

I. INTRODUCTION

The mucosa of the colon is generally considered a less complex structure than that of the small intestine. It is often described as a flat surface containing large numbers of closely spaced crypts,[1] but in this way the role of the haustra in causing a huge increase in the absorptive surface is neglected. In the rat, mucosal folds, presumably equivalent to the haustra of the human colon, assume varying forms at the different levels of the colon (Figs. 1–3); they are not easily quantified on histological preparations, particularly in the cecum and ascending colon, where cutting the tissue strictly perpendicular to the folds is especially difficult. For this reason, these structures tend to be ignored in the description of adaptive changes in this organ.

The surface patterns of the colonic mucosa, as seen under a scanning microscope, are also not uniform.[2] The individual crypts of the rat colon are projected into the lumen as papillae (Fig. 4), whereas the human rectal mucosa exhibits a geometrical pattern of polygonal units, the mouth of the crypts being visible at the center of the polygons, at the bottom of funnel-like depressions.[3] The upper quarter of the individual crypts is composed of fully mature, differentiated cells (Fig. 5). Specht[2] has suggested that when the colon is full or distended, this upper region of the crypt, with its mature cells, may be spread out in the plane of the mucosal surface; in this way the absorptive area will be increased but the depth of the crypts will be correspondingly reduced.

Cell proliferation takes place throughout a relatively broad zone in the lower two-thirds of the crypts, and the cells migrate slowly to the surface, where they are desquamated into the gut lumen at the end of their lives.[1,4] The most complete study of the proliferation kinetics of the colonic mucosa at different levels of the same organ is that

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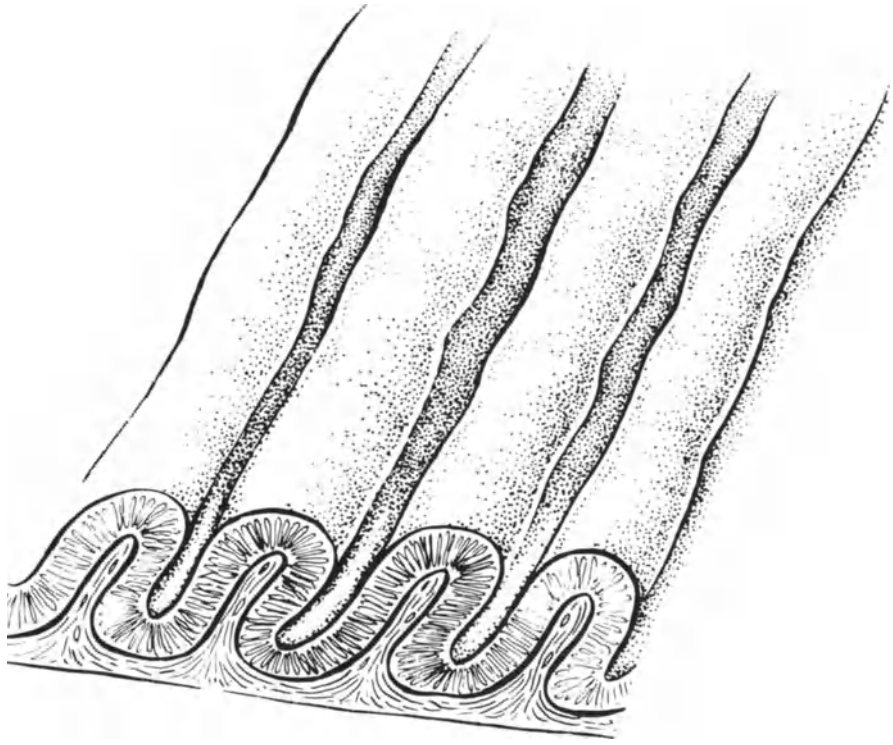

Figure 1. Diagrammatic representation of the mucosal folds in the surface of the rat distal colon. The approximate height of the individual folds is 800 μ.

of Sunter et al,[5–7] who examined the cytokinetic organization of the crypts. These investigators found in the mouse that the total number of cells was greatest in the individual crypts of the descending colon, that this parameter declined progressively along the transverse and ascending regions of the colon, and that it rose again in the cecum.[6] In addition, they described marked differences in the distribution of the proliferating cells, the length of the cell cycle, and the proliferation rate at different sites along the colon, the details of which are beyond the scope of this review. Suffice it to say that the mean cell cycle time in the rat descending colon has recently been estimated as 50–60 hr, whereas this period is reduced to 25 hr in the cecum.[7,8]

Another problem facing investigators studying the colon is the important heterogeneity of the functional characteristics of the organ among different species[9] and the consequent difficulty in extrapolating from one species to another. Herbivorous animals, for instance, possess large colons with important segmental differences[10]; in omnivores, such as the rat, despite important cytokinetic differences from site to site along the organ, regional variations in function seem less pronounced, with the exception of the rectum.[11] The dog represents a species apart, since the colonic mucosa of the adult possesses sodium-dependent active transport mechanisms for sugars and amino acids, analogous to those of the small intestine.[12] In herbivores, at least in the

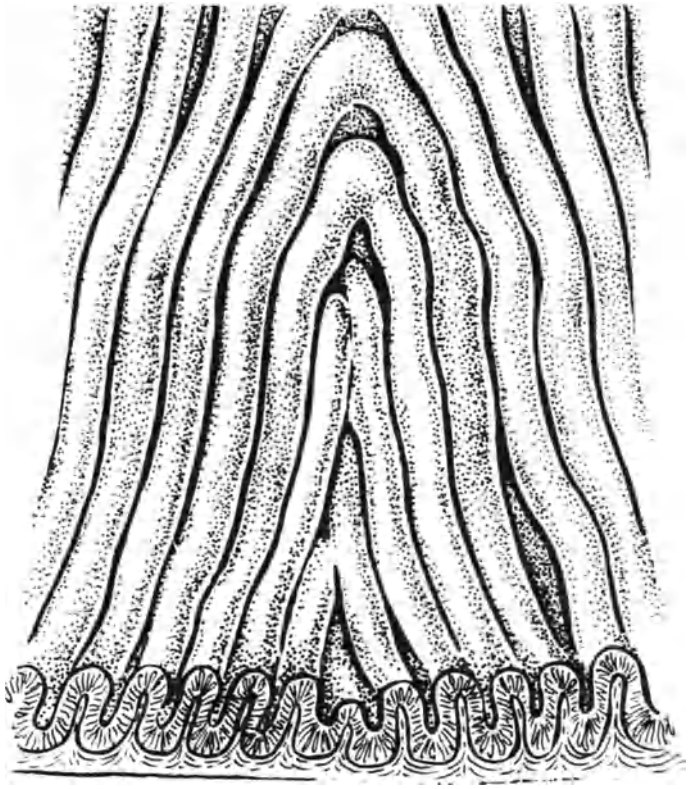

Figure 2. Diagrammatic representation of the mucosal folds in the surface of rat proximal colon.

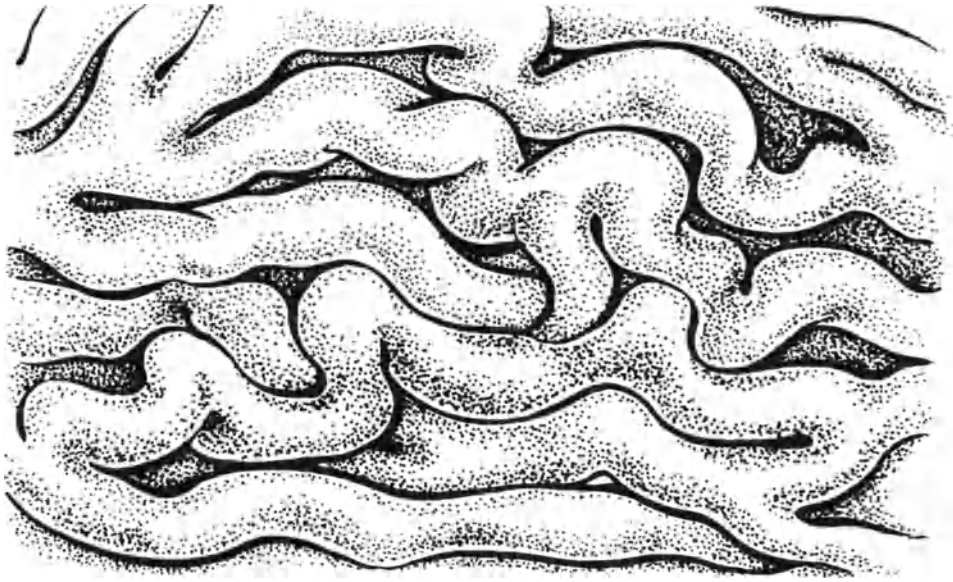

Figure 3. Diagrammatic representation of the folds of rat cecal mucosa.

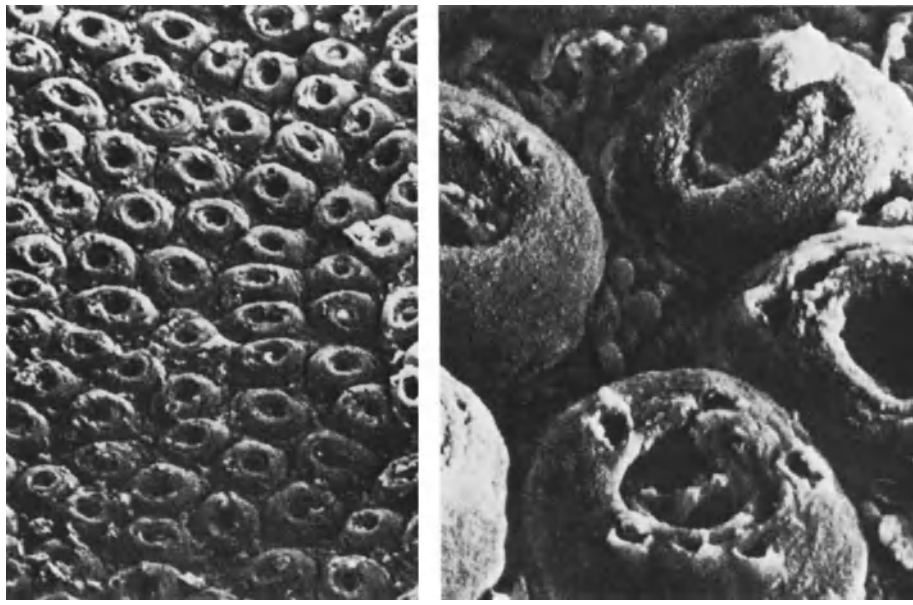

Figure 4. Scanning electron micrographs of the surface of rat colonic mucosa (left, × 370, right, × 1850; both reproduced at 75%). From Specht.[2]

distal regions, amiloride-sensitive sodium transport accounts for the entire short-circuit current across the epithelium *in vitro*[13]; in the rat, net sodium transport is much greater than the short-circuit current[14] and is insensitive to amiloride[11]; in the dog, in view of the presence of cotransport mechanisms, sodium influx is sensitive to harmaline.[15] Such important differences between species have not been demonstrated in the small intestine.

This functional heterogeneity of colons of different species not only hampers the development of general hypotheses to account for the response of the organ to pathophysiological stress, but also clearly influences the choice of techniques for the exploration of the functional characteristics of the stressed organ. The number of studies with a perfused colon *in vivo* is strictly limited, partly because the relatively small size of the organ in many species raises technical problems, and partly because of the difficulty of rinsing the organ sufficiently to permit an efficient perfusion. The use of cecal sacs *in vivo* has been more widespread. The examination of sodium transport capacity *in vitro* is a relatively simple test to apply to large intestinal mucosa, since a substantial net flux of this cation occurs there.[16,17] Furthermore, the discovery that dog colonic mucosa is capable of active transport of sugars and amino acids[12] provides a much easier functional test in this tissue (see Tables 1 and 2), namely, the assessment of the distribution ratio established between tissue and incubation medium during long incubation periods. Nevertheless, such tests on surviving tissue *in vitro* have relatively seldom been applied to large intestinal tissue.

Figure 5. Semischematic diagram of a mouse colonic crypt, showing the distribution of cell types and their development, together with the pericryptal sheath of fibroblasts. From Specht.[2]

Table 1. Functional Characteristics of Dog Colon after 1 hr Ischemia[a]

	Control loop	Ischemic loop	Recuperated loop
Net sodium transport ($\mu eq/cm^2 \cdot h$)	8.7 ± 1.42	1.7 ± 0.40	7.7 ± 2.06
Phenylalanine accumulation (distribution ratio)	5.9 ± 0.45	2.3 ± 0.29	5.7 ± 0.58
β-methylglucoside accumulation (distribution ratio)	6.6 ± 0.38	4.3 ± 0.81	5.3 ± 0.31
Oxygen consumption ($\mu l/min \cdot$ gram of dry tissue)	79 ± 4.8	58 ± 5.4	72 ± 3.6

[a]The dog colon was divided into three segments. On the 1st day, one segment was subjected to 1 hr of ischemia; then the clamps were released and the animal was allowed to recover. On the next day, a second loop was subjected to the same period of ischemia, and then all three loops were excised for examination *in vitro*. Net sodium transport across the emusculated mucosa was determined in flux chambers with ^{22}Na, the two unidirectional fluxes being determined on parallel samples; organic solute accumulation was measured on incubation of mucosal fragments for 1 hr in a solution of 0.1 mM substrate; tissue respiration was determined in a differential respirometer. Results (means ± S.E.M. of nine dogs) are taken with permission from Robinson et al.[30]

Such considerations emphasize the dangers inherent in extropolating from one animal species to another and in treating the colon as a single homogeneous organ, in an attempt to explain its adaptation to pathophysiological stress. In this review, we underline these points, since they could explain, at least in part, the varied responses described by different authors apparently investigating the same stress situation. Most studies on the adaptation of the small intestine in pathophysiological states have concentrated primarily on the changes in the proliferative rate of the epithelium, and the authors have generally attempted to interpret their findings on both structure and function of the mucosa in terms of changes in cell turnover rate and maturation.[18,19] Such an approach has seldom been applied to the colon, where research on the adaptation of the epithelium to pathophysiological stress has not often progressed beyond the descriptive phase. It is our intention, in this chapter, to examine studies on the regeneration of the colonic mucosa following ischemic or mechanical damage, to review the roles of bacteria and dietary stress on the characteristics of the colon, and to describe the effects of resection on the structure and function of the large-intestinal mucosa. Certain other specialized adaptive processes, such as the response of the colon to hormones or bile acids, will not be considered here.

II. RESPONSE OF THE COLONIC MUCOSA TO ISCHEMIA

In the clinic, ischemic accidents of the colon are much more common than those affecting the small intestine. This is because the capacity of the intestinal collateral circulation to compensate for any loss of mesenteric blood flow is very great, so that the blood supply to small-intestinal loops is rarely reduced to a sufficient extent to cause ischemic damage. In contrast, there are regions, at least in the human colon, that have precarious collateral circulation, notably in the splenic flexure,[20,21] so that they are particularly sensitive to any changes in blood flow that occur, for instance, in arteriosclerotic patients. Another potential cause of ischemic damage is the obligatory clamping of the inferior mesenteric artery during surgical interventions on the lower

aorta.[22] In view of the relative importance of colonic ischemia in the clinic, it is surprising that the number of experimental studies on this phenomenon is rather restricted.

Ischemic colitis in the clinic generally follows one of three courses: (1) after temporary and relatively short periods of ischemia, such as arise after clamping the inferior mesenteric artery during vascular surgery, the mucosa may regenerate spontaneously without leaving any trace of the trauma; (2) when the ischemic attack is more severe and the muscular layers are affected, the lesions may evolve toward fibrotic stenoses of the colonic wall, which generally need not be removed surgically unless danger of obstruction arises; (3) prolonged acute ischemia can lead to necrosis of the entire colonic wall, which may then perforate and cause peritonitis. In this last case, immediate surgical intervention to remove the damaged segment is the only way to save the patient.[21]

These three stages in the development of colonic ischemia have been reproduced in experimental animals, and indeed all investigators working with dog colon have emphasized the parallelism between the results obtained in this species and observations made in the clinic.[23–25] In a radiological study, Boley et al[26] injected microspheres of different sizes to obliterate the afferent arteries of the colon and were able to show different lesions in the organ, corresponding to the size of the injected spheres and the consequent extent of the arterial obstruction. Marston et al[24] succeeded, by ligating different vessels, alone or in combination, in reproducing the different stages of ischemic colitis observed in the clinic, according to histological and radiological criteria.

Table 2. Alterations in Functional Characteristics of Dog Colon 14 Days after the Creation of a Mechanical Occlusion in the Descending Colon[a]

Parameter	Control loop (n = 11)	Loop above occlusion (n = 9)	Statistical evaluation t	p
Net movements *in vivo* (expressed per hour and per gram of dry tissue)				
Water (ml)	3.4 ± 0.19	0.4 ± 0.34	7.70	< 0.001
Sodium (μeq)	575 ± 31	111 ± 48	8.12	< 0.001
Chloride (μeq)	498 ± 25	119 ± 48	7.00	< 0.001
Potassium (μeq)	1 ± 1.9	−14 ± 5.1	2.76	< 0.02
Glucose (μmole)	32 ± 4.8	10 ± 3.0	3.89	< 0.001
Solute accumulation *in vitro* (expressed as distribution ratios)				
L-phenylalanine	7.7 ± 0.67	5.4 ± 0.71	2.36	< 0.05
β-methyl-*D*-glucoside	8.0 ± 1.09	4.0 ± 0.75	3.02	< 0.01

[a]A mechanical occlusion was created in the descending colon of dogs. Fourteen days later the animals were reanesthetized; the loop above the occlusion was drained, then perfused for 1 hr with Krebs bicarbonate buffer containing 0.2% glucose, using a technique modified from that applied to the small intestine.[29] A negative sign implies net movement into the perfusate. After the perfusion, the colonic loops were excised, and the mucosa was separated from the underlying muscular layers. Fragments of mucosa were then incubated for 1 hour in a solution of 0.1 mM [^{14}C]-*L*-phenylalanine or [^{14}C]-β-methyl-*D*-glucoside in Krebs bicarbonate buffer. Parallel samples were incubated in [^{14}COOH]inulin for the assessment of the extracellular space; other fragments were desiccated overnight for the determination of tissue water. From these results, the accumulations could be expressed as distribution ratios between intra- and extracellular compartments. The results are compared, using the t test, with values obtained with normal colonic loops from a different series of dogs.

The characteristic early radiological change in the colonic mucosa following ischemia is "thumb-printing" caused by spasm, mucosal edema, and hemorrhage; in cases when the mucosa recovers spontaneously, this sign disappears. If a stenosis develops, it will be clearly visible on barium enema. Small ulcerations that may be seen on sigmoidoscopy are generally not detected by radiological examination. A somewhat different picture arises when the ischemia is produced by venous occlusion.[27] In this case, the primary lesions occur in the muscle layers, loss of mucosa being only a secondary feature. Nevertheless, severe congestion occurs in the mucosal layer and thumb-printing is particularly evident on radiological examination of dogs treated in this manner.

Examination of biopsies from patients with ischemic colitis and from dogs recovering from experimental colonic ischemia has led to the division of the reaction to moderately severe ischemia into three general phases,[28] namely, the acute response, the reparatory phase, and residual pathology. Clearly the acute response involves the development of edema, hemorrhage, and necrosis. The depth of the latter will determine the course of the lesions. The reparatory phase is characterized by the formation of granulation tissue. If islands of mucosa have survived, they will be surrounded by a matrix of granulation tissue and inflammatory cells. Macrophages laden with hemosiderin are invariably present and, being indicative of prior ischemic damage, are of diagnostic importance. The late sequelae include fibrosis of the muscular layers, stenosis, and mucosal ulceration. Since mucosal regeneration is slow after severe colonic damage, it is not uncommon to encounter stenotic loops of colon without any mucosal covering at all.[25] The extent of the damage is so great in these cases that it is often unlikely that reepithelialization will ever occur. These situations could be reproduced in all details in animal studies, in which the effects of various periods of total acute ischemia were examined.

In a series of experiments in dogs, we have examined the response of small- and large-intestinal mucosae to defined periods of total, acute ischemia, using functional and histopathological criteria, in an attempt to assess the sensitivity to the trauma and the regenerative potential of each organ. In addition, comparison of these results with clinical observations underlines the parallelism between findings in dogs and man. An acute ischemia of 1 hr causes extensive damage to the mucosa of the small intestine, with loss of all ability to transport sugars and amino acids actively *in vitro*.[29] However, most functional and structural characteristics are restored within 24 hr of the trauma. In the colon, a similar period of ischemia causes only slight surface damage and a small reduction in functional capacity, as assessed *in vitro*.[30] These scant changes are readily reversible, as shown in Table 1.

In a cell kinetic study in rats, Rijke and Gart[31] examined the effect of 90 min of ischemia on crypt cell proliferation in the colon. The desquamation caused by the ischemia affected principally the nonproliferating zone in the upper third of the crypts, just as the villus cells are more sensitive to ischemia in the small intestine.[32] During the recovery stage, there was a marked increase in proliferative activity, which normalized when regeneration was complete, 48 hr after the original trauma. A similar enhancement of proliferative activity in small-intestinal crypts has been observed during the recovery stage after brief periods of ischemia.[33,34]

In a study designed specifically to explore the relative regenerative potentials of the ileal and colonic mucosae, we subjected loops of each organ in the same dogs to a total acute ischemia of 2 hr[17] and followed their functional and structural recoveries over a period of 1 week. Immediately after the ischemia, there was massive desquamation of the ileal villi with moderate disruption of the crypts, whereas the damage to the colonic mucosa was considerably less pronounced. In both tissues, all active transport function *in vitro* (accumulation of amino acids and sugars for the ileum, net transmural flux of sodium for the colon) was abolished, and there was a significant reduction in the level of Na^+,K^+–ATPase in microsomal suspensions derived from mucosal homogenates. Many of the ileal segments had regained structural and functional integrity 3 days after the trauma, and all parameters had normalized 7 days after the ischemia. In the colon, however, all ischemic loops still had reduced function at the 3-day stage, and in many cases extensive histological lesions were still visible. After 1 week, although no significant reduction in sodium transport or $Na,^+K^+$–ATPase activity remained, histological examinations revealed, in most of the specimens, the presence of small ulcers of granulation tissue covered by a cubic epithelium. The results of these experiments permit the general conclusion that the colonic mucosa is less sensitive to the effects of ischemia than that of the small intestine, but that once destroyed, it regenerates more slowly.

The damage caused by a total ischemic trauma lasting 3 h is considerably more extensive, and the response of dog colons subjected to this treatment was rather heterogeneous.[16] Although certain mucosae recovered within 2–4 weeks of the original trauma, according to both functional and morphological criteria, others evolved toward stenosis of the colonic wall, in which the mucosa is replaced by a sea of granulation tissue. Yet another group of animals died of perforation of the the colonic wall, generally in the region in which the clamps had originally been placed. Postmortem examination of these specimens revealed widespread necrosis and gangrene, as may also occur in the clinic. Thus this study disclosed the development in dogs of the three types of ischemic colitis described in clinical practice. The heterogeneity of the response in this study may have been partly due to the varying degrees of damage to the vascular walls provoked by clamping for a relatively long period. It may be assumed that if complete revascularization had taken place on removal of the clamps, the organ eventually would have recovered. This interpretation is upheld by the findings from an earlier study[35] in which several dogs survived after 4 h of total ischemia of the colon where the blood had been replaced by a buffer solution perfused through of the vascular network; in some animals, complete recovery of mucosal structure was observed.

III. HEALING OF MUCOSAL ULCERS IN THE COLON

The reepithelialization of the colonic mucosa following excision of mucosal biopsies or after burning small areas of mucosa has been studied in dogs[36,37] and monkeys[38]; the observations reported have some bearing on the regeneration of the mucosa after ischemic traumas. In all these experiments, small ulcers of 1 cm^2 or less were produced in the mucosa, and the healing of the wound was followed for a period

of up to 4 weeks. According to each of these studies, the regeneration of the epithelium to cover this small area took nearly 3 weeks. The healing was initiated by a single layer of flat cells which advanced from the edge of the healthy tissue, with progressive changes from flat to columnar cells before invaginations could develop for the formation of new crypts. The relative sluggishness of this process readily explains the frequency of stenoses without mucosal covering that develop after prolonged colonic ischemia.

One other observation in these studies is worthy of comment, namely, that if the muscularis mucosae is excised at the time of the biopsy, this structure does not regenerate.[36,38] We noted a similar phenomenon in one dog colon regenerating after prolonged ischemia[16]: In this unique specimen, a functionally normal mucosa was found reposing directly on the submucosa, in the absence of any muscularis mucosae, which must have been destroyed by the ischemic trauma.

IV. MECHANICAL OCCLUSION OF THE COLON

Mechanical obstruction of the colonic lumen may be encountered in clinical practice under a number of circumstances,[39] particularly in cases of colonic carcinoma, but no detailed study of the functional and morphological alterations in the organ under such conditions seems to have been undertaken. Once again, the corresponding situation in the small intestine has been the subject of more extensive investigation. Studies in dogs have revealed the development of a net secretion of sodium and water across the mucosa of the small intestine above the obstruction.[40,41] This phenomenon has been attributed to the effect on the epithelium of the huge colony of bacteria that proliferates extensively in the obstruction fluid[41]; it is assumed that these organisms produce enterotoxins that stimulate the secretory flux of sodium toward the lumen.

In a recent preliminary communication,[42] we described similar experiments performed on the obstructed dog colon. The principal functional results are reproduced in Table 2. In the absence of any serious histological lesions to the mucosa, the obstructed loop lost most of its ability to absorb sodium, chloride, and water during a perfusion *in vivo,* but net secetion toward the lumen did not develop, even 14 days after the creation of the occlusion. Glucose absorption *in vivo* was diminished, and there was a significant reduction in the accumulation of sugars and amino acids by tissue slices *in vitro.* Thus the functional changes in the colonic mucosa appear to be similar but less pronounced than those observed in the jejunum: If bacterial toxins are held to be responsible for the alterations in net transport, as in the small intestine, it must be mooted that the secretory flux of sodium can be increased to a sufficient extent to neutralize the absorptive flux of this ion but not enough to induce a net secretion toward the lumen. This interpretation presupposes, of course, that the colon, like the small intestine,[43] possesses both absorptive and secretory fluxes of sodium, but the evidence for this state of affairs is conflicting.[9,44] So this discussion must be considered speculative until more information on the effects of bacteria and their toxins on colonic mucosa has been obtained.

One further observation in this model is of interest. As in small-intestinal occlusions, the gut proximal to a colonic obstruction is greatly distended, yet the

intraluminal pressure never exceeds 10 cm of water. The mucosa exhibits considerably increased proliferative activity, as shown in mice by Stragand and Hagemann,[45] a fact that would account, at least in part, for the change in dimensions of the organ. Thus the intriguing situation is created of an organ adapting its turnover rate as a result of stasis of the luminal contents, despite low intraluminal pressures. A similar phenomenon arises in self-filling blind loops of small intestine,[46] where again stasis *per se* has been implicated as the factor triggering an increase in epithelial turnover. However, the bacteria which accumulate in the obstruction fluid or in self-filling small-intestinal blind loops may act directly on the epithelium by destroying surface cells and inducing increased turnover to compensate for this loss (see Section V) and may therefore be responsible for the hypertrophy of the organ.

V. INTERACTIONS BETWEEN BACTERIA AND THE FUNCTION OF COLONIC MUCOSA

In view of the abundant bacterial population of the large-intestinal lumen, it is surprising how few studies have been devoted to the effect of different bacteria on colonic function. Even investigations with cholera toxin, whose action on the small-intestinal mucosa has been the subject of so many studies, are rare and have given rise to conflicting results: Whereas certain authors failed to find an effect of crude cholera toxin on sodium and water fluxes across canine colon,[47,48] Donowitz and Binder[49] recently demonstrated that purified cholera toxin caused an increase in intracellular cyclic-AMP of rat cecal mucosa, and concomitantly induced net secretion of water and sodium into a cecal sac *in vivo*. In the same paper, however, the authors showed that the toxins of enteropathogenic strains of *Escherichia coli* and *Shigella dysenteriae,* at concentrations known to induce secretion in rabbit ileal loops, did not influence sodium and water movements in rat cecal sacs, nor did the *Shigella* enterotoxin cause histological damage as it does in rabbit ileum. Thus it is clearly impossible to extrapolate from small to large intestine, or from the colon of one species to another; it seems nonetheless inherently probable that the colonic epithelium possesses both secretory and absorptive fluxes for sodium,[44] though confirmation of this statement, particularly for the dog, must await further research.

The use of germ-free animals provides an opportunity to study the interactions between bacteria and the function and structure of the mucosa, but for the colon—with the exception of the phenomenon of cecal enlargement in gnotobiotes—this is an area that has largely escaped attention up to the present time. In a single study, Heneghan et al[50] examined the structure of colons from conventional and germ-free piglets and concluded that although the mucosal surface area was similar in the two groups, there was a distinct reduction in the crypt surface area. This observation agrees with the general conclusion that fewer proliferative cells are required in the intestinal tract of germ-free animals to maintain an adequate functioning epithelium[51] and therefore that cell turnover is accelerated in the presence of bacteria, perhaps due to a direct effect of the latter on the epithelium.

One feature of the large intestine that has been extensively studied is the enlarged cecum of germ-free animals, a trait recently reproduced in normal rats on treatment with high doses of antibiotics.[52] One obvious explanation for the increase in cecal size relates to its function as a reservoir for material unabsorbed from the small intestine. In normal animals, the cellulose that accumulates in the cecum is largely catabolized by the bacteria that reside there. In the absence of microorganisms, the amount of roughage stored in the cecum is likely to increase, and so the organ must adapt to receive it. This adaptation does not explain why the germ-free rodent continually passes liquid stools, nor why the cecum is filled with semiliquid material, a situation that has intrigued a number of investigators. It has been shown that the cause of liquid stools lies within the contents of the cecum, rather than in the properties of the cecal mucosa: Perfusion of the enlarged cecum with a physiological buffer solution leads to sodium and water absorption of the same order as that of normal rats[53] or even larger,[54,55] but perfusion of normal ceca with a supernatant prepared from the contents of a germ-free cecum leads to secretion of sodium and water into the lumen. Examination of the contents of the enlarged ceca has revealed that they contain a low concentration of sodium ions, an increased osmolality, and an extremely small concentration of exchangeable anions.[56] In an attempt to define which of these factors could be responsible for the inhibited transport of sodium and water, Donowitz and Binder[53] prepared a number of solutions simulating cecal contents and perfused them through the cecum of normal rats. In this way, they were able to show the importance of the presence of exchangeable anions for net cecal absorption: When sulfate-containing solutions were used, net fluid secretion invariably occurred. The rationale for this situation is not difficult to find: It is known that sodium absorption in the rat colon *in vitro* is dependent on the presence of chloride in the bathing solution[14] and that removal of chloride strongly inhibits the absorptive flux of sodium. If we accept, as discussed above, that the net flux of sodium and water across the large-intestinal wall is the result of two opposing fluxes, then inhibiting one flux without affecting the other will clearly have a profound effect on the net movement, as has been demonstrated in the dog small intestine under different circumstances.[32] Thus the sequence of events in the hypertrophic colon of germ-free rats can be envisaged as follows. The organ becomes enlarged because of the delivery of greater quantities of bulk material from the small intestine. This enlargement is accompanied by ultrastructural changes,[57] an increase in certain enzyme levels, notably Na^+,K^+–ATPase[58] and alkaline phosphatase,[59] and particularly by a greater overall absorptive capacity; the quantity of chloride in the cecal contents then becomes a limiting factor. When the chloride concentration falls below a critical level, sodium influx is inhibited, but sodium and water secretory fluxes continue unabated, with the result that there is a net loss of sodium and water into the lumen of the organ. If there is no concomitant loss of chloride, this sodium and water will not be reabsorbed further down the colon. In contradiction to this model is the suggestion of Frizzell and Heintze[60] that active chloride secretion is the primary ion movement toward the lumen in the descending colon of the rabbit. However, as already mentioned, there is an enormous difference between the functional properties of the rat colon and that of the rabbit, and extrapolation from one to the other is not permissible in the absence of direct experimental evidence in its favor.

VI. CECAL HYPERTROPHY AS A RESULT OF BULK FEEDING

In Section V, it was pointed out that the enlarged cecum of germ-free animals probably results primarily from the accumulation of material that cannot be degraded in the absence of bacteria. If this is true, it should be possible to provoke cecal enlargement by feeding nonmetabolizable bulk. This situation has been achieved by Loeschke and his co-workers,[61-65] who administered to rats polyethylene glycol (PEG) in their drinking water; the investigation of the development of hypertrophy in the cecal mucosa under such circumstances provides the most far-reaching study of large-intestinal adaptation yet undertaken.

The administration of high concentrations of PEG resulted in a rapid increase in the weight and the surface area of the cecum; the enlargement was significant after treatment for 1 week and progressed linearly over a period of 8 weeks.[61] There was a proportional increase in total protein and total DNA (Table 3), so that throughout the entire experiment the ratios of protein to DNA, protein to RNA, and RNA to DNA remained constant.[62] It could thus be concluded that the enlargement of the organ occurred by hyperplasia rather than by cellular hypertrophy.

The functional adaptation was more precocious than the morphological changes. Two days after the beginning of the experiment, net sodium and water absorptions, determined in cecal sacs *in vivo* and expressed per unit surface area, were significantly increased, and they reached a plateau after 1 week's PEG administration.[61] More detailed studies revealed that the principal functional change involved a rise in the active sodium absorption (Table 3) against an electrochemical potential gradient; chloride and potassium movements were hardly affected. The changes in sodium transport were accompanied by a parallel rise in specific activity of Na^+,K^+–ATPase, but no consistent alterations could be detected in the levels of other marker enzymes (lactic dehydrogenase, alkaline phosphatase, glucose-6-phosphatase, and glutamate dehydrogenase). These observations suggest that the primary change occurring in the

Table 3. Effect of Feeding PEG-4000 to Rats for 1 Week on Morphological and Functional Characteristics of Cecum[a]

	Normal cecum	Hypertrophic cecum
Wet weight (mg)	421 ± 19	668 ± 31
Protein content (mg)	46 ± 1.9	74 ± 3.4
DNA content (mg)	4.8 ± 0.2	7.4 ± 0.1
$Na,^+ K^+$–ATPase specific activity (μmole/hr · milligram of protein)	17.9 ± 0.7	34.9 ± 0.6
Sodium absorption *in vivo* (μeq/cm^2 · hour)	5.2 ± 0.4	9.7 ± 0.4

[a]Ceca were removed from normal rats or animals that had consumed PEG-4000 (added at a concentration of 160 g/liter to their drinking water) for measurement of wet weight, DNA, and protein. $Na,^+ K^+$–ATPase was determined in microsomal fractions derived from mucosal homogenates. Sodium absorption from cecal sacs *in vivo* is expressed per unit surface area. Results are means ± S.E.M. of at least five determinations. Results are taken with permission from Loeschke et al,[61] Loeschke and Resch,[62] and Schiffl and Loesche.[63]

mucosa, even before the development of hyperplasia, is the induction of new molecules of Na^+,K^+–ATPase into the basolateral cell membrane, an interpretation supported by kinetic studies demonstrating an increase in the V_{max} of the enzyme.[63] Measures of cyclic nucleotides in the cecal mucosa revealed that, whereas cyclic-AMP levels did not change, cyclic-GMP levels gradually declined, and the time course of this change was a mirror image of the rise in Na^+,K^+–ATPase-specific activity.[64] It therefore seems possible that the factor that triggers off the enhancement of Na^+,K^+–ATPase activity, and consequently of sodium transport, is in fact the change in intracellular cyclic-GMP levels.

In a further study designed to examine whether the rise in sodium transport in the hyperplastic cecum could be prevented if the basal level of sodium absorption were modified, Loeschke and Müller[65] raised the latter by aldosterone treatment or depressed it by adrenalectomy, hypophysectomy, or volume expansion. Following all these maneuvers, PEG administration continued to stimulate sodium tranport; none of the interventions succeeded in abolishing the difference in transport rates between the normal and adapting cecum. Thus it could be concluded that at least the hormonal influences investigated could not be directly responsible for the hyperplastic transformation.

VII. REVERSIBLE COLONIC ATROPHY AS A RESULT OF DECREASED INTRALUMINAL LOAD

The most appropriate experimental model for the study of possible effects of the absence of intestinal contents on the structure and function of the mucosa is the self-emptying blind loop, developed for the small intestine by Menge et al.[66] The principal features of a small isoperistaltic loop, connected to the intestinal thoroughfare only at its distal end, are that the mucosa never comes into contact with ingested material but that desquamated cells are readily evacuated into the intestine in continuity. It is important that the excluded loop should remain relatively small, so that compensatory changes do not occur in the remaining intestine, which should be capable of absorbing its nutrients in a normal manner.

An analogous experimental model has been applied to the descending colon of the rat by Rijke et al,[67] who reported that the number of cells per crypt and particularly of cell columns per crypt was reduced in the blind loop, 6 weeks after is creation. These observations are commensurate with atrophy of the colonic mucosa, though morphometric data concerning the size of the individual cells would be required for the complete characterization of such a transformation. In contrast, the authors demonstrated that 1 hour after injection of radioactive thymidine both the percentage of labeled cells and their distribution within the crypts were the same as in normal colon. Since this parameter may be considered an expression of DNA synthesis and therefore of cell turnover, these observations beg the question as to how the cell number in the blind loop can be decreased in the face of normal cell turnover.

A similar mucosal atrophy arises in animals fed exclusively by an intravenous route, though clearly some biliary or pancreatic secretions and desquamated cells may reach the colon under these circumstances. Ryan et al[68] reported that following 10

days' intravenous nutrition, the weight of the rat colon was reduced by 25% and the incorporation of radioactive thymidine into the DNA of the mucosa was diminished by about 50%. They thus concluded that the loss of weight of the colon was a direct consequence of mucosal atrophy resulting from reduced cellular proliferation. This interpretation was supported by the results of Morin et al,[69] who observed a reduction in protein and DNA content per unit length of the colon, following 8 days' exclusive parenteral nutrition. The oral administration of an elemental, bulk-free diet that is almost completely absorbed at the level of the jejunum induced similar changes in the colon.[68–70]

Starvation also leads to atrophy of the colonic mucosa, though in this condition, the structural changes may be attributed not only to the absence of material from the lumen but also to the caloric deficit to which the animal is subjected. The first detailed study of proliferation kinetics in the intestinal mucosa of fasted animals was that of Wiebecke et al,[71] who showed that in the mouse, after 24 h of starvation, the size of the proliferative zone in the descending colon was significantly decreased. Similar changes occurred in the ascending colon after 3 days' starvation. Some of these observations were not confirmed by Hagemann and Stragand,[72] who were unable to show any change in the size of the proliferative compartment. However, both groups of investigators demonstrated that radio-thymidine incorporation was severely reduced on fasting; this was reflected in a prolongation of the cell cycle, primarily in the G_1 and S phases.[71,72] Strangely, the reduction in cell production was not accompanied by any significant change in the number of cells per crypt[72]; presumably the duration of the starvation was not sufficient for the proliferative changes to have affected the cell number. These different results show in various ways that cell turnover in the colon is strongly diminished in the absence of luminal contents or after fasting. Unfortunately, the morphological, enzymological, and functional consequences of this situation have not yet been examined in any detail.

The dependence of colonic cellular proliferation on the presence of luminal contents raises the interesting question as to which factor within the lumen could be responsible for the control of this process. At the level of the small intestine, it has been demonstrated that the nutritional properties of the luminal contents play a role in the maintenance of cell turnover,[73,74] but this is unlikely to be the case in the colon, since in most species no absorption of nonelectrolytes takes place there.[12] The problem was first examined in mice by Hagemann and Stragand,[72] who explored the time course of proliferation following the introduction of different factors into the fodder after a fasting period of 3 days. Refeeding normal food led to a burst of proliferative activity in the ascending colon within the first 24 h. DNA synthesis increased 4 h after the onset of refeeding, that is to say, as soon as ingested material started to arrive in the colon, and, 8–12 h later, attained a maximum value twice that of a normal colon. These changes were accompanied by a shortening of the cell cycle time, that is to say, by a release of the block in the G_1 phase, by an expansion of the proliferative zone of the individual crypts, and by a slight increase in the height of the crypts. After 5 days, the turnover rate had returned to normal. If a low-bulk (but not a bulk-free) diet was fed under similar circumstances, there was no overshoot in the proliferative rate, but this parameter attained normal values within 24 h of the onset of refeeding. The administration of a

bulk-free glucose solution did not induce a normalization of the turnover rate. These observations strongly suggest that the bulk content of the diet regulates the proliferative activity of the colonic crypts.

Nevertheless, the same investigators showed that bulk is not the only factor involved in this regulation, since the hyperproliferation observed on refeeding a fasted animal requires the presence of sugar, protein, and minerals in the diet[75]; it is not yet known which of these components release the G_1 block induced by fasting and which permit the blocked cells to initiate DNA synthesis. The role of serum factors in this process appears to be minimal, as shown by another series of experiments by Stragand and Hagemann,[45] in which they placed a ligature around the colon of the fasted mouse. First, they observed a distension proximal to the obstruction; this was accompanied by increased proliferative activity, which, as discussed above, was probably caused by distension alone. Second, when they refed animals with an occluded colon, there was a second burst of proliferative activity in the loop of colon proximal to the obstruction, but no change in the mucosa distal to it. This elegant experiment appears to exclude the participation of blood-borne agents such as nutrients or hormones in initiating the return to normal turnover rates.

Somewhat different conclusions were reached by Mak and Chang[76] and Ryan et al,[68] who examined the influence of gastrin on colonic turnover, since it had been demonstrated previously that fasted rats had diminished serum and antral gastrin levels[77] and that this hormone had trophic effects on the small intestine.[78] These authors[68,76] showed that pentagastrin administration to rats that had been starved or fed an elemental diet can reverse the atrophic changes in the colonic mucosa, although the hormone has no effect on colons of normal animals. On the other hand, the same investigators also showed that the reduction in DNA synthesis caused by feeding an elemental diet could be reversed simply by the addition of nonabsorbable bulk (cellulose and petroleum jelly) to the fodder; this effect was obtained in the absence of any change in antral or serum gastrin.[68] Thus under the experimental conditions that have been discussed, it appears that both serum gastrin and physical stimulation by bulk material are independently capable of stimulating colonic growth; whether any interaction between these two factors can occur remains to be determined.

VIII. COLONIC ADAPTATION FOLLOWING RESECTION

Extensive small-intestinal resection is clearly associated with a larger delivery of nutrient material into the colon because of the reduced absorptive capacity of the short small bowel. Barium enemas have often disclosed marked distension of the colon in such patients.[79,80]

The effect of distal small-intestinal resection on colonic structure has been examined in experimental animals on several occasions. Nygaard[81] noted that following 75% distal resection of the rat intestine, the colon increased in weight, length, and circumference. Perry[80] remarked that the cecum was enlarged to a greater extent than the colon under such conditions and that bile acid absorption was also enhanced in the cecum but not in the colon. Rather similar results were reported by Scarpello et al[82]; they found cecal growth after distal small-bowel resection, but no change in the colon

with respect either to structure or to water absorption *in vivo*. A more detailed analysis was undertaken by Michaud et al,[83] who studied cell kinetics in the mucosa of the ascending colon on the 1st, 3rd, and 10th day after 50% midgut resection. This early postopertive period was characterized by significant and progressive increases in labeling index, crypt height, and size of the proliferative compartment. Nundy et al,[84] however, showed that the responses of the different sections of the colon were not identical and suggested that hyperplasia occurred principally in the ascending loop; they also mooted that the situation was complicated at other sites by cell hypertrophy, as opposed to hyperplasia. Finally, McDermott and Roudnew[85] were unable to detect any proliferative changes in the descending colon of the rat after proximal small-bowel resection. It should be emphasized, however, that the conflicting results reported by the different groups may simply be due to the fact that different segments of the small intestine were resected; ileal resection will lead to the delivery of more bile acids to the colon, whereas jejunal resection will permit the advent of more nutrient material.

Removal of part of the large intestine itself is likely to cause hypertrophy of the remaining segments, though the number of studies on this phenomenon is strictly limited. Perry[80] reported that mucosal thickness in the rectum was increased in patients who had undergone hemicolectomy. In animal studies, cecectomy has been shown in rats to induce an increase in the length and surface area of the remaining colon but no change in weight per unit length[86]; such changes were more pronounced after combined ileocecectomy.[82]

All the experiments reported so far have concerned the rat, which possesses a relatively small colon with only minor variations along its length. The large intestine of herbivores is a much bigger organ with greater regional differences in both structure and function.[10] In particular, the colon of the rabbit possesses the unusual characteristic of undergoing circadian changes in function, with the resultant formation of different types of feces; hard feces are rejected, but soft feces (cecotrophes) are reingested and supply the animal with important dietary factors, such as vitamins.[10] Certain investigators have examined the role that different segments might play in this complex process by performing partial resections. Cecectomy in the rabbit leads to enlargement of the proximal region of the colon; animals that survive this operation continue to excrete two types of feces, but they no longer reingest the cecotrophes.[87,88] This observation suggests that normal cecotrophes contain an odoriferous substance of bacterial origin that may no longer be fabricated after cecectomy. In contrast, ablation of the proximal colon of the rabbit abolished both the formation of cecotrophes and the practice of coprophagy.[89,90] This situation appears to be irreversible over a 10-week follow-up period, indicating that the production of cecotrophes is a specific property of the proximal colon of the rabbit and that this tissue cannot regenerate, nor can other sections of the colon take over its functional characteristics.

IX. CONCLUSIONS

There is compelling evidence that the nutritive value of the luminal contents plays a regulatory role in controlling the proliferative activity of the small-intestinal mucosa.[18] The central theme of the present review is that a similar role might be played

by roughage in the large intestine. This conclusion seems reasonable when it is considered that the principal function of the colon is to extract as much salt and water as possible from the material that passes through it; if the lumen is empty, the colon becomes functionally idle.

Compatible with this hypothesis is the observation that mucosal atrophy with reduced cellular proliferation develops when animals are starved or fed bulk-free diets, and that the alterations can be reversed simply by adding bulk to the fodder.[68] The opposite situation arises when excess bulk is added to the diet; the cecum in particular—other sections of the intestinal tract have not been examined—undergoes a hyperplastic transformation, with concomitant enhancement of its functional characteristics.[61] In germ-free animals, where the roughage that arrives in the cecum cannot be readily degraded, a similar hypertrophy of the organ ensues.

How the proliferative changes are initiated remains a matter of some conjecture. It would appear, from the elegant experiments of Stragand and Hagemann,[45] that the triggering factor is not the actual presence of roughage in the large bowel but the distension of the organ caused by the presence of bulk. In this respect, the suggestion[2] that on distension, all the mature cells of the colonic crypt are spread out in the plane of the mucosal surface, may be of relevance. However, the studies of Loeschke et al[61] with the hypertrophic cecum have indicated that the initial response is a functional one, the proliferative changes starting only after the shift in transport capacity has begun. Unfortunately, no functional studies of the colon of fasted rats or animals nourished intravenously are available; it is therefore not known whether the same evolution occurs in the case of the opposite adaptation. Future work should be aimed at determining whether this sequence of events is a general one, and if so how it occurs. If the sodium transport capacity is controlled by cyclic-GMP[64] or some other messenger, how can this respond to simple physical stimulation?

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14

Drugs and the Colon

R. Wanitschke and K. Ewe

I. INTRODUCTION

It is not our intention to review drug effects in the colon, which was done extensively and profoundly by Forth and Rummel[1] and Powell.[2] Our contribution will present studies of drug effects on physiological absorption and secretion processes obtained in the intact human colon and based on results of intestinal perfusion studies of the whole colon and measurements of transepithelial electrical potential difference (PD) in the rectosigmoid colon. These are at present the practiced and—regarding perfusion studies—most accurate techniques in the hands of clinicians to directly study drug effects on a local dose–response scale in the intact human colon. Since these methods are relatively new and their use practically limited to clinical research, no extensive data on this topic are yet available.

II. METHODOLOCIAL REMARKS

A. Intestinal Perfusion of the Colon

A four-lumen polyvinyl tube is swallowed and driven by peristalsis down to the ileocecal valve (X-ray controlled). In place, the lumen of the terminal ileum is blocked by inflation of an occlusive rubber balloon. Above the balloon intermittent suction removes fasting intestinal contents. Distally to the balloon constant-volume-controlled infusion of test solution enters the cecum and is collected in 15-min intervals by a rectal tube. After equilibration to reach approximately steady-state conditions (the amount of volume marker—polyethyleneglycol, mean mol. wt. 4000—that enters the colon equals the amount of polyethyleneglycol leaving the colon at a given time) from changes in polyethyleneglycol (PEG) and solute concentrations between infusion and

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sampling point at a known perfusion rate, net fluid and solute absorption (or secretion) can be calculated. This method, unpleasant and time-consuming for both parts, has given the most reliable information on colonic fluid and electrolyte handling in healthy, diseased, and drug-influenced stages. Essentially a research procedure, this method is the best way to study fluid and electrolyte transfer more confidently in intact man.

Based on intestinal intubation studies by Blankenhorn et al[3] Levitan et al[4] first applied this method to measure water and salt absorption in the intact human colon. The validation of PEG as a suitable dilution indicator on the human stomach and small bowel[5] and the human colon[6] actuated studies in this field. Colonic perfusion studies performed at a 10 or 15 ml/min perfusion rate give reproducible results and tolerable steady-state conditions after 1 hr equilibration time. It is quite obvious that such perfusion rates do not establish physiological conditions and that fasting intestinal contents flow rates are available and approximately 2.5 ml/min in the jejunum and 1.3 ml/min in the proximal ileum.[7]

Furthermore, changes of perfusion rate influence mean transit time and mean volume of the human colon as well as fractional and total absorption rates of sodium and water.[8]

There are many other often more theoretical objections to the use and manifold advice for the corrections of undeniable problems, e.g., contamination of the test segments from above, especially of disadvantage in electrolyte and fluid absorption studies; proximal reflux of the infused fluid increasing the absorption-surface area; possible disadvantages by the use of any occlusive balloon such as altering local mucosal blood flow or motility patterns. But if one knows the pitfalls or limitations of colonic perfusion studies, this method gives reproducible results.

Regarding drug studies on colonic water and electrolyte handling by the intestinal perfusion method, some special problems should be mentioned.

1. Luminal concentration of the drug at the perfusion side decreases along the segment due to drug absorption and/or dilution of the secreted fluid. Measurements of the collected rectal fluid allow only approximate information on the drug concentration within the perfusion segment.

2. Because of the high reabsorption capacity of fluid and electrolytes in the colon significant drug effects in the proximal portion of the colon can be fully compensated for along the more distal part, extinguishing pronounced local effects on the mucosa. Perfusion rates within physiological flow rates would therefore be unsuitable in drug effect studies of compounds being absorbed more rapidly. Perfusion rates of 10 or 15 ml/min are necessary to overcome these difficulties and to increase the overall sensitivity of the study system.

3. High perfusion rates have their limitation by substances that are quantitatively or highly absorbed and peak there their maximal systemic dosage.

4. Recycling of absorbed drugs into the study segment via the enterohepatic circulation has to be considered. Suction above the study segment and the use of an occlusive balloon minimizes this problem.

5. Existing segmental differences in absorptive function of the human colon[9] and regional differences in drug-induced secretory function are usually not considered for technical reasons. No doubt any use of an additional tube along the colon would in-

crease qualitative information on absorption–secretion processes and concentration profile of the drug within the segment but is limited in behalf of volunteers.

6. In order to minimize the error due to contamination with fasting intestinal contents it is important concerning electrolyte and water transfer studies not to lower sodium concentration and perfusion rate and to control the correct position of the inflated occlusive balloon at the beginning and end of each study.

7. After cleansing during the equilibration period with control solution, perfusion should last at least 60 min. After the test period is finished, perfusion with the control solution and sampling every 10 or 15 min should be continued in order to look for functional reversibility of the drug effect.

8. Changes of net absorption and/or secretion at a 10% level are functional variations and not drug effects.

B. Measurement of Transepithelial Electrical Potential Difference in the Intact Rectosigmoid Colon

The existence of a transepithelial electrical potential difference (PD) in the gastrointestinal tract was first demonstrated by Donné.[10] With the validation of the technique by Soergel et al[11] Geall et al,[12] Dalmark,[13] and Edmonds et al,[14] absolute measurements of PD in the intact human colon became obtainable. This method is a noninvasive, fast, and low-risk procedure. The experimental design of PD measurement and registration is illustrated in Fig. 1 in a modification of the technique used by Davenport.[15] The exact nature of the 25- to 40-mV (lumen negative versus blood) PD at the human rectal mucosa is still not yet known. Its magnitude is characterized mainly by the passive permeability properties for electrolytes at the site of intercellular ("tight") junctions,[16] the luminal electrolyte concentrations,[17] and the active sodium absorption. Under control conditions, therefore, measurements of rectal PD provide an easy tool for clinicians to demonstrate effects of drugs on electrolyte and, consecutively, on water transfer, otherwise difficult to obtain. But it should be stressed that PD measurements themselves, without any further knowledge of processes involved, are not meaningful and do not contribute substantially to the clarification of the mode of action. Unfortunately, in our hands results of simultaneous measurements of PD and unidirectional and/or net electrolyte fluxes in the rectosigmoid colon were found of no reasonable correlation. This was most likely for methodological reasons, because length, duration of effect, and luminal drug concentration cannot be determined and, therefore, results of these flux measurements cannot be compared to local PD values at the rectosigmoid site. However, in connection with known effects obtained otherwise, PD measurements in the human rectum offer an easy and convenient tool to document drug-induced changes and to contribute to the knowledge of their possible mode of action.

III. DRUG EFFECTS ON THE INTACT HUMAN COLON

Any more detailed knowledge about the structure–activity relationship of antiabsorptive and/or secretagogue compounds of endogeneous (dihydroxy bile acids)

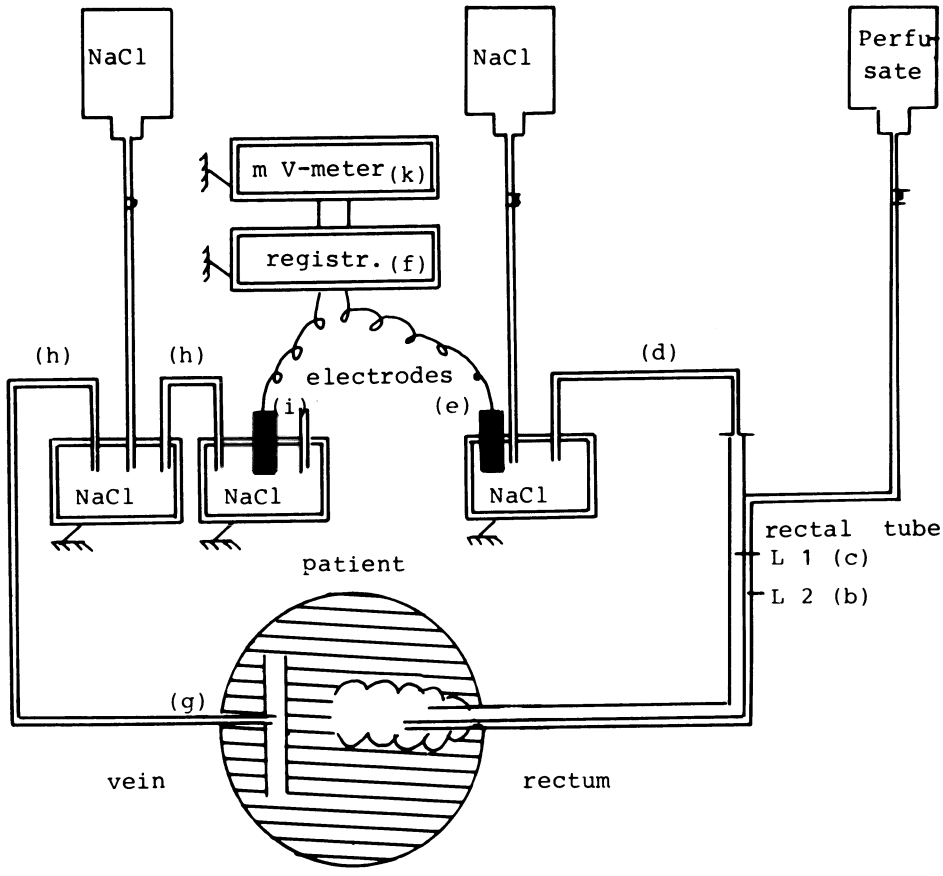

Figure 1. Experimental device for measuring potential differences on human rectum. L 1 and L 2 are tubes leading into the rectum; L2 is connected with the perfuion, L1 serves as a flowing electrode connected (a) over a saline-filled bottle to the calomel electrode (b). The indifferent flowing electrode (c) in the anticubital vein is similarly connected (d) to the other electrode (e).

and exogeneous (cathartic drugs) sources would imply qualitative progress. However, despite the various structural affinities of different secretagogue compounds (see Fig. 2), a unique chemical (or sterical) structure for inducing intestinal secretion cannot yet be defined. Its demonstration would provide for refined methods for further research. In Section III. A, effects of secretagogue agents on electrolyte and water net transfer and PD in intact human colon will be reported.

A. Bile Acids

The effect of secretagogue dihydroxy bile acids will be mentioned here because they can be considered to be endogeneous laxatives inducing the clinical entity of cholerrheic enteropathy.[18] Cholic acid as the dominant primary bile acid in man is converted by customary colonic bacteria (7α-dehydroxylation) to deoxycholic acid,[19] a potent secretory agent. As shown by Mekhjian et al,[20] at a perfusate concentration of 3 mmole/liter (perfusion rate 15 ml/min) consistent colonic secretion of water could be observed; cholic acid up to 10 mmole/liter had no effect, and chenodeoxycholic acid at

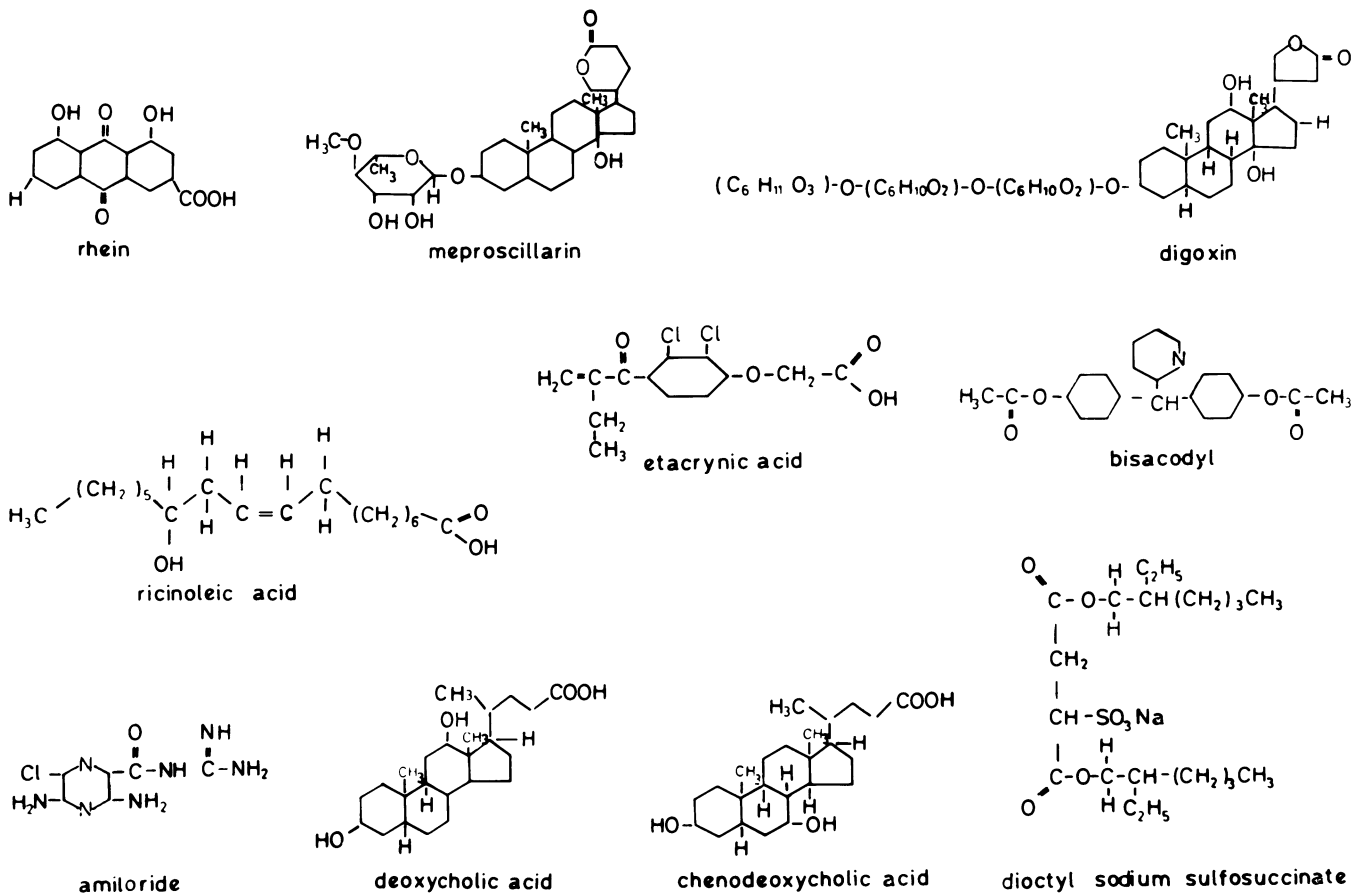

Figure 2. Chemical structure of antiabsorptive and/or secretagogue drugs in human colon.

5 mmole/liter an inconsistent effect, on water secretion (derived from figures by Mekhjian[20] listed in Table 1). It should be noted that structural requirements of bile acids for their secretory action are two α-hydroxyl groups in either the 3, 7, or 12 positions.[21]

Deoxycholic acid absorption within the perfused colon was measured as 9.1 μmole/min, which amounts to 20% of the perfusion load. The secretagogue effect of deoxycholic acid was first demonstrated by Forth et al[22] in rat jejunum and colon and has been since shown in many species on different sites of the intestine using a variety of solutes.[1]

To the mode of laxative action three mechanisms have been proposed: (1) increase of the intracellular amount of cyclic-AMP (cAMP) due to stimulation of the adenylate cyclase system[23–27]; (2) inhibition of the sodium pump[28,29]; (3) increase in mucosal electrolyte and water permeability.[30–32] There is experimental evidence as well as less consistent findings for each of these hypotheses.[1,2,33,34] The question remains whether these experimental findings are causative linked to, and quantitatively meaningful in, intestinal secretion. Dihydroxy-bile-acid-induced permeability changes for water and water-soluble compounds have clinical importance since increased oxalate absorption from the affected colon[35] results in hyperoxaluria and nephrolithiasis.[36]

The results of bile-acid-induced changes in PD in the intact rectosigmoid colon are in concordance with perfusion results. Nine mmole/liter cholic acid did not influence PD, whereas deoxycholic acid did (Table 2). There were, however, differences in effective concentrations: 0.5 mmole/liter, 1.5 mmole/liter, and 4.5 mmole/liter of deoxycholic acid did not alter rectal PD. Considering the possibility that bile acids may act by increasing colonic permeability at the site of the tight junctions[30,37] and the fact that the rectal region is less permeable for water along the intestine,[38] differences in effective concentrations may be explainable. The transepithelial PD of epithelia is characterized predominantly by the shunt resistance of the paracellular pathway.[1] A drop in PD may reflect decreased resistance by the tight junctions, and in the relatively tight rectal mucosa higher local deoxycholic acid concentrations (or longer exposure of lower concentrations) may be necessary to become effective.

B. Diphenolic Laxatives

Most studies are done with bisacodyl (for the chemical structure see Fig. 2). Luminal concentrations of 3×10^{-5} mole/liter (approximately 1 mg%) had a pronounced effect on colonic electrolyte and water transfer, as shown by Ewe and Hölker.[39] Net absorption of sodium and water was reversible into net secretion (Table 3). After bisacodyl was omitted from the perfusion solution this effect was not fully reversible within 4 hr of observation. As in detail presented elsewhere,[39] this prolonged effect is caused by bisacodyl still present in the lumen after omission of bisacodyl from the perfusion solution. This phenomenon, observed in our studies, with other compounds too, might be a common finding during colonic perfusion and might influence the effect of the luminal concentration of a drug; but this is changed more by the colonic absorption of the secretagogue compound itself. During colonic perfusion bisacodyl was absorbed by more than 70% of the infusion load. Within 18 hr, 10.8 ± 2.5% (S.E.M.) of the absorbed bisacodyl was excreted by the urine; most of the substance absorbed underlies

Table 1. Electrolyte and Water Transfer in the Perfused Human Colon (Whole Colon) Calculated from Figures Given in the Literature

Compound	Perfusate concentration (mole/liter)	Perfusion rate (ml/min)	n	Rate of water transfer (ml/min)		Rate of sodium transfer (μmole/min)		Rate of chloride transfer (μmole/min)		Rate of potassium transfer (μmole/min)	
				Control	Test	Control	Test	Control	Test	Control	Test
Deoxy-cholate[a]	3×10^{-3}[b]	15	4	1.54±0.54	−0.44±0.16	202±89.8	−13.5±19.2	271±99.6	67±264	+35.5±26	−61±35.7
Cheno-deoxycholate[a]	5×10^{-3}	15	3	2.05±1.46	−1.46±131	292±237	−219±191	335±141.6	−102±105	41.7±8.1	−36±16
d-Aldosterone[c]	Perfusion 24 hr after 1 mg i.v.	10	12	2.24±0.36	3.77±0.14	410±32	670±12	620±17	790±57	25±1	24±0.0
Antidiuretic hormone[d]	1 U/hr i.v. during perfusion	15 31	29	−3.26±1.4 ($p < 0.01$)	2.15±1.42	440±210 ($p < 0.02$)	290±220				
Ricinoleic acid	2×10^{-3}	15	4	2.67±0.17	0.36±0.38	316±23	119±49	344±17	201±28	68±3	5±5

[a]Composition of perfusate (μmole/liter): NaCl 82, KCl 19.7, $NaHCO_3$ 49 ($\bar{x}$ ± S.D.). From Mekhjian et al.[20]
[b]Mean absorption rate of deoxycholate within the segment: 9.1 μmole/min ≈ = 20%.
[c]Composition of perfusate (mmole/liter): Nacl 147 ($\bar{x}$ ± S.D.). From Levitan and Ingelfinger.[62]
[d]Composition of perfusate 0.85% NaCl ($\bar{x}$ ± S.D.). 1 Number of collection periods, each lasting 20 min. From Levitan and Mauer.[68]
[e]Composition of perfusate: Na 130, K 20, Cl 100 ($\bar{x}$ ± S.D.). 1 single but representative out of 4 subjects. From Ammon and Phillips.[58]

Table 2. The Influence of Antiabsorptive and/or Secretagogue Compounds on the Transepithelial PD in the Intact Human Rectosigmoid Colon

Compound	Perfusion concentration	n	PD (mV) lumen negative versus blood	
			Control	Test
Bisacodyl	3×10^{-5} mole/liter	11	34 ± 3	8 ± 4[a]
Digoxin	2 mg%	5	38 ± 5	40 ± 6
Digitoxin	2.5 mg%	5	39 ± 4	21 ± 3[a]
Meproscillarin	0.5 mg%	5	43 ± 3	23 ± 3[a]
Deoxycholic acid	9×10^{-3} mole/liter	4	47 ± 5	25 ± 9[a]
Ricinoleic acid	9×10^{-3} mole/liter	5	35 ± 11	34 ± 3

[a]Values are given as $\bar{x} \pm$ S.D., $p < 0.05$ versus control. The values under the inscription test are obtained 30 min after the start of the perfusion with test solution. For details see Ewe and Wanitschke.

glucuronidation and biliary excretion.[40] The observed effect of bisacodyl on rectal PD (Fig. 3) parallels the results of colonic electrolyte changes. The drop of PD was prompt to a 23.6% level of the initial value ($n = 11; p < 0.001$). After perfusion with standard solution the PD went up to 56.2% of the initial value within 120 min. For diphenolic laxative compounds the mode of action seems to be similar to that of dihydroxy bile acids. It has been shown that mucosal Na^+, K^+–ATPase activity is decreased in rat small intestine[41] and colon and that intestinal permeability changes for water and solutes at the site of zonulae occludentes take place.[32,43–46] Support of the third hypothesis of cAMP-mediated intestinal secretion in the current discussion on the mechanism of intestinal secretion was brought by Loeschke[42] in rat colonic loops *in vivo* in acute but not chronic experiments with bisacodyl and oxyphenisatine.

C. Anthraquinones

Rhein[47–49] is a laxative anthraquinone derivate (for its chemical structure see Fig. 2). As shown in Table 3 it has a distinct secretagogue effect in the large (and small) bowel of man. During colonic perfusion rhein was found to be absorbed [63.3 ± 11.5% ($n = 6$; $\bar{x}$ ± S.D.) of the infusion load]. This and the pattern of functional reversibility are similar to bisacodyl, but compared on a molar level it seems less effective than bisacodyl. This could also explain that rhein up to 20 mg% concentrations at the luminal border had no effect on rectal PD in man. Alternatively, anionic secretion could counteract active sodium absorption, but the mode of action of rhein needs further studies. In rat colon rhein is effective similarly[50]; luminal concentrations of 6 mg% on the stripped rat colon inhibit the Na^+, K^+–ATPase activity.[34] Studies on alternative modes of action such as hydraulic permeability changes or alterations of cyclic nucleotides of the mucosal tissue are not available yet.

D. Cardiac Glycosides

Ever since Cooperstein and Brockman demonstrated[51] that cardiac glycosides also influence intestinal transfer processes, these compounds were used for intestinal

Table 3. Effect of Drugs on Colonic Water or Electrolyte Transfer in Intact Human Colon

Compound	Perfusate concentration (mole/liter)	Perfusion rate[a] (ml/min)	n	Rate of water transfer (ml/min)		Rate of sodium transfer (μmole/min)		Rate of chloride transfer (μmole/min)		Rate of potassium transfer (μmole/min)	
				Control	Test	Control	Test	Control	Test	Control	Test
Bisacodyl	3×10^{-5}	10	5	0.85±0.1	−2.18±1.6	180±23	−204±203			−24±2.0	−180±28
Rhein	1.2×10^{-3}	6	5	1.61±1.0	−6.16±7.9	272±81	−199±292	396±72	−67±44	0±6.0	−78±75
Ectacrynic acid	5.5×10^{-4}	5	5	1.69±0.5	−1.40±1.9	389±112	−118±277			−22±19	−39±14
Digoxin	1.9×10^{-5}	5	5	1.48±0.9	−2.17±0.9	361±141	−240±143			−40±6.0	−73±53
Mepros-	1.9×10^{-5}	6	7	1.76±1.1	−2.60±2.1	319±122	−206±170	470±143	−109±87	0±6.0	−19±18

[a]Composition of perfusate ($\bar{x}$ ± S.D.): Bisacodyl (mmole/liter): jejunum: NaCl 138, $NaHCO_3$ 12, KCl, 2.5, glucose 16.7; colon: NaCl 138, $NaHCO_3$ 12, KCl 2.5. Etacrynic acid (mmole/liter): jejunum: NaCl 138, $NaHCO_3$ 25, KCl 5, glucose 11.2; colon: NaCl 138, $NaHCO_3$ 25, KCl 5. Digoxin (mmole/liter): jejunum: NaCl 138, $NaHCO_3$ 25, KCl 5, glucose 11.2; colon: NaCl 138, $NaHCO_3$ 25, KCl 5. Rhein (mmole/liter): jejunum: NaCl 90, $NaHCO_3$ 25, KCl 5, glucose 11.2, xylose 11.2; colon: NaCl 116, $NaHCO_3$ 25, KCl 5. Meproscillarin (MMole/liter): jejunum: NaCl 90, $NaHCO_3$ 25, KCl 5, glucose 11.2, xylose 11.2; colon: NaCl 122, $NaHCO_3$ 25, KCl 5.

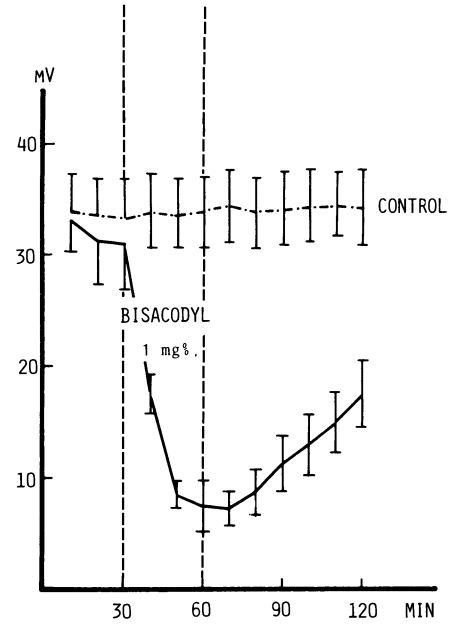

Figure 3. Effect of bisacodyl on electrical PD in intact human rectum.

studies in different parts of the intestine on different species using a variety of solutes.[1] We performed perfusion studies and PD measurements in the intact human colon with two cardiac glycosides: digoxin and meproscillarin, which was introduced for cardiac treatment of patients with renal failure because of its mainly biliary excretion and its approximately 30% daily elimination. It differs from digitoxin in a shorter half-life time and provides an advantage in certain clinical situations.[52] Digitoxin was used by us for PD studies so far. Our results show that digoxin and meproscillarin (Table 3; Fig. 2) exert a distinct and sustained effect on colonic electrolyte and water net transfer in intact human colon and change net absorption into net secretion. Compared on a molar basis they possess an even higher local secretagogue potency (digoxin!) than pharmacologically applied laxatives (bisacodyl or rhein). Their effect on sodium and water net secretion is prompt. As shown in Fig. 4 the effect of digoxin was fully reversible after 90 min perfusion with control solution. Meproscillarin showed a more prolonged return to functional reversibility, which was not fully restored following a 220-min perfusion period with control solution. Considering the almost complete digoxin absorption (measurements of digoxin concentration in the effluent were not performed) and the demonstrated almost 100% absorption of meproscillarin (measurements of the effluents were performed as described by Lowenstein and Corrill[53]) in the colon, the local and not the systemic effect of these compounds is demonstrated clearly for digoxin and seems most likely for meproscillarin by the demonstrated functional reversibility of electrolyte and water net secretion after complete absorption of the drugs.

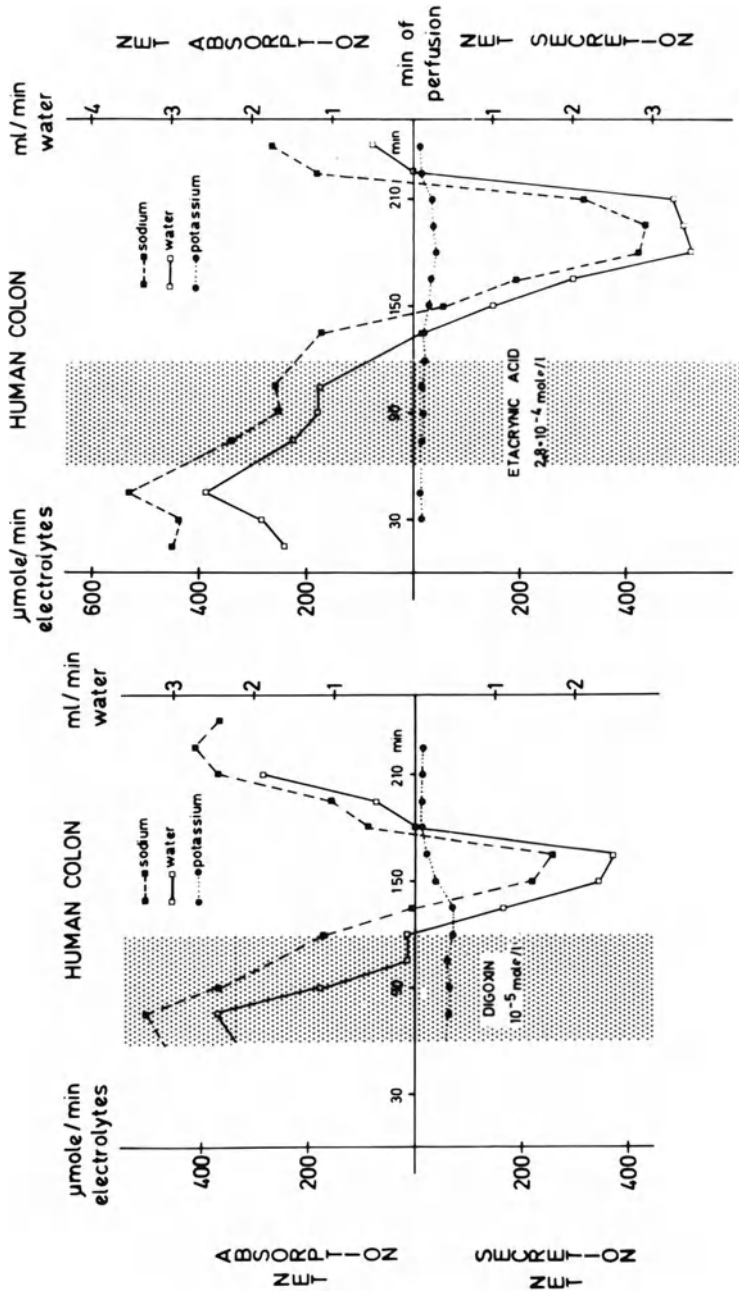

Figure 4. Effect of digoxin and etacrynic acid on intestinal electrolyte and net water transfer in the human colon.

The effects of different glycosides on rectal PD are summarized in Table 2. Whereas meproscillarin (53%) and digitoxin (49%) decreased rectal PD to a similar degree, digoxin had no effect. The return to the control level after omitting meproscillarin from the perfusate was prolonged in a comparable fashion, as observed during perfusion studies. The absence of any effect of digoxin on rectal PD could be at least partially explained by the different lipid solubility of cardiac glycosides. Digoxin is less lipid soluble than digitoxin,[54] and the lipophilic property of the latter compound is comparable to that of meproscillarin, which was almost 100% absorbed during colonic perfusion in man. But whether the possible differences in time course and local concentration at the basolateral membrane-located ATPase system due to distinct lipid solubility can explain the observed lack of PD effect of digoxin in the rectum remains an open question. Intestinal perfusion studies and PD measurements in intestinal human intestine are not suitable to draw firm conclusions to the mode of action of these substances. However, the results shed some light on the relative importance of the mechanisms of drug-induced fluid secretion. In the authors' opinion the most interesting result is the induction of net electrolyte and water secretion by the cardiac glycosides digoxin and meproscillarin when applied to the luminal side of the mucosa in intact human colon. Systemic effects of cardiac glycosides on intestinal transfer processes are unlikely because functional reversibility of their secretagogue effect was observed within 2 hr after almost complete systemic absorption of meproscillarin. Similar effects with ouabain in the rabbit small intestine have been reported.[55] In contrast to these *in vivo* results, cardiac glycosides decrease *in vitro* exclusively the unidirectional sodium flux from the mucosal to the serosal side and do not elicit secretion.[56] The implication of our observation in man and of the results in the rabbit intestine[55] is that *in vivo* inhibition of the sodium pump alone is sufficient to cause intestinal electrolyte and water secretion.

The perfusion results obtained with meproscillarin and digoxin can be used to demonstrate the importance of the pharmacokinetics of a drug concerning its adverse effect, i.e., diarrhea. Both compounds have a distinct and pronounced local secretagogue effect in the intact human colon. Clinical observations, however, show marked differences in frequency and severity of unwanted diarrhea during treatment with these cardiac glycosides. Digoxin is eliminated predominantly by the kidney. Its local secretagogue potency in the intestine becomes ineffective. This is, however, the case for meproscillarin, which is in higher proportion excreted biliary. In certain situations effective secretagogue luminal concentrations may occur, resulting in diarrhea. Digitoxin, on the other hand, is excreted biliary in a proportion of approximately 40% of the total load but does not result in diarrhea. The daily excretion rate of digitoxin is about one-fourth that of meproscillarin. Therefore, only extreme conditions such as digitoxin intoxication or more rapid biliary elimination may result in effective luminal secretagogue concentrations leading to diarrhea as a reversible side-effect.

E. Diuretics

It has been quite obvious to study the effect of diuretics on the intestinal mucosa because of behavior patterns similar to those of the renal tubular epithelium. The side effect of diarrhea during etacrynic acid therapy, and its approximately one-third excre-

tion by the liver supports this view and led us to study this compound. Etacrynic acid had a marked effect on intestinal electrolyte and water transfer and changed net absorption of sodium and water into net secretion (Table 3). As with all other compounds studied, it should be noted here that the physiologically existing hypertonic absorption of sodium (compared to the sodium concentration of the perfusate) in the human colon changes in a fairly typical fashion (Fig. 4): whereas the absorbed fluid is hypertonic with respect to sodium, the secreted fluid becomes hypotonic with respect to sodium. The effect of etacrynic acid was functionally nearly completely reversible within 2 hr after omitting the compound from the perfusion solution (Fig. 4). This rules out the systemic effect. Compared on a molar basis it is at least as equally potent secretagogue agent for intestinal secretion as the laxative bisacodyl or rhein when applied locally to the luminal border of intact human colon. The mean effective concentration within the study segment cannot be given, since etacrynic acid concentrations of the effluents were not measured yet. The absorption rate of etacrynic acid in the colon must be assumed to be almost 100%.

There are several studies on the effect of etacrynic acid on intestinal transfer processes in different species and a variety of *in vitro* and *in vivo* animal studies on the effect of other diuretic compounds on intestinal fluid and electrolyte transfer,[1] but heterogenity of experimental conditions and results do not favor one of the current concepts of intestinal secretion.

In the intact human colon one study on the effect of the diuretic agent amiloride (for its chemistry see Fig. 2) on the rectal PD is reported by Rask-Madsen and Hjelt.[57] They showed in 13 subjects on exponential fall in PD from -46 ± 0.8 mV to -27 ± 0.3 mV following rectal instillation of 1 mmole/liter amiloride. The half-life time required for this effect was less than 13 sec; the effect was prompt and fully reversible within minutes.

F. Ricinoleic Acid

This compound is the active principle of castor oil. In the perfused human colon,[58] 2 mmole/liter concentration reduced the net electrolyte and water absorption by approximately 75% (Table 1). Measurements of ricinoleic acid concentrations of the effluent showed approximately a 70% absorption rate of ricinoleic acid. The inhibition of intestinal absorption seems to be a general property of C_{18} fatty acids, since hydroxystearic acid and oleic acid also reduced the net absorption in the colon.[58] The inhibitory effect on absorption sustained. Within 2 hr after omission of ricinoleic acid from the perfusion solution no control values for electrolyte and water absorption could be obtained.

Rectal potential difference, obtained during 30-minute perfusion periods with increasing ricinoleic acid concentrations (0.5, 4.5, 9.0 mmole/liter), was not influenced by this cathartic agent. The difference between effective luminal concentration during perfusion (2 mmole/liter) and effective concentration during PD measurements (9 mmole/liter) is remarkable and at present explained by the existing marked differences in absorption characteristics and "tightness" along the colon.[9,59]

The mode of action of ricinoleic acid has been attributed to altered intestinal motility.[60] This mechanism does not explain net intestinal secretion in rat colon.[61]

Changes in hydraulic permeability of the colonic mucosa might be involved and have clinical consequences regarding the higher rate of oxalate absorption in the affected colon.[35]

G. Adrenal Steroids

Adrenal steroids should be mentioned in this chapter because the following phenomena reported in the literature are related to pharmacological concentrations.

Based on indirect evidence from animal studies, Levitan and Ingelfinger[62] demonstrated that systemic bolusapplication of 1 mg *d*-aldosterone increased sodium, chloride, and water net absorption from the intact healthy colon in 12 volunteers. Drug effect was evident 24 hr after administration. The magnitude of effects is shown in Table 1; the figures there are taken von Levitan and Ingelfinger.[62] Similar changes in net water, sodium, and chloride absorption from the human colon after administration of 9α-hydrocortisone are reported.[63] The activation of sodium absorption by mineralocorticoids in the human colon has been confirmed by Shields et al.[64] Unlike Levitan and Ingelfinger, they found (probably because of differences in electrolyte composition of the perfusate) increased potassium secretion too.

The profound effect of adrenal steroids on colonic electrolyte and water absorption has been demonstrated in increased PD also.[14,65,66] The observed increase in rectal PD was so striking that trials were made to use rectal PD measurements as a screening procedure in hyperaldosteronism.[14] Although the average PD was statistically higher in the group with hyperaldosteronism than in the control group, broad interindividual scattering limits its clinical use as a diagnostic tool in hyperaldosteronism.

According to studies by Sharp and Leaf,[67] aldosterone-induced metabolic processes lead finally to a reduction of the sodium resistance on the mucosal side of the enterocyte. It is followed by an increased passive sodium influx from the lumen, which is actively extruded into the intercellular spaces involving the Na^+, K^+–ATPase system.

H. Antidiuretic Hormone

Intravenous administration of pharmacological doses (1 U ADH over a period of 1 hr) caused a significant decrease in net absorption of water, sodium, and chloride in the intact human colon of healthy volunteers. This effect was obtained within the first 20 min after antidiuretic hormone (ADH) administration and sustained for 1 hr after ADH administration was discontinued.[68] As stated by the authors, the observed effect was unexpected, contrary to observations in animal models and not concordant with the current theoretical conception of ADH effects in biological membranes. Similar inhibitory action of sodium and water net transfer of ADH is also reported from studies in human small intestine.[69]

I. Drugs That Influence Intestinal Electrolyte Transfer Most Likely Due to Altered Intestinal Motility

No systematic studies of changes in electrolyte and water absorption on a dose–response relationship are available on this clinically important group of drugs

such as opioids, anticholinergics, and psychoactive drugs. Whether these compounds have a systemic or local effect on absorptive or secretagogue functions of the intestinal mucosal tissue or only indirect functions due to inhibition of colonic propulsive movement is not yet clearly demonstrated in the human intestine. There is still a lack of information on the relationship between changes in propulsive intestinal motility and intestinal water and solute absorption too. A recent review of antidiarrheal agents is given by Fingl and Freston.[70]

IV. SOME FINAL CONSIDERATIONS

Our current findings on intestinal secretion processes do not allow us to draw any far-reaching conclusions as far as the mode of action is concerned. This is especially true for the action of drugs. Some progress has been made with respect to the knowledge on structural requirements for secretory activity at least within a defined structure group, e.g., bile acids or laxative-acting diphenoles. But in looking at the different chemical structures of secretagogue agents in the colon (Fig. 2), no further conclusive structure–activity relationship can be reached yet.

The importance of the pharmacokinetics of a certain drug to affect the colonic mucosal tissue has been demonstrated by cardiac glycosides.

The mode of action of antiabsorptive and/or secretagogue drugs in the colon might not be unique. Altered intestinal motility, cAMP-mediated anionic secretion, and increased hydraulic mucosal permeability are discussed and supported by experimental evidence. But the question is whether these mechanisms are causally linked to or "epiphenomena" in intestinal secretion. In assuming that cardiac glycosides have no other than a selective inhibitory effect on Na^+, K^+–ATPase activity, it is concluded that sole inhibition of the basic function absorption in the intestine results in net secretion *in vivo*. This seems to be remarkable since *in vitro* exclusive inhibition of absorptive function by cardiac glycosides never results in net secretion. What, in this model, is the real difference between *in vivo* and *in vitro*? The question at issue on the nature and location of the driving force for secretion remains open.

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15

Summary

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I. INTRODUCTION

As noted in the introduction to this book, a better knowledge of physiology is the basis for further progress in physiopathology and clinical practice.

This last chapter is intended to summarize what the authors have considered advances in different aspects of colonic disease, made possible by a clearer understanding of large-bowel structure and functions. Our review will be divided as follows: (1) alterations caused by exeresis of the colon; (2) a discussion of what may be regarded as "breakthroughs" in the physiopathology of the colonic disease due to a better understanding of its physiology.

II. ALTERATIONS CAUSED BY EXERESIS OF THE COLON

A. Discomfort

The function of the colon is to store for several hours the unabsorbed dietary residues together with an abundant colonic flora and intestinal secretions. Some of this intestinal content is absorbed, and the rest is eliminated without discomfort for the host. The upsets that the absence of this subtle evacuation mechanism entail for ileostomized patients are well known.

B. Possibility of a Negative Balance of Water, Sodium, and Electrolyte

1. Water

In Chapter 2, Kerlin and Phillips report that the colon is capable of absorbing up to 5 liters of water daily, as long as the inflow rate is kept contant and does not overwhelm the absorptive capacity of the organ.

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This water recovery ability of the large bowel explains why in some cases removal of the organ can result in an excessive loss of water and the ensuing dehydration of the patient.

2. Sodium and Chloride

As sodium and chloride are absorbed in the colon against concentration gradients, it may be presumed that the organ is actively involved in the conservation of these ions and that patients face a higher electrolyte descompensation risk after the colon has been removed.

C. Possibility of a Reduced Energy Supply

It has been traditionally accepted that in herbivorus animals an important energy supply is provided by the organic acids resulting from bacterial hydrolysis in the rumen; humans would utilize only the energy derived from the diet by absorbing monosaccharides, amino acids, fatty acids, and glycerol from the small bowel.

Recently, it has been shown that short-chain fatty acids (propionate, acetate, and butyrate) can be absorbed from the colon. The extent to which these absorption mechanisms may contribute to the energy balance in humans remains to be established.

Soergel has estimated that the human colon can absorb approximately 500 kcal/day in the form of short-chain fatty acids.

D. Possible Suppression of a Protein Synthesis Mechanism

It seems possible that in normal conditions the protein requirements of the body are met by amino acid absorption from the small intestine. In Chapter 12, Hill puts forward the interesting suggestion that in uremic patients the blood-to-colon transport of urea, as well as the normal recycling of nitrogen, may be enhanced. The increased quantity of ammonium returning to the liver possibly contributes to a greater protein synthesis.

E. Suppression of the Endocrine Functions of the Colon

According to Polak and co-workers (Chapter 9), four peptides are present in the colon in significant quantities: vasoactive intestinal polypeptide (VIP), substance P, enteroglucagon, and somatostatine. Other peptides, such as enkephalins, bombesin, cholecystokinin (CCK), and gastrin-like peptide, are also present in the colon. VIP and substance P are found mainly in the autonomic nerves, enteroglucagon and somatostatine in the mucous cells.

Although these neuropeptides present in the colon may play a role in control mechanisms of the organ's own functions, it may be supposed that some of them are possibly involved in the activities of the other alimentary canal organs or in tissue outside the gastrointestinal tract.

III. PROGRESS IN THE PHYSIOPATHOLOGY OF SOME FUNCTIONAL AND ORGANIC DISORDERS OF THE COLON

A. Diarrhea

Diarrheas may be classified into malabsorptive and secretory. The colon may participate in malabsorptive diarrheas when the influx of liquids from the small bowel is in excess of approximately 5 liters per day. This is not likely to be the case, except in choleriform diarrhea. Kerlin and Phillips (Chapter 2) have reported that in short-lived small-bowel diarrheas, an influx of over 500 ml can occur in a short period of time and provoke diarrhea even where the overall daily influx is under the above-mentioned 5-liter threshold level.

When the colon is affected by organic diseases, its absorptive capacity may be impaired. Thus diarrhea occurs even though the water flow in the colonic lumen may be lower.

The colon can act as a secretory organ and induce diarrhea when intraluminal stimulants such as invasive microorganisms, laxatives, deconjugated bile acids, and un-ionized organic anion elicit a release of water and electrolytes into the lumen.

In newborns there is a type of watery diarrhea with metabolic alkalosis. The stools of these babies have low pH levels, a diminished bicarbonate concentration, and a high concentration of chloride. The changes seem due mainly to an alteration of the chloride–bicarbonate exchange.

B. Constipation

According to studies carried out by Devroede[1] and discussed by Phillips in Chapter 2, no conclusive evidence has been found that in these patients there is a primary increase in colonic absorption. The rise in absorption might be explained by a delay in intestinal transport.

In the past few years numerous studies have discussed the relation between the quantity and quality of the fiber intake and the occurrence of constipation. Satisfactory results have been observed with an intake consisting of bran, apple, and orange.

Rat studies carried out in our laboratory have shown that a fiber-rich diet enhances the hexosamine content of the stools. The quantity of the enhancement is such as to suggest that secreted mucus may be involved, through its high hydrophilia, in fecal weight (unpublished observations).

C. Irritable Bowel Syndrome

After the landmark studies of Almy and co-workers, little has been contributed to the knowledge of the physiopathology of this disorder, a highly common one in gastrointestinal practice.

It is generally accepted that an abnormal change in motor activity is primarily involved.

Studies carried out in healthy humans have demonstrated two types of frequency for the basic electric rhythm (BER): a higher one of 6 cycles/min and a lower one of 3

cycles/min. The latter frequency occurs in approximately 50% of individuals affected by the irritable bowel syndrome and in only 10% of normal individuals. What determines an abnormal BER in these patients has not been elucidated, but it seems clear that a major change in the motor physiopathology of the colon has taken place. The reader is referred to Chapter 7 for further insights into this disorder.

As neuroendocrine control seems likely to be implicated in the motor activity of the colon, a review of Chapters 4, 9, and 10 should prove helpful.

Most studies on the irritable bowel syndrome have failed to consider the role of the colonic contents in the motor and secretory response of the colon. In Chapter 8 Eastwood refers to a statistical investigation comparing patients on a fiber-rich diet and a control group of patients who were given the orthodox low-residue diet usually prescribed for treatment of this disorder.

After 6 weeks it was observed that the patients on the fiber-rich diet showed significantly improved symptoms and evident changes in the motor activity of the colon. The individuals to whom a low-fiber diet had been administered registered no improvement in their symptoms and no changes in motor activity.

D. Megacolon

Megacolon or Hirschprung's disease has been attributed to the absence or degeneration of ganglion cells in the affected gut wall. Interestingly, Polak and colleagues (Chapter 9) report that the number of vipergic nerves is markedly reduced in Hirschsprung's disease and that this reduction is accompanied by hypo- or aganglionosis, vipergic fibers being absent in denervated areas.

A similar observation has been made with substance P and somatostatin. Since in these cases a decrease in the number of mucous cells has been observed in intestinal areas where the autonomic nervous system is either absent or reduced, it has been proposed that these nerves may exert a trophic effect on the cells of the mucosa, which is in agreement with the enhancement of mitotic activity noted after stimulation of the nerves.

E. Chronic Inflammatory Bowel Disease

Although there is a good deal of clinical knowledge about ulcerative colitis and Crohn's disease, the pathogenesis of these disorders remains poorly understood.

It may be assumed that at least two major factors are implicated in the development and maintenance of the disease: a failure of the mucosal barrier against the aggressive action of microorganisms and other antigens and an immunopathological mechanism in the colon wall itself.

In Chapter 3, Allen argues that if a sufficient quantity of mucus is present, it may form a continuous protective layer over the mucosa. The effectiveness of such a barrier depends on the dynamic relation between secretion and its degradation by proteolytic enzymes. Allen maintains that any factor reducing mucus secretion or enhancing its bacterial degradation might induce a weakening and possibly the destruction of this surface mucus gel, thereby contributing to the etiology of the colon disease.

Jewell (Chapter 5) points out that the colon has not been considered an immune organ despite even though it is exposed to a marked antigenic stimulation. Its role in

the immunological protection of the body may be as important as that of the small bowel. Jewell further says it is to be hoped that more comprehensive studies on immunological effector mechanisms involved in the inflammation of the mucosa in ulcerative colitis and Crohn's disease will afford new insights into the etiology of these disorders.

Recently, Heaton and his co-workers (personal communication) have reported a significant role for dietary quality in the genesis and treatment of Crohn's disease. These investigators have found a high consumption of refined carbohydrates with a very low fiber content in the dietary history of patients affected by the disease.

F. Drug-Induced Functional Disorders of the Colon

Independently of the well-established action of various laxatives, Wanitschke and Ewe (Chapter 14) studied the effect of some cardiotonics and diuretics on colonic electrolyte transport.

These investigators performed experiments with among other cardiotonics, two substances: digoxin and meprosillarin. Surprisingly, both substances compared on a molar basis have a higher secretion-inducing ability than laxatives (e.g., bisacodyl).

As regards diuretics, ethacrynic acid is known to elicit diarrhea. It seems likely that the diuretic exerts this undesired effect because it is eliminated by biliary tract and induces a reversal from absorption to secretion in the large bowel.

G. Vascular Pathology of the Colon

In Chapter 13, Menge and Robinson state that the risk of ischemic disorders is higher in the colon than in the small bowel. The small bowel seems to have a better collateral circulation capable of preventing the occurrence of those disorders. Contrariwise, some areas of the human colon, e.g., the splenic flexure, have poor collateral circulation and are commonly the site of serious vascular insufficiency in arteriosclerotic patients.

In Chapter 11, Lundgren and Jodal point out that blood circulation in the colon has received less attention than that of the skeletal muscle or even that of the small bowel. The blood flow in the human colon has been calculated to be 240 ml/min in repose. This level is increased 10-fold after meals or as a result of drug action. In the case of fulminating ulcerative colitis the blood flow in man has been estimated to be as high as 1500 ml/min.

H. Malignant Tumors of the Colon

A possible relationship of colonic cancer and the intestinal microflora is the subject of an interesting and detailed study by Simon and Gorbach in Chapter 6. These investigators provided the evidence that the incidence of colon cancer is much higher among the population of Western Europe and North America than of Asia, Africa, and South America. These epidemiological studies seem to indicate that the difference is due not to genetic factors but rather to environmental conditions, particularly to dietary patterns high in proteins and fats.

Numerous experiments have shown that dietary variations do not determine any changes in the bacterial pattern. However, recent investigations by Gorbach's team have indicated that although diet only slightly affects the composition of fecal flora it may induce major changes in its metabolism, an aspect neglected by traditional bacteriological studies.[3]

The following enzymes have been studied and found to be possibly affected by the diet; β-glucuronidase, β-glucosidase, nitroreductase, azoreductase, 7 α-dehydroxylase, and cholesterol dehydrogenase. All of these enzymes seem to have in common the ability to transform procarcinogenic substances into carcinogens in the colon lumen.

The role of bacterial enzymes in these types of neoplasias has been demonstrated with cycasin. This substance, the β-glucoside of methylazoxymethanol, is converted in the colon lumen to an active carcinogenic substance, methylazomethanol. The neoformative ability of cycasin was not demonstrated when administered to germ-free animals.

Hill and his co-workers (Chapter 12) have researched the metabolism of bile acids and the genesis of cancer of the colon. They maintain that the ingestion of animal fat determines an increase in the bile flow and, hence, an enhanced output of conjugated bile acids passing into the intestine. The presence of these substances induces the colonic bacteria to produce a greater quantity of dehydroxylase, an enzyme involved in bile-acid degradation. Reddy et al[2] have reported that the diet eaten in the United States and, in general, in Western countries has a higher content of degraded bile acids, particularly deoxycholic and litocholic, than the diet of American vegetarians and Orientals. This seems to support the hypothesis that diet induces changes in the bacterial enzymes of the colon, which in turn promotes the transformation of non-carcinogenic substances into other products with a greater ability to generate cancer of the colon.

Finally, mention should be made of the information furnished by Gorbach in Chapter 6 about a possible interaction between lactobacilli and colonic cancer. In 1977 statistical studies revealed that groups of Finnish individuals with a low risk of cancer of the colon consumed a higher quantity of dairy products and their stools had a higher content of lactobacilli than the high-risk groups. Significantly, in rats fed a meat-rich diet, the concentration of procarcinogenic enzymes such as glucuronidase, nitroreductase, and azoreductase was decreased after an intake of lactobacilli.

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Index