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Digestive System

MOUTH AND OROPHARYNX

The oral mucosa in humans may manifest local or systemic disease and derangements produced by therapeutic agents. A significant proportion of therapy-related oral drug reactions appear to be lichenoid reactions, erythema multiforme and bullous lesions similar to idiosyncratic or immune-mediated skin reactions.^{1,2} Inflammation of the oral mucosa (oral mucositis) is a particular side effect of conventional anticancer drug treatment and this has also been a feature of targeted anticancer therapy both monoclonal antibodies and small molecules.³ Systemic disorders produced by anticoagulants or anticancer therapy may also be evident by bleeding or ulceration in the oral cavity. Buccal ulceration is also described as part of a generalized hypersensitivity reaction to drugs.⁴

Excessive contact by therapeutic agents such as aspirin, potassium supplements, captopril, nicorandil, a potassium-channel activator and corticosteroids has been reported to produce local ulceration in the mouth.^{1,2,4–6} The increased use of mouthwashes over the last 20 years has also resulted in a number of reported adverse effects to the buccal mucosa in people.⁷

While the buccal route offers some advantages for drug delivery because intestinal and first pass hepatic metabolism can be avoided, this is limited by the low adsorption across the oral mucosa and the irritant nature of many proposed permeation enhancers.⁸

The major and minor salivary glands and their secretions also represent an integral part of the protective mechanism of the oral cavity and derangement of saliva production may lead to loss of integrity of the oral mucosa (see below). Many drugs enter the saliva by simple passive diffusion.⁹

Drugs that effect motor coordination can give rise to drooling and disruption of cricopharyngeal coordination.¹⁰ Drug-induced abnormalities of taste sensation are also well-described phenomena occurring in patients. Indeed, many alterations in the oral mucosa are those that are more readily detected by careful clinical observation in people rather than exhaustive histopathological examination of the buccal mucosa in laboratory animals.

Oral mucosa irritation studies

Oral irritation studies are used in the testing of products for use in the oral cavity. Many of these are for surgical, dental and hygiene purposes but some therapeutic agents are also developed for administration by the sublingual or buccal routes. These routes may be selected for substances that are broken down in the stomach or show a rapid first pass effect. As it is technically not feasible to perform full preclinical toxicity studies by the sublingual or buccal routes, conventional oral or parenteral routes are used for systemic toxicity studies on such compounds. The choice of the best route will to a large extent be dictated by pharmacokinetic considerations. However, it is usually deemed necessary to assess local irritancy potential to oral mucosa using a laboratory animal model.

Test species for oral irritation studies are usually rats, hamsters (cheek pouch), guinea pigs, dogs or primates using gross and histopathological assessment. A similar scheme to that employed in the histological assessment of skin irritancy is appropriate.

Inflammation (mucositis)

Inflammation of the oral cavity (*stomatitis*) may involve the buccal mucosa, the gingiva (*gingivitis*), the tongue (*glossitis*) and the peridontal tissues (*peridontitis*). Inflammatory lesions are found sporadically in untreated laboratory rodents, dogs and primates. Gingivitis due to *Shigella* infections is reported in macaque colonies where intestinal shigellosis is endemic.¹¹ Acute necrotizing ulcerative gingivitis affects the interdental papilla which occurs in primate colonies is strongly suggestive of endemic type D retrovirus infection.¹¹ WBN/Kob rats made severely diabetic with a single dose of alloxan were shown to develop an increase in inflammation in the oral cavity associated with mucosal hyperplasia distributed in the hard palate, central part of tongue and gingiva. The cause was uncertain although alloxan can reduce saliva flow or there may have been an increase in oral commensal microorganisms.^{12–14}

Stomatitis can be induced by systemic administration of high doses of therapeutic agents of different classes. Anticancer and antimetabolic agents are particularly liable to induce stomatitis both in experimental animals and in cancer patients. A notable example is bleomycin that is capable of producing stomatitis as part of its general effect on squamous cells.¹⁵ A number of other cancer chemotherapeutic agents can produce mucositis involving the oral cavity, including some of the newer targeted anticancer therapies. For example, monoclonal antibodies and small molecules targeting the epidermal growth factor receptor have been reported to produce oral inflammatory alterations in both preclinical toxicity studies and in patients.^{3,16,17}

In humans, the adverse effects of therapeutic ionizing radiation on the salivary glands with ensuing reduction in the secretion of protective saliva may also give rise to inflammatory changes in the oral cavity.¹⁸

Diuretics and other agents that are capable of producing severe electrolyte disturbances and uremia at high doses can also produce stomatitis when they are administered to laboratory animals.¹⁹ These lesions may be analogous to the well-described association of ulcerative stomatitis and uremia in humans and laboratory animals. The dog appears very sensitive to the ulcerogenic effects of uremia in the oral cavity, although as there is a poor correlation between actual levels of blood urea and stomatitis, other factors are undoubtedly involved.

Pigmentation

Compounds that alter skin pigmentation can produce similar changes in oral mucosa. A number of drugs including chlorpromazine, quinacrine, chloroquine, amodiaquine and pyrimethamine are associated with pigmentation of the oral mucosa in humans. Prolonged ingestion of silver salts may cause a slate gray pigmentation not only of the skin but also of the oral mucosa as a result of silver deposits around the basement membrane.²⁰ In patients infected with human immune deficiency virus (HIV) pigmentation is also reported following therapy with clofazimine, zidovudine and ketoconazole.¹

Similar effects may occur in laboratory animals. Chloroquine and pyrimethamine have been shown to significantly increase numbers of active melanocytes within the palatal mucosa of pigmented DA rats when treated orally for 12 weeks.²¹ Melanocytes in treated rats were shown to be enlarged and packed with melanin pigment and to possess extensive arborization of cell processes between squamous cells.

An experimental inhibitor of platelet aggregation, which produced pigment loss in the dark hair of Long-Evans rats and the skin of beagle dogs, also induced pallor of the normally pigmented oral mucous membranes in dogs.²² Apart from loss of pigment, the histology of the mucous membranes and skin was normal.

Tongue

The tongue is conveniently sectioned for histological study, although reliance is often placed on careful visual inspection because the usefulness of systematic histological examination of the tongue in preclinical safety studies has not been clearly established. A few lesions occur which are fairly specific to the tongue. Amyloid may become deposited in the muscular and connective tissue of the tongue in amyloid-prone species, particularly mice. Mice, especially DBA and DBA/2NCrj strains, are liable to develop calcification in the lingual muscle spontaneously, even at a young age. This is associated with myocardial and aortic mineralization.^{23,24} Calcified lesions are seen in the longitudinal muscle under the dorsolateral epithelium and the central part of the tongue, which, when severe, are associated with inflammation, granulation tissue, polypoid change, hyperplasia of the overlying squamous epithelium and ulceration. The histogenesis of this lesion is uncertain.

Therapeutic agents, particularly anticancer drugs, can induce inflammatory lesions in the tongue. An example is provided by the investigational anticancer immunotoxin, ZD0490, a mouse monoclonal antibody (C242) against colorectal carcinoma antigen conjugated to recombinant ricin A-chain. When administered to Wistar rats, this agent produced myocyte necrosis and inflammation specifically located below the ventral subepithelial surface of the tongue.²⁵ As the changes were different to the low grade myositis seen elsewhere in treated animals, these authors speculated that the changes in the tongue may have been related to the particular receptor profile of this area targeted by the monoclonal antibody.

In common with other changes induced in the digestive tract of rats and cynomolgus monkeys by the administration of recombinant human epidermal growth factor, the tongue showed squamous epithelial hyperplasia. This was characterized by a uniform increase in the thickness of the squamous epithelium in both species.^{26,27} At high doses, the squamous epithelium of the tongue of the primates was twice the thickness of control

mucosa and was associated with elongation of rete pegs. Conversely, interference of growth factor activity by inhibition of tyrosine kinases associated with the epidermal growth factor receptor appears to be associated with mouth inflammation and ulceration in some patients although this is found less commonly than skin changes.^{28–30}

Teeth

Teeth are usually only inspected by naked eye in conventional toxicity studies and this is appropriate for the assessment of a mature dentition. However, there has been an increasing awareness of dental lesions in toxicity studies, particularly as the teeth are visualized when the maxilla is examined histologically in inhalation studies. Study of the rodent dentition in inhalation studies has shown that spontaneous lesions of the dentition are quite common. In one laboratory, malformations (dental dysplasia) of the maxillary incisors were observed in 3% of female and 9% of male CD-1 mice and 14.5% female and 10.5% Sprague-Dawley rats in 24- and 18-month inhalation studies respectively.³¹

Unlike in humans, the rodent incisor continues to grow and differentiate throughout life and is renewed every 40–50 days. By contrast rodent molars grow little once root development is complete, although dentine production continues at a slower rate. Located at the center of the tooth is the vascular pulp. This is surrounded by proliferating odontoblasts which form predentin which when calcified becomes dentin. Surrounding ameloblasts when induced by the presence of dentin produce the overlying enamel layer. It is these active cellular layers which can be modified or damaged by drugs, vitamin deficiencies, calcium, phosphate or magnesium deficiency, parathyroidectomy, hypophysectomy, hyperparathyroidism, adrenal insufficiency and fluorosis.³² In the rat these odontogenic cells have been shown by immunocytochemistry to express high levels of parathyroid hormone-related peptide and its receptor.³³ Studies in genetically modified mice have shown that parathyroid hormone-related peptide, a member of the parathyroid hormone family, is important in normal tooth development as well as cartilage and a number of other tissues and organs.³⁴

Drug-induced changes to teeth

Although in humans the mature dentition ceases to grow, in children the dentition is in a growth phase that starts *in utero* and lasts into the second decade. As increasing numbers of children survive malignant disease, damage to the mature dentition can occur as a result of cytotoxic therapy during childhood.

Clinical study of the teeth of children treated for malignancy have shown increased incidence of dental defects such as enamel hypoplasia and missing teeth.^{35–37} Histological examination of teeth from children treated with vincristine or combination chemotherapy for malignant disease has demonstrated prominent incremental lines in dentine correlating with the number of times the intravenous cytotoxic agents were administered.³⁸ These changes can be reproduced in rodents.³⁹ It has been shown that vincristine which interferes with the assembly of microtubules and reduces secretory activity in a number of cells including osteoblasts and chondroblasts alters dentine formation in the rat incisor. Two weeks following a single intravenous dose of vincristine to young adult rats, a faint incremental line in the dentine was shown to occur, probably a reflection of a direct effect of the drug on the dentinogenic tissue at the time of injection.^{40,41} At higher doses, focal

niche-like or punched-out defects in dentine were also observed, expression of more severe injury to highly sensitive dentinogenic populations at the time of injection. The precise mechanism of damage is not fully understood although decreased secretion of dentine matrix by odontoblasts was demonstrated. Administration of the alkylating anticancer agent cyclophosphamide or a single exposure to ionizing radiation also produces localized niche-like or punched-out defects in the rat incisor.^{42,43}

Anticonvulsant drugs may also produce changes in the dentition of humans and experimental animals. In children, reported alterations include tooth root resorption, small teeth, delayed shedding of deciduous teeth and retarded eruption of permanent teeth, features similar to those found in hypoparathyroid or pseudohypoparathyroid conditions.⁴⁴ Tooth root alterations were also reported in a study in which young male Wistar rats were treated with diphenylhydantoin for one month.⁴⁵ Treated rats showed evidence of molar root resorption lacunae that penetrated the cementum and involved the dentine. The lacunae contained a dense infiltrate of cells contiguous with similar cells in the surrounding periodontal ligament. Changes were similar to those occurring in parathyroid-ectomized rats but not those in rats made hypocalcemic with a calcium-deficient diet. It has therefore been suggested that the changes induced by diphenylhydantoin in rats are similar to those in pseudohypoparathyroidism in which resistance of tooth roots to resorption is reduced.⁴⁵ High circulating levels of parathyroid hormone-related peptide has also been shown to disrupt odontoblastic cell function and normal tooth growth in the rat.⁴⁶

Numerous drugs have been reported to produce tooth *discoloration* in humans and it may be either *intrinsic* or *extrinsic* in nature.⁴⁷ Extrinsic discoloration occurs in teeth that have erupted into the mouth and it represents a superficial discoloration which can be removed by cleaning. Offending agents include chlorhexidine and orally administered iron salts. Intrinsic tooth discoloration is a permanent change relating to interference with odontogenesis, notably produced by fluoride, tetracycline, doxycycline and minocycline. Discoloration of teeth and bone is a well-described side effect of tetracycline administration and it has also been reported in patients treated with doxycycline and the semisynthetic derivative minocycline.^{48,49}

The ameloblastic epithelium of the enamel-forming tissues of growing incisors in Wistar rats treated with high doses of human recombinant epidermal growth factor developed *hyperplasia*. This was characterized by pseudostratification, increased nuclear–cytoplasmic ratio and increased cytoplasmic eosinophilia.²⁶ This finding is consistent with the presence of epidermal growth factor receptors in the cells of the enamel organ.⁵⁰ Administration of inhibitors of the vascular endothelial growth factor receptor or fibroblast growth factor receptor also has been shown to alter tooth formation in the rat.^{39,51} After several weeks' administration teeth become whitened and fractured. Histologically the teeth show reduced dentine formation with degeneration of odontoblasts, presumably as a result of defective vascularization in a manner similar to that seen in the epiphyseal growth plate (see Chapter 5).

Ameloblast and odontoblast degeneration with thickening of incisors and teeth fractures was also reported in rats given high doses of sitagliptin phosphate, an orally active inhibitor of the dipeptidyl peptidase-4 enzyme used in diabetes mellitus, although its causation was unclear.⁵²

Periodontitis

Periodontitis is a common and important disease in humans and animals although overt cases are not usually seen in toxicity studies. However, periodontitis can be severe enough to disrupt chronic rat toxicity and carcinogenicity studies. Periodontitis characterized by erosive granulomatous cavities adjacent to molar teeth with fistulas opening into the nasal cavity has been linked to penetrating food fibers in the gingival sulcus in rat studies.⁵³ It has been suggested that the presence of long pointed food fibers in the powdered diet is the main cause for its occurrence. Periodontitis in rodents also results from the effects of dental pathology such as fractures, malformation or malposition of incisors.³¹

Gingival overgrowth, hyperplasia

Drug-induced overgrowth of the gingival tissues is a well-described phenomenon in both humans and laboratory animals including dogs, cats, and rats. In humans these changes have been associated with administration of diphenylhydantoin (phenytoin) nifedipine and other calcium channel blockers, cyclosporin A, valproic acid and very occasionally trexenic acid, erythromycin, sodium valproate, phenobarbitone (phenobarbital) and vigabatrin.^{1,54–57} Cyclosporin A, diphenylhydantoin and calcium channel blockers have been associated with similar changes in laboratory animals.^{58–60} In most instances there is swelling of the gingiva by firm nodular overgrowths around the teeth. Histologically, these overgrowths are characterized by marked acanthosis of the squamous epithelium overlying connective tissue that is infiltrated by large numbers of chronic inflammatory cells. In a rat model of cyclosporin A-induced hyperplasia the epithelium shows increased proliferative activity.⁶¹ Fibrovascular proliferation may be marked. In patients treated with cyclosporin, myxomatous degeneration is described in association with dense infiltration of plasma cells and lymphocytes.⁵⁵ Secondary acute inflammation in association with food debris and hair shafts is described in dogs treated with oxodipine.⁶⁰

The forces behind these changes are unclear. Studies of changes induced by nifedipine and hydantoin have shown increases in extracellular ground substance and increased numbers of fibroblasts containing sulfated acid mucopolysaccharides.^{62–64} These drugs may alter fibroblastic proliferative and synthetic activity, possibly by selection of a subpopulation of fibroblasts.⁶³ It has also been suggested that an underlying mechanism in phenytoin-induced gingival hyperplasia involves the decrease in salivary IgA that develops in some patients.⁵⁴ Study of cyclosporin A-induced changes have suggested that impairment of T lymphocyte function may permit overgrowth of oral bacteria and bacterial products which may influence fibroblast function.⁵⁵

A spontaneous form of gingival hyperplasia has been described in non-human primates (*Macaca mulata*). This is characterized by an enlargement of the marginal and alveolar gingiva by connective tissue consisting of relatively poorly cellular bundles of collagen fibers. The lesions show little inflammatory alterations and the overlying squamous epithelium shows mild hyperkeratosis only.⁶⁵ This pathology is similar to hereditary gingival fibromatosis in humans.

Neoplastic lesions

Papillomas and carcinomas of the oral cavity

Sessile or pedunculated squamous papillomas and infiltrating squamous carcinomas are occasionally found in the oral cavity of most laboratory animals including rodents, rabbits, beagle dogs and monkeys.^{66–73} Most oral cancer in humans is squamous cell carcinoma which afflicts individuals in low income communities where it is mainly a problem of older men exposed to risk factors of tobacco with or without alcohol.⁷⁴

The microscopic structure of these neoplasms in rodents resembles those occurring in squamous epithelium in other sites. Squamous carcinomas are generally uncommon spontaneous lesions in laboratory animals. However, some strains of rodent develop squamous neoplasms more commonly. For instance, in lifetime studies *ad libitum* fed Brown Norway rats, 21% of males and 32% females developed oral squamous cell carcinomas although only 9% and 10% in food-restricted animals respectively.⁷⁵ It was suggested that certain pedigrees possessed a genetic predisposition to these neoplasms.

A number of mutagenic and non-mutagenic industrial chemicals have been reported to induce squamous carcinomas in the oral cavity of the rat in carcinogenicity studies.^{76,77} Few therapeutic agents are able to show a significant tumorigenic effect in the oral cavity in rodent bioassays. However, local cytotoxic or irritant effects following oral administration of high doses of drugs for long periods may produce small increases in the number of these tumors in predisposed rodent strains.

Papillomas occurring in rabbits and dogs are of note because they can occur in quite young animals, apparently as a result of infection with viruses of the papilloma group. Viral inclusions may be seen in histological sections. The implications of papilloma viruses in laboratory species are that the progression of virally induced papillomas to malignant squamous carcinomas can be potentiated by non-viral factors including application of xenobiotics.⁷⁸

In rabbits, the prevalence of oral papillomas varies considerably but they are quite common in some laboratory strains. They are overlooked because of their small size and location on the ventral surface of the tongue.⁶⁹ Microscopically, they are typical squamous papillomas composed of irregular acanthotic squamous epithelium and a fibrovascular stalk of variable size. Squamous cells at the margins of papillomas at the junction with normal mucosa often show large, oval nuclei, marginated chromatin and central, basophilic, intranuclear inclusions, which electron microscopic examination shows to contain viral particles.

Oral papillomas in dogs develop as multiple growths, regressing spontaneously after a few months. They are also caused by a virus of the papilloma group, which possesses a high degree of specificity for the mucosa of the oral cavity and adjacent skin.⁷² They are composed of proliferating masses of epithelial cells, keratinized on the surface and resting on an irregular connective tissue stroma or pedicle. Large vesicular cells with basophilic intranuclear inclusions are also found in the granular cell layer, identifiable as virus arrays by electron microscopy.⁷⁹ Malignant change has been described in these canine lesions and this can occur in young beagle dogs.⁷²

Although many types of papilloma viruses have been identified in both humans and animals, common antigenic determinants exist between viruses in different species.⁸⁰ This

immunological cross-reactivity can be exploited in the immunocytochemical localization of papilloma viruses in epithelial lesions of many animal species. Papilloma virus antigen has been demonstrated in oral papilloma of dogs and rabbits using antisera to bovine papilloma virus type I.⁷¹ Cells positive for virus and viral inclusions are located in the upper layers of the epithelium, especially within cells of the granular layer.

Odontogenic neoplasms

Spontaneously developing odontogenic tumors are rare in rodents but they have been induced in laboratory animals given carcinogens such as nitrosoureas or exposed to ionizing radiation.⁸¹ A range of tumors originating from dental tissues with epithelial, mesenchymal or mixed appearances has been reported in rodents.³² The classification of odontogenic tumors is complex and confusing. They range from benign anomalies and cystic structures through to malignant neoplasms. The *ameloblastoma* comprises cords, nests, anastomosing strands or islands of odontogenic epithelial cells within a fibrous stroma. The tumor cells resemble ameloblasts with the cords of spindle-shaped cells similar to the stellate epithelium bounded by a peripheral layer of cuboidal or columnar cells resembling the inner enamel epithelium. Other tumors of the odontogenic epithelium show induction of the mesenchymal elements or develop a complete sequence of odontogenic epithelium, odontogenic mesenchyme and dental hard tissues including dentine, enamel and cementum. These have been classified as *odontoma* characterized by the presence of dental hard tissues and *odontogenic fibroma* composed of undifferentiated or primitive mesenchymal cells of developing dental tissue.^{67,82}

Odontogenic tumors developing in Fischer rats treated with aflatoxin were located in the upper jaw associated with the incisor teeth and were composed of proliferating fibroblast-like cells within which ovoid calcified bodies resembling cementum were seen.⁸³ Occasional inclusions of solid epithelial nests were also seen. No metastatic deposits were found although the neoplasms were locally aggressive.

In addition, squamous tumors and neoplasms of mesenchymal origin typical of other organs and soft tissues are found in this region.

SALIVARY GLANDS

Although salivary glands may not represent vital organs in the same sense as the kidneys or heart, severe derangement of their secretions can alter both the quality and quantity of saliva. The autonomic nervous system plays an important role in the release of saliva. β -adrenergic stimulation tends to produce a low volume of saliva of high viscosity, α -adrenergic stimulation a low volume fluid of low viscosity whereas cholinergic stimulation produces a saliva of high volume but low viscosity.⁹

The severe oral complications of irreversible salivary damage and dysfunction which occurs in patients with head and neck cancer as a consequence of local irradiation may have a significant impact on the efficacy of therapy, quality of life and survival.^{18,84} Although a large number of drugs are associated with dry mouth, particularly in patients treated for hypertension or mental disorders, this does not seem to always correlate with diminished salivary gland function.^{1,18} Drugs that appear to be associated with

measurable glandular hypofunction are those with anticholinergic properties. Some drugs may occasionally cause painless bilateral swelling of salivary glands in patients.²

A protective layer of mucus, a visco-elastic material containing high molecular weight glycoproteins produced by the major and minor salivary glands, covers the stratified squamous mucosa of the oral cavity. These mucins usually contain more than 50% carbohydrate in the form of neutral and acidic oligosaccharide chains, O-glycosidically linked to threonine or serine. Mucins possess several roles including mechanical flushing of the oral cavity, protection and lubrication of soft and hard tissues, modulation of oral microbial flora, buffering activity, regulation of calcium/phosphate equilibrium, digestion and extracellular post-translation processing of molecules present in saliva.⁸⁵ The heterogeneity of salivary glycoproteins suggests that they act as a defense against pathogenic microorganisms by competing with microbial binding sites of similar structure on the surface of cells lining the digestive tract.⁸⁶ Minor salivary glands may also play an important part in the local immuno-surveillance of the oral cavity for their ducts are anatomically closely associated with lymphoid tissue.^{87,88} Salivary secretions also possess digestive enzyme activity, although in herbivores and carnivores, it is usually low in contrast to high digestive enzyme activity in omnivorous species.⁸⁹

The phylogenetic association of the salivary glands with the thyroid gland is evident functionally because salivary glands are capable of concentrating iodide in their secretions, although this is not under control of thyroid-stimulating hormone. It has been shown that thyroxin accelerates the differentiation of the granular convoluted tubule cells and the appearance of epidermal growth factor in the submandibular gland of the neonatal mouse.⁹⁰

The structure of the salivary glands differs among laboratory species, between different glands in the same species and between sexes. It is usually considered that there are three major salivary glands, the *parotid*, the *sublingual* and the *submandibular (submaxillary)* glands. Minor salivary glands are scattered in other locations throughout the mouth and oropharynx. There is a high degree of structural variability in human anterior lingual salivary glands but their precise role remains uncertain.⁹¹ In dogs and other carnivores, the zygomatic (infraorbital) gland, located just below the zygomatic arch and the buccal (molar) gland are also often referred to as major salivary glands.

Microscopically, salivary glands are composed of secretory glands or *endpieces* attached to a connecting system of intralobular and extralobular (secretory) ducts. Secretory endpieces may be acinar or tubulo-acinar in nature. The secretory cells have been subdivided into serous, mucous, seromucous and special serous types. Controversy remains about the precise nature of the secretory cells found in the various salivary glands of different species and this makes critical interspecies comparisons difficult.⁹² The duct system is less complex. This comprises an intercalated duct which leads from the secretory endpiece into a striated (secretory or intralobular) duct, so termed because their lining cells are striated by delicate eosinophilic cytoplasmic rods. The striated ducts converge into interlobular ducts and a main excretory duct system.

Rodent salivary glands

In rats, mice and hamsters, an overall similarity in gross and microscopic anatomy of the various salivary glands exists although there are histochemical differences.⁹²⁻⁹⁵

Salivary glands in rodents as well as a number of other species show morphological and histochemical sexual dimorphism.⁹⁴ The sublingual gland in rats, mice and hamsters is composed principally of mucous acini, with indistinct serous demilunes. Acini open into fairly long intercalated ducts lined by flat or cuboidal cells devoid of granules. The parotid gland is composed of serous-type secretory cells containing zymogen granules and prominent hyperchromatic basal cytoplasmic poles.

The submandibular gland is anatomically the most complex salivary gland in rodents. Secretory endpieces are composed of small or moderately sized cells with foamy cytoplasm and basophilic basal poles. The most striking feature is the presence of an additional duct segment interposed between the intercalated and striated ducts. This segment is lined by cylindrical epithelium with basal nuclei and eosinophilic cytoplasm containing secretory granules. This duct segment is termed the *granular duct* or *granular convoluted tubule*. The granules in these duct cells stain particularly well with the chromotrope-aniline blue stain but also orange G and PAS.⁹⁶ These granular cells are of special interest because they contain a large number of heterologous biologically active peptides including nerve growth factor, epidermal growth factor, renin, and kallikrins.⁹⁷⁻⁹⁹ Epidermal growth factor is secreted in saliva and believed to promote wound healing. However, the precise physiological role of many of these peptides in salivary tissue remains uncertain. Epidermal growth factor was originally isolated from the mouse salivary gland where it was shown to produce premature eyelid opening and incisor eruption when injected into neonates. This led to the isolation not only of epidermal growth factor but also its tyrosine kinase-active receptor.^{100,101} Testosterone, thyroxin and growth hormone have been shown to regulate the concentration of epidermal growth factor in the rat submandibular gland.⁹⁹ Moreover, testosterone is responsible for the male pattern of the gland notably the larger volume of secretory tubules and cytoplasmic granules. Castration causes their regression and androgens their hypertrophy in castrated males and intact females.¹⁰² Granular cells stain with antisera for epidermal growth factor.⁹⁶ The intracellular distribution of epidermal growth factor in acini and ducts has been confirmed in other species including humans.¹⁰³

Study of the mouse submandibular gland has shown that both epidermal growth factor and nerve growth factor are released into saliva following the administration of phenylephrine, sympathomimetic amine acting mainly on α receptors and isoprenaline (isoproterenol), a β adrenergic agent.¹⁰⁴ Immunohistochemical study also demonstrates that epidermal growth factor becomes depleted in mouse salivary tissue following administration of phenylephrine and similar agents.¹⁰⁵ Phenylephrine has been shown to cause marked secretory activity accompanied by loss of granules from granular cells, as well as loss of immune reactive carbonic anhydrase, an enzyme that participates both in membrane transport of bicarbonate ions into saliva and glandular secretion.¹⁰⁶ Morphological studies have shown that both acinar and granular tubular cells participate in this response to adrenergic agents. This is in contrast to the effects of pilocarpine, a cholinergic agent, which elicits the secretion of saliva deficient in serous proteins with little or none of the growth factors, as its effects are more limited to acinar cells.

The abundant glycoprotein secretion of rodent salivary glands has stimulated histochemical study using both conventional mucin histochemical techniques and labeled lectins which possess affinity for specific sugars or sugar sequences. Studies of rat, mouse

and hamster salivary glands using batteries of labeled lectins have shown a greater heterogeneity of oligosaccharides in salivary glands than seen by classical histochemical techniques.^{86,107,108}

Dog salivary glands

Less attention has been paid to the structure and cytochemistry of the dog salivary glands. There appears to be relatively little variation between the structure and mucin histochemistry of salivary tissues between beagles and other strains although variation with age has been reported.^{109–111} The dog parotid is of seromucinous type secreting both acidic and neutral mucosubstances in contrast to the more neutral mucosubstances secreted by rodent glands.⁹³

Primate salivary glands

The salivary glands of non-human primates are similar to those in humans. They possess parotid glands of serous or seromucous type, submandibular glands with both serous and mucous acini and sublingual glands of mainly mucous type. The salivary glands of the non-human primate react to adverse stimuli such as ionizing radiation in a similar manner to human salivary tissue.^{112,113}

Non-neoplastic lesions

Inflammation and necrosis

Focal chronic inflammation of the salivary glands occurs sporadically in untreated rats, mice, hamsters, dogs and primates employed in toxicology although severity and prevalence is variable. Acute and chronic inflammation may also develop in glands affected by vasculitis, whether spontaneous in nature or induced by xenobiotics.¹¹⁴

Sialoadenitis as a result of the sialodacryoadenitis corona virus is a well-known and fairly ubiquitous condition in rats, first described by Innes and Stanton in 1961.¹¹⁵ The condition is characterized histologically by edema and congestion of submandibular and parotid salivary glands as well as extra-orbital lachrymal and harderian glands (Figure 8.1). It is accompanied by inflammation of variable severity and chronicity in both glandular and connective tissue as well as degeneration and necrosis of duct epithelium. Depletion of salivary gland epidermal growth factor also occurs during the infection. The regenerative hyperplasia of the duct epithelium may be quite intense about a week after infection but all changes regress after about two weeks and glands are essentially normal after three or four weeks.^{116–118} There may be a delay in the appearance of inflammatory cells and the onset of repair in rats treated with immunosuppressive drugs such as cyclophosphamide.¹¹⁹

Suppurative infections in the neck region of the rat such as those produced by *Klebsiella aerogenes* also cause acute and chronic inflammation of salivary glands with fibrosis and glandular proliferation of salivary tissue.¹²⁰

Sialadenitis occurs spontaneously in autoimmune-prone strains of mice such as the NZB/NZW and SL/Ni strains and it has been reported in aging female but not male BDF1 mice.¹²¹ The non-obese diabetic mouse known for its spontaneous insulin-dependent

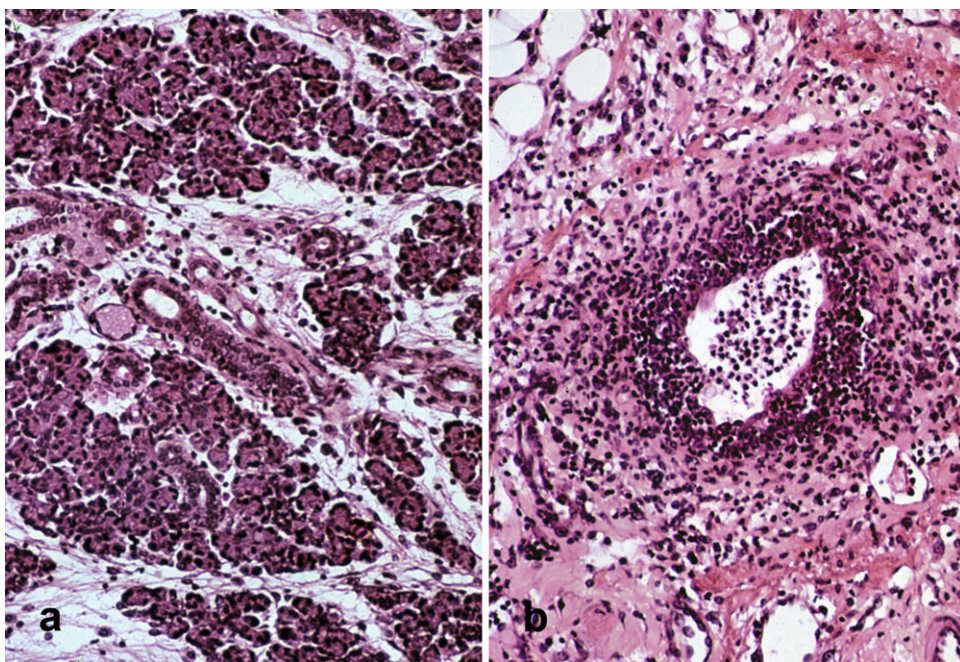


FIGURE 8.1 Salivary tissue from a Sprague-Dawley rat during an infection with the sialodacryoadenitis virus. *Panel a:* The interstitium is expanded and the parenchyma infiltrated by a light scattering of inflammatory cells. *Panel b:* Intense ductular inflammation (H&E×110).

diabetes mellitus also develops immune-mediated damage to submandibular glands.^{122,123} In aging BDF1 females the submandibular gland was shown to be involved by a destructive inflammatory process characterized by an intense infiltration by small and medium-sized lymphocytes, associated with mild inflammation in other organs such as the parotid and sublingual glands, pancreas and kidney. Immunocytochemistry showed that most of the lymphocytes were T cells of the helper/inducer subset (CD4 positive) and less than 10% were of suppresser/cytotoxic (CD8 positive) type. Circulating antisalivary duct antibody of IgG was also detected in afflicted mice. It was suggested that helper/inducer T cells played a key role in the production of this change, unlike induced autoimmune sialoadenitis in which cytotoxic T cell subsets may directly destroy glandular tissue. It has been suggested that this process in aging females is related to the decline in the number of suppresser/cytotoxic T cells in mice with advancing age.¹²¹

In the non-obese diabetic strain of mouse derived from JcL-ICR mice, a periductal chronic inflammatory infiltrate is found in the submandibular gland at about the same time that immune-mediated insulinitis is most marked. This suggests that there is an extension of the autoimmune process to salivary tissue.¹²² It is probable that helper/inducer CD4 T cells are essential components of this infiltrate and a number of cytokines and their receptors such as IP-10 (interferon γ inducible protein 10) and RANTES (regulated upon activation normal T cell expressed and secreted) may have an important role.¹²³

An autoimmune type of sialoadenitis can also be experimentally induced in certain strains of mice. CRJ:CD1 mice, thymectomized at three days, a time point at which CD8 suppresser T lymphocytes can be maximally reduced, followed by immunization at 28 and 42 days after birth with homogenates of salivary gland and complete Freund's adjuvant, develop a distinctive sialoadenitis in the submandibular and to some extent the parotid glands.¹²⁴ This sialoadenitis is characterized by degenerative changes in salivary glandular tissue associated with an extensive and intense infiltrate of small and medium-sized lymphocytes mostly suppressor/cytotoxic T (CD8) lymphocytes. These cells appear shortly after immunization but increase in number with time. Plasmacytoid lymphocytes contain immunoglobulin of mainly IgG class appear later.¹²⁴

Immunization of BALB/c mice with short peptides from the 60 kDa Ro (or SSA) antigen, which is a common target of the autoimmunity of Sjögren's syndrome in humans, also develop similar pathology. This human syndrome is a chronic autoimmune disorder of the exocrine glands with associated lymphocytic infiltrates of the salivary glands. Dryness of the mouth and eyes result from functional disturbance of the salivary and lacrimal glands.¹²⁵ Affected mice show lymphocytic infiltrates in salivary tissue consisting of both T and B lymphocytes reminiscent of the human Sjögren's syndrome.¹²⁶

In the hamster salivary glands, interstitial infiltrates of lymphocytes and plasma cell are quite common and may become more marked with advancing age.¹²⁷

Whereas necrosis of the parotid gland of uncertain etiology sometimes occurs in the dog, mild focal chronic inflammation is quite a common incidental finding in canine salivary glands. It has been reported in about 5% of normal beagle dogs.¹²⁸ Inflammatory cell infiltration of the submaxillary and parotid salivary glands is also found commonly in untreated cynomolgus monkeys.¹²⁹

Although the inflammation in salivary tissue which results from ionizing radiation is only indirectly relevant to drug safety evaluation, it is pertinent because it demonstrates species differences in sensitivity. Serous acinar cells in humans and rhesus monkey appear least resistant to the effects of ionizing radiation, where damage is characterized by widespread degranulation and degeneration of acini, infiltration by polymorphonuclear cells followed by lymphocytes, plasma cells and subsequent atrophy and fibrosis.^{112,113} *Xerostomia*, a dryness of the mouth due to salivary gland dysfunction, can develop in cancer patients following local ionizing radiation to the gland.⁸⁴ These changes contrast with the lesser effects of ionizing radiation on the rodent salivary glands in which there is little or no acute inflammatory response.

Atrophy

Like many other glandular organs, the size of the secretory tissue of the salivary gland is responsive to functional demand and is subject to age-related changes. In humans the gland parenchyma frequently becomes atrophic and replaced by connective tissue or fat with advancing age, possibly partly related to vascular changes.^{130,131} In aging rats, the extent and height of granular ducts and their content of mature secretory granules has also been shown to decrease with age.¹³²

Dietary factors influence salivary gland size. Decreased food consumption or protein starvation can reduce the weight of salivary glands in rats. There is shrinking of mucous and serous glands and loss of zymogen granules associated with decreased RNA but

unchanged DNA content, attributable to the reduced requirements for protein synthesis.^{133,134}

As salivary gland function is responsive to adrenergic stimulation, it is not surprising that atrophy occurs following adrenergic blockade. The weights of the submandibular gland in mice were shown to decrease following administration of the β adrenergic blocking agent propranolol.¹³⁵ This was associated with a reduction in stainable neutral mucins and a decrease in the thickness of the acinar cells making the gland lumens appear larger than normal. Although repeated administration of phosphodiesterase inhibitors have been shown to increase the size of salivary glands in rats, a study of the rat salivary gland after a single dose of the phosphodiesterase inhibitor theophylline has shown that this is preceded by a reduction in the size of acini.¹³⁶ Drugs possessing α or β adrenergic blocking effects have also been shown to result in a reduction of salivary flow rate in patients.⁹

The cytotoxic agent alloxan, known primarily for its specific effect on pancreatic B cells, has also been shown to produce weight loss of the rat submandibular gland, associated with lipid inclusions in the acinar cells, capillary basement membrane thickening and reduced salivary flow possibly as a result of cytotoxicity.^{13,14} Methotrexate, a folic acid antagonist, has also been reported to cause vacuolization of acinar and ductular cells with reduction of secretory granules in rat salivary glands.¹³⁴

Ligation of the main excretory ducts has frequently been used as an experimental model for study of salivary gland atrophy as well as the regeneration that follows removal of the ligature. Following ligation there is atrophy of all cell types but particularly the acinar cells through a process of apoptosis. Although overt necrosis has been reported following ligation of the excretory duct, it appears that this may have been the result of constriction of the vasculature for acinar cells, which are relatively intolerant to decreases in oxygen and nutrients.¹³⁷

Weight increase, diffuse hypertrophy and hyperplasia

A number of therapeutic agents increase salivary gland size in humans, although the scarcity of biopsy data precludes a critical assessment of the precise pathology in many cases. Drugs reported to produce salivary gland enlargement in humans include iodide-containing radiological contrast media, isoprenaline, lithium salts, phenytoin and anti-inflammatory drugs phenylbutazone and oxyphenbutazone.^{9,138,139} A number of these drugs are also linked to treatment-related increases in salivary gland secretion in patients.⁹ Salivary gland enlargement may also occur after endotracheal anesthesia and upper gastrointestinal tract endoscopy in humans.¹⁴⁰ Some of these agents and procedures may produce spasm of large salivary ducts and retention of secretions. High circulating growth hormone levels are also associated with salivary gland enlargement in patients which correlates with concomitant raised serum IGF-I concentrations.¹⁴¹

In rodents several pharmacological agents, particularly sympathomimetic amines, have been shown to produce increases in salivary gland size following repeated dosing.¹⁴² There is an intimate relationship of sympathomimetic amines with the control of the secretory process in salivary tissue. A single injection of isoprenaline (isoproterenol) in the range of 20–200 mg/kg induces discharge of preformed secretory granules followed by gradual resynthesis and reconstitution. However, repeated injections produce an increase in the size of salivary glands. The enlarged glands are composed of secretory cells of increased size

that contain increased amounts of secretory substances in the cytoplasm accompanied by DNA synthesis and differential changes in RNA transcription.^{143,144} These histological features are principally those of diffuse cellular hypertrophy. However, the increase in DNA content and radioactive thymidine uptake described in the salivary tissue following repeated administration of isoprenaline suggests that hyperplasia also occurs.¹⁴⁵

These effects do not depend on the integrity of the autonomic nerves because they occur after ablation of the autonomic ganglia.¹⁴⁵ They appear to be mediated by an effect on adrenergic β receptors because the effects can be blocked by propranolol, a β receptor antagonist but not by phenoxybenzamine, an α receptor antagonist.¹⁴² As theophylline and caffeine also elicit salivary gland enlargement in rats preceded by initial discharge of secretions, a role for cyclic 3',5'-adenosine monophosphate (cAMP) in salivary gland enlargement has been postulated.¹⁴² However, as administration of a β receptor antagonist has also been shown to diminish the response to theophylline, β adrenergic activation may also play a role in this effect.

Detailed study of hypertrophy, protein synthesis, and intracellular cAMP activity in the salivary glands of rats treated for ten days with isoprenaline (isoproterenol), a series of β adrenergic receptor agonists and the phosphodiesterase inhibitors, theophylline and caffeine, showed that similar effects occurred with all these agents although differences in the degree of hypertrophy, the nature of protein and glycoprotein synthesis and Golgi membrane enzyme activity were recorded.¹⁴⁶ The parotid gland showed the most pronounced hypertrophy followed by the submandibular gland. The sublingual gland appeared to be unaffected by treatment.

The degree and nature of the changes induced by the various β_1/β_2 receptor agonists suggested that most of these effects were mediated through β_1 receptors which are present in greatest numbers on the parotid and other salivary cells. The effects of β adrenergic agonists on salivary gland are probably the result of receptor-mediated stimulation of adenylate cyclase activity causing an increase in levels of intracellular cAMP.

Over many years selective cardioactive phosphodiesterase inhibitors of both type III and IV have also consistently been shown to produce submaxillary hypertrophy in rat subacute toxicity studies.¹⁴⁷⁻¹⁵⁰ Parotid and submaxillary glands were those most affected by the inotropic phosphodiesterase inhibitor ICI 153,110.¹⁴⁹ As these agents act via selective inhibition of the cardiac phosphodiesterases requiring cAMP as a substrate, it has generally been suggested that the salivary gland hypertrophy is a direct result of phosphodiesterase inhibition.

Other classes of drugs can also produce salivary gland enlargement in rats in repeated dose studies. Doxylamine, a representative of the widely used ethanolamine group of antihistamines, has been reported to produce marked cytomegaly in the Fischer 344 rat parotid gland. Enlarged cells were characterized by a basophilic and coarsely granular or vacuolated cytoplasm.¹⁵¹ The B6C3F1 mouse did not develop these changes after a similar treatment schedule.

In view of the presence of a considerable amount of epidermal growth factor in salivary glands, it is of interest to note the effects of its administration to laboratory animals. Salivary gland weights were increased in rats and cynomolgus monkeys infused with high doses of recombinant human epidermal growth factor.^{26,27} However, histological features seen are primarily those of ductular epithelial hyperplasia (see below under hyperplasia).

Eosinophilic (oncocyctic, oxyphil) cells, oncocytes

Epithelial cells characterized by abundant granular eosinophilic cytoplasm as a result of the accumulation of mitochondria are often referred to as *oncocytes*, a term used by Hamperl to describe similar cells in Hürthle tumors of the thyroid gland.¹⁵² They may be found in various focal nodular and neoplastic states of the salivary glands in both humans and laboratory animals. The precise significance of these cells is uncertain. The mitochondria usually appear unremarkable except for lack of dense granules and it has been suggested that the mitochondrial changes represent an adaptive phenomenon or compensatory hyperplasia.¹⁵³ In human salivary tissue their prevalence seems to increase with advancing age and they can be associated with hyperplastic lesions or neoplasms such as oxyphil adenomas and adenolymphomas.

Eosinophilic cells also occur in the salivary glands of certain strains of aged rats and in mice with experimentally induced autoallergic sialoadenitis.^{154,155}

Hypertrophic foci (foci of cellular alteration, basophilic foci, basophilic hypertrophic foci, giant acini)

Well-defined, unencapsulated foci of enlarged acinar cells occur spontaneously in the salivary glands, particularly the parotid of rats, mice and hamsters although their incidence varies between laboratories.¹⁵⁶ The enlarged cells possess greatly expanded cytoplasmic volume that retains a vesicular, vacuolated or foamy appearance or possesses a pale eosinophilic granular texture. The basal parts of the cells usually stain intensely blue in hematoxylin and eosin-stained sections and contain large, dense, irregular hyperchromatic or pyknotic nuclei showing little evidence of mitotic activity. Although there has been little ultrastructural study of these foci, the cytoplasmic alterations appear to be distinct from those of so-called oncocytes that are characterized by granular eosinophilic cytoplasm packed with mitochondria.

The biological nature of these foci is uncertain. The lack of any prominent mitotic activity, cell proliferation or expansive growth suggests that they are most aptly regarded as hypertrophic lesions rather than neoplastic or preneoplastic.¹⁵⁶ The number of these basophilic foci was reported to be increased by an uncertain mechanism in both rats and mice treated for up to two years with exenatide (Byetta[®]), a synthetic peptide which enhances glucose-dependent insulin secretion by the pancreatic B cell and used to treat diabetes mellitus.¹⁵⁷

Duct hyperplasia and metaplasia

Hyperplasia and squamous metaplasia of the salivary ducts are common features of many inflammatory and reactive conditions in the salivary glands of rodents, dogs, monkeys and humans and can be associated with the presence of stones and calculi with the duct system.

Squamous metaplasia and regenerative change in the ducts occurs in rats afflicted with sialodacryoadenitis.¹¹⁶ It can also be located in the ducts of the sublingual glands in the Wistar rat in the absence of obvious sialodacryoadenitis or evidence of any specific disease. Similar regenerative hyperplastic duct changes are also seen in necrotic and inflammatory conditions in the dog salivary gland.¹²⁸

Morphological examination and immunocytochemical study of epidermal cytokeratins of the rat salivary gland after arterial ligation has shown that the acinar units can also undergo squamous metaplasia.¹⁵⁸ The acinar-intercalated duct complexes appear to rapidly reprogram to produce epidermal cytokeratin filaments in ischemic states.

Hyperplasia of the ductular epithelium was reported to be the principal result of the administration of epidermal growth factor to rats and cynomolgus monkeys. In rats histological features were primarily of ductular epithelial hyperplasia without evidence of significant acinar hyperplasia.^{26,27} In primates the changes were most striking in the interlobular and large intralobular ducts where the epithelium showed multilayered and papilliform projections. However, mitotic activity was evident throughout the duct epithelia. Acinar cells also showed hypertrophy with depletion secretory granules and the presence of large vesicular nuclei.²⁷

A high incidence of ductal hyperplasia has also been described in the submandibular glands of rats treated for 26 weeks with a synthetic steroid possessing both progestagenic and estrogenic activity.⁹⁶ Lesions were characterized by microcystic duct-like or cribriform structures lined by single or multilayered epithelium and surrounded by myoepithelial cells. Immunocytochemistry showed that nuclei of lesions as well as normal gland possessed abundant progesterone receptors but were devoid of estrogen receptors. It was argued that this represented an exaggerated response of the intercalated duct to a high dose of a particular ratio of estrogenic and progestagenic activity.

Neoplasia

Primary neoplasms of salivary glands are uncommon in the usual strains of rats and mice employed in carcinogenicity bioassays. They are uncommonly induced by systemic administration of xenobiotics.¹⁵⁹ However, acinar and tubular adenomas and adenocarcinomas as well as squamous carcinomas are reported in rats, mice and hamsters and they have been the subject of histopathological classification.⁶⁶⁻⁶⁸ Some squamous or glandular carcinomas that originate in other structures of the head and neck region may be observed infiltrating the salivary gland. Occasionally, salivary gland neoplasms show adenomyomatous differentiation. Mixed glandular and lymphoid tissue patterns resembling Wartin's tumor in humans are also sometimes seen. Neoplasms of soft tissues also develop in and around the major salivary gland in rodents (see Integumentary System, Chapter 2).

ESOPHAGUS

In *humans* the esophagus is not considered a common site for drug-induced injury although some studies have suggested that medication-induced changes are more prevalent than previously supposed.¹⁶⁰⁻¹⁶² Although in one hospital series of 88 unselected cases of esophageal ulcers, gastrointestinal reflux accounted for over 65% of cases, 20% were drug induced.¹⁶³ Severe damage can occur following prolonged contact between mucosa and ingested tablets or capsules which adhere to produce local high concentrations of potentially irritant substances.¹⁶⁴⁻¹⁶⁷ Damage as a result of local contact may be more common in elderly subjects as the amplitude of esophageal contractions decrease with age and capsules more liable to lodge in the lumen of the esophagus. However, patients of all ages may be affected. Women have been injured more frequently than men probably because of the greater likelihood of their being treated with potentially injurious drugs.¹⁶⁶ The shape and

surface coating of tablets may influence their tendency to adhere to the mucosa and lodge in the esophagus.¹⁶⁸ A wide variety of drugs have been implicated. In the United States as well as other countries many cases appear to be caused by ingestion of tetracycline or doxycycline.^{167,169} Some of the causative agents such as potassium chloride, aspirin and other non-steroidal anti-inflammatory drugs are also implicated in ulceration lower in the gastrointestinal tract.¹⁷⁰ Over recent years the bisphosphonate alendronate has been a common cause of adverse effects in the esophagus and severe injury has been reported. Injury is linked to ingestion without water or failing to remain upright after swallowing the medication as alendronate is particularly caustic.¹⁶⁶ However, this risk is now better known and only a low incidence of esophageal damage is now reported.¹⁷¹

Esophagitis due to *Candida albicans* is a well-described complication of antibiotic therapy particularly in patients being treated with immunosuppressive drugs or in HIV-infected patients.¹⁶¹ A number of agents affecting neuromuscular coordination may also predispose to gastroesophageal regurgitation and reflux esophagitis.¹⁶⁴

In laboratory *rodents* spontaneous lesions of the esophagus are occasionally seen. *Esophageal impaction* has been described in untreated SrL:BHE rats. This is characterized by massive dilatation of the esophagus with food or bedding.¹⁷² The muscle fibers in the wall of the esophagus show varying degrees of degeneration including swelling or shrinking of fibers, myofibrillar fragmentation, cytoplasmic vacuolation and mineralization. So-called *megaesophagus*, characterized by enlargement of the esophagus, degeneration of muscle fibers and ganglion cells in the myenteric plexus, has also been described as spontaneous lesions in some strains of rats and mice.^{173,174} Megaesophagus is reported in both animals and humans as a result of congenital or acquired motility disturbances affecting the esophagus.

A lesion reported in Fischer 344 rats of all ages is *esophageal hyperkeratosis*. This has been reported to occur more commonly in rats fed a protein-restricted, calorie-unrestricted diet than in rats fed *ad libitum* with normal diet. In one rat study a particularly high prevalence of esophageal hyperkeratosis was ascribed to acidification of drinking water.¹⁷⁵ It has also been reported in rats made severely diabetic with alloxan.¹²

Another pathological finding in rodents is perforation of the esophagus as a result of a gavage accident. Under these circumstances there is a variable inflammatory and purulent exudate localized around the perforation or spread within the pleural or occasionally the pericardial cavities. The esophagus and surrounding tissues need careful examination by the pathologist for it is not always clear from clinical findings that esophageal damage has occurred.

Spontaneous esophageal lesions are uncommon in laboratory beagles, even though emesis and vomiting are frequent responses of this species following dosing in toxicity studies. A small proportion of laboratory cynomolgus monkeys are reported to show foci of inflammation in the esophagus.¹²⁹

Drug-induced changes in toxicity studies

Systemic administration of drugs with radiomimetic or antimetabolic activity can cause hypoplastic changes (*atrophy* or *hypoplasia*) in the esophageal mucosa in addition to the well-known pathological changes in the gastric and intestinal mucosa.¹⁷⁶

Hyperplasia with increased keratinization has been reported in the esophagus of the rat following chronic high dose administration of vehicles such as alcohol.¹⁷⁷ Acanthosis with hyperkeratosis and parakeratosis has been reported in the esophagus but not stomach of rats treated for up to 18 months with mesuprine hydrochloride, a β adrenergic receptor stimulator.¹⁷⁸ As part of its general effects on the gastrointestinal mucosa, the esophagus in rats and primates has been reported to develop uniform hyperplasia of the squamous epithelium following infusion of recombinant epidermal growth factor.^{26,27,179}

Local esophageal *irritancy* potential of drugs has been assessed in a number of animal models, notably the cat, pig and dog.^{180–183} In these models, the test drugs are placed in the upper esophagus using endoscopic techniques for standard periods of several hours to allow dissolution of the preparation. Subsequently, the animals are followed for 3–6 days and histopathological assessment performed on the esophagus. The degree of inflammation, erosion of mucosa or deep ulceration is recorded in a semiquantitative manner. The degree of ulcerogenic activity of drugs in these models seems to correlate with reported ulcerogenic activity in the human esophagus.¹⁸¹

FORESTOMACH

In the rat, mouse and hamster the forestomach occupies about two-thirds of the proximal area of the stomach and is lined by cornified stratified squamous epithelium. The *limiting ridge* is a distinct elevated mucosal fold at the junction between the forestomach and the mucosa of the glandular part of the stomach. The minipig has a small zone covered by squamous epithelium adjacent to the cardia. As humans lack a forestomach, the relevance of changes produced by drugs and chemicals in the rodent forestomach is disputed.

Studies in rats in which the forestomach has been removed have suggested that the forestomach acts as a storage organ releasing relatively undigested food into glandular stomach in response to energy demand.¹⁸⁴ Hence, the forestomach mucosa may be exposed to xenobiotics mixed in undigested food for longer periods than elsewhere in the gastrointestinal tract. The interpretation of forestomach changes should take into account physiological factors, residence time and exposure differences to drugs between the rodent forestomach and human esophagus. However, it is likely that the squamous mucosa lining the esophagus in species without a forestomach reacts to xenobiotics in a similar way to the forestomach mucosa of rodents if equivalent exposure levels are attained.

Non-neoplastic lesions

Inflammation, erosions, ulceration

Inflammation and ulceration of the forestomach mucosa are some of the commonest spontaneous gastrointestinal lesions in laboratory rats, mice and hamsters. The prevalence of these gastric lesions varies between species and strains of laboratory rodents as well as between different laboratories. The cause of forestomach ulceration often remains unclear although a variety of factors have been associated with its development including advanced age, infection, parasitism, diet, feeding regimens and stress. In rats, conflict-induced

ulceration occurs in the forestomach and there is an age-related susceptibility; older rats developing more ulcers than younger rats.¹⁸⁵

In rats and mice dying of spontaneous disease, ulceration of the forestomach is quite frequently observed. Protein restriction or starvation has also been shown to produce forestomach ulceration in rats.¹⁸⁶ WBN/Kob rats made diabetic with a single dose of alloxan develop an increase in forestomach inflammation associated with the increase in the presence of *Candida albicans* and bacteria and eventually proliferative lesions (see below) which were shown to be alleviated by treatment of diabetes with insulin or administration of antifungal drugs.^{12,187,188}

Histological features of ulcers and inflammatory lesions of the forestomach are similar in rats, mice and hamsters. In mild cases, a scattering of acute inflammatory cells is seen in the intact squamous mucosa. Ulcers can be single or multiple and are characterized by loss of squamous epithelium with a variable accumulation of neutrophils, mononuclear cells, cellular debris, fibrin and hair fragments in the ulcer crater (Figure 8.2). The inflammatory process may extend deeply into the stomach wall and be associated with intramural inflammation, edema, endarteritis and fibrosis. Some agents appear to induce primarily a submucosal or intra-epithelial inflammatory process giving rise to pustule formation suggesting that the effects are not mediated by local direct irritancy.¹⁸⁹

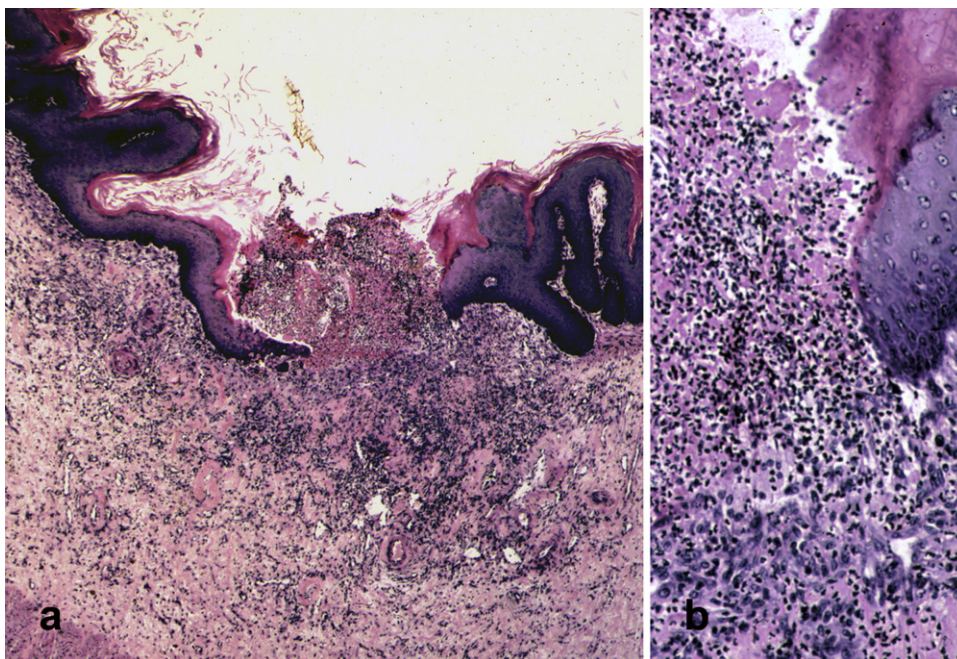


FIGURE 8.2 Panel a: A long-standing ulcer of the forestomach showing exudate and underlying inflammation in the submucosa with hyperplasia, acanthosis and hyperkeratosis in the surrounding epithelium of an aged female Sprague-Dawley rat. Panel b: Higher magnification showing exudate and underlying vascular proliferation (H&E×400).

Hemosiderin pigment can also be found in the ulcer margins. Profuse hemorrhage may follow erosion of large blood vessels and complete perforation of the stomach wall with peritoneal involvement also occurs.¹⁹⁰ In long-standing cases of ulceration, hyperplasia of the adjoining squamous epithelium occurs, characterized by irregular acanthosis and down-growths of squamous epithelium into the submucosa.¹⁹¹

In rodent toxicology studies ulceration of the forestomach is often sporadic and its distribution among animals shows no clear relationship to dose. If ulceration is limited to high dose groups, it may be a result of non-specific toxicity. However, some chemicals have direct local effects of sufficient severity to cause focal damage to the forestomach mucosa.

Xenobiotics may also produce inflammatory changes in the forestomach mucosa following initial dosing but subsequently repair and adaptation occurs even though treatment continues. An example of this phenomenon is illustrated by butylated hydroxyanisole.¹⁹² After one week of administration of this agent in a 2% mixture in diet to rats, a vesicular inflammatory reaction characterized histologically by the presence of subepithelial vesicles containing inflammatory cells and exudate was seen. After further treatment, only hyperplasia of the squamous epithelium was evident, presumably an adaptive response to the effects of the continued insult.

Hyperplasia (hyperkeratosis, parakeratosis, acanthosis, papillomatosis)

Hyperkeratosis associated with hyperplasia of the squamous epithelium is seen sporadically in untreated aged rodents. These changes may be localized to the margins of chronic forestomach ulcers or they can be associated with diffuse inflammation of the mucosa. Occasionally, the forestomach mucosa of untreated, aged rodents exhibits hyperkeratosis with hyperplasia without inflammation. Such changes may be diffuse or focal, but they are often localized to the zone adjoining the glandular stomach mucosa. There may be evidence of basal cell proliferation and down-growth of the epithelium into the underlying stroma.

The cause of these changes is often unclear. Dietary factors have been shown to influence the thickness of the forestomach mucosa. Vitamin A deficiency linked to squamous metaplasia in glandular tissues may produce forestomach hyperplasia and hyperkeratosis in rats. When SPF Fischer 344 rats were maintained in a vitamin A deficient state for over three months, hyperplasia with hyperkeratosis, not unlike that produced by carcinogens, was reported.¹⁹³ Diabetic rats have also been shown to develop squamous hyperplasia more prominently in the greater than the lesser curvature and most marked near the limiting ridge. This was associated with inflammation in the forestomach and the presence of *Candida albicans* which may have been a significant pathogenetic factor.¹⁸⁸

Administration of a wide range of genotoxic and non-genotoxic industrial chemicals and therapeutic agents may produce hyperkeratosis and hyperplasia of the forestomach epithelium. The extensively used antibiotic ampicillin produces inflammation, ulceration, acanthosis and hyperkeratosis of the forestomach mucosa of mice but not rats when administered for two years.¹⁹⁴ Sodium saccharin is also reported to produce hyperplasia without neoplasia in the forestomach in Fischer 344 rats.¹⁹⁵ Cytoprotective prostaglandins also appear capable of inducing hyperkeratosis and hyperplasia without neoplasia presumably through a mechanism related to their trophic activity.¹⁹⁶ This occurs in rats

treated with misoprostol, a synthetic prostaglandin E₁ analog with gastric antisecretory and anti-ulcer activity and other synthetic analogs of prostaglandin E₁ and E₂ types.^{197–199} Among its wide range of pharmacological effects on the gastrointestinal tract, the forestomach also responds to recombinant epidermal growth factor when infused into rats.^{26,179}

Histologically, the changes are characterized by hyperkeratosis and parakeratosis with varying degrees of acanthosis and papillomatosis.¹⁹⁰ The changes can be florid and it may be difficult to make a clear distinction between severe hyperplasia and neoplasia. Nevertheless, it has been shown that the florid hyperplasia of the forestomach epithelium without evidence of cellular atypia can be completely reversible following the withdrawal of an inciting stimulus, ethyl acrylate.²⁰⁰ Hence, an important feature is the presence of cellular atypia in view of its association with agents with potent (genotoxic) carcinogenic activity, particularly if it occurs rapidly following initiation of treatment.

Neoplasia

Neoplasms arising in the forestomach of rodents are usually *squamous cell papillomas or carcinomas*.^{67,68,201–203} Basaloid cellular features are occasionally seen.²⁰³ Squamous carcinomas, as at other sites, show variable differentiation being composed of proliferating squamous epithelium with moderate to marked cellular atypia, pleomorphism and mitotic activity with clear evidence of invasion into the muscularis. Although they are relatively uncommon spontaneous lesions in aged rodents, they can be induced in rodents by administration of nitroso compounds as well as a number of genotoxic and non-genotoxic chemicals.

Human relevance of proliferative lesions induced by drugs in the rodent forestomach

A large number of genotoxic and non-genotoxic compounds have been reported to produce hyperplasia and carcinoma in the forestomach of rats, mice or hamsters.^{76,204–206} They are the sixth most commonly induced neoplasm in the *National Toxicology Program* of rodent carcinogenicity studies.²⁰⁶ A proportion of these are genotoxic chemicals that are associated with rapid development of atypical hyperplasia and neoplasia in the forestomach as well as tumor development at other sites. By contrast some are non-genotoxic chemicals that induce an adaptive response to chronic cytotoxicity eventually followed by neoplasia after long periods. In addition some agents appear to produce hyperplasia by their inherent trophic activity on epithelial cells.

Examples of non-genotoxic agents include butylated hydroxyanisole (BHA), an important food antioxidant, structurally related phenols and acids,^{207,208} ethyl acrylate used in the production of materials for dental and medical devices,²⁰⁰ SK&F 93479, an experimental histamine H₂ receptor antagonist,²⁰⁹ and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or statins.^{210–212} Aristolochic acid stands alone as a genotoxic medicinal agent that induces forestomach tumors although it is no longer used as an anti-inflammatory drug.^{213,214}

The data from a large body of studies performed with butylated hydroxyanisole is instructive for it illustrates the various factors that influence the development of treatment-induced hyperplasia and neoplasia of the rodent forestomach. Butylated hydroxyanisole

possesses little or no mutagenic activity *in vitro* but when administered to rats for two years as a 2% mixture in the diet, it was shown to produce inflammation followed by squamous hyperplasia, squamous papillomas and squamous carcinomas of the forestomach. At 0.5% in the diet butylated hydroxyanisole induced only hyperplasia.²¹⁵

Studies in which butylated hydroxyanisole was fed in the diet to rats for shorter periods have shown that squamous epithelial hyperplasia occurs after only one week of treatment mainly over the lesser curvature, the site at which carcinomas developed in the two-year studies.¹⁹² After 13 weeks of treatment, mucosal hyperplasia characterized by pronounced hyperkeratosis, parakeratosis and acanthosis most pronounced over the lesser curvature was present in rats given 2% butylated hydroxyanisole in diet but not in rats given 0.5, 0.25 and 0.1% mixtures.²¹⁶ Abundant mitoses were found in the basal cells layers and tritiated-thymidine labeling confirmed that hyperplasia was accompanied by a high rate of cell proliferation. Following cessation of administration of butylated hydroxyanisole after 13 weeks, the tritiated-thymidine labeling index rapidly reverted to control levels within about one week although hyperplasia took longer to regress. Nearly complete regression of the hyperplasia occurred after about nine weeks of normal diet.²¹⁶

The distribution of squamous hyperplasia induced in the rodent stomach by butylated hydroxyanisole is influenced by the mode of administration. Following feeding of rats with butylated hydroxyanisole mixed in the diet, lesions tended to be located near the limiting ridge whereas gavage in corn oil produced similar changes at the apex of the forestomach.¹⁹² It therefore has been suggested that such differences are due to incomplete mixing of butylated hydroxyanisole in the stomach lumen when given by gavage and prolonged contact of the gavage mixture with the upper segment of the forestomach. Moreover, it has been shown that Fischer 344, SHR, Lewis and Sprague-Dawley rats differ in their response to the hyperplastic and carcinogenic effects of 2% butylated hydroxyanisole in pelleted diet. The most sensitive appears to be the SHR strain followed by the Fischer 344 rats. The differences correlate with the cytotoxic effects of butylated hydroxyanisole in the different strains.²¹⁷ The presence of vascular damage in the stomachs of the SHR rats might contribute to the response to cytotoxicity and subsequent carcinogenicity.

Residence time of administered compounds in the forestomach may influence the development of lesions. Although it has been demonstrated that butylated hydroxyanisole does not produce hyperplasia in the esophagus of animal species without a forestomach, high doses given to primates are capable of producing an increase in mitotic activity in the lower end of the esophagus similar to that occurring at equivalent exposure levels in the rat forestomach.²¹⁸ The implication is that these interspecies differences may simply be a question of differences in exposure of the squamous mucosa to compound. This underlines the fact that mechanisms of action and exposure levels of xenobiotics attained in the gastrointestinal tract of rodent and non-rodent species as well as of humans need to be assessed when hyperplasia and neoplasia are induced in the forestomach mucosa of rodents. Such information can clearly be helpful in facilitating decisions made by government's regulatory authorities in this area.²¹⁹ However, in the case of butylated hydroxyanisole the evidence suggests that the tumor development in rodents represents an epigenetic phenomenon related to largely reversible cytotoxicity, inflammation and increased cell proliferation.²⁰⁷ In view of the low levels of exposure to butylated hydroxyanisole that occurs with the usual use of this agent, carcinogenic hazard for the human stomach is therefore very small.

Similar phenomena have also been reported in studies of phenols and acids that are structurally related to butylated hydroxyanisole.²⁰⁸ These agents include n-butyl and n-propyl-4-hydroxybenzoic acid esters, propionic acid and 4-methoxyphenol. However, these studies suggested that certain areas of the forestomach epithelium react differently to structurally related chemicals, possibly due to the variable levels of activating enzymes within different zones of the forestomach epithelium. Co-administration of acetylsalicylic acid was shown to abrogate some of these effects, suggesting that prostaglandin synthetase may be involved in the hyperplastic response.²⁰⁸

A number of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) with different chemical structures including marketed products such as lovastatin, simvastatin and fluvastatin are also associated with the development of squamous hyperplasia of the rodent forestomach. Hypolipidemic agents that inhibit oxidosqualene have also been reported to produce squamous hyperplasia in the forestomach of mice and hamsters.^{220,221} The hyperplasia produced by the HMG-CoA reductase inhibitors is time and dose dependent and may be associated with edema and inflammation of the submucosa. Some but not all of these agents are also capable of producing squamous neoplasia of the forestomach mucosa of rats, or mice or both after long-term treatment.^{210–212,222,223} The mechanism of action remains unclear, although this effect seems correlated to their pharmacodynamic activity. Detailed morphological study of the forestomach in rats treated with lovastatin suggested that it is related to the effects on the assembly of cholesterol and other lipids into the lamella bodies and intracellular lipid sheets.²²⁴ Their tumorigenic potential in rodent bioassays does not seem to relate to the degree of hyperplasia in short-term studies.²⁰⁴ Moreover, the development of hyperplasia depends on local high concentrations of drug because when administered by non-oral routes, hyperplasia does not occur.²¹⁰ As most of these drugs are non-mutagenic, these findings are presumably also epigenetic in origin and possess relatively little risk for humans when given in the usual therapeutic doses.

The importance of inflammation as a factor in the development of forestomach tumors is illustrated by studies in which WBN/Kob rats made diabetic with a single dose of alloxan developed an increase in forestomach inflammation, squamous hyperplasia prominent near the limiting ridge and invasive squamous cell carcinomas in 20% of animals after periods of 50 weeks or more. These changes were linked to the presence of *Candida albicans* and gastric bacterial infection. The proliferative changes and inflammation was shown to be alleviated by treatment of diabetes with insulin or administration of antifungal drugs and the number of neoplasms also reduced.^{12,187,188}

A contrasting example is provided by aristolochic acid, a nitrophenanthrene derivative of the ancient medicinal plant *Aristolochia clematis* which was used as an anti-inflammatory component in a number of medicinal preparations in Germany until 1982.^{213,214} Aristolochic acid is found in the *Aristolochia* and *Asarum* species of plants, both in the *Aristolochiaceae* family, and is a direct-acting mutagen in *Salmonella typhimurium*. When fed to rats at doses of 1.0 and 10 mg/kg/day, aristolochic acid produced severe papillomatosis of the entire forestomach within a period of three months. This was characterized histologically by the presence of branched squamous papillomas up to 6 mm high with focal dysplastic features. Invasive squamous carcinomas with metastases were found subsequently three or six months later without further treatment.²²⁵ Even at a low dose of 0.1 mg/kg/day papillomas and squamous carcinomas developed nine months after a three-month period of treatment.

These findings preclude the safe use of aristolochic acid for therapeutic purposes. Indeed the use of aristolochic acid has been associated with the development of a specific nephropathy and with urothelial cancer in humans.²²⁶

A framework for the assessment of the relevance for human cancer risk assessment has been developed for these forestomach neoplasms.²²⁷ It has been proposed that tumors associated with chronic irritation, particularly those induced by repeated oral gavage dosing, should not form the basis for carcinogenic classification or quantitative cancer potency estimates for humans. By contrast genotoxic chemicals and those that produce neoplasms at multiple sites in the absence of forestomach irritation are considered to be more likely to have human relevance.

STOMACH (GLANDULAR)

There is a relatively good correlation between drug-induced effects in the stomach as well as intestines in rodents and non-rodents and those found in humans.²²⁸ Safety pharmacology studies in rodents appear to reflect general adverse effects such as vomiting in patients.²²⁹ Studies of anticancer drugs has also suggested that the dog is a particular good predictor of adverse gastrointestinal effects in humans.^{230,231}

Unlike the mouth and esophagus through which tablets, capsules, gavage fluids and drug/diet mixtures pass relatively rapidly, the human stomach mucosa remains in contact with high local concentrations of administered compounds for much longer periods of time. However, the gastric mucosa is protected by a number of defense mechanisms including a mucus-bicarbonate layer and an epithelial barrier in a constant state of renewal.

In the fasted state there is a cyclical pattern of motility consisting of three main phases. The first is a quiescent phase. This is followed by a phase of irregular contractions that increase in amplitude and frequency to reach a maximum in a third phase. Feeding results in the replacement of this cyclic pattern by regular tonic contractions that move food towards the antrum and mix it with gastric secretions. These patterns have been well studied in both dog and humans and appear to be qualitatively similar in the two species.²³² These motility patterns may have an impact on the length of time drugs remain in contact with stomach mucosa. For instance, the residence time of large non-disintegrating capsules or tablets administered in the fasting state is more dependent on the frequency of powerful phase III contraction than if drugs are given as fluids or mixed with diet. For dosage forms released in the stomach, gastric residence time will influence drug supply to the main absorptive surfaces in the small intestine, which in turn may affect drug absorption.²³³ Gastric acid is important in making ingested salts soluble. Although the presence of food in the stomach is a stimulus for acid production, the pH in the stomach of rats is highest in full stomachs and lowest when empty, presumably as a consequence of the buffering action of food.²³⁴

Epithelial morphology and physiology

The glandular stomach is conveniently subdivided into the fundus characterized by mucosal folds or rugae and the smoother antrum, which opens into the pylorus and

duodenum. In species devoid of a forestomach, the proximal stomach mucosa or cardia is also lined by glandular mucosa. Although the stomach of monkeys is broadly similar to the human stomach, a detailed study of the cynomolgus monkey stomach has shown that unlike humans the fundus lacks parietal cells.²³⁵

The glandular mucosa is covered by surface epithelium of regular columnar cells that extends downwards to form small gastric pits or foveolae. The gastric glands are simple tubular structures usually considered to comprise three segments. The base is the deepest part, the neck the mid-region, and the most superficial is the isthmus, continuous with the gastric pit. The upper part of the gastric gland contains mucous neck cells. Small cuboidal chief or zymogenic cells, which secrete pepsinogen and stain blue or purple in hematoxylin and eosin sections, are located in deeper parts of the gland. The eosinophilic-staining parietal (oxyntic) cells, which produce hydrochloric acid, are distributed more randomly throughout the gastric glands. Parietal cells can also be visualized by immunocytochemical staining with antibodies directed at H^+K^+ -ATPase.²³⁶ It has been suggested that gastric parietal cells may have an endocrine role because in rats it has been shown that they possess aromatase activity and an ability to synthesize estrogen.²³⁷

The gastric glands situated near the limiting ridge in rodents show a simplified structure. In species not endowed with a forestomach, the mucosa near the cardia is composed of simplified branched glands lined by columnar epithelium. The antral mucosa is covered by a surface epithelium with gastric pits similar to that of the fundus but mucous secreting columnar glands line the glands.

The stomach mucosa is richly endowed with endocrine cells, not all of which have been well characterized. In the rat at least seven endocrine cell types have been demonstrated based on their structural and histochemical features. Enterochromaffin cells are quite numerous in the basal parts of the gastric glands of the fundus, particularly in the rat.²³⁸ They are generally argyrophilic, staining with silver techniques such as that of Grimelius that utilize exogenous reducing agents.^{239,240} These cells contain histamine and histamine-related enzymes such as histidine decarboxylase. Endocrine cells which are argentaffin in type are also reported in the mucosa of the fundus of some species including humans but apparently not in rats.²³⁸ These cells stain with silver preparations such as that reported by Masson in 1914 because of the presence of endogenous reducing substances including 5-hydroxytryptamine and catecholamines.²⁴¹ Enterochromaffin cells are characterized ultrastructurally by the presence of numerous rounded or oval, vesicular, electron-lucent granules frequently containing a small eccentric electron dense core.

Gastric enterochromaffin cells are now more reliably stained by immunocytochemical techniques using antisera to histamine and histidine decarboxylase as well as to non-specific enolase and chromogranin A.²⁴²⁻²⁴⁴ Enterochromaffin cells can be recognized in the basal zones of the oxyntic mucosa by their expression of histidine decarboxylase and histamine. Immunocytochemical study shows a variety of other peptides in cells in different areas of the glandular stomach including somatostatin, glucagon, gastrin and serotonin reactivity.²⁴⁵

Increased gastric acid secretion is initiated by activation of central vagal efferent pathways but acid secretion is maintained by both neural and endocrine reflexes activated by the presence of food in the stomach. Gastrin secreted from the G cells of the antrum is the main stimulant of acid secretion. Somatostatin is secreted from antral D cells when the

luminal pH falls to below 3.5 to act by a paracrine mechanism to suppress G cell function thus forming a negative feedback loop.²⁴⁶ The two main endocrine cell types from the body mucosa integrate neurohumoral stimuli rather than respond to luminal chemicals. Although gastrin is capable of stimulating parietal cells directly, it has an even greater effect through stimulation of enterochromaffin cells to release histamine, a potent paracrine stimulator of parietal cells.²⁴⁷ Gastrin stimulates release of histamine from enterochromaffin cells of the body mucosa, which increases acid secretion through activation of parietal cell histamine H₂ receptors. Both parietal and enterochromaffin cells are inhibited by somatostatin released from the D cells of the body mucosa in response to a variety of neurohumoral stimuli such as norepinephrine (noradrenalin), vasoactive intestinal peptide, calcitonin gene-related peptide, and cholecystokinin. Gastrin acts at the gastrin/cholecystokinin B receptor that is expressed by gastric epithelial cells as well as by neurons in the central nervous system.^{248,249} The gastrin receptor is a cholecystokinin B type receptor located in the stomach. The other cholecystokinin receptor, cholecystokinin A, has high affinity for cholecystokinin. Stimulation of this receptor in the stomach mediates secretion of pepsin from gastric chief cells and release of somatostatin from D cells resulting in inhibition of acid secretion.

Kinetics of the gastric mucosa

Generative cells in the gastric mucosa as shown by uptake of tritiated thymidine are distributed principally in the superficial zones of the gastric glands or isthmus.²⁵⁰ Tracing of cells using thymidine labeling have shown that most of the cells in the generative zone migrate in a successive manner to the mucosal surface to form columnar epithelium. The lifespan of surface epithelium in the stomach of rats, mice and hamsters has been calculated to be about three to four days. Studies of cell cycle and DNA synthesis time in the proliferative zones in the stomach have suggested that the generative cells in the isthmus undergo mitoses at about 30 hour intervals in rodents and 40 hour intervals in humans.²⁵⁰

Although this process of migration from the proliferating cell zone of the isthmus renews surface epithelial cells rapidly, cell migration to the lower parts of the gastric glands is much slower and more complex. Detailed studies have shown that undifferentiated cells in the region of the isthmus represent a common source for surface mucous cells and mucous neck cells.²⁵¹ Studies in transgenic mice have shown that mature parietal cells influence the fate of other gastric epithelial cells because targeted degeneration of parietal cells is associated with loss of chief cells suggesting interactions between these cell populations in determining their differentiation.^{236,252}

Labeling experiments in the hamster stomach have shown that both chief and parietal cells possess a similar but quite long lifespan of about 200 days.^{253,254} It has been suggested that the relative distribution of chief and parietal cells in the gastric gland represents an expression of their different migration patterns downwards from the proliferative zones in the isthmus. This type of migration pattern in which cells are able to overtake each other has been termed a *stochastic flow system*.²⁵⁰

Mucus layer

The mucus layer represents the first line of mucosal defense. This is a layer of mucus gel along with bicarbonate and hydrophobic phospholipids which maintains a near

neutral pH as well as a physical barrier.²⁵⁵ Much of our knowledge about mucins produced by the epithelial cells lining the gastrointestinal tract has been obtained using histochemical techniques and these approaches can be helpful in the understanding of spontaneous and drug-induced gastrointestinal disease.^{256–258} The physiochemical properties of gastrointestinal mucins are dependent on their glycoprotein constituents. These glycoproteins are high molecular weight compounds with large numbers of sugar chains attached to a polypeptide backbone by O-glycosidic linkages between N-acetylgalactosamine and serine or threonine.²⁵⁹ The principal monosaccharides present are fucose, galactose, N-acetylgalactosamine, N-acetylglucosamine and sialic acid. Due to this extensive glycosylation, mucins have a filamentous conformation, which is often negatively charged. This is believed to be important in forming a protective barrier to the cell. However, this property is a two-edged sword because when opposing cells have specific receptors for mucins, adhesion may become the predominant factor.²⁶⁰

There are considerable regional variations in glycoprotein constituents in the gastrointestinal tract and these differences are probably related to physiological and functional factors. Furthermore, synthesis and secretion of glycoproteins alter with changes in cell differentiation. Alterations also occur in mucins in various inflammatory and neoplastic disease states as well as following administration of drugs and chemicals.²⁶¹

Gastrointestinal mucins are usually stained using PAS and alcian blue as well as the high iron diamine technique for sulfated mucins. Terminal sugars or sugar sequences can be demonstrated histochemically by the use of labeled lectins, mostly plant proteins which combine non-enzymatically with particular sugar molecules.^{262–265}

When gastrointestinal mucins were studied in several species using histochemical techniques under uniform conditions, species differences were most obvious in the stomach and duodenum.²⁵⁶ Neutral mucins generally predominate in the stomach, contrasting with acid mucins in the small intestine and sulfated mucins in the colon. In the stomach, neutral mucins staining purple with the PAS/alcian blue stain predominate in the surface and foveolar mucosa, whereas mucous neck cells and antral glands contain acidic mucins that stain blue with PAS/alcian blue procedure. Sulfated mucins, which stain black with the high iron diamine technique, are also found in the deep glandular mucosa of the antrum in rats, mice and humans.^{257,266,267} Extremely heterologous staining patterns are seen in the gastric mucosa with labeled lectins, each lectin staining different cell populations.^{203,268,269}

Non-neoplastic lesions

Inflammation, erosions and ulceration of the gastric glandular mucosa

HUMANS – DRUG-INDUCED CHANGES

A variety of therapeutic agents produce gastric damage in humans. These include potassium chloride, iron salts, alendronate, and sodium polystyrene sulfonate (Kayexalate), a cationic exchange resin, and above all non-steroidal anti-inflammatory drugs.^{170,270} Low dose aspirin used in elderly patients to prevent cerebral and myocardial ischemia is also associated with gastrointestinal damage.^{271,272} Non-steroidal anti-inflammatory drugs are some of the most commonly prescribed drugs worldwide and peptic (gastric and upper small intestine) ulceration is one of their well-known adverse effects.²⁷³

A systematic analysis of the published data suggested that one-third of patients taking non-steroidal anti-inflammatory drugs for long periods develop upper gastrointestinal tract injury.²⁷⁴ Serious injury is less common, although gastrointestinal bleeding following non-steroidal anti-inflammatory therapy is a significant cause of serious and sometimes fatal adverse drug reactions.^{275–280} In one prospective study, gastrointestinal bleeding caused by these compounds accounted for 10% of those hospital admissions due to adverse drug reactions.²⁷⁸ Predisposing factors in human patients with peptic ulceration include *Helicobacter pylori* infection of the antrum, cigarette smoking and alcohol use.^{271,281} Peptic-ulcer disease has been shown to be more common in *Helicobacter pylori*-infected patients taking non-steroidal anti-inflammatory drugs than in those without the infection, suggesting a possible interaction between drug use and this infection in the development of peptic ulcer.²⁷⁴ *Helicobacter pylori* is a micro-aerophilic, Gram-negative organism that possesses potent urease activity crucial for its survival at acidic pH. Genome sequence analysis has shown that *Helicobacter pylori* has well-developed sequences for motility, scavenging iron and DNA restriction and modification systems used by bacteria to degrade foreign DNA. The link between *Helicobacter pylori* infection and peptic ulceration has been related to increases in gastrin release, perhaps through bacterial products or cytokines released from activated lymphocytes.²⁸²

Although gastric pathology represents a significant cause of morbidity and mortality in humans following therapy with non-steroidal inflammatory agents, the mechanisms leading to injury are not fully understood. The theory that has gained widespread acceptance is that the ulcerogenic potential of non-steroidal anti-inflammatory drugs is related to their pharmacological activity. John Vane proposed that the ulcerogenic potential of these agents is largely a result of their ability to inhibit prostaglandin synthetase, thereby reducing the protective effects of prostaglandins.²⁸³ By inhibiting cyclooxygenase these agents not only reduce pro-inflammatory cytokines but also prostaglandin synthesis. Indeed, there is a good correlation between time and dose dependency of induced suppression of gastrointestinal prostaglandin synthesis and potential for peptic ulceration.²⁸⁴ Why this should lead to ulceration is less clear but it probably is a result of effects on several components of mucosal defense. Local irritancy of some formulations may be important and there may be alterations to the microcirculation, impairment of repair and changes in mucin or bicarbonate production.^{273,284}

The discovery of two different cyclooxygenases led to the development of drugs that preferentially inhibited the cyclooxygenase 2 (COX-2) isoform. This was based on the postulate that constitutively expressed cyclooxygenase 1 (COX-1) protects gastric mucosa whereas the inducible isoform COX-2 is responsible for inflammation and pain. While the benefit of selective COX-2 inhibitors for protection of the gastrointestinal tract is generally accepted, the situation turns out to be far more complex than originally envisaged. Many different mediators have been shown to contribute to the resistance of the gastrointestinal mucosa to injury.^{273,285} Indeed, in some circumstances COX-2 produces a highly potent gastro-protective substance 15-R-lipoxin A₄. Moreover, one of the most dramatic pharmaceutical events over recent years has been the withdrawal from the marketplace of certain of these selective COX-2 inhibitors, e.g. Vioxx™ (rofecoxib) and Bextra™ (valdecoxib), because of concerns over their cardiovascular safety and skin effects which have been shown in major clinical trials.^{286,287}

LABORATORY ANIMALS – SPONTANEOUS CHANGES

In laboratory animals in toxicity studies inflammatory changes in the mucosa as well as gastric erosions and ulcers in the glandular mucosa may be seen. *Erosions* represent mucosal breaks superficial to the muscularis mucosa (Figure 8.3a). *Ulcers* are lesions that extend through the muscularis mucosa. While the histopathological features of gastric erosions and ulcers are themselves relatively non-specific, it is important to look for any associated pathology in the stomach such as mucus depletion, epithelial hyperplasia or dysplasia, intestinal metaplasia and vascular lesions (see below).

The gastric mucosa, particularly in the antrum, may show inflammatory infiltrates along with the presence of lymphoid follicles. In dogs this may be accompanied by the presence of spiral organisms of *Helicobacter* type in the gastric glands.²⁸⁸

Monkeys may also show a variety of spiral organisms in the gastric mucosa, some of which have associated lymphocytic gastritis with intestinalization of mucosa or atrophy.¹¹ *Gastritis*, more prominent in the antrum than in the fundus, is one of the most commonly observed pathologies in laboratory cynomolgus monkeys.¹²⁹ It can be also often found in association with the presence of *Helicobacter* organisms.²⁸⁹ As in the analogous condition in humans, the stomach of affected animals shows infiltration of the mucosa by small

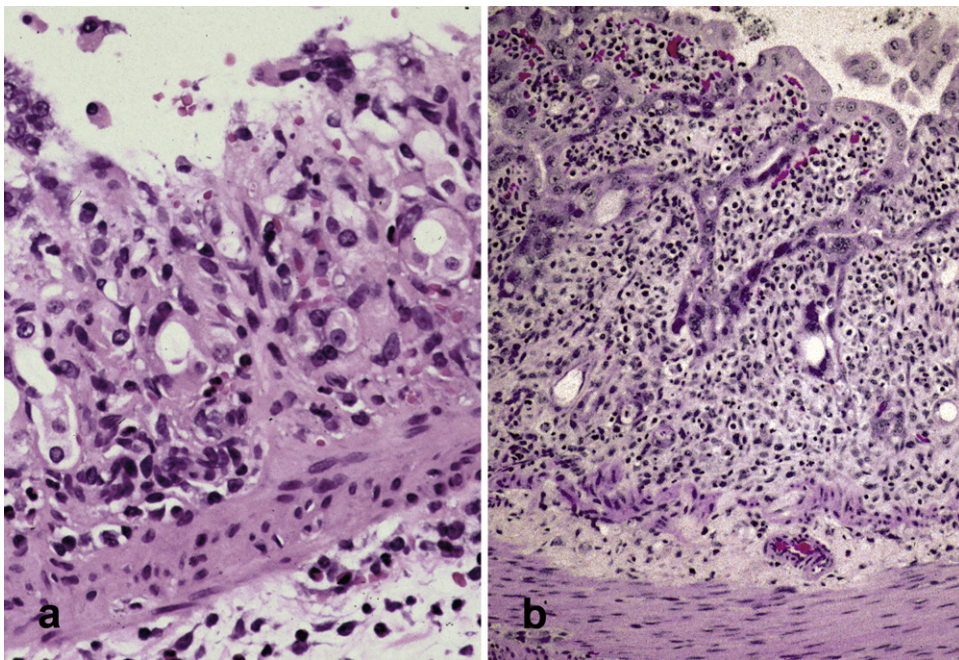


FIGURE 8.3 *Panel a:* Glandular stomach from a rat treated with a high dose of an angiotensin II antagonist that shows superficial degeneration and ulceration (erosion) of the mucosa (H&E $\times 280$). *Panel b:* Small intestinal mucosa of a rat treated with a colchicine-like anticancer drug. There is loss of the epithelium and residual cells show a striking increase in nuclear pleomorphism including the presence of large atypical nuclei. The mucosa is infiltrated by numerous chronic inflammatory cells (H&E $\times 100$).

lymphocytes and plasma cells, associated with reactive or atrophic changes and the presence of small curved bacteria in glands, seen best with the Warthin-Starry stain.

Although *Helicobacter pylori* can infect rodents, they may not develop the inflammatory response seen in primates.²⁹⁰ An exception appears to be the Mongolian gerbil which is considered the model of choice in the study of gastritis, gastric ulcer and metaplasia in response to *Helicobacter pylori* infection.²⁹¹ C57BL mice and some other strains also appear to develop similar alterations in response to *Helicobacter felis*.²⁹² The minipig may also show scattered foci of inflammation and microabscesses in the gastric mucosa.²⁹³

LABORATORY ANIMALS – INDUCED CHANGES

Gastric ulceration induced experimentally by stress by cold or restraint is a particularly well-studied phenomenon in rodents.²⁹⁴ There are differences in sensitivity to this form of stress among laboratory animals. For example, Sprague-Dawley rats appear less susceptible to the ulcerogenic effects of cold-restraint stress than Wistar rats.²⁹⁴

There is generally a good correlation of drug-induced toxicity in the gastrointestinal tract in laboratory animals and humans although prediction of the precise ulcerogenic potential of drugs in humans based on animal models is clouded by the lack of good comparative data in patients.²²⁸ There are extensive differences in side effect reporting and proper comparisons require not only equivalent therapeutic doses but also comparable dosage forms.^{165,295} Moreover, it can be quite difficult to determine whether inflammatory lesions in the stomach of treated animals indicate a real ulcerogenic risk for the test compound or some non-specific effect related to experimental conditions.

There is usually little that is histologically very specific about drug-induced ulceration of the gastric glandular mucosa, although morphological evidence of crystals, fragments of drug or other associated features may be helpful. Erosions and ulcers develop in laboratory animals following stress, reflux of intestinal contents and bile, changes in acid secretion and hypoxia, all of which may develop under the conditions occurring in high dose toxicity studies. The requirement to give the test compound in high doses may also dictate the need to administer exceedingly high concentrations of test agent which may simply damage the mucosa as a result of local irritancy in a manner not relevant to therapeutic doses used in clinical practice. For example, it has been demonstrated that oral administration of hyperosmolar solutions of quite innocuous substances such as glucose or sodium chloride can cause gastric inflammation, hemorrhage, erosions and ulcers of the rat gastric mucosa.^{296,297} Hyperosmolar solutions of sodium chloride have also been shown to induce increased expression of cytochrome P450 enzymes of *CYP1A* and *CYP3A* subtypes in the surface and foveolar cells of the gastric mucosa of rats, which could also modify the expression of xenobiotic activity.²⁹⁷ The well-known association of gastric erosions and hemorrhage with uremia may also be manifest following administration of high doses of drugs such as diuretics which severely derange fluid and electrolyte balance.¹⁹ Synergism between the ulcerogenic action of drugs and stress is also a well-described phenomenon.^{298,299} Protein depletion and starvation is also capable of inducing gastric ulceration in rats.³⁰⁰

In laboratory animals, a variety of different patterns of drug-induced gastric damage have been described. A study of different anti-inflammatory drugs administered to fasted Sprague-Dawley rats under identical conditions suggested the drugs could be

divided into three groups based on their profiles of gastrointestinal toxicity.³⁰¹ Immunological drugs and antiproliferative anticancer agents such as azathioprine, cyclophosphamide, methotrexate and d-penicillamine produced gastric mucosal hemorrhage whereas aspirin and related agents produced gastric mucosal hemorrhage and ulcers. The powerful non-steroidal anti-inflammatory drugs indomethacin and phenylbutazone produced gastric mucosal erosion and ulcers as well as small intestinal damage. However, novel targeted therapies for treatment of cancer may not fall into these simple categories. For example, a cyclin-dependent kinase inhibitor which produced single cell necrosis in the intestine in treated rats also produced cell damage (apoptosis) in the gastric glands of the fundus and pyloric regions most markedly in the basal zones and isthmus regions.³⁰²

Comparative single oral dose studies of several non-steroidal anti-inflammatory agents at three different dose levels in the rat using histology evaluation and measurement of fecal blood loss with ⁵¹Cr-labeled blood cells have also shown that different patterns of ulceration can be produced by different agents when administered under identical conditions.³⁰³ Single oral doses of some non-steroidal anti-inflammatory drugs including aspirin produced widespread superficial damage and desquamation of gastric epithelium with little or no inflammation at six hours following dosing which completely healed two weeks later. This damage was associated with transient fecal blood loss. By contrast, indomethacin and ibuprofen produced both gastric damage and circumscribed, penetrating ulcers along the mesenteric border of the jejunum and ileum. Furthermore, ulcers were still present after two weeks and were associated with prolonged or biphasic blood loss. Pharmacokinetic factors may also be important. Lipid solubility in the low pH environment of the stomach may influence local penetration into the mucosa.³⁰⁴

In addition, feeding conditions influence the distribution of erosions and ulcers in laboratory animals. In fasted rats, erosions due to indomethacin treatment are found in the body of the stomach while in conventionally fed rats they are most prominent in the small intestine. One detailed study showed that rats fed for one hour after a 24-hour fast and given a single dose of indomethacin within two hours of refeeding developed erosions and ulcers in the antrum primarily along the lesser curvature. Indomethacin given to fasted rats produced erosions in the body mucosa.³⁰⁵

A further factor that needs to be kept in mind is that chronic administration of ulcerogenic compounds may produce quite different pathological appearances to those found following single dose administration. Administration of aspirin to rats for four weeks has been shown to stimulate epithelial proliferation of the gastric body but not antral mucosa, possibly by an effect on cyclic adenosine 3',5' monophosphate (cyclic AMP) or through increasing the rate of epithelial exfoliation.³⁰⁶ Such a response may be the basis for increased resistance of the gastric mucosa to the chronic effects of these agents. It also may explain the tendency for ulcers to occur in the antrum following chronic administration of aspirin-like drugs as the proliferative response and presumably the adaptive potential appears less in this part of the gastric mucosa. A reactive proliferative change has also been described in humans treated with non-steroidal inflammatory drugs (see below).

Both interspecies variations and strain differences have been reported in the response to ulcerogenic compounds. Extravasation of red blood cells and greater vascular damage was observed in rats treated with aspirin or benoxprofen than in pigs given similar doses.³⁰⁷

In animal pharmacology models, notably those using the rat, the COX-2 inhibitors have generally appeared to be less ulcerogenic than the conventional non-steroidal anti-inflammatory agents, consistent with their reported effects in patients.^{308–312} However, the simple concept that COX-1 inhibition causes gastrointestinal damage and selective COX-2 inhibition is not ulcerogenic has been clouded by a number of experimental observations.³¹³ For example, inhibition of COX-2 delays gastric ulcer healing through interference with cell proliferation, angiogenesis and maturation of granulation tissue.³¹⁴ Moreover, high dose toxicity studies revealed that COX-2 inhibitors can cause gastrointestinal ulceration. For example, rats, dogs and mice all developed gastric or pyloric ulceration when treated with the COX-2 inhibitor celecoxib, although the distal small intestine seemed to be more markedly affected.³¹⁵ It has been shown by immunocytochemistry that the distribution of COX-2 expression in interstitial cells (monocytes, macrophages, fibroblasts and endothelial cells) of the lamina propria predominates in the distal ileum which has led to the suggestion that this might be a factor in the greater sensitivity of the ileum than the stomach to the ulcerogenic effects of COX-2 inhibition.³¹⁶

Diuretics and some angiotensin converting enzyme (ACE) inhibitors and angiotensin II antagonists have been associated with the development of gastric erosions and ulceration when administered in high doses to laboratory animals (Figure 8.3a).^{19,317} However, these effects appear related to the severe electrolyte disturbances produced by excessive doses of these drugs. This is perhaps analogous to the well-known association of gastrointestinal tract erosion and hemorrhage with uremia. Dogs appear predisposed. Microscopic examination may show that this form of ulceration is associated with deposition of basophilic ground substance and mineral in connective tissues and blood vessels in the mucosa.³¹⁸ The vascular damage which may occur in laboratory animals treated with high doses of cardioactive drugs such as the phosphodiesterase inhibitors may also occur in intramucosal blood vessels of the stomach and the inflammatory process can spill over into the glandular mucosa.¹¹⁴

Infiltration of the stomach by lymphocytes in rats treated with human recombinant interleukin 2 without ulceration was reported as part of a multisystem involvement induced by this agent.³¹⁹

Mucus depletion

Decrease in gastric mucus secretion may accompany both spontaneous inflammatory conditions and drug-induced lesions in the stomach of humans and experimental animals. Mucus depletion is characterized histologically by the presence of an intact epithelial layer in which cells show loss of the normal clear cytoplasm replete with mucous substances and replacement by more basophilic cells that contain little or no mucin.

Qualitative changes in mucus composition can also accompany mucus depletion. Gastric epithelium in humans shows decreases in sulfated mucosubstances following stress, high alcohol consumption or after aspirin and non-steroidal inflammatory drug administration.^{170,257} Similar changes occur in laboratory animals subjected to ulcerogenic regimens. Stress ulceration in the rat is accompanied by decreased sulfation of gastric glycoproteins.³²⁰ Administration of aspirin and other anti-inflammatory agents including adrenocortical steroids to laboratory animals also reduces the content of sulfomucins in the gastric mucosa, probably by reducing their synthesis.^{261,321–323} It has also been

suggested that these agents modify phospholipids within the luminal aspects of the mucus gel layer which normally provides it with non-wettable properties, thereby reducing the protective hydrophobic barrier properties of the upper gastrointestinal tract.³²⁴

Administration of histamine H₂ receptor antagonists and proton pump inhibitors that reduce gastric acid output and increases in gastrin secretion have also been associated with alterations in gastric mucus. Administration of omeprazole or famotidine to rats for four weeks was shown to inhibit prostaglandins PGE₂ as well as the synthesis of both total and sulfated glycoprotein along with histochemical evidence of reduction in PAS staining of the surface mucus.³²⁵ Although the mechanism for this change is unclear, the reduction in mucus, particularly sulfated mucus that is believed to be particularly resistant to peptic digestion, may have implications for mucosal defense.

Intestinal metaplasia

Intestinal metaplasia of the stomach is characterized by the presence of differentiated epithelium which resembles the small intestine on the basis of light microscopic and ultra-structural morphology, mucin patterns and enzyme histochemistry.^{326–330} It develops in human gastric mucosa altered by chronic atrophic gastritis. Its significance is due to the fact that intestinal metaplasia is associated with the presence of gastric cancer. Although intestinal metaplasia is found much less commonly in laboratory animals, it has also been reported to occur in association with gastric neoplasia induced by polychlorinated biphenyls.³³¹ In view of this association with gastric cancer, it has been suggested that intestinal metaplasia represents a pre-neoplastic lesion. However, over recent years prospective clinical studies and experimental data have suggested that it is an epiphenomenon, coexisting with, but unrelated to, the development of cancer.

In humans, several forms of intestinal metaplasia have been described. These variants fall into two main groups, a so-called *incomplete* type and a *complete* form.^{266,332–334} Others have proposed that it is best regarded in terms of *gastric*, *intestinal* and *mixed* phenotypes.³³⁵ Complete intestinal metaplasia is characterized by the presence of goblet cells, Paneth cells and absorptive cells with brush borders and variably developed intestinal villi. Incomplete forms are more heterogeneous, characterized by goblet and mucous columnar cells but no absorptive cells and variable patterns of mucin. The routine alcian blue stain performed at pH 2.5 and periodic acid-Schiff stain distinguishes between the intestinal acid mucins (blue) and the neutral mucins of gastric type. However, variable sialomucin and sulfomucin staining patterns are also seen in intestinal metaplasia in humans if staining with the high iron-diamine/alcian blue stain is performed. The incomplete form of intestinal metaplasia, showing marked sulfomucin secretion, has been found more commonly in association with gastric cancer in people.^{266,332,334} However, it is probable that intestinal metaplasia itself represents an adaptive response to long-standing chronic inflammation and reduced acid secretion. It has also been suggested that it represents an adaptive defensive response to long-standing *Helicobacter pylori* infection because intestinal mucosa is more resistant to these organisms.^{336,337} Immunocytochemical study has shown the presence of intestinal endocrine cells in the various forms of intestinal metaplasia that appears to follow the phenotype of the particular mucous cell differentiation.³³⁵

Fischer 344 rats treated with the polychlorinated biphenyl Aroclor 1254, mixed in the diet for two years, developed foci of intestinal metaplasia in the stomach epithelium in

association with gastric adenocarcinomas.^{331,338} These lesions were characterized by abundant mucin-containing cells with alkaline phosphatase activity typical of the small intestine. Similar, but more diffuse intestinal metaplasia was reported in the stomach of primates exposed to polychlorinated biphenyls, although this was not associated with gastric neoplasia.^{339,340} Intestinal metaplasia has also been inconsistently reported in the stomachs of laboratory animals treated with powerful genotoxic gastric carcinogens. For example, one study of rats treated with N-methyl-N'-nitro-N-nitrosoguanidine has demonstrated only hyperplasia and foci of atypical changes (dysplasia) with little or no intestinal metaplasia whereas another has shown intestinal metaplasia after administration of the same agent.^{203,258}

Despite this link with carcinogen administration, intestinal metaplasia can be induced in rodents by a variety of different procedures that are not associated with the development of gastric cancer. Intestinal metaplasia can be induced in the glandular stomach of rodents by fractionated, localized, ionizing radiation, injection of xenogenic stomach antigens as well as propantheline bromide and the non-carcinogen iodoacetamine.^{330,341–344} Intestinal metaplasia has also been shown to occur as part of the response to *Helicobacter pylori* infection in the small animal model, the Mongolian gerbil, as well as in C57BL mice infected with *Helicobacter felis*.^{291,292}

The characteristics of intestinal metaplasia in laboratory rodents are similar to those seen in humans with early increases in intestinal enzyme activity (alkaline phosphatase, lactase, trehalase, sucrose and maltase), development of goblet cells containing neutral, sialo-, or sulfomucins, and intestinal crypts with or without Paneth cells. Both the fundus and antrum can show changes, although, as in humans, males appear more prone to develop intestinal metaplasia than females.³³⁰

Based on these findings, it has been proposed that intestinal metaplasia is not a precancerous condition but an adaptive response to a chronic elevation in pH in gastric secretion due to the early loss of parietal cell mass brought about by these various procedures.³³⁰ On balance therefore, the evidence suggests that although intestinal metaplasia is associated with cancer and may consequently be considered a helpful morphological feature in the evaluation of human gastric biopsies, the finding of isolated intestinal metaplasia in safety studies does not indicate a preneoplastic state.

Hepatic metaplasia

A form of metaplasia in which *hepatocytes* have been found has been reported as rare incidental findings in the glandular stomach of mice sacrificed at the end of two-year carcinogenicity bioassays.³⁴⁵ Focal accumulation of well-differentiated hepatocytes were found in the submucosa and lamina propria adjacent to the limiting ridge with dilated adjacent gastric glands showing epithelial hyperplasia and mineralization with herniation into the submucosa. While these foci were not believed to be related to treatment with xenobiotics, it is not clear whether they represented metaplasia or congenital ectopia.

Mineralization

The gastric glandular epithelium is predisposed to the deposition of calcium possibly as it is a site at which marked ion exchange normally takes place. Focal aggregates or concretions of densely blue-staining mineral are fairly commonly observed in hematoxylin-stained

sections from the stomachs of aged rats where they are associated with cystic dilatation of the gastric glands.¹⁹⁰ Mice and hamsters occasionally show similar changes. Small concretions are also observed in gastric glands in the beagle dog. These appear to represent aggregates of calcium around mucoid material.

Gastric mineralization may become marked in rodents and dogs when there is disturbance of mineral metabolism, particularly in association with renal pathology. This has been well described in rats with severe renal disease (glomerulosclerosis) and parathyroid hyperplasia as well as in vitamin D toxicity.³⁴⁶ A similar phenomenon has been described in the stomach of dogs in uremic states.³⁴⁷ Identical changes result from the administration of drugs that induce prolonged azotemia or electrolyte disturbances. These changes are characterized by diffuse deposition of mineral in the intestinal tissue of the mucosa of the gastric body but usually not cardia, antrum or pylorus. Mineral deposits develop around basement membranes surrounding epithelium and blood vessels. The lamina propria becomes expanded by edema and fibroplasia of the interstitium also develops. The gastric glands themselves become distorted with swelling and degeneration of parietal cells and atrophy of chief cells. Erosion of the glandular epithelium with hemorrhage occurs presumably as a result of the ischemia caused by diffuse vascular injury and altered parietal cell function.

Mineralization of the gastric mucosa in the fundus, the muscularis and submucosal arteries has also been reported in rats treated with a selective inhibitor of fibroblast growth factor tyrosine kinase.³⁴⁸ This appeared to be part of a generalized effect on calcium balance with changes to the bone growth plates, elevated serum phosphorus levels and soft tissue mineralization. Another example was reported in rats following intravenous administration of gadolinium chloride.³⁴⁹ Gadolinium compounds are widely used as contrast agents in magnetic resonance imaging. Treated animals developed a discrete band of interstitial mineralization in the fundic glandular mucosa composed of calcium and phosphate in the form of hydroxyapatite with little or no gadolinium present, findings consistent with a form of metastatic mineralization.³⁴⁹

Atrophy

Focal atrophy of the gastric glandular mucosa is a sporadic occurrence in laboratory rodents, usually as a result of previous focal gastric inflammation, ulceration, mineralization or vascular occlusion. These changes, characterized microscopically by focal fibrosis of the mucosa, gastric glandular dilatation and atrophy accompanied by polymorphonuclear cells and mast cells, are common in certain strains of rats when two years or more in age.³⁵⁰

Whereas diffuse mucosal atrophy occurs following severe inflammatory insult, diffuse atrophy of the stomach glandular mucosa without inflammation can be a result of surgical or drug-induced reduction in trophic factors necessary for the maintenance of normal gastric morphology and function. This is observed in humans and experimental animals following antrectomy because this removes the peptide-producing cells of the antrum.^{351,352} In the rat, antrectomy is accompanied by hypogastrinemia, reduced weight and height of the oxyntic mucosa and a reduced number of argyrophil cells.^{238,353} This is in contrast to procedures such as antral exclusion that lead to hypergastrinemia and increased thickness of the oxyntic mucosa.

Mice with genetic deletion of the gastrin gene also show reduction in the thickness of the gastric mucosa. While all cell types are present, there is a most pronounced decrease in the numbers of parietal and enterochromaffin cells associated with an increase in surface mucous cells. These changes are linked to a profound decrease in acid secretion, which becomes unresponsive to histaminergic, cholinergic and gastrinergic stimulation.²⁴⁷ Analogous atrophic changes have been reported following pharmacological removal of trophic stimuli. For instance, administration of the cholecystokinin-B/gastrin receptor antagonist CI-988 to cynomolgus monkeys for periods of up to 13 weeks was associated with an initial phase of multifocal degeneration of gastric glands primarily in the fundus followed by diffuse reduction in the thickness of the glandular mucosa with little or no qualitative changes to the cell populations.³⁵⁴

Although bilateral vagotomy produces profound functional changes in the stomach, notably reduction of gastric acid secretion, morphological changes in the fundal mucosa are not marked either in experimental animals or in humans.^{355,356} Studies in the rat have shown that diffuse atrophy of the gastric glands characterized by a decrease in the number and size of parietal, chief and mucous cells occurs transiently following truncal vagotomy but histological features return to normal by about one month after surgery.³⁵⁷ By contrast, unilateral vagotomy in the rat leads to marked and persistent atrophy of the oxyntic zone on the denervated side. This is characterized histologically by reduced height of the mucosa and reduced numbers and staining intensity of argyrophil cells.³⁵⁸ It has been argued that this unilateral atrophy is due to the removal of the trophic action of the vagus. The lack of lasting atrophy after bilateral but not unilateral vagotomy has been explained by the subsequent rise in gastrin that occurs after bilateral vagotomy as a result of lack of acid feedback inhibition of gastrin release.³⁵⁸

Removal or reduction in extra-gastric trophic factors or hormones may also reduce the thickness of the gastric mucosa. This has been demonstrated in the rat by hypophysectomy which causes a reduction in thickness of oxyntic and antral mucosa, compared with pair-fed controls. Although there was little or no change in peptic:parietal cell ratios, a significant decrease in cell volume and secretory activity of gastric glandular cells were demonstrated which suggested a widespread disturbance of synthesis and secretory mechanisms.³⁵⁹

Atrophic changes in the chief cells were observed in rats treated for six months with high doses of omeprazole, an inhibitor of acid secretion. The findings were considered to represent disuse atrophy secondary to the inhibition of acid secretion.³⁶⁰ Another inhibitor of gastric acid secretion, the tricyclic agent pirenzepin, also produced atrophy of the fundic mucosa of rats following three months but not one month of treatment.³⁶¹ The atrophy was characterized by reduction in parietal cell numbers associated with lower numbers of gastrin-containing cells in the antrum, features unlike those following prolonged treatment with histamine H₂-receptor antagonists.

Diffuse hypertrophy and hyperplasia of glandular mucosa

An increase in the thickness of the gastric mucosa can result from hypertrophy or hyperplasia of the mucosal cells and this occurs both spontaneously or following administration of drugs and chemicals. In view of the different cell populations in the gastric mucosa and the variety of morphological alterations that occur, it is difficult to make a clear distinction between hypertrophy and hyperplasia without morphometric techniques.

These techniques have shown that hypertrophy of some mucosal cells can coexist with hyperplasia of other gastric cell populations. A distinction also needs to be made between diffuse or uniform hyperplasia involving one or more of the cell populations from the hyperplasia associated with focal proliferative or 'adenomatous' overgrowth.

Hyperplasia should be evaluated for atypical cytological features (dysplasia) which are linked to development of gastric carcinoma (see below). The difficulty in making the distinction between an exuberant reactive hyperplasia from a dysplastic or premalignant change is an issue in all parts of the gastric glandular and intestinal mucosa particularly in the presence of chronic inflammation and fibrosis. Moreover the effects of compounds such as colchicine and taxol that arrest mitosis through inhibition of tubulin polymerization into microtubules may mimic gastrointestinal dysplasia (Figure 8.3b).³⁶²⁻³⁶⁴

Like the mucosa of other parts of the gastrointestinal tract, the stimulus of high concentrations of bulky or irritant substances can cause hyperplasia of the stomach mucosa. An example of this in both rats and mice was observed following administration of high doses of poorly absorbed lanthanum carbonate used therapeutically as a phosphate binder in patients with compromised renal function. At high doses, rats and mice showed glandular hyperplasia of the stomach mucosa associated with submucosal chronic inflammation and hyperplasia of the squamous mucosa at the limiting ridge.³⁶⁵

Cells of gastric glandular mucosa also undergo increases in size or number in response to the effects of gastrointestinal trophic hormones or their synthetic analogs. Similar changes also follow administration of compounds that inhibit gastric acid secretion or modify other trophic hormones or growth factors. Likewise transgenic hypergastrinemic mice also develop gastric mucosal hyperplasia. It has been shown in this model that cyclooxygenase 2 (COX-2) within interstitial cells contributes to the gastric hyperplasia.³⁶⁶

When gastrin or its synthetic analog pentagastrin is administered subcutaneously to rats and mice for several weeks, there is an increase in both the number and size of parietal cells without concomitant increase in zymogenic chief cells.³⁶⁷⁻³⁶⁹ In addition, diffuse hyperplasia of enterochromaffin cells also occurs. By contrast, cholecystokinin, a trophic peptide found in the duodenum and sharing the same C-terminal tetrapeptide sequence as gastrin, increases in the number of chief cells but not parietal cells when administered to mice under similar conditions.³⁶⁹

Drugs, which inhibit or neutralize gastric acid secretion such as histamine H₂-antagonists, proton pump inhibitors and antacids, also induce hypertrophy or hyperplasia of the parietal cell population.^{242,368,370-374} This occurs not only in laboratory animals treated with these drugs but also in humans.¹⁷⁰ These agents are associated with a rise in serum gastrin levels, probably as a result of loss of feedback inhibition of low antral pH on gastrin-producing G cells. Not all histamine H₂ antagonists produce identical effects. Other cytological changes have been reported with famotidine, another H₂-receptor antagonist. This agent produced a dose-related increase in the prevalence and degree of eosinophilic granularity in chief cells of the stomach in toxicity studies in rats but not dogs.³⁷⁵ Electron microscopy showed an increase in electron density of zymogen granules and it was argued that these effects were the result of secondary inhibition of pepsin secretion or turnover due to inhibition of acid secretion.

Cytoprotective agents of prostaglandin type produce yet different forms of diffuse gastric hyperplasia. Rats treated with 16,16-dimethyl prostaglandin E₂ hourly for three weeks

developed not only forestomach alterations (see above) but also thickening of both the body and antral mucosa. In the body mucosa, these changes were the result of a proportional increase in the total mass of surface and foveolar mucous cells, mucous neck cells, chief cells, parietal and endocrine cells as well as connective tissue. This was largely as a result of increase in cell number, although parietal cells also increased in size.¹⁹⁹ Unlike treatment with gastrin and gastrin analogs there was an increase in number of surface and foveolar mucous cells associated with increase in mucus content.

Misoprostal, a synthetic prostaglandin E₁ methyl ester analog, also produced diffuse glandular hyperplasia, characterized by lengthening of gastric pits and increased mucous secretion in the preclinical safety studies in dogs and rats.¹⁹⁷ This glandular hyperplasia affected not only the body but also the antral mucosal. Studies with tritiated thymidine showed that the labeling index was reduced in rats treated with misoprostal, suggesting hyperplasia following administration of prostanoids is a result of an increase in cell survival and decrease in cell shedding rather than an increase in cell proliferation.³⁷⁶ Other prostaglandins of the E series have also been shown to produce a uniform hyperplasia of the surface mucous cells and an increase in the depth of the foveolae in the body and antrum of the stomach in dogs and rodents.¹⁹⁶

A dose-related diffuse hyperplasia of the gastric glandular mucosa has been reported in both rats and cynomolgus monkeys given human recombinant growth factor. The gastric mucosa was thickened and there was an increase in the number of undifferentiated cells particularly in the neck region and upper part of the gastric glands.^{26,27} Mitotic figures were also numerous in the upper reaches of the mucosa. The lower parts of the gastric glands were generally less affected. The large increase in the number of undifferentiated cells may have a functional effect on gastric acidity and function.¹⁷⁹ Administration of recombinant growth hormone has also been reported to induce thickening of the gastric glandular mucosa in dogs along with typical growth hormone-induced changes in other organs, body weight increases and insulin-like growth factor.³⁷⁷ The pyloric and fundic mucosa showed histological evidence of hyperplasia of the mucous neck cells.

Gastric hyperplasia with proliferative or adenomatous features (adenomatous hyperplasia, giant hypertrophic gastritis, hypertrophic gastropathy) adenomatous polyps, adenoma

Thickening of the gastric glandular mucosa as a result of various forms of irregular, polypoid or regional proliferation and cystic dilatation of gastric glands associated with inflammation characterizes a number of non-neoplastic conditions in the stomach of humans and laboratory animals. Cystic change with chronic inflammation and foveolar hyperplasia is observed in biopsies taken from the edge of chronic gastric ulcers in humans.³⁷⁸ Hyperplastic or cystic-type benign fundic polyps arise in 7–10% of the patients taking proton pump inhibitors for 12 months or more but these regress with cessation of therapy.^{379,380}

Ménétrier's disease (*polyadenomes en nappes*), a rare condition found primarily in middle-aged men, is also characterized by enlarged gastric folds, foveolar hyperplasia and glandular cystic dilation.^{381–383} It is associated with decreased acid secretion, hypoproteinemia due to selective loss of serum proteins across the gastric mucosa and the development of gastric cancer. Although its pathogenesis remains elusive, increased expression of

transforming growth factor α (TGF α) and the epidermal growth factor receptor has been described.³⁸⁴ TGF α is an epithelial cell mitogen that inhibits gastric acid secretion and increases gastric mucin. Transgenic mice that over-express TGF α in gastric mucosa develop a similar condition (see below) and it has been suggested that TGF α might have an important role in this condition.³⁸⁴ Mutant histamine H₂ receptor deficient mice also develop a similar condition which is associated with over-expression of TGF α .³⁸⁵ As TGF α is one of several ligands that bind to the epidermal growth factor receptor, it is of interest to note that treatment of patients with Ménétrier's disease with a monoclonal antibody against the epidermal growth factor receptor ameliorates the condition.³⁸³

Similar changes have been observed in animals, even camels, sometimes in association with infestation of the gastrointestinal tract by parasites.^{386–388} Laboratory rodents may develop a similar pattern of changes spontaneously with advancing age, although the cause of these changes remains uncertain. In rodents the distinction between this form of exuberant adenomatous hyperplasia and adenoma is not clear cut. However, it is customary in laboratory animal pathology to define adenomas as *localized* or focal proliferative lesions with well-ordered glandular patterns with a clear boundary with the surrounding normal mucosa. They are usually exophytic or polypoid in nature.

MOUSE

Marked proliferation of the gastric glandular mucosa has been well characterized in the laboratory mouse for over 60 years because certain strains have a particular tendency to develop this condition spontaneously with advancing age.^{389,390} Hyperplasia occurs spontaneously in conventional laboratory strains employed in carcinogenicity bioassays. Its prevalence can be influenced by environmental factors such as housing, food restriction and the administration of diverse xenobiotics, but notably histamine H₂ receptor blockade and other agents that cause hypergastrinemia.^{391–394} Similar gastric changes have also been reported to occur in mice thymectomized shortly after birth, in mice that overproduce TGF α and in histamine H₂ receptor deficient mice.^{384,385,395,396}

Microscopically, these changes in mice are characterized by hyperplasia of the foveolar and neck regions of the body mucosa (Figure 8.4). In advanced cases this is accompanied by elongated, tortuous, or dilated glands lined by simple columnar or cuboidal epithelium, devoid of parietal or chief cells. The abnormal cells show only mild cellular pleomorphism and mitotic activity. The abnormal glands displace normal glandular tissue and may penetrate through the muscularis mucosa to reach the muscularis externa and serosa. Step sections demonstrate continuity between these glandular elements and a total absence of metastatic spread in the adjacent tissues and lymph nodes. The lamina propria also shows increased amounts of smooth muscle and collagen accompanied by variable numbers of lymphocytes and other chronic inflammatory cells. Edema may be observed and blood vessels are often dilated. The antral mucosa remains relatively unaffected. Histochemistry has shown variable mucin secretion of the altered glands. Some glands are devoid of mucin; others show an increase in sulfomucin.³⁹⁷

The etiology of the spontaneous condition in the mouse remains uncertain. It has been suggested that these features are similar to Ménétrier's disease in humans and might have a similar pathogenesis.^{383,384,395} The occurrence of similar lesions in thymectomized mice has given rise to the suggestion that autoimmune damage to the gastric mucosa may be

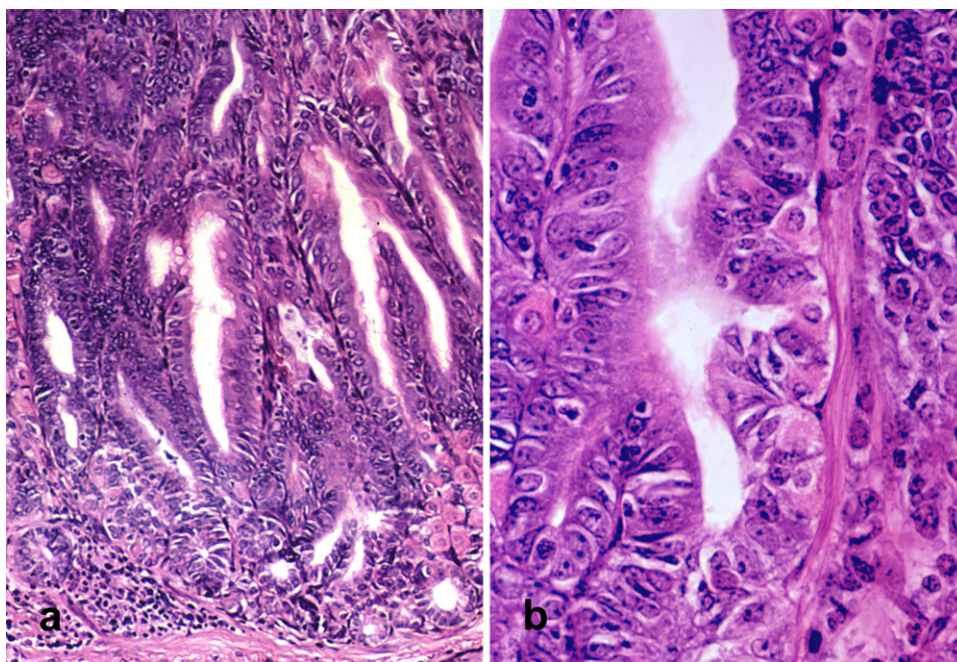


FIGURE 8.4 Gastric glandular mucosa from an untreated 18-month-old CD-1 mouse showing moderate hyperplasia of the gastric glands of the body mucosa. *Panel a:* Low power view (H&E×110). *Panel b:* High power view shows the hyperplasia and loss of the specialized cells in the gastric glands (H&E×425).

responsible.³⁹⁸ The presence of circulating antiparietal antibodies and the decrease in the number of parietal cells in thymectomized mice suggested that autoimmune damage can occur to parietal cells with compensatory chronic stimulation and proliferation of the generative zones.³⁹⁶ However, evidence from studies in female Han NMRI mice suggests that this proliferative condition can develop in mice in the absence of antiparietal antibodies but is associated with an increase in the number of antral gastrin cells, raising the possibility of a hormone or paracrine mechanism.³⁹⁴

As a similar proliferative form of gastropathy has been reported in mice that over-express transforming growth factor α (TGF α), a potent mitogen and member of the epidermal growth factor family of peptides; it is possible that TGF α is a key mediator in the development of this condition. TGF α acts by binding to and activating the tyrosine kinase of the epidermal growth factor receptor. Transgenic mice over-expressing TGF α develop severe adenomatous and cystic hyperplasia of the gastric glandular mucosa starting from about two months of age along with loss of mature parietal cell numbers and a diminution in gastric acid production.³⁹⁵ The degree of change was shown to be somewhat dependent on the genetic background of the mouse.

An increased prevalence of similar changes has been reported in CD-1 mice treated with the novel histamine H₂ receptor antagonist SK&F 93479 for 21 months.^{242,393} Although treated mice developed hyperplasia of gastric neuroendocrine cells similar to that observed in rodents treated with other antisecretory agents, they also showed an

increase in the severity of glandular hyperplasia. Like the spontaneous condition, these changes were characterized by thickening of the mucosa by hyperplasia of the foveolar and neck regions, and downward proliferation of glandular elements into gastric glands.²⁴² Similar glandular hyperplasia in the mouse stomach alongside neuroendocrine alterations has been associated with histamine H₂ blockade with the agent ioxitidine.³⁹²

Adenomatous polyps have also been reported in the pyloric antrum of C57Bl/10J mice treated for 52 weeks with the synthetic progestin cyproterone acetate.³⁹⁹ These were single, pedunculated and well-differentiated lesions showing little evidence of dysplasia. The mechanism for the induction of these polyps is unclear although they may have been hormonally mediated as both progesterone and estrogen receptors have been identified at low levels in gastric tissue.⁴⁰⁰ However, similar glandular polyps have also been described in male but not female mice following oral administration lanthanum carbonate for up to 99 weeks at a dose of 1.3 times the maximum recommended daily dose in renal compromised patients to bind phosphate.^{401,402} However, lanthanum carbonate is particularly inert, poorly soluble and not well absorbed suggesting that the polyps may also develop as a response to local mechanical factors.

RAT

Although usually less prevalent and less exuberant than in mice, the aged rat also develops proliferative gastric glandular changes spontaneously. These changes are characterized by hyperplasia of the foveolar and mucin-secreting cells of the body mucosa, development of cystic glands lined by simple mucous or flattened cells, accompanied by chronic inflammatory cells, prominent blood vessels and smooth muscle in the lamina propria.¹⁹⁰ The antrum remains relatively unaffected.

Proliferative alterations can be induced by administration variety of xenobiotics as well as following surgical procedures that induce chronic reflux of normal intestinal contents. For instance, hyperplasia of the gastric mucosa, notably over the lesser curvature, has been described in rats following the so-called Bilroth II gastrectomy that allows reflux of intestinal and biliary secretions into the stomach.⁴⁰³

A proliferative condition of the gastric mucosa has been shown to develop following long-term treatment of rats with an ulcerogenic regimen of aspirin. Female Sprague-Dawley rats given 250 mg/kg of aspirin in 1% methylcellulose once daily orally by gavage for six months followed for periods of up to 18 months without treatment developed focal proliferative changes at the sites of healed ulcers, mainly in the mucosa of antrum or antral-body junction.⁴⁰⁴ These lesions were characterized by the presence of proliferating gastric glands lined by columnar, cuboidal or flattened epithelial cells in the mucosa, which also extended through the muscularis mucosa. Mucus content of these glands was variable but when present was principally acidic in type, as shown by staining with alcian blue at pH 2.5. The lesions were accompanied by increased collagen in the lamina propria, endarteritis and an infiltration of lymphocytes, plasma cells and mast cells. The lesions were not associated with the development of carcinoma following 18 months' observation and it is probable that they were the result of the chronic damage and repair induced by aspirin treatment.

Polychlorinated biphenyls such as Arochlor 1254, which produce intestinal metaplasia and adenocarcinoma in the stomach of rats, also induce proliferative alterations

characterized by proliferative cystic lesions in the mucosa associated with inflammation and fibrosis.³³⁸ These changes are found primarily in the antrum and pyloric regions, zones of predilection for the development of gastric carcinoma in humans and experimental animals.

Gastric atypical hyperplasia (dysplasia)

It is important to distinguish between the various hyperplastic and adenomatous conditions found in the gastric glandular mucosa in laboratory animals that are not associated with neoplasia from those which precede the development of carcinoma. This distinction is complicated by the fact that proliferative changes associated with the development of cancer both in humans and laboratory animals possess features in common with lesions which are not associated with neoplasia. It is probable that there has been some overdiagnosis of gastric neoplasia in laboratory rodents. Atypical but non-neoplastic gastric glands may penetrate deep into the muscularis in inflammatory conditions which can mirror invasive cancer.²⁹¹ However, a key distinguishing feature is the presence of atypical epithelium (*dysplasia*).

Dysplasia is considered to be the lesion common to gastric conditions in humans such as atrophic gastritis and gastric polyps that have been linked with a significantly increased risk of gastric cancer. Although the term dysplasia may be less widely employed in experimental pathology, similar dysplastic changes to those occurring in humans have been characterized in laboratory animals in which precancerous gastric lesions have been studied.²⁵⁸ It therefore represents a unifying concept in the assessment of proliferative changes in the gastric glandular mucosa of laboratory animals.

As defined by an international group concerned with the diagnosis of preneoplastic conditions in the stomach of humans, three principal features of dysplasia have been defined.^{405,406}

1. Cellular atypia
2. Abnormal differentiation
3. Disorganized mucosal architecture

Cellular atypia is characterized by nuclear pleomorphism, hyperchromasia and stratification of nuclei, increased nuclear–cytoplasmic ratio and loss of cellular and nuclear polarity. Abnormal differentiation is shown by reduction or alteration in the normal secretory products of the mucosa. Disorganized mucosal architecture is shown by irregularity of crypt structure, back-to-back glands, budding and branching of crypts and intraluminal and surface papillary growths.

It is important to assess gastric mucosa very carefully for the features of dysplasia when hyperplastic gastric changes are found in treated animals. In the rat gastric cancer model employing the carcinogen N-methyl-N'-nitro-N-nitrosoguanidine, dysplastic changes were shown to start in the proliferating neck region of hyperplastic zones.²⁵⁸ These changes were characterized histologically by irregular growth patterns of glandular cells showing reduced mucin secretion, numerous mitoses and enlarged pleomorphic nuclei. These atypical glands were observed to extend downwards, eventually replacing normal gastric glands and ultimately penetrating the muscularis mucosa forming infiltrating adenocarcinomas of variable differentiation. The antrum developed these changes earlier than the

body mucosa. Involvement of the antrum is quite unlike the spontaneous hyperplasia of the rat gastric mucosa.

These considerations were important in the safety evaluation of the histamine H₂ receptor antagonist tiotidine (ICI 125,211), a guanidino-thiazole derivative that also produced proliferative gastric lesions in the stomach of rats in a 24-month carcinogenicity study.^{407,408} These changes were found mainly in the pyloric region and were characterized histologically by superficial erosions and irregular pyloric glands lined by cells with basophilic cytoplasm and enlarged hyperchromatic nuclei. Some atypical glands penetrated the muscularis mucosae. Dysplastic lesions situated primarily in the pyloric region were also associated with the development of invasive carcinoma in some rats. Extensive histological sectioning of the stomach in rats treated with tiotidine for only six months also revealed evidence of early proliferative changes. Therefore, these lesions produced by tiotidine possessed more in common with those induced by powerful carcinogens such as N-methyl-N'-nitro-N-nitrosoguanidine than the benign, species-specific proliferative change of little or no relevance for human safety. Interestingly, mice treated for 18 months with tiotidine were devoid of dysplastic changes in the gastric mucosa.⁴⁰⁸

Certain compounds may produce changes that mimic gastrointestinal dysplasia. This has been well described in human gastric biopsies in patients being treated with compounds such as colchicine and taxol that interfere with tubulin and inhibit its polymerization into microtubules.^{362–364} This effect produces mitotic arrest characterized by prominent ring mitoses associated with epithelial pseudostratification, loss of polarity and increased apoptosis, features that may mimic true dysplasia. Similar features have also been reported in laboratory animals (Figure 8.3b).^{363,409,410}

Hyperplasia and neoplasia of gastric endocrine cells, carcinoid tumors

One of the most remarkable examples of drug-induced gastric alterations reported in rodent bioassays has been the hyperplasia of enterochromaffin cells and development of carcinoid-like neoplasms in the stomach of rats treated with omeprazole.^{411,412}

Omeprazole is a substituted benzimidazole which inhibits gastric acid secretion by blocking the enzyme H⁺, K⁺-ATPase, the proton pump of the parietal cells.⁴¹³ At that time, now 30 years ago, there was much concern about the findings in the rat and their potential implications for humans. Similar findings have now been reported with other drugs of the same type so it has become to be regarded widely as a *class effect*.^{414–416} These proton pump inhibitors have been extensively used safely for many years. There is a mild hypergastrinemia and gastric biopsies in long-term clinical trials in humans have suggested an increased incidence enterochromaffin hyperplasia with prolonged dosing. However, the evidence for enterochromaffin hyperplasia is not convincing.^{379,380} Only benign fundic glandular polyps have been convincing related to treatment with proton pump inhibitors and these regress with cessation of therapy.^{379,380} Carcinoid tumors, dysplasia or gastric carcinomas have rarely been detected.⁴¹⁴ Even when hypergastrinemia is marked and prolonged, such as in patients with Zollinger-Ellison syndrome, gastric carcinoids are rare. Thus, these rodent findings are widely understood to be an effect of exaggerated pharmacodynamic activity not directly relevant to the therapeutic use of these drugs.

Although in rats there is an increase in number of gastric argyophilic cells with increasing age, rats treated with omeprazole for 104 weeks showed a marked, dose-related and

diffuse increase of argyophilic, non-argentaffin cells in the basal half of the oxyntic fundal mucosa.⁴¹¹ These changes were more marked in female than in male rats. Moreover, they were not observed in the bioassay in which CD-1 mice were treated with similar doses of omeprazole for 78 weeks.

These diffuse changes in the rat stomach were associated with focal hyperplasia of argyophilic cells. These focal lesions were also associated with a dose-related increase in larger focal nodular lesions of argyophilic cells, some of which were undoubtedly locally infiltrating carcinoid tumors. These nodular argyophil lesions posed the usual problems of differential diagnosis of endocrine hyperplasia and neoplasia (see Endocrine Glands, Chapter 13), the precise distinction between hyperplasia and neoplasia being unclear.

Histologically, nodular lesions are composed of multifocal anastomosing solid or pseudoacinar cords of proliferating, regular cells with uniform nuclei and moderately abundant fine granular pale cytoplasm (Figure 8.5). These nodules show little or no cellular pleomorphism or mitotic activity but clear evidence of submucosal infiltration without involvement of the muscularis externa was observed in some cases. The overall light microscopic features are similar to those of gastrointestinal carcinoid tumors reported in humans. The incidence of gastric carcinoids was reported to be as high as 40% in females in the high dose group but only a few cases observed in similarly treated males.^{411,412}

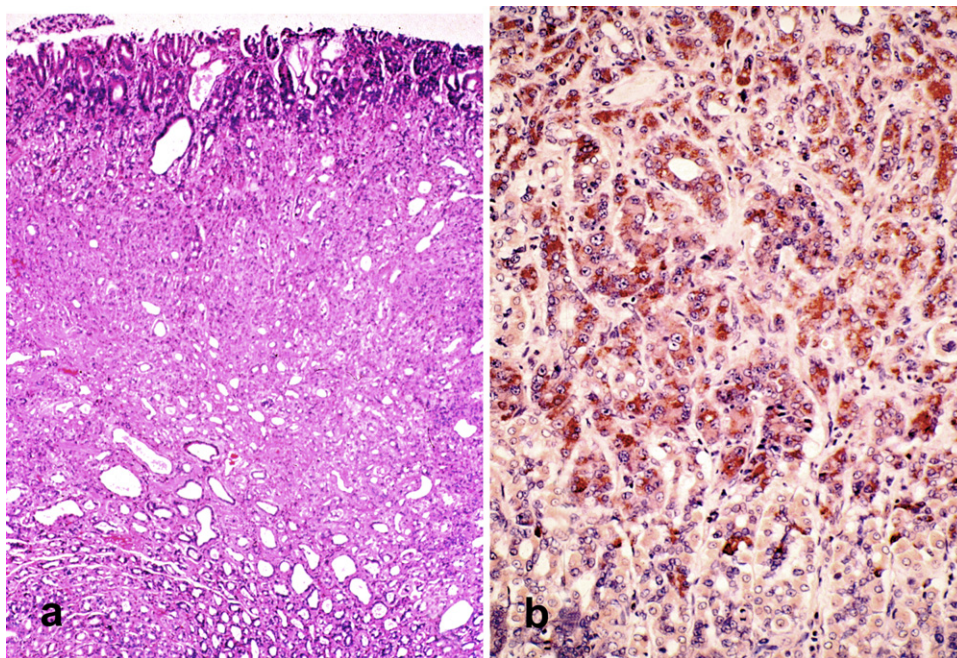


FIGURE 8.5 Gastric glandular mucosa from a rat treated with a high dose of a histamine H₂ antagonist for two years. *Panel a:* A well-defined benign nodular zone showing both solid and glandular proliferation (H&E×50). *Panel b:* Higher power view of same case stained for chromogranin A revealing the neuroendocrine nature of the cell proliferation (hematoxylin, immunoperoxidase×140). Illustrations by courtesy of Dr Graham Betton.

Electron microscopy of the altered argyrophil cells confirmed the presence of electron-lucent, vesicular granules, frequently with small irregular dense cores characteristic of enterochromaffin cells of the stomach. Immunocytochemical study showed that these cells contained histidine decarboxylase, a normal enzyme in gastric enterochromaffin cells that produce and store histamine.⁴¹⁷ Other findings reported in rats treated with omeprazole have been a proportional increase in the number and size of non-endocrine cells of the fundus, an increase in the number and immunostaining properties of the antral gastrin-containing G cells and hypergastrinemia.^{245,418,419} All functional and morphological changes following treatment for 60 days were fully reversible after 42 days of drug withdrawal.

As a result of the treatment-related increases of these normally rare gastric carcinoids in the rat bioassay with omeprazole, clinical trials with this agent were suspended until it was agreed that the endocrine alterations were a result of prolonged drug-induced achlorhydria. It was postulated that omeprazole causes a prolonged inhibition of acid secretion in the rat which causes activation, and subsequently hyperplasia of antral gastrin cells and marked hypergastrinemia. Hypergastrinemia in turn stimulates enterochromaffin cells of the fundus, which in time results in enterochromaffin hyperplasia.²³⁸ This argument is supported by the fact that similar morphological findings are reported in chronic atrophic gastritis and other achlorhydric sites in humans and that antrectomy in the rat prevents the appearance of enterochromaffin hyperplasia following treatment with omeprazole.^{420–422}

Although mild dose-related gastric argyrophil cell hyperplasia was noted in dogs treated with omeprazole for one year, neoplasms of the stomach were not observed during this time period. Why mice developed neither argyrophil hyperplasia nor gastric carcinoids with a similar treatment regimen is not clear, as the mechanism of action of omeprazole is similar in rat, dog and mouse. However, as the duration of action of omeprazole was shorter in the mouse, it was postulated that sustained inhibition of gastric acid secretion over 24 hours is necessary to activate increased gastrin secretion from antral cells.⁴¹¹ It has also been suggested that the mouse possesses fewer gastric enterochromaffin cells than the rat and shows a much lower serum gastrin response to omeprazole treatment.⁴¹² Nevertheless, gastric neuroendocrine tumors have occasionally been reported to develop spontaneously in old laboratory mice where they can be an issue in carcinogenicity studies because of their association with raised gastrin levels.⁴²³

Duration of action or potency may also be the explanation for the lack of reports of carcinoid neoplasms in rats following inhibition of gastric acid secretion by the histamine H₂ receptor blockers cimetidine and ranitidine. Neither of these drugs completely inhibits gastric acid secretion in the rat for 24 hours.^{422,424} However, while neoplasia has not been reported, mild gastric neuroendocrine hyperplasia has been described in cimetidine-treated rats.⁴²⁵ Moreover, the long-acting H₂ receptor antagonist SK&F 93479 produced gastric carcinoid neoplasms when administered at a high dose (1,000 mg/kg) to rats for two years (Figure 8.5).^{242,393} Although this dose level of SK&F 93479 did not entirely suppress gastric acid secretion and control gastric pH over 24 hours, plasma gastrin levels remained elevated at three to four times control values over this period. In a 21-month oral carcinogenicity study in CD-1 mice at the same dose level (1,000 mg/kg), a diffuse neuroendocrine cell hyperplasia and multifocal glandular hyperplasia and neoplasia was also observed.^{242,393} Similarly, loxidine, a potent, non-competitive, insurmountable histamine H₂ antagonist,

produced hyperplasia of neuroendocrine cells and carcinoid tumors in the gastric fundus of both rats and mice after two years' treatment in diet and drinking water, respectively.^{392,426} Other histamine antagonists, BL-6341 and ICI 162846, have been reported to produce neuroendocrine neoplasms in the stomach of rats and rats and mice, respectively.^{408,425}

Drugs of other classes also cause hyperplasia of gastrin-containing cells. Immunocytochemical study using antigastrin antibody revealed increased gastrin cell numbers in the antral mucosa of dogs given high doses of adrenocorticosteroids for four weeks and these changes were accompanied by enhanced serum and tissue gastrin levels.⁴²⁷ These results suggest that corticosteroids have a trophic effect on gastrin-containing cells.

Gastric carcinoma and nitrosation

A confounding factor in drug safety evaluation is the association of gastric cancer in both humans and laboratory animals with N-nitroso compounds. Some of the most effective stomach carcinogens in laboratory animals have proved to be N-nitroso compounds, particularly since Sugimura and Fujimura induced gastric adenocarcinomas in rats with N-methyl-N'-nitro-N-nitrosoguanidine dissolved in drinking water.⁴²⁸ Furthermore, epidemiological evidence associating N-nitroso compounds with human cancer is also fairly strong for the stomach.^{429,430}

In the past this has been considered a potential safety issue for new drugs because the formation of N-nitroso compounds is theoretically possible with a number of compounds that contain amino groups.

Some drugs in widespread clinical use have been shown to produce N-nitroso products in acidic aqueous media, although the extent to which this occurs in actual therapeutic use is unclear.^{431–433} Some evidence suggests that nitrosation of therapeutic agents can occur in clinical practice. For instance, piperazine, a cyclic secondary amine widely used as an antihelminthic drug, has been shown to form small quantities of N-mononitrosopiperazine in the human stomach measured by gas chromatography-thermal energy analysis.⁴³⁴

The possibility of nitrosation is not usually taken into account in the testing of carcinogenic potential of novel drugs as bioassays are usually only performed with parent compound. However, concerns about nitrosation have arisen in subsequent clinical practice. An example of this was the proposal that a few gastric cancers found in patients while being treated with the histamine H₂ receptor antagonist cimetidine were the result of treatment.^{435–437} It now seems likely that all those observed cancers associated with cimetidine were incidental.⁴³⁸ However, at that time concerns were increased by the theoretical possibility that cimetidine has the potential to be nitrosated *in vivo*.⁴³⁵ A further factor was the concept that the treatment-induced gastric secretory inhibition with subsequent bacterial colonization of the stomach rendered the conditions conducive to the generation of N-nitroso compounds from normal dietary constituents.^{436,438} All these concerns appear to be unfounded. Long-term surveillance studies with cimetidine have shown no causal link between its clinical usage and gastric malignancy.^{439,440} In addition, carcinogenicity bioassays performed with cimetidine, cimetidine plus nitrite and nitrosocimetidine have not shown any tumorigenic effect in the gastric mucosa.⁴⁴¹ A seven-year study in dogs in which multiple gastric biopsies were taken at intervals of approximately six months has

also shown no indication of gastric hyperplasia, dysplasia, intestinal metaplasia or neoplastic change.⁴⁴² Although complacency is certainly not warranted with respect to the nitrosation of therapeutic agents *in vivo*, the risks of development of gastric malignancy from such drugs when administered on a short-term basis are probably very small.⁴⁴³ Even for gastric antisecretory agents administered for longer periods of time, the balanced view would also permit development of novel agents provided they are not obviously mutagenic or carcinogenic in the usual preclinical studies and are not particularly liable to undergo rapid nitrosation.

Gastric carcinoma – histopathology

Most carcinomas of the glandular mucosa are adenocarcinomas, whether induced by the potent genotoxic carcinogens or therapeutic agents. They range from those with well-differentiated tubular or papillary features to poorly differentiated forms with trabecular, mucoid or signet ring features.^{67,68,203} Squamous metaplasia within adenocarcinoma can also be observed. Stroma may be abundant with pronounced chronic inflammatory infiltration and hyalinization. Metaplastic cartilage and bone has also been described.^{67,444} Gastric adenocarcinomas induced in dogs by N-methyl-N'-nitro-N-nitrosoguanidine show similar histological features although their reported distribution in the stomach appears more variable than in rodents.⁴⁴⁵

Histological criteria for the diagnosis of invasive adenocarcinoma in experimental animals may vary between individual pathologists. Some retain the old criteria of Stewart and co-workers who defined invasive cancer as a neoplastic growth reaching the *serosa*.⁴⁴⁶ It is now considered more appropriate to apply criteria of use in human diagnostic pathology. Unequivocal invasion of the *submucosa* is sufficient evidence of an invasive and therefore malignant process. However, it is very important to make the distinction between invasive adenocarcinoma from penetrating hyperplastic but non-neoplastic gastric glands that occurs quite commonly in rodents, particularly mice. These hyperplastic lesions do not show dysplastic features: glands are not 'back to back', nuclei are not stratified, the nuclear–cytoplasmic ratio is not overly increased and there is no loss of cellular and nuclear polarity.

SMALL INTESTINE

The small intestine is of major importance in drug safety evaluation for it represents the primary site of drug absorption. In view of its length and the presence of villi, it possesses an enormous surface area of specialized absorptive epithelium. Ingested substances have an extended residence time in this part of the gastrointestinal tract so it is comforting to note that there is a reasonable correlation between gastrointestinal findings in preclinical studies performed in rodent and non-rodent species and gastrointestinal adverse effects in patients.

The dog appears to be a better predictor of adverse gastrointestinal drug effects in patients than rodents.²²⁸ The canine model has been one of the most popular for the study of drug absorption because the dimensions of the canine gastrointestinal tract permit administration of dosage forms intended for clinical use in humans. For this reason factors that influence drug absorption have been better studied in dogs and humans than in many other species.

Residence time is of particular importance for drugs which are incompletely absorbed because differences in mucosal contact time can be expected to result in differences in the fraction absorbed. Dressman has shown using the Heidelberg capsule technique that small intestine transit time in dogs varies between 15 and over 200 minutes whereas in humans equivalent times are between 180 and 300 minutes.²³³ These results suggest that absorption of poorly absorbable drugs is likely to be quantitatively less although more variable in dogs than in people. However, these differences do not explain why some poorly lipophilic drugs such as chlorothiazine, acyclovir and phosphalinic acid are more extensively absorbed in dogs than in humans.

Intestinal pH is consistently higher in dogs than in humans so that drugs with half maximal absorption pH in the range pH 5 to 7 may also be expected to be absorbed at different rates in humans and dogs.²³³ Physiological and anatomical differences in the small intestine of other test species are also likely to have an impact on drug absorption although many of these factors are still poorly understood. More recent studies have suggested that intestinal transit time and intestinal pH in the minipig is closer to humans than other laboratory animal species.⁴⁴⁷

In addition to the small intestine acting as an absorptive surface, it plays an important part in the metabolism of drugs.⁴⁴⁸ Although monooxygenase activity is relatively low in the gut compared with the liver, conjugation mechanisms are efficient and activity of UDP-glucuronosyltransferase and glutathione-S-transferase are as high as or even higher than in the liver.⁴⁴⁹ In addition, the gastrointestinal microflora not only possesses metabolic capacity itself but also can influence the turnover rate of mucosal cells and subsequent exfoliation and release of enzymes into the lumen.

One study in untreated rats has shown that the concentration of total cytochrome P450 in small intestinal microsomes is about 10% of that found in liver microsomes.⁴⁵⁰ There appears to be differences between the expression of different subfamilies between P450 enzymes expressed in the liver and intestine as well as significant species differences. In mice *CYP3A* appears to be the principal subfamily present, similar to the human small intestine.⁴⁵¹ Likewise in the beagle dog, *CYP3A* is the most abundant P450 expressed in small intestine predominantly in mature epithelial cells in the upper villus mostly in the duodenum and jejunum but less in the terminal ileum.⁴⁵² Gene expression data from the rat suggest that there is strong expression of *CYP2C* in this species.⁴⁵³ It has been demonstrated in the rat that the concentration of cytochrome P450 and drug metabolizing enzyme activity also increases in intestinal epithelial cells as they move from crypt to villous tips.^{450,454}

As in the liver, the activity of cytochrome P450 can be induced or inhibited by drugs.^{451,452} The phenobarbitone-inducible form of cytochrome P450 which normally represents less than 5% of total P450 in the small bowel can be increased to about 50% of total cytochrome P450 in small intestine cells with phenobarbitone treatment. Furthermore, it has been shown that drug metabolizing activity in the tips of the villi in the duodenum is greater in rats fed a conventional diet than a semi-synthetic diet and that the activity depends critically on the absorption of iron from the intestine.⁴⁵⁴

Glutathione is also present throughout the entire mucosa. In rats, cells at the tips of the villi contain less glutathione than cells located more basally, whereas related enzymes γ -glutamyl transpeptidase and glutathione-S-transferase show highest activity in the

villous tip region.⁴⁵⁵ The fact that these enzyme activities are highest in the duodenum and lowest in the terminal ileum suggests that detoxification systems for exogenous compounds are greater in the proximal small intestine.

Structural and histochemical characteristics

The small intestinal mucosa is constructed to act not only as an absorptive surface but also as a barrier to potentially pathogenic substances and microorganisms. Although the main cell population of the epithelium is composed of absorptive cells, other major epithelial cell types, the mucous (goblet) cells, Paneth cells and endocrine cells have important protective functions. In addition, specialized epithelial cells, the microfold (membranous or M) cells, are located in the epithelium over Peyer's patches. These cells form part of the other important protective system of the intestine, the gut associated lymphoid tissue (GALT) or mucosal associated lymphoid tissue (MALT).

The mucosal lining is in a constant state of renewal. Enteric epithelium possesses the fastest rate of turnover of any tissue exceeded only by a few rapidly growing neoplasms.^{456,457} In normal circumstances, the constant turnover of small bowel mucosa is maintained by equilibrium between cell production in the crypts and cell loss at the tips of the villi. Exogenous substances, intraluminal secretions, mechanical and neural factors as well as alterations in blood flow all possess potential to influence mucosal cell kinetics.^{456,457}

All main epithelial cell types are believed to arise from undifferentiated columnar cells at the crypt base although mucous cells may also arise by proliferation of partly differentiated mucous cells in the crypts.⁴⁵⁸ The cells in the crypts have high activities of enzymes such as thymidine kinase that are involved in nucleic acid synthesis.⁴⁵⁹ The complete cell cycle lasts about 10 to 17 hours in rodents and at least 24 hours in humans. Enteric epithelium is completely replaced within two to three days in mice and rats and within three to six days in humans.^{456,457} After two or more divisions in the crypt cells migrate to the villus, lose ability to incorporate thymidine and differentiate into mature cells equipped with enzymes associated with nutrient absorption.⁴⁵⁹ Cell migration in the rat is completed more rapidly in the ileum than in the jejunum principally as a result of the lower villous height in the ileum.⁴⁶⁰ Migration terminates by loss of cells from the tip of the villi. Surrounding the crypt is a sheath of fibroblastic cells. These cells also undergo synchronous division and migration with the epithelial cells, maintaining the intimate relationship between the epithelium and supporting tissues.⁴⁶¹

Mature absorptive cells are important in the active and passive transport of nutrients as well as in the endocytosis of macromolecules. They are characterized by the presence of a striated or brush border which is seen in hematoxylin and eosin-stained sections as a refractive bi-laminar band. The inner, wider lamina corresponds to the microvillous region that is associated with the presence of neutral mucins in most species. The outer, thinner band corresponds to the glycocalyx, which is composed principally of acidic mucosubstances.²⁵⁶ This outer band of the brush border shows histochemical staining predominantly for sulfomucins in most species including mouse, hamster, dog and rhesus monkey, although in the duodenum of the rats and in the entire human small bowel sialomucins predominate in this layer. Electron microscopy of the absorptive cells shows that the surface of absorptive cells is covered by tightly packed and well-developed microvilli

approximately 1 μm long and 0.1 μm wide. These are considered the first site of entry of food substances into the cell.

An important aspect of the absorptive cell membrane is its high concentration of disaccharidases such as sucrase, maltase and lactase, related to the absorption of sugars. Alkaline phosphatase activity is also abundant on the surface of absorptive cells. Immunocytochemical demonstration of alkaline phosphatase can be used as a tool to examine the effects of xenobiotics on intrinsic membrane glycoproteins in the small intestine.⁴⁶² Enterokinase, the glycoprotein enzyme, which initiates the activation of pancreatic zymogens by converting trypsinogen to trypsin, is also present in the brush border and glycocalyx of the small intestinal epithelium, both in humans and animals. Immunocytochemical studies have demonstrated that in humans this enzyme is located in the duodenum and proximal jejunum but not ileum, colon and stomach.⁴⁶³

Goblet cells are much fewer in number than absorptive cells in the small intestine but they increase in number from the duodenum to the lower ileum. They are important in the production of mucus, which remains on the surface of the mucosa as a viscous layer and acts as the first line of defense against intestinal pathogens. Goblet cells are characterized by the presence of abundant mucous droplets formed by the Golgi complex which accumulate in the apical part of the cell cytoplasm. Histochemical study shows that neutral mucins are present in the goblet cells found in crypts and on the villi in the entire small bowel mucosa of most species including humans but there is an interspecies variation in the population of sialo- and sulfomucins.²⁵⁶ In the mouse, sulfomucins predominate but among rats considerable individual variation in the proportion of sialo- and sulfomucins is reported. In the hamster, sulfomucins are more prominent in the proximal and sialomucins in the distal small bowel. In the dog, both sulfomucins and sialomucins are found with predominance of one or other in individual animals. Staining for acidic mucins is less intense in the goblet cells of the small intestine in humans compared with non-human primates but sialomucins are predominant in both species.

Paneth cells are located near the crypt base throughout the small intestine in rodents and humans but typically not in carnivores such as dog and cat.^{464–466} They are characterized by the presence of numerous eosinophilic cytoplasmic secretory granules between about 1.0 and 2 μm diameter that contain various enzymes and mucosubstances. Particular care is needed in fixation and staining for optimal demonstration of Paneth cells for they rapidly degranulate after death and granules are destroyed by acetic acid fixation. Formalin and mercuric fixatives appear appropriate methods and they permit staining with methylene blue, Lendrum's phloxine-tartrazine and Masson's trichrome.⁴⁶⁷ The apical parts of Paneth cells show glucose-6-phosphatase, carbonic anhydrase and monoamine oxidase activity and they have been shown to contain lysozyme and immunoglobulins, particularly IgA.^{465,468–470} The Paneth cell granules also contain antimicrobial peptides such as secretory phospholipid A₂, α defensins also called cryptins. These are believed to possess not only antimicrobial activity but are also important in the regulation of cell volume, chemotaxis, mitogenesis and inhibition of natural killer cell activity.^{471–474} Studies of transgenic and knockout mice have supported a pivotal role of Paneth cell defensins in protection from oral bacterial pathogens. Other observations suggest that Paneth cell dysfunction may contribute to the clinical manifestations of Crohn's disease and necrotizing enterocolitis in humans.⁴⁷⁴

Endocrine cells are also scattered throughout the small intestinal mucosa. They are of both argentaffin and argyrophil types and are situated predominantly in crypts. Immunocytochemical study shows that they contain a variety of different peptides although gastrin, secretion and serotonin-containing cells have been those most extensively studied.⁴⁷⁵

In addition to the barrier formed by mucus and epithelial cells, lymphocytes, plasma cells, macrophages, dendritic cells and mast cells also form part of the protective function of the small intestine. Some lymphocytes are located within the epithelium mostly above the basal lamina but below epithelial nuclei.⁴⁷⁶ These lymphocytes are termed intraepithelial lymphocytes and are predominantly of T suppresser/cytotoxic type in humans and laboratory animals.^{476,477} Most lymphocytes in the lamina propria are also T cells but T helper (CD4 positive) cells outnumber the T suppresser/cytotoxic (CD8 positive) phenotype.^{476,478,479} Many plasma cells present in the lamina propria produce IgA, the major immunoglobulin of mucosal secretions representing another important component of the mucosal barrier.^{480,481} Morphometric analysis of IgA-containing immunocytes in the rat ileal mucosa using immunocytochemical staining has shown that the number of these cells varies with alterations in the microbiological status of intestinal contents.⁴⁸²

Peyer's patches are prominent aggregates of lymphoid tissue in the gastrointestinal tract and constitute important sites at which antigens from the gut lumen encounter immune competent cells which are responsible for the initiation of immune responses.⁴⁸³ Peyer's patches are located on the ante-mesenteric wall of the small bowel and consist principally of collections of lymphoid follicles. In humans, Peyer's patches are more common in the ileum but in mice they are more uniformly distributed.^{484,485} In rats the number of patches is more common distally.⁴⁸⁶ The number of follicles within individual patches usually varies from two to six but there are strain differences. They may show considerable variation in size between individual animals.⁴⁸⁷ A comparative study showed that in Fischer 344 rats they are smaller than those in Wistar rats.⁴⁷⁹ Particular care in selection and orientation of tissue blocks is therefore essential for any form of critical assessment of Peyer's patches. In Peyer's patches lymphoid follicles are surrounded by a corona of small lymphocytes principally of B cell type. The interfollicular area contains post-capillary venules and T lymphocytes.^{486,488,489}

The epithelium overlying the Peyer's patch follicles (dome area) contains specialized epithelial cells, called microfold, membranous or simply M cells. These cells have been identified in many species including rats, mice, hamsters, dogs, monkeys and humans.^{490,491} These cells differ functionally from other enterocytes by their ability to transport large molecules such as ferritin and horseradish peroxidase and particulate matter from the lumen to the underlying lymphoid tissue.^{492,493} They have also been shown to be the site of penetration of reoviruses into the epithelium and they can transport *Vibrio cholerae* and other organisms.^{491,494} M cells therefore form points in the intestinal wall which transport intact antigen and macromolecules to the follicles where they can be processed and be transported to lymph nodes with consequent IgA immune responses. This contrasts with the uptake of soluble antigens, which can be taken up by ordinary epithelial cells and transported in the circulation of the villi to be ultimately trapped in the spleen, possibly to evoke an IgM/IgG response.⁴⁹³ Understanding of these cellular and molecular characteristics is critical for the design of mucosal vaccines for pathogens that exploit this pathway.⁴⁹⁵

Mucosal mast cells also appear to be involved in the immunological defense of the gastrointestinal tract. They respond by proliferation, migration and discharge of granules during nematode infestations.⁴⁹⁶ It has been shown in the rat that mucosal mast cells of the gut differ in several ways from connective tissue mast cells. These differences result in poor preservation of mast cells of the gut if the usual metachromatic staining techniques are employed for the demonstration of mast cells in tissue sections.⁴⁹⁷ Histochemical study suggests that mucosal mast cells differ from connective tissue mast cells by a lower degree of sulfation of glycosaminoglycans and different spatial relationships of protein and glycosaminoglycans in their granules. These cross-link following formalin fixation to prohibit cationic dye binding and prevent histological staining. However, staining can be achieved in tissues fixed in formalin by prolonged incubation in toluidine blue (5–7 days), a procedure which allows adequate penetration of the toluidine blue molecule.⁴⁹⁷

Histological techniques

Optimal histopathological study of the small intestine is complicated by its length and mucosal fragility. It is important to avoid vigorous washing procedures or any form of excessive manipulation of the unfixed bowel, as artifacts caused by washing may confound interpretation of changes induced by xenobiotics. Combination of artifacts due to washing, autolysis and the presence of neutrophils can produce a histological appearance that mimics *in vivo* damage.

Although careful visual inspection of the intestine and sampling of appropriate segments for histological examination is usually sufficient for routine examination, various forms of *Swiss roll* techniques are helpful for more complete study, not only for the mucosa but also for detailed examination of the associated lymphoid tissue.^{487,498} Rolling the unfixed, opened rodent intestine around a wooden stick prior to freezing or fixation is one proposed method, although this method risks undue manipulation of the unfixed tissue.⁴⁹⁹ Another more versatile technique applicable to rodent, large animal and human intestine can be performed after fixation. The unfixed opened bowel is pinned flat on a cork or board and fixed in a bath of formal saline. After fixation, the full thickness of rodent intestine can be rolled, transfixed by a pin and embedded in paraffin wax. Likewise the mucosa of the intestine of large animal species or humans can be rolled after fixation by separating it from the muscularis externa.⁵⁰⁰

Non-neoplastic lesions

Inflammation and ulceration of the small intestine (duodenitis, jejunitis, ileitis)

Inflammation and ulceration of the mucosa occurs as a result of stress, infection with bacteria, viruses, and infestation by parasites, as a direct result of the effects of xenobiotics or ionizing radiation. Erosions, ulcers and strictures have been associated with a number of therapeutic agents in people.¹⁷⁰ Antimitotic or radiomimetic agents as well as ionizing radiation are liable to adversely affect the rapidly dividing cells of the small intestine with resulting breakdown of the mucosal barrier. Newer targeted anticancer drugs have also been associated with intestinal damage.³ The ulcerogenic activity of non-steroidal anti-inflammatory drugs is also expressed in the small bowel mucosa, most commonly in

the distal ileum. Moreover, different agents also act in synergy to enhance damage to the small bowel mucosa. Important examples are drugs that depress the immune system or antimicrobial agents that permit the development of pathological infections by microorganisms of the opportunistic type in the small intestine. Vasoconstriction may also result in intestinal ulceration. For example, life-threatening gastrointestinal ulceration is reported as a result of cocaine abuse. Although gastrointestinal complications are less common than cardiac damage, cocaine abuse may induce mesenteric ischemia as a result of vasoconstriction leading to gastric or intestinal ulceration and perforation of the entire wall.^{501,502} It appears that the abuse of the free base form of cocaine (crack) is more liable to produce more upper gastrointestinal intestinal damage than oral cocaine abuse which tends to induce more distal intestinal perforation. Although the exact mechanism leading to intestinal ischemia is not certain, cocaine blocks the reuptake of norepinephrine (noradrenalin) which is believed to be the cause of mesenteric vasoconstriction and ischemia.

The histological features of the inflammatory process in the small intestine are not often specific for a particular agent. It is important to search for evidence of microbiological organisms and viral inclusions which can indicate the cause of intestinal inflammation and ulceration. Associated features in non-ulcerated mucosa such as morphology of the villi, accumulation of abnormal cells, presence of drug crystals of debris and changes in lymphoid cells or blood vessels are also important in the assessment of these changes.

Infections and infestations

A number of organisms including those which are normal residents of the gastrointestinal tract can cause inflammatory changes in the intestinal mucosa of laboratory animals. With the notable exception of non-human primates, inflammatory bowel disease caused by microbiological organisms is not usually evident or of concern in most toxicity or carcinogenicity studies. However, when animals are treated with antibiotics, immunosuppressive agents or other drugs which alter the normal intestinal flora, conditions may favor the proliferation of potentially pathogenic organisms in sufficient quantities to cause overt damage to the mucosa.

In non-human primate colonies, gastrointestinal disease remains one of the important health problems such that many indoor-housed monkeys commonly have histological evidence of intestinal inflammation, notably micro-abscesses and erosions rather than overt ulcers.^{11,129,503} Although the majority of potentially pathogenic organisms affect the primate colon, a number of bacteria, protozoa and metazoan, occur in the small intestine.⁵⁰⁴ A useful aid for identification of metazoa in histological sections remains the publication of Chitwood and Lichtenfels.⁵⁰⁵

BACTERIAL INFECTIONS

Bacillus piliformis, the agent responsible for Tyzzer's disease, produces intestinal inflammation and ulcers in rats, mice and hamsters. Susceptibility of different species and strains to experimental infection with *Bacillus piliformis* is variable. For instance, C57BL, BALB mice and Fischer 344 rats appear more resistant to infection than outbred Syrian hamsters.⁵⁰⁶ Lesions of variable severity usually occur in the ileum but may also extend into the cecum and colon. Severe infections are characterized histologically by ulceration of the mucosa, edema and acute inflammation of the submucosa and muscle coats. Muscle may

also show focal necrosis. Non-ulcerated mucosa is typically infiltrated by polymorphonuclear cells and crypt abscesses form. There is blunting and fusion of villi and reactive hyperplasia and mucin depletion of the overlying epithelium.⁵⁰⁷ Mucosal lymphoid tissue may also show reactive changes or hyperplasia. Filamentous bundles of *Bacillus piliformis* can usually be found in the cytoplasm of both epithelial cells and smooth muscle cells at the edges of necrotic zones. Methylene blue, Giemsa or silver impregnation techniques such as Warthin-Starry or Levaditi stains are the best stains for the demonstration of these organisms, although with care they can be visualized in hematoxylin and eosin-stained sections. They are Gram negative and PAS positive.

Intestinal infections due to salmonella species are relatively common in the mouse but also occur in the hamster and rat.⁵⁰⁸ *Salmonella typhimurium* and *Salmonella enteritidis* are regarded as the organisms typical of murine salmonellosis. Lesions occur in the ileum and may extend into the jejunum and cecum. They are characterized by the presence of ulcers covered by fibrinous exudate and associated with diffuse infiltration of the adjacent mucosa by macrophages, neutrophils and lymphocytes. Intact crypt epithelium shows mucin loss and reactive proliferative changes. A characteristic feature is the presence of poorly defined granulomatous lesions composed of macrophages mainly in associated lymphoid tissue or Peyer's patches.

Clostridia species, especially *Clostridia difficile*, which cause pseudomembranous colitis in humans and laboratory animals (especially hamsters) may also produce inflammation and ulceration in the terminal ileum with histological features similar to those found in the colon (see following).

Proliferative ileitis (transmissible ileal hyperplasia) is a striking lesion of hamsters affecting the distal segment of the ileum that is associated with intracellular invasion of the intestine mucosa epithelium by bacteria. The condition is representative of a number of enteric conditions in animals referred to under the general heading of proliferative enteropathy.⁵⁰⁹ The definitive causative agent has not been cultivated and the disease has been associated with various organisms.^{510–512} Although it is characterized by hyperplasia of the ileal mucosa in its early stages, an inflammatory phase intervenes in which there is focal necrosis and hemorrhage of the mucosa, crypt abscesses and infiltration of the lamina propria by acute inflammatory cells and macrophages. The histological features of the associated hyperplasia are characteristic. The mucosa is covered by immature, mucin-depleted pseudostratified hyperchromatic epithelium with mitoses extending to the tips of villi and densely basophilic intracytoplasmic inclusions.

Helicobacter jejuni (*Campylobacter jejuni*) is a common cause of diarrhea in humans and may be the causative agent in small intestinal inflammation in laboratory dogs and primates. *Campylobacter* species may be more prevalent in beagle dogs and primates than is commonly appreciated. It is important to recognize that animals colonized with these agents may be susceptible to stress-induced, acute onset gastroenteritis.^{289,513} In humans this form of bacterial disease is characterized histologically by mucin depletion, flattening and reactive changes in the small bowel epithelium, crypt abscesses, edema and infiltration of the mucosa by neutrophils, lymphocytes and plasma cells. Similar histological findings have been reported in dogs infected with this organism.⁵¹⁴ The organisms are Gram-negative curved, slender rods, which can be most easily visualized in tissue sections with the Warthin-Starry stain, a recognized technique for spiral bacteria. The carbol

fuchsin technique of Gimenez first used for the identification of *Rickettsiae* in yolk sac culture and a cresyl fast violet technique is also a useful method for the identification of *Campylobacter* species in paraffin sections.^{515–517}

PROTOZOAN PARASITES

Spiroucleus muris (*Hexamitis muris*) is also a cause of inflammation in the small bowel of rats, mice and hamsters. During overt infestation, organisms are seen extracellularly in crypts and intervillous spaces associated with blunting of intestinal villi, epithelial degeneration and mucin depletion, reactive epithelial hyperplasia, edema and leukocyte infiltration.^{518,519}

Giardia species represent marginally pathogenic flagellates, which are found in the upper gastrointestinal tract. They are opportunistic agents that can become important in both animals and humans with depressed immune function. Studies in mice infected experimentally with *Giardia muris* have shown that an early response is an increased infiltration of the epithelium by lymphocytes, predominantly T cells.⁵²⁰ Depression of the immune response by treatment with corticosteroids has been shown not only to increase parasite numbers in murine giardiasis but also to cause recrudescence of occult infections.⁵²¹ *Giardia muris* (*Lamblia muris*) is sometimes found in the small intestine of rat, mouse and hamster (Figure 8.6). Trophozoites appear in histological sections as crescent-shaped or kite-like structures on the brush border of the intestinal mucosa or in the

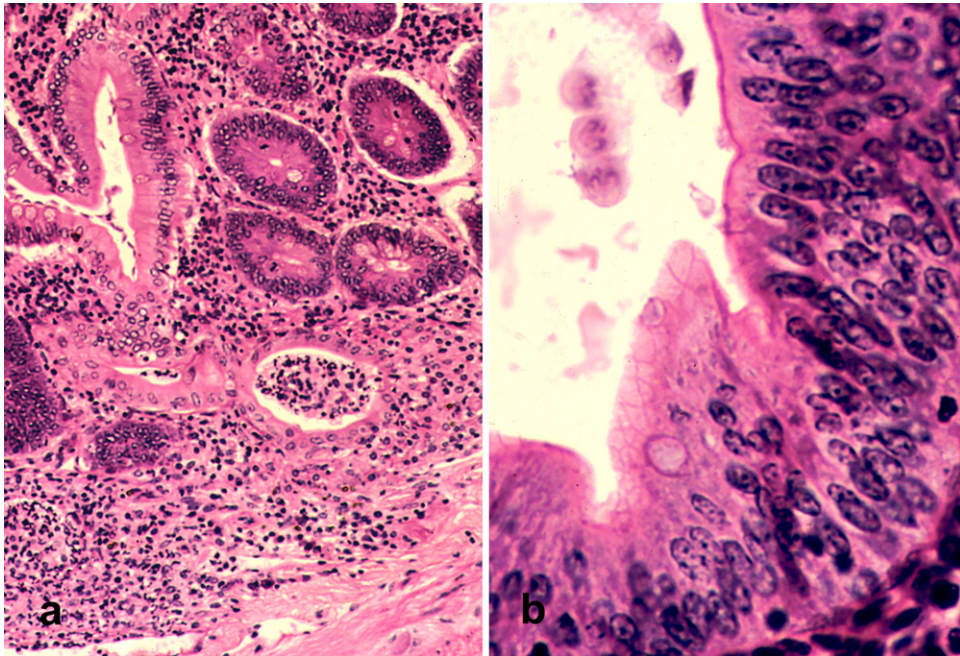


FIGURE 8.6 Duodenal mucosa of an untreated hamster infested with *Giardia muris*. Panel a: Inflammatory infiltrate and a crypt abscess can be seen along with reactive hyperplasia of the luminal epithelium (H&E×110). Panel b: Several kite-shaped organisms can be seen in the lumen just above the epithelial surface (H&E×425).

adjacent lumen. Mucosal lesions may be totally absent or there may be blunting of villi, reactive epithelial hyperplasia. A typical feature is increased infiltration of the epithelium and lamina propria by mononuclear cells. Another finding is that lactase, sucrase and maltase levels decrease in the small intestine in mice infested with *Giardia muris*.⁵²⁰

Giardia lamblia may colonize the small intestine of monkeys and humans and produce similar morphological appearances to those found with infestations in rodents by *Giardia muris*. Other flagellates such as *Tritrichomonas muris* are also found in the small intestine of mice, rats and hamsters.

The coccidian protozoan parasite cryptosporidium represents a striking example of the close relationship between some human and animal diseases. This organism was first recognized in the gastric glands of mice by Tyzzer in 1907 and has since been confirmed as a cause of diarrhea in animals and as a human pathogen. It causes mild diarrhea in normal subjects especially children and young adults but it can produce severe intestinal disease in immunocompromised individuals.⁵²² Histological examination of the small intestine of laboratory animals infested by cryptosporidium reveals the presence of organisms attached to the mucosal surface, often associated, as in humans, with other parasites or infections. They are rounded, weakly basophilic structures 1–4 μm diameter in hematoxylin and eosin-stained sections but are strongly basophilic following Romanowsky staining. Transmission electron microscopy reveals the detailed internal structure of cryptosporidium attached to the microvillous surface of the epithelial cells. The various stages in the life cycle have been visualized by light and electron microscopy. Infection starts with ingestion of an oocyst containing four sporozoites, which are probably released by the action of digestive enzymes. These attach themselves to the intestinal mucosa and undertake their life cycle attached to the epithelial cells. These organisms have been demonstrated in most laboratory species including mice, hamsters, rabbits, dogs and monkeys.^{504,523–526}

METAZOA

Hymenolepis nana (dwarf tapeworm) and *Hymenolepis diminuta* (rat tapeworm) are described in the intestine of rats, mice, hamsters, non-human primates and humans.⁵²⁷ A variety of other metazoan patients are found in the small intestine of non-human primates.⁵⁰⁴

VIRUSES

A number of viruses produce inflammatory small bowel changes in mice. For example, mouse hepatitis virus (lethal intestinal virus of infant mice) can cause mucosal epithelial necrosis and inflammation with characteristic compensatory epithelial hyperplasia and the formation of epithelial syncytia.⁵²⁸ Murine rotavirus (epidemic diarrhea of infant mice) produces swollen enterocytes of small and large bowel with fine cytoplasmic vesiculation with little or no inflammation but dilated lymphatics and vascular congestion. Cytoplasmic acidophilic inclusions, 1–4 μm diameter, are characteristic findings.⁵²⁹

A variety of viruses have been isolated from the gastrointestinal tract of non-human primates including viruses of humans, although overt pathology is not usually seen.⁵³⁰ However, goblet cell hyperplasia, villous blunting, and enteritis are reported in the gastrointestinal tract of monkeys infected with simian type D retrovirus.⁵³¹ Viral antigen can also be demonstrated in tissues throughout the gastrointestinal tract and as lesions can be

observed in the absence of any other detectable enteric pathogens a primary pathogenic role for simian type D retrovirus in gastrointestinal tract has been proposed.

Drug-induced inflammation and ulceration

Many of the drugs that are associated with intestinal damage in humans, particularly non-steroidal inflammatory agents and anticancer drugs, also produce similar effects in laboratory animals. As in other parts of the gastrointestinal tract histopathological changes are usually fairly non-specific being characterized by loss of mucosa and submucosa, acute or chronic inflammation and a variable fibroblastic response. The reactive responses of adjacent epithelial cells may be intense and in some cases may even mimic pre-neoplastic alterations.

Non-steroidal anti-inflammatory agents such as indomethacin and phenylbutazone not only produce gastric ulceration but also penetrate ulcers of the small bowel of both patients and laboratory animals.^{170,272,301} The newer inhibitors of cyclooxygenase 2 (COX-2) have also shown a tendency to produce penetrating ulcers of the distal small intestine rather than gastric damage in toxicity studies.^{315,316} Imaging studies with ¹¹¹indium-labeled leukocytes in humans have also suggested that subclinical intestinal inflammation is associated with long-term therapy with non-steroidal anti-inflammatory drugs.⁵³² It has been postulated that indomethacin-induced intestinal ulcers in rats and dogs may be produced by a prostaglandin-independent mechanism, different from the manner in which gastric ulceration is induced.^{533,534} It has been argued that damage may be related to concomitant effects of contact irritancy of drug and bile.

In the rat similar mechanisms may be responsible for both gastric and intestinal ulceration because dosing regimen can influence the distribution of ulceration and a good temporal correlation between the development of intestinal ulcers and inhibition of prostaglandin synthesis has been demonstrated.³⁰⁵ It has also been shown that potent intestinal ulcerogens such as indomethacin inhibit the incorporation of radioactive ³⁵S sulfate into glycoproteins of the stomach and upper intestinal mucosa of rats which may decrease the capacity of the mucus in the intestine to act as a buffer for hydrogen ions.⁵³⁵ The mechanisms are undoubtedly complex and multifactorial and may include not only prostaglandin suppression and local irritancy but also the effects of drug disposition.

Single dose studies with indomethacin and ibuprofen in rats have demonstrated differences in pathological expression of the induced damage between the stomach and small intestine.³⁰³ Gastric damage was superficial, occurred within six hours and was fully repaired two weeks after dosing. Ulcers in the jejunum and ileum reached a maximum area at 48–72 hours after dosing, occurred on the mesenteric border, penetrated the muscularis mucosa and were accompanied by inflammation and edema. Ulcers were still present two weeks later. In dogs, indomethacin given orally in doses of 2.5 mg/kg/day for one to 23 days was also shown to produce deep, punched-out ulcers in the small intestine, many of which were situated over Peyer's patches.⁵³⁶ Some ulcers involved the whole circumference of the small intestine wall. Histologically, the ulcers were associated with an intense inflammatory response principally of mononuclear cells, which infiltrated the bowel wall down to the serosa, particularly adjacent to Peyer's patches. It was suggested that this distribution of ulcers was a result of an exaggerated immune response to normal intestinal antigens following depression of prostaglandin synthetase by indomethacin.

Special dye techniques, scanning and transmission electron microscopy have also shown that non-steroidal anti-inflammatory drugs produce more generalized but mild morphological effects on the small intestine mucosa without overt erosions or ulcerations being evident by light microscopy. Following administration of aspirin to mice for several weeks, shortening and erosion of microvilli and increased numbers of goblet cells were demonstrated in the duodenum and jejunum by scanning and transmission electron microscopy.^{537,538} Morphometric studies of the intestinal mucosa of indomethacin-treated mice have also shown widespread alterations to columnar cells, goblet cells and Paneth cells suggesting generalized effects on mitotic activity and crypt loss.⁵³⁹

Although non-steroidal, anti-inflammatory drugs are the best-known drugs with adverse effects on gastrointestinal mucosa, small intestinal inflammation and ulceration can also be produced by other drugs through different mechanisms. Antimitotic anticancer drugs, high doses of antiviral drugs that depress cell proliferation and immunosuppressive agents may also produce intestinal mucosal apoptosis, necrosis, hemorrhage, inflammation and opportunistic gastrointestinal overgrowth when administered to dogs or rodents in high doses (Figure 8.7).^{540–545} Drugs include the older anticancer drugs of diverse structural and pharmacological types as well as new antimitotic agents and so-called ‘targeted’ anticancer therapies such as those acting on vascular endothelial growth factor or microtubules.³ Acute disruption of the upper small intestinal mucosa is considered to be an important factor in the development of nausea and vomiting during

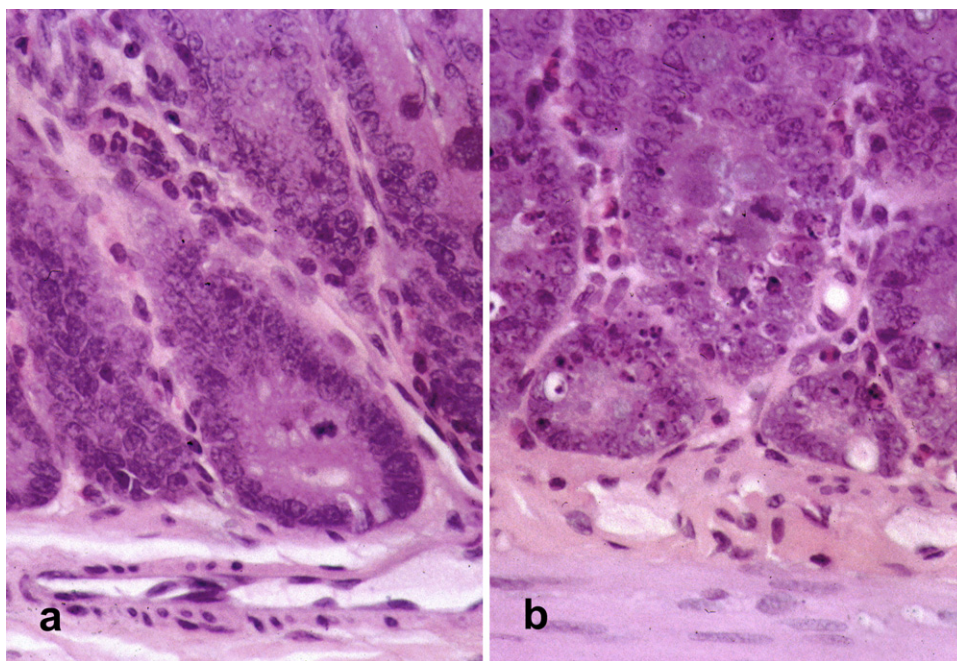


FIGURE 8.7 *Panel a:* Small intestinal mucosa from an untreated rat. *Panel b:* Small intestinal mucosa from a rat treated with a single dose of the antiproliferative cancer drug ET-743 12 hours previously. By three days the changes had disappeared (both panels H&E×425).

anticancer therapy. Chemotherapy is believed to induce free radicals formation leading to localized exocytotic release of 5-hydroxytryptamine from the enterochromaffin cells which interacts with 5-hydroxytryptamine₃ receptors on vagal afferent terminals in the wall of the bowel. These link to the dorsal brain stem and the area postrema to produce nausea and vomiting.⁵⁴⁶

Particularly well studied in human patients and laboratory animals have been the effects of methotrexate on the small intestinal mucosa.^{547,548} It induces loss of crypts, crypt and villus atrophy and flattening of epithelial cells as a consequence of apoptosis and inhibition of cell proliferation. However, treatment seems to spare goblet cells and Paneth cells as well as the epithelium associated with Peyer's patches.⁵⁴⁹

Colchicine and the anticancer drug taxol (paclitaxel), which interfere with tubulin and inhibit its polymerization into microtubules, have also been reported to produce intestinal damage.^{362–364} This effect produces mitotic arrest characterized by large pleomorphic nuclei, prominent ring mitoses associated with epithelial pseudostratification, loss of polarity and increased apoptosis, features that may mimic true premalignant changes (dysplasia). Similar features also occur in laboratory animals (Figure 8.3b).^{363,409,410}

Antiviral drugs may also show radiomimetic effects on the gastrointestinal mucosa such as seen in dogs treated with high doses of acyclovir.¹⁷⁶ There are also reports of experimental drugs of other therapeutic classes also producing intestinal damage as a result of their generalized antimetabolic effects.⁵⁵⁰

The small intestine is very radiosensitive and changes are seen in patients after ionizing radiation therapy. The terminal ileum appears to be more sensitive by virtue of its being a more fixed structure than the other parts of the intestine.⁵⁵¹ Three stages of damage are described. There is an acute phase characterized by hyperemia, edema and inflammation, crypt abscesses and ulceration, a subacute phase two to 12 months after radiation where there is partial tissue repair but with varying degrees of arteriolar sclerosis and a chronic phase where progressive fibrosis occurs.^{552–554} Whereas the acute epithelial alterations resemble changes following administration of antiproliferative anticancer drugs, ionizing radiation is typically associated with a fibrous thickening of blood vessel walls, not normally seen after drug therapy.

Lymphoid infiltrates without tissue damage were also reported in the small intestine of rats treated with human recombinant interleukin 2 as part of a multisystem involvement.³¹⁹

Other agents of general toxicological interest are cysteamine, propionitrile and their structural analogs as well as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which are capable of producing ulcers of chronic type in the duodenum of rats and mice.⁵⁵⁵ These compounds vary in their ulcerogenic capacity but they are all able to produce ulcers of chronic type with crater formation, granulation tissue and reactive changes in adjacent mucosa in the anterior and posterior wall of the proximal segment of the duodenum of rodents. Although these different agents influence gastric acid secretion in different ways, structure–activity relationships suggest that they produce duodenal dysmotility, decrease bicarbonate production and reduce its delivery from the distal to proximal duodenum. These factors decrease the neutralization of gastric acid in the first part of the duodenum which may contribute to the development of ulceration.⁵⁵⁵ Furthermore, these effects can be attenuated or prevented by dopamine agonists or their precursors whereas dopamine

antagonists can potentiate their effects suggesting dopamine-mediated actions of these agents may be involved in the pathogenesis of duodenal ulceration.^{556,557}

Fatty change (lipidosis)

Using appropriate fixation and staining procedures, fine granular lipid droplets can be visualized in the apical parts of epithelial cells covering the upper third of normal small intestinal villi. Administration of drugs and chemicals may produce an excessive accumulation of lipid through specific effects on lipid metabolism or as part of general cellular toxicity.

For example, in the preclinical toxicity studies with 2,6-di-tert-butylamino-3-acetyl-4-methylpyridine (Sa H51-055), an inhibitor of glucose transport intended for use as an anti-obesity drug, lipid accumulation occurred in the lamina propria of the small intestinal villi of Sprague-Dawley rats and guinea pigs but not in dogs or primates.⁵⁵⁸ After administration of this agent to rats, there was progressive accumulation of lipid droplets in the epithelial cells over the tips of the duodenal villi demonstrable by osmium tetroxide staining. Electron microscopic examination revealed uniform electron-lucent droplets within profiles of the smooth endoplasmic reticulum and Golgi apparatus. Lipid droplets increased with time and accumulated to form large droplets in the lamina propria. Larger droplets were phagocytosed by macrophages in the lamina propria but there was no evidence of epithelial damage or necrosis. Changes were most pronounced in the duodenum but were also noted to a lesser extent in jejunum and ileum but not in colon or stomach. Sequential studies using electron microscopy showed that lipid rapidly accumulated within several hours in the profiles of smooth endoplasmic reticulum and Golgi apparatus of the epithelial cells and formed droplets or chylomicra in the intercellular space. The absence of any other subcellular changes or evidence of derangement of protein synthesis suggested that Sa H51-055 altered the pathways of lipid resynthesis or transport. This was consistent with the distribution of the lipid in the upper third of the jejunal villous epithelium, a zone reported to be most active in lipid absorption, resynthesis and transport.⁵⁵⁹ It was suggested that fatty change might have taken place because of alterations in the sugar moiety of chylomicra brought about by interference with glucose transport.

Lipid droplets which stained with oil-red-O in formalin-fixed frozen sections and showed uniform electron density under the electron microscope, characteristic of neutral lipid, were also observed in the epithelial cells and macrophages in the lamina propria of jejunum and duodenum and mesenteric lymph nodes in rats given a synthetic 2'-dodecyl glutaramide ester of erythromycin.⁵⁶⁰ Unlike the erythromycin base, rats poorly tolerated this ester. It appeared that the ester was absorbed unhydrolyzed and converted to chylomicron-like droplets, which then accumulated in the macrophages of the lamina propria and local mesenteric lymph nodes, without overt damage to epithelial cells.

Accumulation of lipid in epithelial cells of intestinal villi has been observed in rats following administration of puromycin and ethionine.^{561,562} Both agents have inhibitory effects on protein synthesis. Detailed morphological studies of the intestinal epithelial cell in rats treated with puromycin have shown that there is concomitant accumulation of lipid with a decrease in the quantity of rough endoplasmic reticulum and Golgi membranes.⁵⁶¹ These changes were in keeping with the concept that lipid accumulates as a result of

inhibition of the synthesis of membrane components of the Golgi by the rough endoplasmic reticulum, thus compromising transport of lipid.

In addition to lipid droplets forming as a result of altered lipid metabolism, they may form in the epithelial cells of the intestinal mucosa as a result of a direct toxic effect of the ingested drugs on the small intestinal mucosa. In such instances atrophy of villi and degenerative changes in the epithelial cells are typically observed (see following).⁵⁶³

Phospholipidosis (myelin figures, myeloid bodies, myelinoid bodies)

The small intestinal mucosa is also one of the many sites at which systemic drug-induced accumulation of polar lipids form laminated structures (myeloid bodies) or crystalloid structures within lysosomes. This form of lipid storage disorder is produced by diverse amphiphilic cationic drugs in laboratory animals and occasionally in patients usually as a result of drug interaction with polar lipids rendering them difficult to digest (see Respiratory Tract, Chapter 6). Species differences in susceptibility and tissue distribution of phospholipid are probably of diverse origin. They are related not only to physicochemical characteristics of the inducing drugs which influence their ability to permeate selective biomembranes and react with different lipids but also to local tissue concentrations of drugs and the ability of particular organs to metabolize parent drug to less amphiphilic products. In general terms, this disorder is characterized by membrane-bound, acid phosphatase-positive cytoplasmic inclusions, which on ultrastructural study are seen as lamellar crystalloid structures in lysosomes. These appearances are characteristically reversible on cessation of treatment with the inciting agent.

Chloroquine was noted to produce an accumulation of foam cells in the stroma of small intestinal villi in rats treated for two years as part of generalized phospholipid accumulation back in 1948.⁵⁶⁴ Another example of this phenomenon occurring in the small intestine is also provided by the iodinated amphiphilic drug amiodarone, which has been used clinically for the past 20 years in the treatment of angina and more recently in the control of supraventricular cardiac arrhythmias. Although its adverse effects in patients have been linked to accumulation of drug in lysosomes, particularly in liver, skin and eye, toxicity does not appear to be a direct result of the accumulation of phospholipids. After high doses of amiodarone were administered orally to rats and beagle dogs, multilamellar lysosomal inclusion bodies accumulated first in the jejunal mucosa and mesenteric lymph nodes before becoming widely distributed in other organs, particularly in the lungs.⁵⁶⁵ In both rats and dogs the small intestinal lesions were characterized by the presence of foamy macrophages with pale finely vacuolated cytoplasm and condensed eccentric nuclei within the lamina propria of the jejunal villi. Mesenteric lymph nodes were also involved early after the onset of treatment. In the dog, jejunal villi were somewhat flattened and widened or showed a variable degree of villous atrophy, most marked in the proximal and middle jejunum.⁵⁶⁶ Electron microscopy confirmed the presence of lamellar lysosomal bodies distending macrophages.

The early accumulation of foam cells in the jejunal macrophages was probably a reflection of the disposition of drug following oral absorption. Although similar phospholipidosis was seen in many organs following intravenous administration in dogs, more phospholipidosis was seen in the jejunum after oral dosing. Moreover, there were species differences in sensitivity to these changes, baboons being relatively insensitive compared

to dogs. Fischer 344 rats were very sensitive to these changes compared to Sprague-Dawley rats and Wistar rats which were more resistant to phospholipidosis induced by amiodarone under similar conditions.⁵⁶⁵

Villous atrophy, hypoplasia

Villous shortening or stunting results when the proliferative activity of the crypt epithelium is reduced or crypt cell proliferation is insufficient to compensate for increased cell loss following mucosal cell damage. Decreased cell proliferation can be seen following decreased food intake, parenteral nutrition, hypophysectomy, thyroidectomy or in bowel segments which have been surgically bypassed.^{359,456,457}

As adrenergic factors are important in the control of small intestinal epithelial cell division, drugs that alter α or β adrenoceptor activity may influence the proliferative capacity of the epithelium. In mice, increased α_1 or β receptor stimulation by appropriate agonists (e.g. phenylephrine) diminishes proliferation of crypt cells. Proliferation is increased by stimulation of α_2 receptor activity.⁵⁶⁷ Yohimbine, an α_2 antagonist, also reduces cell proliferation in the same animal model. Some of the effects of these agents may be mediated by changes in splanchnic blood flow.

The detailed morphological study of the small intestinal mucosa in the rat following hypophysectomy has shown a reduction in the height of the small intestinal villi associated with reduction in mitoses in the crypt epithelium.³⁵⁹ The number of goblet cells was shown to fall particularly in the jejunum and the number of Paneth cells increase in the ileum. Examination of ultrastructure showed decreased height of the microvilli of absorptive cells and a lower number of intracytoplasmic organelles and ribosomes. There were also significant decreases in brush border enzyme activities of alkaline phosphatase, aminopeptidase, maltase and lactase about one week following hypophysectomy.

Substances which reduce mitotic activity and therefore lower regenerative capacity of the intestinal epithelium also produce shortening or stunting of small intestinal villi and eventually flattening of the mucosa. A wide variety of anticancer agents and antiviral drugs with radiomimetic properties interfere with cell division in the crypts thereby reducing the number of epithelial cells produced. Histologically, the effects of such agents are characterized by blunting, shortening through to complete atrophy of villi. Mitotic activity is reduced in the crypts and the crypts become dilated and lined by flattened cells. The overlying epithelium loses its normal regular arrangement and cells show pleomorphic nuclei with irregular chromatin patterns. Increased numbers of inflammatory cells may infiltrate the lamina propria and epithelium. Ulceration, hemorrhage and secondary infection of the gut wall ensue if there is overwhelming cell damage.

Comparisons of the gastrointestinal toxicity expressed by antimetabolic anticancer drugs of different classes in rodents, dogs, monkeys and humans have suggested that there is a higher degree of correspondence between effects in humans and dogs than between humans and other species.^{231,545} In studies with the antiviral agent acyclovir, a radiomimetic effect was noted in the gastrointestinal tract of dogs at high doses but not rodents.¹⁷⁶

Another example is the villous atrophy described in rats following treatment with an experimental antibacterial agent ICI 17,363, which was believed to arise as a result of both interference with cell division and a direct effect on the surface epithelial cells.⁵⁶³ The effects of ICI 17,363 were characterized by atrophy of villi with dilatation of crypts and

atypical features in the crypt epithelium suggestive of an effect on mitotic activity. In addition, vacuolated lipid-laden epithelial cells were observed over the tips of villi accompanied by reductions in the numbers of goblet cells and reduced activity of acid and alkaline phosphatase, esterase, adenosine triphosphatase, glucose-6-phosphatase and succinic dehydrogenase, compatible with a direct adverse effect on superficial mature epithelial cells.

Hypertrophy and hyperplasia

A variety of factors stimulate cell proliferation in the small intestinal epithelium. These include partial enterectomy, increased feeding, stimulation of autonomic nerves, and administration of neurotransmitters, thyroxin, growth hormone, corticosteroids, testosterone, gastrin, glucagon, glucagon-like peptide 2 and epidermal growth factor.^{456,568,569} Key determinants of feeding-induced intestinal adaptation appear to be non-specific luminal stimulation, functional workload induced by polymeric nutrients, stimulation of pancreatic or biliary secretion as well as diverse humoral mediators and induction of intestinal hyperemia.⁵⁷⁰ In the *short bowel syndrome* which occurs when there is insufficient length of the small intestine to maintain adequate nutrition following surgical resection of the intestine, the extent of adaptation depends on the anatomy of the resected bowel and the amount of bowel remaining. These changes have also been shown to be mediated by multiple factors, including intraluminal and parenteral nutrients, gastrointestinal secretions, hormones, cytokines, and growth factors.⁵⁷¹ Understanding these processes has led to the use of growth hormone and glucagon-like peptide 2 in intestinal rehabilitation in patients.⁵⁷² Many of these mechanisms have been explored in laboratory animals over several decades.

In rats hypothalamic damage, hyperthyroidism, tube feeding, diabetes mellitus and insulin injections have been shown to produce intestinal hyperplasia.^{573–576} Most causes of greater cell production lead to increased villous height and mucosal hyperplasia, although intense crypt cell proliferation as a compensatory regenerative response can be associated with villous atrophy.

Intestinal hyperplasia can be seen as a physiological process to allow an increase in the capacity of intestinal absorption because numerous studies in rodents as well as other species have shown that small intestinal mass increases up to 200% during lactation, most marked in its proximal two thirds.⁵⁷⁷ This is associated with histological evidence of increased villous height in the jejunum and ileum and a generalized hypertrophy and hyperplasia of the jejunal, ileal and cecal mucosa.

The compensatory response to the surgically resected or bypassed intestine has been the focus of the most detailed studies of cell renewal in the small intestine. Partial resection in both rats and humans is accompanied by increased villous height and crypt length.^{578,579} This is primarily the result of hyperplasia for it has been shown that the numbers of cells per unit length of villus remains unchanged but there is an overall increase in the cell population of villus and crypt. DNA/RNA ratios remain largely unaltered.⁴⁵⁷ No gross changes in villous shape have been reported after resection and the total number of crypts remains constant. Although increased intestinal uptake of substances from the bowel lumen occurs in hypertrophied segments per unit length of bowel, disaccharide and dipeptidase activities are normal or even decreased after resection suggesting a

comparative immaturity of cells in the residual mucosa. Functional adaptation therefore is largely achieved by a larger number of cells but their individual absorptive capacity is not increased.⁴⁵⁷ Increased numbers of specific goblet cell populations are also seen in hyperfunctional states. Following jejuno-ileal bypass operations in rats, increased numbers of PAS-positive goblet cells develop in the villi and crypts of the hyperfunctional segments of the duodenum, jejunum and ileum.⁵⁸⁰ Mucin histochemistry has shown that the goblet cells in the hyperfunctional segments contain increased sialomucins in the villi and crypts of the jejunum and ileum but not in the duodenum and increased sulfomucins in the distal ileal segment. As sialic acid conveys more viscoelastic properties to mucin, it has been suggested that the goblet cells change following intestinal bypass and fulfill a protective function against the increased flow of gastrointestinal contents.⁵⁸⁰

Interestingly partial hepatectomy (70%) of rats has also been shown to produce persistent trophic changes in the proximal jejunum characterized by thickening of the jejunal wall and villous hypertrophy.⁵⁸¹

A number of nutritional factors, particularly dietary fiber, have been shown to influence the proliferative characteristics of the small bowel mucosa. Carefully controlled studies in rats given different forms of dietary fiber have shown that the proliferative characteristics of the small intestine can be modified by both the quantity and the precise nature of the fiber. These different effects may be the result of differences in solubility, gel formation, water holding capacity, effect on transit time and ion exchange activity or bile acid adsorption of the different fibers. However, interactions between dietary constituents are complex. For instance, the histological effects in the rat small intestine after administration of 2% dietary cholestyramine, a non-absorbable ion exchange resin, has been shown to depend on interaction with other dietary factors.⁵⁸²

Administration of an inhibitor of cholesterol biosynthesis, 5 α -cholest-8-(14)-en-3 β -ol-15-one, to rats for up to nine days was also shown to produce enlargement of the small intestine in a way which was morphologically similar to the changes found following intestinal bypass.⁵⁸³ The enlargement was most marked in the proximal segment of the small intestine and progressively diminished towards the ileo-cecal junction, sparing the stomach, cecum and colon. Histological examination and morphometric analysis revealed an increase in smooth muscle mass, lengthening of the villi as well as an increase in the depth and cellular proliferation in the crypts of Lieberkühn without evidence of cell damage or fatty change. Like the changes following jejunal bypass procedures, there was also an increase in acid mucosubstances in the goblet cell population overlying the villous mucosa. The mechanism for this change in the rat was unclear, particularly as intestinal hyperplasia was not seen in baboons treated with this 15-ketosterol for long periods. However, it was suggested that it was an adaptive response, possibly related to inhibition of cholesterol metabolism and cholesterol absorption from the diet as the laboratory diet employed in the rat study was particularly low in cholesterol.

Local and systemic changes in hormones and various transmitter substances also influence the number of cells in the small intestinal epithelium. Morphometric studies of the small intestinal mucosa in mice following gastrin administration have shown increases in villous area associated with decreases in microvillous area, increased number of goblet cells and Paneth cells.³⁶⁹ Studies in which rats were treated with the prolactin inhibitor ergocryptine have shown that the total number of mucous cells and the number staining

with alcian blue at pH 1.0 increase in the ileal crypts, possibly as a result of increased synthesis of sulfated mucosubstances.⁵⁸⁴ Administration of human insulin-like growth factor I (IGF-I) to rats for five days has also been reported to produce a significant increase in the weight of small intestine along with mucosal hyperplasia.⁵⁸⁵

Chronic treatment with the *Rauwolfia* neuroleptic reserpine, which depletes adrenergic nerves of norepinephrine (noradrenalin), causes an increase in the sulfation of goblet cell mucin in the small intestine as demonstrated by alcian blue staining at pH 1.0 and the high iron diamine technique without changes in goblet cell number.⁵⁸⁶ Other agents which affect activity of the sympathetic nervous system can also alter epithelial cell proliferation in the small (and large) intestine. Whereas treatment of rats with epinephrine (adrenalin), isoprenaline, phenylephrine, phentolamine and yohimbine result in decreased mitotic activity of jejunal and colonic crypt cells, administration of metaraminol, clonidine, propranolol, prazosin and labetalol as well as simultaneous injection of propranolol and epinephrine (adrenalin) result in an increased rate of crypt cell proliferation.^{567,587} These results suggest that agents that stimulate α_2 adrenergic receptor activity and those that are α_1 antagonists and β adrenergic receptor antagonists increase proliferative activity in the rodent intestinal mucosa.

Phosphodiesterase inhibition and resultant increases in intracellular cAMP may also produce thickening of the mucosa of small intestine. Rats treated for periods of up to six months with high doses of the inotropic vasodilator, ICI 153,100, a phosphodiesterase inhibitor intended for treatment of congestive cardiac failure, produced not only salivary gland hypertrophy but also marked thickening of the small and large intestinal mucosa. This was characterized by an increase in villous length and deepening of intestinal glands, with a relatively unchanged number of epithelial cells per unit length of gland or villus.¹⁴⁹

Although prostaglandin E analogs produce most of their effects in the stomach, increased thickness of the small intestine characterized by longer villi, deeper crypts and increase in cell size have been reported in rats treated with these agents.¹⁹⁶

Focal hyperplasia, focal avillous hyperplasia, focal atypical hyperplasia, duodenal plaque, polypoid hyperplasia, polyp – mouse

Irregular, atypical single or multiple foci of glandular hyperplasia may be found in the small intestinal mucosa of several strains of aged, untreated mice. The lesions are usually located in the first part of the duodenum where they form discrete, raised plaques composed of elongated, irregular or branched glands which replace the normal villous structure of the mucosa. The glands are lined by hyperchromatic columnar cells characterized by marked pseudostratification and proliferative activity. Paneth cells and mucin-secreting goblet cells may also be prominent. Some glands are cystic and the stroma is fibrous and infiltrated by chronic inflammatory cells. The lesions become pedunculated or polypoid in appearance and show a fibrovascular core that is infiltrated by inflammatory cells. They resemble adenomatous polyps described in humans. Typically they show no severe dysplastic features that suggest they are malignant or premalignant lesions (see neoplasia, below).

The cause of these changes in the mouse small intestine is unknown but their prevalence can be altered by dietary fiber, purified diets particularly when deficient in pantothenic acid as well as by administration of drugs and chemicals.^{390,588–591} In their

original study of DBA mice, Hare and Stewart considered that the lesions were not genuine neoplasms since they were composed of a mixture of cell types, which normally populate the mucosa.⁵⁸⁸ Furthermore, they suggested that the presence of an inflammatory component in the stroma and the fact that the prevalence of these lesions was increased in mice fed a high roughage diet were consistent with the concept that they represent an inflammatory adenomatoid hyperplasia. Panthothenic acid deficiency was also associated with inflammation and deep penetrating chronic ulcers of the duodenum in affected mice, compatible with an inflammatory etiology of the lesions.

An increase in the prevalence of these duodenal changes was described in CD-1 mice treated with the synthetic prostaglandin E₁ analog misoprostol for 21 months.⁵⁹² It was suggested that the findings posed no real concerns for the safety of patients treated with misoprostol on the grounds that the mouse was unique in this aspect of the response to misoprostol because it had a particular liability to develop such changes in the small intestine. The proliferative lesions were found in a few control CD-1 mice in the same study. In addition, it was also argued that the lesions were neither neoplastic nor preneoplastic in nature. Similar lesions were not seen in rats treated with misoprostol for two years.⁵⁹³

Lesions characterized by such intense proliferative activity may be difficult to distinguish from neoplastic lesions. Indeed chronic administration of hydrogen peroxide, widely used as an antiseptic and in dental products, to C57BL/6J mice in drinking water was reported by Ito and colleagues not only to potentiate the development of a similar type of duodenal hyperplasia but also to produce a small number of adenocarcinomas after a period of two years.^{591,594} These were non-metastatic atypical (dyplastic) flat lesions associated with erosions and inflammation. It has been argued that as hydrogen peroxide is unlikely to reach the duodenum intact, these results were related to an interaction with greatly decreased water consumption and the resultant abrasion of the luminal lining by dry pelleted rodent feed.⁵⁹⁵ However, this may simply be another example of neoplastic development following prolonged periods of chronic inflammation in the gastrointestinal mucosa at doses irrelevant for the usual use of hydrogen peroxide in humans (see neoplasia, below).

Some compounds may produce proliferative reactions that mimic intestinal dysplasia. This has been well described in human biopsies in patients being treated with compounds such as colchicine and taxol that interfere with tubulin and inhibit its polymerization into microtubules.^{362–364} This effect produces mitotic arrest characterized by prominent ring mitoses associated with epithelial pseudostratification, loss of polarity and increased apoptosis, features that may mimic dysplasia. Similar features may occur in laboratory animals (Figure 8.3b).^{363,409,410}

LARGE INTESTINE

In humans and in monkeys the large intestine can be divided anatomically into cecum, appendix, ascending colon, transverse colon, sigmoid colon, rectum and anal canal. Like the small bowel, the wall of the colon comprises mucosa, submucosa, muscularis mucosa and serosa. Mucosal plicae are only found in the rectum although plicae semilunaris, formed by folds of the entire thickness of the bowel wall, are found in the colon. The large

intestine of the dog resembles that of humans more than that of most other domestic species. It is a simplified tubular structure only slightly larger in diameter than the small intestine. The colon of the dog is divided anatomically into ascending, transverse and descending parts, but there is no well-defined sigmoid segment. The cecum in dog is a small diverticulum, similar to that found in other carnivorous species and it communicates directly with the colon. The cecum and colon are larger in minipigs than in humans and arranged in a series of coils with a vascular arcade in the subserosa rather than mesentery.⁴⁴⁷

The colon of the rat and mouse is shaped like an inverted V that can be divided into ascending and descending segments. There is no clearly defined transverse colon. A characteristic feature in both rat and mouse is the presence of a curved kidney-shaped cecum. Its size is intermediate between the large and anatomically complex cecum of herbivores such as the rabbit and the small cecum of carnivorous species. This probably reflects the omnivorous nature and flexibility of the rat and mouse in their dietary habits, particularly their ability to break down cellulose.⁵⁹⁶

The cecum of the rat and mouse is a blind pouch from which the colon and ileum exit in close proximity and in which antiperistaltic movements occur. This structure and the presence of bacteria undoubtedly contribute to its ability to function as a fermentation organ in which breakdown of substances can occur in a controlled milieu.⁵⁹⁷ Anatomical study has shown that the mouse cecum is less voluminous than the rat cecum. The ampulla, the region between the colon and cecum, is also narrower in the mouse than in the rat.⁵⁹⁷ The cecum is the site of absorption of many substances including calcium, magnesium, water and electrolytes vitamin K and fatty acids in these species. Cecectomy has been shown to decrease digestion of carbohydrates and protein and increase loss of fecal water in these species.⁵⁹⁸ These anatomical and functional features of the rodent cecum with its peristaltic and antiperistaltic movements may expose this segment of the large bowel to potentially irritant orally administered xenobiotics for more prolonged periods.

The activity of intestinal microflora in the metabolism of both endogenous and exogenous substances has been demonstrated in the rodent cecum.^{599,600} The usual stock diets for rodents contain abundant plant fiber which provides bulk and fermentable carbohydrate for the microbial population in the cecum. Rats fed stock diets have been shown to possess high levels of reductive and hydrolytic enzyme activity (e.g. azoreductase, nitroreductase, nitrate reductase, β -glucosidase and β -glucuronidase) in their cecal contents compared with rats fed purified fiber-free diets.⁶⁰¹ Intestines of animals with reduced microflora have thinner lamina propria, lower cell turnover, enlarged cecum, altered metabolism of cholesterol, bilirubin and bile salts and larger amounts of mucin in feces compared with animals possessing normal gastrointestinal microflora.⁶⁰²

Histological and histochemical characteristics

The colon and cecum in humans and laboratory animals are lined by a fairly uniform mucosa devoid of villi. Columnar cells of two main types cover the surface epithelium. These are absorptive and mucous cells similar to those found in the small intestine. Intestinal glands or crypts extend downwards from the surface generally as simple, unbranched tubules lined principally by mucous cells with smaller populations of absorptive, endocrine and undifferentiated cells.

The mucosa in humans and laboratory animals shows a slightly corrugated or uneven pattern which varies with the particular site within the colon. In histological sections of the colon in humans, this corrugated pattern is seen as anthemion-like structures of crypts reminiscent of a Greek architectural feature.⁵⁰⁰ This is also seen in larger laboratory animal species. In rats and mice the crypts of the cecal mucosa are wider near the lumen than in the crypt base and crypts may be branched, features which may be related to the absorptive function of this zone.⁵⁹⁷

The proliferative zone in the large bowel is normally located in the lower part of the gland. As in the small bowel, multipotent, undifferentiated stem cells situated in the gland base give rise to the principal cell types which migrate to the cell surface with subsequent differentiation and alteration of their enzyme activities and morphological features.⁶⁰³ In studies with mouse aggregation chimaeras it has been demonstrated that the entire epithelium of each adult gland descended from a single progenitor cell.⁶⁰⁴ The single progenitor may itself give rise to several stem cells which are responsible for the cell renewal in the complete crypt.

Absorptive cells are found most commonly in the surface epithelium but also to a lesser extent in the glandular epithelium. They are morphologically similar to those in the small intestine each possessing apical plasma membranes with uniform microvilli and a well-formed glycocalyx.

There are species and regional differences in the glycoconjugates found in the brush border of the large intestine, although they generally contain predominantly acidic mucosubstances. In the mouse and rat, sialomucins with some neutral mucins are found. In hamsters, dogs, non-human primates and humans both sulfomucins and sialomucins may be seen in the brush border.²⁵⁶ Some compounds may induce qualitative and quantitative changes in mucin content in the colonic mucosa without marked morphological alterations. An example of this phenomenon was described in rats treated with reserpine for seven days. The colonic mucosa showed an increase in sulfomucin (high-iron diamine positive) containing goblet cells in the surface epithelium.⁵⁸⁶ The glycocalyx is important in the protective function of the colonic mucosa for its disruption by agents such as salicylates has been shown to increase absorption of xenobiotics from the rat rectal mucosa.⁶⁰⁵

The colon, like many other tissues, also possesses drug metabolizing activity, although much less than in the liver, while among humans there is substantial individual variability.^{606–608}

The lamina propria of the large bowel is arranged in a similar way to that of the small bowel. By virtue of the presence of lymphocytes, plasma cells, macrophages and dendritic cells as well as scattered small lymphoid aggregates or patches, it forms an integral and important part in the mucosal immune defense system. Most of the lymphocytes in the lamina propria of the human colonic mucosa, like that of the ileum, have been shown to be T cells with helper T cells outnumbering the T suppresser phenotype.^{476,478} This contrasts with intra-epithelial lymphocytes of the human colonic mucosa which are also T cells but more than 80% of which possess characteristics of the suppresser/cytotoxic phenotype and only 10–20% being helper T cells. Distribution of lymphocyte subsets in the rat colon also shows the presence of T lymphocytes in the lamina propria and most of these are of CD4 helper phenotype.⁴⁸⁹ Few T lymphocytes in the lamina propria are of suppresser/cytotoxic (CD8) type in contrast to the higher proportion within the colonic

epithelium. Mature, small B lymphocytes are relatively uncommon in the colonic lamina propria of humans and laboratory animals although the lamina propria contains large numbers of plasma cells mainly of IgA subtype.

A feature of the colonic mucosa is the presence of lymphoid aggregates, also called lymphoid nodules, patches, lymphoid–glandular complexes or microbursa.⁴⁸³ These are similar to Peyer's patches of the small intestine as they are composed principally of lymphoid cells of the B cell series arranged in follicles with germinal centers with interfollicular and perifollicular zones composed of T cells.⁴⁷⁶ They are distributed along the entire length of the colonic mucosa although they are generally smaller than Peyer's patches. In Sprague-Dawley rats lymphoid aggregates are usually about 5 mm in diameter except in the distal colon where they attain sizes of up to 10 mm in maximum diameter.⁴⁸⁶

Unlike Peyer's patches which are characteristically not associated with crypts or villi, the colonic lymphoid aggregates frequently contain irregular atypical mucosal glands which may enter deeply in the lymphoid tissue and penetrate below the muscularis mucosa both in humans and laboratory animals.^{609,610} These glandular structures, which are intimately associated with lymphoid tissue, may be important in the immune protection of the colonic mucosa, perhaps by acting as a special local receptor for antigens. Colonic carcinomas induced by dimethylhydrazine in the rat also appear to develop more commonly in the lymphoid aggregates than in other zones.⁴⁸⁶

Inflammation, ulceration, colitis, proctitis

Although microorganisms are important causes of inflammatory disease in the large intestine of humans and animals, among laboratory animals over recent years they have only been significant problems in non-human primates and hamsters. In the strains of rats and mice and in beagle dogs commonly employed in drug safety evaluation, spontaneous disease of the colon as a result of infectious agents is uncommon.

A form of chronic colitis characterized by submucosal round cell infiltration, shortened or irregular crypts, crypt abscesses, mucin depletion and reactive mucosal changes was reported to occur in significant numbers of cynomolgus monkeys in one laboratory.¹²⁹ No specific pathogens were usually identified although it was associated with clinically evident diarrhea.

Treatment with some therapeutic agents may alter the normal bacterial flora to permit overgrowth of pathogenic organisms or disturb the normal balance between antigens in the lumen or control mechanism to evoke inflammation. Inflammation induced by microorganisms may also confound the histological assessment of drug-induced changes in the colon.

Ulceration and inflammation of the colon as a direct result of systemic administration of potential therapeutic agents is reported in humans, although less commonly than in the small intestine. It has been suggested from studies of the effects of anticancer compounds on neoplastic cells that colonic cells possess inherent protective properties in the form of an accelerated efflux pump which can serve to protect them from potentially damaging agents.⁶¹¹ Ulceration and inflammation can be induced by the local application of drugs and vehicles to the rectal mucosa. Assessment of these effects in an appropriate animal model is important in the safety evaluation of preparations designed for use in human patients as rectal suppositories.

Inflammation of the large intestinal mucosa usually has no specific histological features. In early or mild inflammation, the surface and glandular mucosa remains intact but shows mucin depletion. This is characterized by reduction in the mucus in goblet cells and increased cytoplasmic basophilia. Scattered neutrophils may be seen in the epithelium and adjacent lamina propria. In more severe cases, crypts become filled or distended with acute inflammatory cells (crypt abscesses). The lamina propria is variably hyperemic and congested and contains increased numbers of mononuclear cells.

Severe changes are characterized by attenuation or frank erosion of the epithelium and the formation of penetrating ulcers filled with fibrinous exudate and surrounded by intense inflammation, granulation tissue and eventually fibrosis. Residual glands may be dilated and lined by flattened epithelium or show reactive changes and mitotic activity. Regenerative hyperplasia, which can become florid in chronic ulcerative conditions, is characterized by lengthening, irregularity and cystic dilatation of glands which are often lined by hyperplastic epithelial cells and goblet cells distended with mucin. When extremely florid the reactive changes may appear atypical and mimic dysplastic (preneoplastic) changes (see neoplasia, below). Where ulcerative damage has destroyed glands and supporting stroma, regeneration of glands may not occur in the normal regular fashion and branching of crypts may be evident.

Infections and infestations

BACTERIAL INFECTIONS

Clostridium difficile may cause inflammatory changes in the colon of laboratory animals, particularly hamsters, and this may extend into the distal ileum. As in humans this form of colitis, often referred to as *pseudomembranous colitis*, is usually associated with antibiotic therapy. In humans it was originally associated with lincomycin and clindamycin therapy but other antibiotics have been implicated. *Clostridium difficile* is believed to be responsible for approximately 10% of cases of antibiotic-associated diarrhea.⁶¹² However, the precise cause of much antibiotic-associated diarrhea in humans is less well defined. Other microorganisms have also been implicated in antibiotic-associated diarrhea, notably *Klebsiella oxytoca*, *Clostridium perfringens* type A, *Staphylococcus aureus*, *Candida* and *Salmonella* species although the evidence for these is less strong.⁶¹² In addition, alterations to microflora may also reduce bile acid metabolism or reduce fermentation of carbohydrate.

It has been shown that in both humans and hamsters *Clostridium difficile* produces a toxin that induces enteritis.^{613–615} In humans this condition is characterized by the presence of plaques or pseudomembranes on the colonic mucosal surface. The pseudomembrane is composed of mucus, fibrin, blood cells, inflammatory cells and cell debris, which has an appearance of streaming from the underlying mucosa. The mucosa may be partly necrotic or mucosal glands are dilated and lined by flattened or hyperplastic cells. The ileal mucosa may show similar changes.⁶¹⁵

Similar features can occur in antibiotic-treated laboratory animals.^{616–619} In the hamster, the condition is characterized by erosion of the colonic epithelium and the presence of a pseudomembranous plaque of mucin and cell debris. Intact but affected mucosa is thickened with reactive changes accompanied by mucin loss in the epithelium and infiltration of a hyperemic and edematous lamina propria and submucosa by polymorphonuclear

cells. Although most instances of this form of clostridia colitis in the hamster have been associated with antibacterial therapy, it has also been reported in untreated hamsters and those treated with antineoplastic drugs.^{616,620} Similar changes have been reported in antibiotic-treated guinea pigs and rabbits.^{616,618} As in humans, these drugs are believed to alter the intestinal flora, permitting overgrowth of *Clostridium difficile* resulting in a severe and sometimes fatal enterocolitis.

Citrobacter freundii, a Gram-negative, short, plump rod and member of the family of Enterobacteriaceae, is the causative agent of naturally occurring transmissible colonic hyperplasia of mice.^{621–623} This agent usually produces mild or even asymptomatic enteritis in susceptible mouse populations, although it is a cause of rectal prolapse.⁶²⁴ Marked strain differences have been noted in mice infected with this organism. NIH Swiss mice show the most severe histological changes, C57BL/6J mice appear the least affected and rats and hamsters seem to be resistant.⁶²³ Microscopic changes are found primarily in the descending colon, although proximal segments of the colon and the cecum may also be affected. An important morphological feature is epithelial hyperplasia, which occurs maximally two to three weeks after experimental inoculation with *Citrobacter freundii*. The colonic glands are elongated and lined by cells that show mucin depletion or loss of goblet cells, considerable immaturity and mitotic activity. The surface epithelium may be covered with numerous coccobacilli, which can be visualized in routine hematoxylin, and eosin-stained sections. Crypt abscesses, inflammatory cells in the lamina propria, mucosal erosions and ulceration are also features. In regressing lesions there is a rebound increase in goblet cells, which are often distended with mucin. The colonic glands may be branched or irregular.⁶²²

Most laboratory animals are naturally resistant to shigella infections but this is not the case for non-human primates.¹¹ In infections with shigella, the colon shows a superficial acute inflammatory reaction comprising edema, congestion, hemorrhage and infiltration by acute inflammatory cells. The surface epithelium shows mucin loss and formation of small ulcers where total destruction of the epithelium has occurred. Ulcers can extend into the lamina propria but in general terms the inflammatory process remains relatively superficial.⁶²⁵ Organisms are also located predominantly in the superficial epithelium.

Another bacterial infection of the gastrointestinal tract, which affects the colon in primates, is that produced by non-tuberculous mycobacteria. Large intestinal lesions are characterized by massive accumulation of epitheloid macrophages in the lamina propria, which may extend into the submucosa and muscular layers and along lymphatics to involve mesenteric lymph nodes. Small intestinal lesions may also occur, characterized by the presence of similar large macrophages in the lamina propria of the tips of villi. Superficial ulcers may occur in severely affected segments of intestine.⁶²⁶ Acid-fast bacteria are typically found within macrophages. Other organs, including spleen, liver, bone marrow and lungs, may also be involved by focal accumulations of bacteria-laden macrophages or occasionally discrete granulomas with multi-nucleated giant cells.

In New World monkeys, *chronic idiopathic colitis* is common and may occur as part of a marmoset wasting syndrome. Early features include neutrophil infiltration of the lamina propria, crypt abscesses, erosions and ulceration with regeneration, mononuclear inflammation, micro-herniation of glands, dysplasia and occasionally carcinoma. The cause is uncertain although a number of infective agents have been implicated.¹¹

PROTOZOA AND METAZOAL INFECTIONS OF THE COLON

Numerous protozoa and metazoa have been described as inhabitants of the cecum and colon of non-human primates although they are less prominent in current colonies of laboratory monkeys.^{129,504} Far fewer are observed in the usual laboratory rodents and beagle dogs.

Amoebiasis caused by *Entamoeba histolytica* is a widespread disease among non-human primates. It is characterized histologically by the presence of necrotizing ulcers, which reach the muscularis mucosa to form typical flask-shaped ulcers containing or surrounded by trophozoites. Extensive hemorrhage may be seen as well as an inflammatory infiltrate composed of neutrophils and mononuclear cells.

The ciliate, *Balantidium coli*, can also cause an ulcerative process in the colon of primates, characterized by ulcers which extend down to the muscularis mucosa accompanied by lymphocytic infiltrate and *Balantidium coli* trophozoites of up to 150 μm in greatest diameter.⁵⁰⁴

A variety of metazoan parasites can be observed in the primate colon and usually can be reasonably well identified in tissue sections.⁵⁰⁵ The nematode of species *Strongyloides* is an important parasite, which may be observed in the intestinal mucosa of primates. Oxyurids commonly known as pinworms are essentially innocuous parasites seen in humans, monkeys and rodents. *Enterobius vermicularis* is found in the large intestine and appendix of humans and non-human primates, *Syphacia muris* and *Syphacia obvelata* in rodents.

Oesophagostomum species (nodular worms) are especially common nematode parasites of non-human primates forming characteristic nodules up to 5 mm diameter most frequently on the serosal surface of the large intestine and cecum and adjoining mesentery as well as in other sites in the peritoneal cavity. It is also a parasite of ruminants and pigs and it has been found in humans in some parts of Africa.^{627–629} Histologically, the nodules are composed of parasite cell debris surrounded by fibrous tissue and a variable mantle of chronic inflammatory cells and occasional foreign-body giant cells. They are frequently found in close proximity to small arteries and arterioles in the submucosa and subserosa of the colon and may be associated with a local granulomatous arteritis.⁶³⁰ The inflammatory process may spread to surrounding or draining tissues, particularly if nodules rupture. Mild periportal hepatic chronic inflammation is sometimes associated with the presence of this parasite in the mesentery, which may confound interpretation of drug-induced hepatic changes in the monkey.

Drug-induced inflammation, erosions, ulcers

HUMANS

A diverse range of xenobiotics are reported to damage the colonic mucosa in humans, although in comparison to stomach and the small intestine the colon appears to be remarkably resistant to drug-induced damage.

Although long-term use of non-steroidal anti-inflammatory drugs is associated the beneficial effect of a decrease in colorectal cancer incidence and regression of adenomatous polyps, these drugs are occasionally the cause of mucosal inflammation and ulceration followed by focal scarring of the submucosa with constriction and formation of a mucosal

diaphragm.^{631–635} Damage occurs mainly in the right side of the colon and is believed to be related to inhibition of prostaglandins rather than contact damage.¹⁷⁰ Potassium chloride therapy may also produce colonic damage characterized by segmental full thickness scarring and constriction.⁶³⁶

Another form of induced colon damage has been reported in children with cystic fibrosis, many of whom take high strength pancreatic-enzyme supplements to control malabsorption.^{637,638} In these patients there is fusiform stenosis of a long segment of ascending colon, primarily as a result of submucosal thickening by mature collagen. The mucosa appears relatively spared but may show some ulceration and reparative change.⁶³⁶ Although it has been suggested that the changes may be due to the methacrylate copolymer used for enteric coating of the high strength preparations, a case-control study showed a strong relation between high daily doses of the enzyme supplements, accentuated by availability of high dose forms.^{638,639} In view of their usage for over 50 years, pre-clinical data on this material are scarce.

Cellular degeneration, with loss of mitotic activity and mucin depletion, can also occur in the colon following treatment with antimetabolic drugs.

Cocaine abuse can also lead to an ischemic form of colitis.⁶⁴⁰ Although the exact pathophysiology this form of ischemia is not certain, cocaine blocks the reuptake of norepinephrine (noradrenalin) which can lead to mesenteric vasoconstriction and tissue ischemia which is the likely cause of perforation. Digoxin and diuretics have also been linked to ischemic colitis in individuals predisposed to low mesenteric blood flow such as occurs in heart failure.¹⁷⁰ This form of colitis is characterized by superficial epithelial loss with sparing of crypt bases along with hemorrhage and hyalinization of the lamina propria.

The rectal administration of therapeutic agents and surfactants may also induce ulcerative and inflammatory changes. Chemical colitis resembling pseudomembranous colitis has been reported in humans as a result of chemical cleaning agents accidentally induced by endoscopic examination.⁶⁴¹

LABORATORY ANIMALS

In laboratory animals colonic damage can be induced experimentally by administration of therapeutic agents. Dogs administered 2.5 mg/kg of the non-steroidal, anti-inflammatory drug indomethacin orally each day for periods of up to 23 days developed not only gastric and small intestinal ulceration but also scattered hemorrhagic erosions in the colon and rectum. Microscopically, these lesions were characterized by loss of superficial epithelial cells, mucus depletion of glandular epithelium and crypt abscesses, frequently with acute inflammation in adjacent lymphoid aggregates in the submucosa.⁵³⁶

Another example of chemically induced colitis of relevance to the pathology of drug safety assessment is that induced by degraded carrageenans or synthetic sulfated dextrans. Carrageenans are a heterogeneous group of sulfated polysaccharides composed mainly of long chains of D-galactose subunits (D-galactan) derived from red seaweed species which are widely used as food emulsifiers, stabilizers, thickeners and gelling agents.⁶⁴² When carrageenans are degraded by acid hydrolysis into smaller molecular weight fragments of about 20 to 40 kDa and administered orally in high doses (e.g. 10% of diet) to rats, mice, guinea pigs, rabbits and rhesus monkeys, colitis results.^{643–647} Similarly, colitis has been induced in rats following administration of a 5% dietary admixture of dextran sulfate

sodium, a sulfated polymer of glucose (α -D-glucose) of molecular weight of 54 kDa and a very high molecular weight D-glucan, amylopectin sulfate.^{642,648}

Although histological features of this form of induced colitis vary between study, species and strain, the colitis in rodents is generally characterized mucosal ulceration mainly in the cecum but also in the distal ileum, distal colon and rectum. There is mucus depletion with variable acute inflammatory infiltrate of the intact epithelium, increased cell proliferation, crypt abscesses and inflammatory infiltrate of the lamina propria with edema, hyperemia and even vascular thrombosis in the submucosa.^{644,648} In the cecum of rats, ulcers are linear but often circulating the entire circumference of the intestinal wall with subsequent scarring and stricture formation. Ulcerating lesions in the rectum and at the anal margin are associated with squamous metaplasia. Both the squamous metaplasia and the regenerative hyperplasia of the columnar epithelium have been shown to progress even after cessation of treatment. Foamy macrophages containing metachromatic material, presumably polysaccharide, are also seen in the lamina propria, submucosa, regional lymph nodes, liver and spleen.^{648,649}

The precise mechanisms involved in the development of this colitis are unclear. Low dose levels, which may be expected to mimic human exposure, do not produce colitis. Dextrans, carrageenans and other polysaccharides of molecular weights outside the range 20 to 60 kDa tend not to incite colitis. An exception to this is the agent amylopectin sulfate, which has a far higher molecular weight. However, amylopectin is composed of polysaccharide chains which can be degraded by amylase, and therefore smaller molecular weight fragments may be formed *in vivo*.⁶⁴² It has been suggested that colonic disease produced by these agents may be linked to induced changes in intestinal microflora or a result of increased intestinal permeability to antigenic or inflammatory substances normally resident in the large intestine.^{645,650}

Long-term administration of high doses of these agents to rats leads to the development of colorectal cancer despite their being devoid of mutagenic activity (see below). The only obvious pathological colonic changes associated with administration of these non-genotoxic agents are chronic inflammation and increased proliferative activity.

Lymphoid infiltrates without tissue damage were reported in the large bowel of rats treated with human recombinant interleukin 2.³¹⁹

Pigmentation

Melanosis coli represents a well-described phenomenon in humans associated with chronic ingestion of anthraquinone purgatives but also bisacodyl, a diphenylmethane stimulant laxative which acts on contact with the mucosa of the large bowel to increase peristaltic activity.^{651–653} *Melanosis coli* is due to the excessive accumulation of lipofuscin-like pigment in the macrophages of the colonic lamina propria.^{654–656} This pigment probably originates from organelles of epithelial cells or macrophages, which are damaged by treatment. On the basis of the study of *melanosis coli* induced in guinea pigs by anthraquinones it has been suggested that the primary process is a treatment-induced increase in apoptotic bodies in the surface colonic epithelium that are phagocytosed by intraepithelial macrophages and transported to the lamina propria.⁶⁵⁷ While pigment deposits are inducible in guinea pigs, they do not seem to occur in rats, mice or dogs.^{658–660} Oral rat toxicity and carcinogenicity studies of the widely used laxative senna have shown colonic

epithelial hyperplasia but no evidence of carcinogenicity which is consistent with the benign nature of melanosis coli and its lack of risk for cancer in human patients.^{659–661}

Lipofuscin and iron pigment is occasionally observed in the lamina propria of untreated rodents, presumably a result of aging, previous inflammatory processes and hemorrhage.

Hyperplasia

As in other glandular epithelial tissues, hyperplasia may be focal or diffuse, with or without atypical cellular features. The term usually used for hyperplasia with atypical cytological features is *atypical hyperplasia* although some use the term *dysplasia*. Thus the pathologists must make a distinction between hyperplasia that represents a simple adaptive response from those showing sufficient atypical features to suggest premalignant state.

Like small intestine, cell proliferation in the large intestinal mucosa can be stimulated by a variety of different factors although these functional adaptive responses have been less well studied. Physical stimulation by distension or increased dietary bulk has long been known to be sufficient to initiate hyperplasia including thickening of the muscle coats.^{662,663}

One of the most clearly documented forms of compensatory hyperplasia is that which occurs as a response to surgical resection or bypass of a segment of the colon. Following resection of a segment of colon in rats it has been shown that the remaining proximal segment of the right side of the colon develops an increase in the thickness of the mucosa and the muscularis externa as well as enlargement of lymphoid aggregates.⁶⁶⁴ Microscopically, the mucosa of the right side of the colon appears uniformly thickened with accentuated folds, elongated mucosal glands and increased height of the surface columnar cells. The changes are marked up to 30 days following surgery but less pronounced after 72 days. It has been shown that a significant increase in the mitotic index in the proximal segment occurs at seven days but by 14 days the mitotic index returns to normal. The distal, downstream segment shows little or no morphological change but rather a long-lived increase in mitotic activity. It was suggested that these differences were related to the different embryological origin of the segments.⁶⁶⁴

A similar form of uniform colonic hyperplasia affecting principally the cecal and right-sided colonic mucosa also occurs in rats following oral administration of sulfated dextrans of molecular weight of approximately 400 kDa (Figure 8.8). Oral administration of large volumes of a wide range of poorly absorbable carbohydrates and osmotically active agents can produce similar changes. Dosing rodents with large amounts of compounds such as raw and chemically modified starches, various dietary fibers, caramels, sugar alcohols (lactitol, sorbitol, mannitol, xylitol), lactose, synthetic polydextrose, cyclodextrins, polyethylene glycol and magnesium sulfate has also been linked to an increase in cecal size and colonic mucosal hyperplasia.^{665–668} The characteristic histological appearance of the colonic mucosa following administration of these agents is lengthening of the mucosal glands which are lined by epithelium composed of increased numbers of enlarged epithelial cells including goblet cells (i.e. hypertrophy and hyperplasia) showing increased proliferative activity and more rapid incorporation of tritiated thymidine.

It should be noted that morphometric studies in rats given pectin and cellulose have shown that the precise nature of the administered substances can influence the degree and

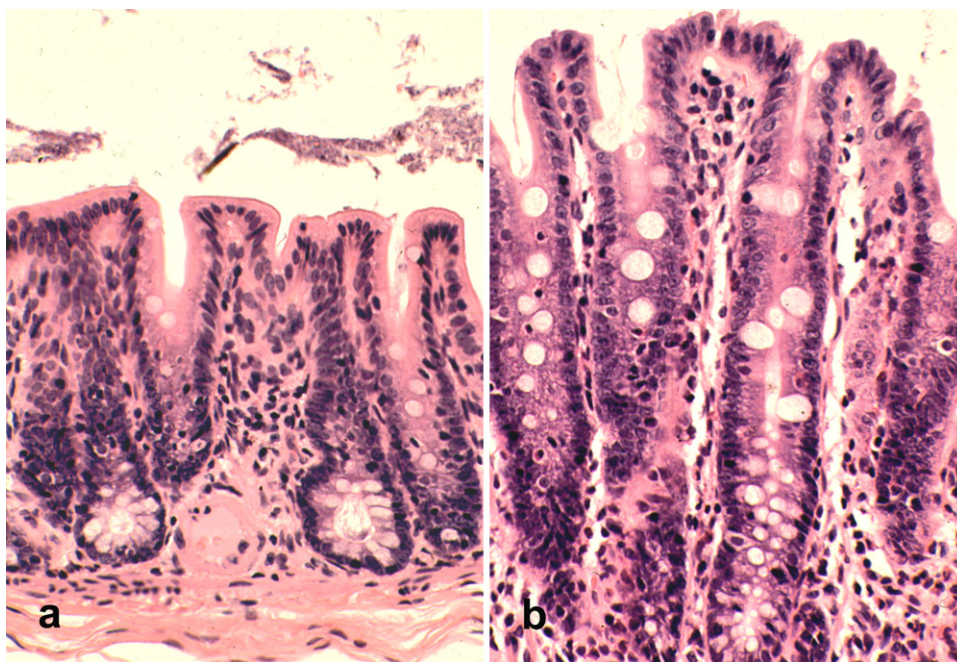


FIGURE 8.8 *Panel a:* Normal colonic mucosa from a Sprague-Dawley rat. *Panel b:* Similar area of colonic mucosa at the same magnification but after dietary administration of 10% dextran (molecular weight 500 kDa) for two weeks. This shows diffuse hyperplasia of the mucosa with elongation of colonic glands lined by relatively normal epithelial cells with abundant mucin and prominent vesicular nuclei (H&E \times 175).

distribution of the mucosal hyperplasia and any associated smooth muscle hypertrophy.⁶⁶⁸ Moreover, some agents may produce additional changes. Mucosal and submucosal edema has been reported in association with the administration of lactose and increased mucosal lymphoid aggregates following lactose or xylitol feeding. There may also be an interaction between dietary fiber content and colonic microflora that influences mucosal growth, although the mechanism is unclear.⁶⁶⁹

It is generally argued that the increase in cecal size and mucosal hypertrophy appears related to the osmotic activity of the cecal contents in rodents treated with large doses of these agents and is therefore not relevant to humans exposed to low doses. Thus the changes represent a physiological adaptation to increased osmotic forces when high doses are given irrespective of the contributing compounds.⁶⁶⁵

Administration of high doses of laxatives has also been reported to produce mild colonic hyperplasia in laboratory rats. It was considered to be an exaggerated pharmacodynamic effect.^{660,670} Treatment of rodents with antibiotics also causes cecal enlargement or dilatation without significant histopathological changes, probably as a result of changes in cecal microflora. It has been suggested that the enlargement relates to accumulation of urea as a result of inhibition of bacterial ureases.⁶⁷¹ However, histochemical studies of the intestinal mucosa of rats treated with neomycin have also shown treatment-related reductions in activities of NAD tetrazolium reductase, succinate dehydrogenase, esterase, alkaline and

acid phosphatase in the distal ileum, suggesting that some antibiotics also possess the potential to directly influence absorption and metabolic functions of mucosal cells.⁶⁷²

Long-term administration of 16,16-dimethyl prostaglandin E₂ and other prostaglandin E analogs to rats also produces thickening of the proximal colonic mucosa, although this is less marked than in the stomach and duodenum.^{196,199}

As in the small intestine, administration of epidermal growth factor to rats and cynomolgus monkeys induces hyperplasia of the colonic mucosa characterized histologically by hyperplasia and increased mitotic activity of crypt cells and reduction in goblet cell numbers with an increase in crypt depth and a slight increase in the numbers of infiltrating neutrophils.^{26,27}

ATYPICAL HYPERPLASIA

In common with other epithelial surfaces, atypical hyperplasia is associated with the development of colonic cancer in both humans and laboratory animals. The early alterations observed in rats treated with colonic carcinogens are similar to those found in the immediate vicinity of human colorectal carcinomas. The changes are characterized by lengthening, dilatation and branching of glands. The epithelium lining these glands shows mucous cell hyperplasia (goblet cell hyperplasia). Goblet cells contain predominantly sialomucin instead of the normal sulfomucin.^{257,500,673} As lesions become more atypical, these dilated, branched glands become more complex and lined by epithelium that shows increasing pseudostratification and vesicular cell nuclei.⁶⁷⁴ In rats treated with the carcinogens azoxymethane or 1,2-dimethylhydrazine, crypts show diminution of mucus secretion, increased cytoplasmic basophilia, prominent, rounded or enlarged nuclei which show variable degrees of pseudostratification and which eventually develop into frankly invasive glands.⁶⁷³ Similar alterations occur in rats treated with azoxymethane. Most studies with animal models for human colorectal cancers and preneoplastic changes have used rats or mice given these chemicals.⁶⁷⁵

Pertinent to the assessment of drug safety is the fact that atypical hyperplasia and eventually neoplasia can be induced by non-genotoxic agents such as dextran sodium sulfate that produce prolonged chronic colonic inflammation.⁶⁶⁹ Potential therapeutic agents designed to poorly absorb may produce similar alterations in the rodent colon when administered in very high doses.

Dosing dextran sodium sulfate to rodents has been used to model human chronic inflammatory disease such as ulcerative colitis and Crohn's colitis that is associated with an increase in the risk of developing colorectal cancer (see neoplasia, below).⁶⁷⁵ The approach to the histological examination of this form of hyperplasia should take into account the fact that in these inflammatory models there is progression of atypical features (dysplasia) which can be polypoid through to flat but infiltrating carcinomas. Grading of the degree of atypical features as performed in humans cases of colitis appears to be the best approach.⁶⁷⁶⁻⁶⁷⁸

Neoplasia

Adenomas and adenocarcinomas of the small and large intestine are infrequent spontaneous neoplasms in laboratory animals compared with humans where colorectal carcinoma is one of the most prevalent neoplasms in the Western world.

Adenomas and adenocarcinomas probably occur spontaneously in older dogs more than in any other animal species and as in humans these are located most frequently in the distal colon and rectum.⁶⁷⁹ In non-human primates glandular neoplasms of the intestine occur with increasing age in the ileum and in the colon with a predilection for the zones near the ileocecal valve.⁶⁸⁰ In one rhesus colony the incidence of colon cancer was reported to be 12% in 21- to 36-year-old animals.¹¹

In conventional rats and mice, spontaneous intestinal neoplasms are uncommon although adenocarcinomas are occasionally observed in the ileum or colon in chronic toxicity studies and carcinogenicity bioassays.^{175,190,681-684} Most of these originate in the distal part of the small intestine, cecum and right side of the colon. They may produce metastases, mostly to liver and lungs. In one series of spontaneous adenocarcinomas developing over a 17-year period in Wistar rats there appeared to be an intimate relationship with campylobacter-like organisms together with diverticulae and chronic inflammation which suggested that the associated inflammation was involved in the pathogenesis of these cancers. Adenomas and adenocarcinomas have been reported to occur in the colon or rectum of up to 0.3% of male and female control Fischer 344 rats and in the cecum in 0.3% of male but not female control B6C3F1 mice in the *National Toxicology Program*.²⁰⁶

Some hamster colonies with inflammatory bowel disease also have a high incidence of small and large intestinal polyps, adenomas and adenocarcinomas.⁶⁸⁵ Poorly differentiated carcinomas may infiltrate local lymph nodes and it may be difficult to locate the primary neoplasm. Polyps are predominantly adenomatous in nature although inflammatory or regenerative polyps are observed.

Chemicals induce colon carcinoma relatively uncommonly in rodent carcinogenicity studies. In the *National Toxicology Program* database, only 7% of rodent tumorigens have targeted the intestine compared with 12% that have produced tumors in the forestomach and 57% in the liver.²⁰⁶ Therapeutic agents have seldom induced intestinal neoplasms in rodent carcinogenicity studies. One of the few cases reported is itraconazole formulated in hydroxypropyl- β -cyclodextrin.⁶⁸⁶ The cyclodextrin vehicle produced cecal and colonic hypertrophy and a small increase in colonic carcinomas in rats given very high oral doses for two years. This was seen as an adaptive high dose effect not relevant to the intravenous use of this vehicle with antifungal drug.

Adenocarcinomas can be induced experimentally in the rodent intestine by the carcinogens 1,2-dimethylhydrazine and azoxymethane. The histogenesis of these induced carcinomas has been extensively studied and it is generally accepted that they resemble human colorectal cancer.^{673,675,687} In view of the importance of colon cancer in humans, a number of new genetic mouse models predisposed to colon cancer have been developed over recent years.⁶⁸⁸ One of the most widely used in chemoprevention assays, the *Apc^{Min}* mouse, heterozygous for the multiple intestinal neoplasia allele, produces intestinal polyps mostly in the small intestine rather than the colon.⁶⁸⁹ However, infection with *Citrobacter rodentium* has been shown to promote colonic tumor formation in these mice, again reflecting the complex interactions involved in colon tumorigenesis.⁶⁹⁰ A study which compared the use of different rodent models in the prediction of efficacy of colonic tumor prevention in human volunteers suggested that while all these models have reasonable predictive ability, the carcinogen-induced rat model performed as least as well as the *Apc^{Min}* mouse model.⁶⁹¹

COLORECTAL NEOPLASIA AND INFLAMMATION

Pertinent to the evaluation of drugs and chemicals in rodents is the close link between chronic inflammation and the development of neoplasms particularly in the large intestine. Over recent years epidemiological studies in patients and genetic evidence in animal models have served to confirm the close link between chronic inflammation and cancer.⁶⁹² Moreover it has been shown that treatment with non-steroidal inflammatory drugs decreases the incidence and mortality from a number of cancers. Two key genes in the inflammatory process, cyclooxygenase 2 (COX-2) and nuclear factor κ B (NF- κ B), also provide plausible mechanistic links between inflammation and cancer.⁶⁹³

In humans chronic inflammatory bowel disease has long been associated with an increased risk of colonic cancer and several animal models using predominately oral administration of dextran sodium sulfate recapitulate these findings.⁶⁷⁵ Neoplasms occurring in the rat colon following administration of high doses of sulfated dextrans or degraded carrageenans usually occur in the distal colon and rectum as well as in the cecum and proximal colon and may be both flat or polypoid adenomas and adenocarcinomas.^{642,648,649} Their distribution is closely linked to areas of chronic inflammation.

The pathogenesis of neoplasms induced by carrageenans and dextrans remains unexplained although the presence of inflammation is essential. It is likely that this provides a microenvironment which aids proliferation and survival of malignant cells, promotes angiogenesis and metastasis, subverts immune responses and alters responses to hormones and other mediators.⁶⁹² Other suggested factors include reduced apoptosis, alterations to crypt cell metabolism and changes in bile acid enterohepatic circulation. It has also been suggested that bacteria flora specific to rodents might also be involved in tumor development.⁶⁹⁴

Although these agents are biologically active compounds, they are non-mutagenic in the usual short-term tests. It has been suggested that carrageenans act as tumor promoters for they have been shown to potentiate the appearance of carcinomas in rats treated with standard intestinal carcinogens.³⁴³ It has also been proposed that these agents are tumor initiators based on the development of carcinomas in rats treated with degraded carrageenans for only two months.⁶⁴⁹ However, despite only a short period of treatment, inflammation, regenerative changes and squamous metaplasia persisted throughout a period of 18 months after treatment was withdrawn before development of cancer in these rats.

Overall the only consistent association of carrageenans and dextran sulfate with development of carcinoma in rodents is that of chronic inflammation. The key point in the evaluation of agents that produce these neoplasms in the context of chronic inflammation in the large intestine in rodents is that neoplasms do not occur in the absence of severe and prolonged inflammation.

Classification

A similar range of neoplasms can be defined histologically in both human and experimental pathology and it is usually appropriate to use the same classification for all species, domestic and laboratory animals and humans.^{67,68,679} Assessment of neoplasia is more difficult in the context of chronic inflammation where large zones of mucosa may have developed atypical features (*field change*) as well as being disrupted by inflammation,

fibroblastic change and fibrosis. Invasive carcinoma may develop in the context of small flat dysplastic changes in the epithelium. In this context grading of the degree of atypical cytological features is useful as severe atypia (dysplasia) may not only be a marker or precursor of carcinoma but may itself be malignant and associated with direct invasion into the underlying tissue.⁶⁷⁶

The generally agreed classification for rodent intestinal tumors is summarized as follows.^{67,68}

ADENOMA (ADENOMATOUS POLYP)

These represent localized, sessile or polypoid neoplasms composed of proliferating tubular glands, which show varying degrees of nuclear hyperchromatism, pseudostratification and cellular pleomorphism (Figure 8.9). A useful scheme for grading the carcinogenic potential of hyperplastic mucosa and adenomatous polyps in humans based on the degree of epithelial pseudostratification has been proposed by Kozuka.⁶⁷⁴ Although experimental neoplasms may not always show the full spectrum of these changes reported in humans, this grading provides a useful baseline concept for the assessment of these non-invasive proliferative lesions. As these polyps progress they show increased nuclear pseudostratification and a typical branching of their glands. If neoplastic cells or glands are seen in the stroma of the stalk or base the diagnosis of carcinoma is made.

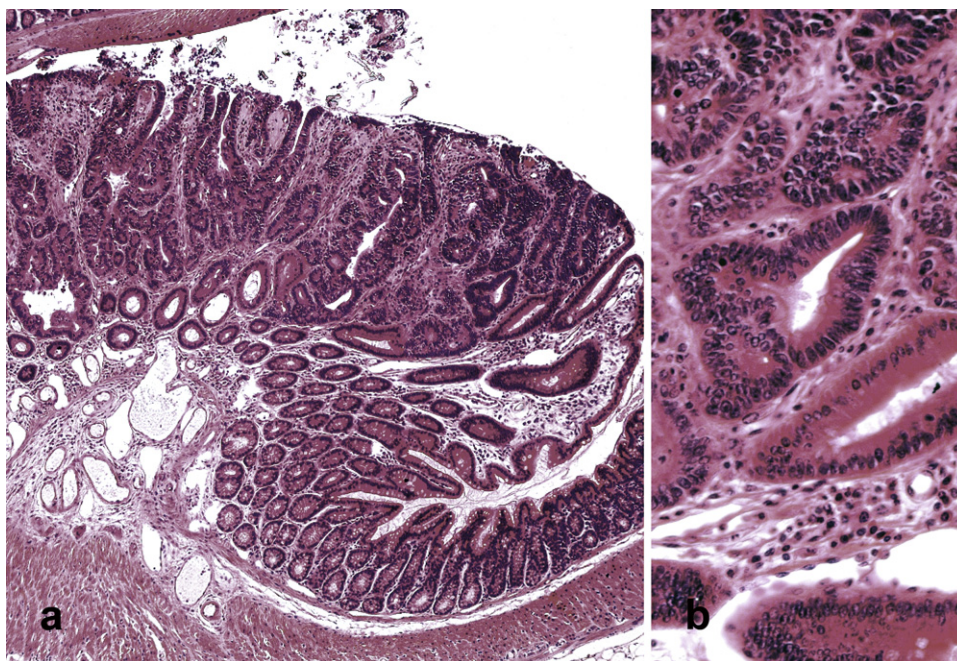


FIGURE 8.9 Panel a: Section of a pedunculated adenomatous polyp (adenoma) in the colon of a *Min* mouse. It is well differentiated and shows no invasion of the fibrovascular stalk. (H&E×60.) Panel b: A higher power view of the same lesion showing the moderate pseudostratification of the nuclei in the glandular cells (H&E×250).

Villous adenoma is a form of adenoma in which the epithelial proliferation takes the form of elongated villi with a sparse fibrovascular stroma. They can be graded in a similar way to other adenomas.

ADENOCARCINOMA

These are glandular neoplasms of variable differentiation, sometimes originating in adenomatous polyps or villous adenomas but which show infiltration of the intestinal wall, i.e. beyond the boundary of the muscularis mucosa.

Squamous carcinomas also occur in the anorectal zone but are similar to those which occur in squamous epithelium elsewhere. Similarly, mesenchymal neoplasms also are found in the small and large intestinal wall (see Integumentary System, Chapter 2).

References

1. Porter SR, Scully C. Adverse drug reactions in the mouth. *Clin Dermatol* 2000;**18**:525–32.
2. Scully C, Bagan JV. Adverse drug reactions in the orofacial region. *Crit Rev Oral Biol Med* 2004;**15**:221–39.
3. Keefe DMK, Gibson RJ. Mucosal injury from targeted anti-cancer therapy. *Support Care Cancer* 2007;**15**:483–90.
4. Zentler-Monro PL, Northfield TC. Drug-induced gastro-intestinal disease. *Br Med J* 1979;**1**:1263–5.
5. Desruelles F, Bahadoran P, Lacour JP, Perrin C, Santini J, Ortonne JP. Giant oral aphthous ulcers induced by nicorandil. *Br J Dermatol* 1998;**138**:712–3.
6. Shotts RH, Scully C, Avery CM, Porter SR. Nicorandil-induced severe oral ulceration - a newly recognized drug reaction. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1999;**87**:706–7.
7. Gagari E, Kabani S. Adverse effects of mouthwash use. A review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995;**80**:432–9.
8. Sohi H, Aluja A, Ahmad FJ, Khar RK. Critical evaluation of permeation enhancers for oral mucosal drug delivery. *Drug Dev Ind Pharm* 2010;**36**:254–82.
9. Aps JKM, Martens LC. Review: the physiology of saliva and transfer of drugs into saliva. *Forensic Sci Int* 2005;**150**:119–31.
10. Wyllie E, Wyllie R, Cruse RP, Rothner AD, Erenberg G. The mechanism of mitrazepam-induced drooling and aspiration. *N Engl J Med* 1986;**314**:35–8.
11. Lowenstine LJ. A primer of primate pathology: lesions and nonlesions. *Toxicol Pathol* 2003;**31**(Suppl. 92–102).
12. Kodama Y, Ozaki K, Sano T, Matsuura T, Akagi H, Narama I. Induction of squamous cell carcinoma of forestomach in diabetic rats by single alloxan treatment. *Cancer Sci* 2006;**97**:1023–30.
13. Reuterving CO, Hägg E, Henriksson R, Holm J. Salivary glands in long-term alloxan-diabetic rats. A quantitative light and electron-microscopic study. *APMIS Sect A Pathol* 1987;**95**:131–6.
14. Sagström S, Scarlett SM, Sagulin GB, Roomans GM. Early effects of alloxan on rat submandibular gland. *J Submicrosc Cytol* 1987;**19**:555–9.
15. Thompson GR, Baker JR, Fleischman RW, Rosenkranz H, Shaeppi UH, Cooney DA, et al. Preclinical toxicologic evaluation of bleomycin (NSC 125 006), a new anti-tumor antibiotic. *Toxicol Appl Pharmacol* 1972;**22**:544–55.
16. Pilaro, AM. Eribitux (Cetuximab). *Pharmacology/toxicology review and evaluation*. Application No: 125084. Maryland, USA: Center for Drug Evaluation and Research. Food and Drug Administration; 2004.
17. McGuinn, WD. Iressa (gefitinib) *Pharmacology/toxicology review and evaluation*. Application No: 021399. Maryland, USA: Center for Drug Evaluation and Research. Food and Drug Administration; 2003.
18. Fox PC. Acquired salivary dysfunction. Drugs and radiation. *Ann N Y Acad Sci* 1998;**842**:132–7.
19. Garthoff B, Hoffmann K, Luckhaus G, Thurau K. Adequate substitution with electrolytes in toxicological testing of 'loop' diuretics in the dog. *Toxicol Appl Pharmacol* 1982;**65**:191–202.
20. Medical Mystery—the answer *N Engl J Med*. 2004;**351**:2349–50.
21. Savage NW, Barber MT, Adkins KF. Pigmentary changes in the rat oral mucosa following antimalaria therapy. *J Oral Pathol Med* 1986;**15**:468–71.

22. Walsh KM, Gough AW. Hypopigmentation in dogs treated with an inhibitor of platelet aggregation. *Toxicol Pathol* 1989;17:549–54.
23. Imaoka K, Hojo K, Doi K, Mitsuoka T. Development of spontaneous tongue calcification and polypoid lesions in DBA/2NCrj mice. *Lab Anim* 1986;20:1–4.
24. Doi K, Maeda N, Doi C, Isegawa N, Sugano S, Mitsuoka T. Distribution and incidences of calcified lesions in DBA/2NCrj and BALB/cAnNCrj mice. *Jpn J Vet Sci* 1985;47:479–82.
25. Westwood FR, Jones DV, Aldridge A. The synovial membrane, liver, and tongue: target organs for a ricin A-chain immunotoxin (ZD0490). *Toxicol Pathol* 1996;24:477–83.
26. Breider MA, Bleavins MR, Reindel JF, Gough AW, de la Iglesia FA. Cellular hyperplasia in rats following continuous intravenous infusion of recombinant human epidermal growth factor. *Vet Pathol* 1996;33:184–94.
27. Reindel JF, Pilcher GD, Gough AW, Haskins JR, de la Iglesia FA. Recombinant human epidermal growth factor₁₋₄₈-induced structural changes in the digestive tract of cynomolgus monkeys (*Macaca fascicularis*). *Toxicol Pathol* 1996;24:669–79.
28. Anon. IRESSA[®] (gefitinib) prescribing information. Wilmington DE: AstraZeneca; 2005.
29. Vincenzi B, Santini D, Rabitti C, Coppola R, Zobel BB, Trodella L, et al. Cetuximab and irinotecan as third-line therapy in advanced colorectal cancer patients: a single centre phase II trial. *Br J Cancer* 2006;94:792–7.
30. Segaert S, Van Cutsem E. Clinical signs, pathophysiology and management of skin toxicity during therapy with epidermal growth factor receptor inhibitors. *Ann Oncol* 2005;16:1425–33.
31. Losco PE. Dental dysplasia in rats and mice. *Toxicol Pathol* 1995;23:677–88.
32. Kuijpers MHM, Van De Kooij AJ, Slootweg PJ. The rat incisor in toxicologic pathology. *Toxicol Pathol* 1996;24:346–60.
33. Kato A, Suzuki M, Karasawa Y, Sugimoto T, Doi K. PTHrP and PTH/PTHrP receptor 1 expression in odontogenic cells of normal and HHM model rat incisors. *Toxicol Pathol* 2005;33:456–64.
34. Strewler GJ. The physiology of parathyroid hormone-related protein. *N Engl J Med* 2000;342:177–85.
35. Welbury RR, Craft AN, Murray JJ, Kernahan J. Dental health of survivors of malignant disease. *Arch Dis Child* 1984;59:1186–7.
36. Marec-Berard P, Azzi D, Chauv-Bodard AG, Lagrange H, Gourmet R, Bergeron C. Long-term effects of chemotherapy on dental status in children treated for neuroblastoma. *Pediatr Hematol Oncol* 2005;22:581–8.
37. Alpaslan G, Alpaslan C, Gogen H, Oguz A, Cetiner S, Karadeniz C. Disturbances in oral and dental structures in patients with pediatric lymphoma after chemotherapy – a preliminary report. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1999;87:317–21.
38. MacLeod RI, Welbury RR, Soames JV. Effects of cytotoxic chemotherapy on dental development. *J R Soc Med* 1987;80:207–9.
39. Fletcher AM, Bregman CL, Woicke J, Salcedo TW, Zidell RH, Janke HE, et al. Incisor degeneration in rats induced by vascular endothelial growth factor/fibroblast growth factor receptor tyrosine kinase inhibition. *Toxicol Pathol* 2010;38:267–79.
40. Stene T, Koppang HS. The effect of vincristine on dentinogenesis in the rat incisor. *Scand J Dent Res* 1976;84:342–4.
41. Stene T, Koppang HS. Autoradiographic investigation of dentine production in rats incisors after vincristine administration. *Scand J Dent Res* 1980;88:104–12.
42. Koppang HS. Histomorphologic investigations on the effect of cyclophosphamide on dentinogenesis of the rat incisor. *Scand J Dent Res* 1973;81:383–96.
43. Vahlsing HL, Kim S-K, Feringa ER. Cyclophosphamide-induced abnormalities in the incisors of the rat. *J Dent Res* 1977;56:809–16.
44. Robinson PB, Harris M, Harvey W. Abnormal skeletal and dental growth in epileptic children. *Br Dent J* 1983;154:9–13.
45. Robinson PB, Harvey W. Tooth root resorption induced in rats by diphenylhydantoin and parathyroidectomy. *Br J Exp Pathol* 1989;70:65–72.
46. Kato A, Suzuki M, Karasawa Y, Sugimoto T, Doi K. Histopathological study of time course changes in PTHrP-induced incisor lesions of rats. *Toxicol Pathol* 2005;33:230–8.
47. Tredwin CJ, Scully C, Bagan-Sebastian JV. Drug-induced disorders of teeth. *J Dent Res* 2005;84:596–602.
48. Cale AE, Freedman PD, Lumerman H. Pigmentation of the jawbone and teeth secondary to minocycline hydrochloride therapy. *J Periodontol* 1988;59:112–4.

49. Ayashoglu E, Erkek E, Oba AA, Cebecoglu E. Doxycycline-induced staining of permanent adult dentition. *Aust Dent J* 2005;**50**:273–5.
50. Martineau DB, Warshawsky H, Dickson K, Lai WH, Bergeron JJ. Localization of epidermal growth factor receptors in cells of the enamel organ of the rat incisor. *Dev Biol* 1991;**148**:590–601.
51. Schaffner J-C, Ernst E, Junker U, Thomas H, Germann P-G. Vascular endothelial growth factor inhibitors (VEGF inhibitors). In: Drommer W, Karbe E, Germann P-G, Morawietz G, editors. *Classic examples in toxicologic pathology*. Hannover: European Society of Toxicologic Pathology; 2005.
52. Bourcier, T. Januvia (sitagliptin phosphate). *Pharmacology/toxicology review and evaluation*. Application No: 021995. Maryland, USA: Center for Drug Evaluation and Research. Food and Drug Administration; 2006.
53. Robinson M. Dietary related periodontitis and oro-nasal fistulation in rats. *J Comp Pathol* 1985;**95**:489–98.
54. Beghi E, Di Mascio R, Tognoni G. Adverse effects of anticonvulsant drugs: a critical review. *Adverse Drug React Acute Poisoning Rev* 1986;**2**:63–86.
55. Barthold PM. Cyclosporin and gingival overgrowth. *J Oral Pathol Med* 1987;**16**:463–8.
56. Lederman D, Lumerman H, Reuben S, Freedman PD. Gingival hyperplasia associated with nifedipine therapy. *Oral Surg* 1984;**57**:620–2.
57. Syrjamen SM, Syrjamen KJ. Hyperplastic gingivitis in a child receiving sodium valproate treatment. *Proc Finn Dent Soc* 1979;**75**:95–8.
58. Latimer KS, Rakich PM, Purswell BJ, Kircher IM. Effects of cyclosporin A administration in cats. *Vet Immunol Immunopathol* 1986;**11**:161–73.
59. do’Nascimento A, Barreto R, de C, Bozzo L, De Almeida OP. Interaction of phenytoin and inflammation induces gingival overgrowth in rats. *J Periodont Res* 1985;**20**:386–91.
60. Waner T, Nyska A, Nyska M, Pirak M, Sela M, Galiano A. Gingival hyperplasia in dogs induced by oxodipine, a calcium channel blocking agent. *Toxicol Pathol* 1988;**16**:327–32.
61. Cetinkaya BO, Acikgoz G, Aydin O, Korkmaz A, Keles GC. The relationship between proliferating cell nuclear antigen expression and histomorphometrical alterations in cyclosporin A-induced gingival overgrowth in rats. *Toxicol Pathol* 2006;**34**:180–6.
62. Kantor MI, Hassel TM. Increased accumulation of sulfated glycoaminoglycans in cultures of human fibroblasts from phenytoin-induced gingival overgrowth. *J Dent Res* 1983;**62**:383.
63. Hassel TM, Page RC, Narayanan AS, Cooper CG. Diphenyldydantoin (dilantin) gingival hyperplasia: drug-induced abnormality of connective tissue. *Proc Natl Acad Sci U S A* 1976;**73**:2902.
64. Lucas RM, Howell LP, Wall BA. Nifedipine-induced gingival hyperplasia: a histochemical and ultrastructural study. *J Periodontol* 1985;**56**:211–5.
65. Schiødt M, Armitage GC, Lackner AA. Gingival fibromatosis, *Macaca mulatta*. In: Jones TC, Mohr U, Hunt RD, editors. *Monographs on pathology of laboratory animals. Nonhuman primates II*. Berlin: Springer-Verlag; 1993. p. 30–1.
66. Takahashi M, Okamiya H. Tumours of the oral cavity, buccal pouch, oesophagus, forestomach and salivary glands. In: Turusov VS, Mohr U, editors. *Pathology of tumours in laboratory animals, Second Edition. Vol. 3, Tumours of the hamster*. Lyon: International Agency for Research on Cancer; 1996. p. 59–77.
67. Mohr U. The digestive system. In: Mohr U, editor. *International classification of rodent tumours, Part 1, The rat*. Lyon: International Agency for Research on Cancer; 1997.
68. Betton GR, Whiteley LO, Anver MR, Brown R, Deschl U, Elwell M, et al. Gastrointestinal tract. In: Mohr U, editor. *International classification of rodent tumours. The mouse*. Berlin: Springer-Verlag; 2001. p. 23–58.
69. Sundberg JP, Junge RE, El Shazly MO. Oral papillomatosis in New Zealand white rabbits. *Am J Vet Res* 1985;**46**:664–8.
70. Sundberg JP, Everitt JI. Diagnostic exercise: lingual growths in rabbits. *Lab Anim Sci* 1986;**36**:499–500.
71. Sundberg JP, Junge RE, Lancaster WD. Immunoperoxidase localization of papillomaviruses in hyperplastic and neoplastic epithelial lesions of animals. *Am J Vet Res* 1984;**45**:1441–6.
72. Watrach AM, Small E, Case MT. Canine papilloma: progression of oral papilloma to carcinoma. *J Natl Cancer Inst* 1970;**45**:915–20.
73. Kaspereit J, Friderichs-Gromoll S, Buse E, Habermann G. Spontaneous neoplasms observed in cynomolgus monkeys (*Macaca fascicularis*) during a 15-year period. *Exp Toxicol Pathol* 2007;**59**:163–9.
74. Scully C, Bagan JV. Oral squamous cell carcinoma: overview of current understanding of aetiopathogenesis and clinical implications. *Oral Dis* 2009;**15**:388–99.

75. Thurman JD, Greenman DL, Kodell RL, Turturro A. Oral squamous cell carcinoma in ad libitum-fed and food restricted Brown-Norway rats. *Toxicol Pathol* 1997;**25**:217–24.
76. Gold LS, Manley NB, Slone TH, Ward JM. Compendium of chemical carcinogens by target organ: results of chronic bioassays in rats, mice, hamsters, dogs, and monkeys. *Toxicol Pathol* 2001;**29**:639–52.
77. Anon. *Report on carcinogens*, 11th ed. U.S. Department of Health and Human Services. Public Health Service, National Toxicology Program Research Triangle Park NC; 2005.
78. Howley PM. On human papillomaviruses. *N Engl J Med* 1986;**315**:1089–90.
79. Cheville NF, Olson C. Cytology of the canine oral papilloma. *Am J Pathol* 1964;**45**:849–72.
80. Pfister H. Biology and biochemistry of papillomaviruses. *Rev Physiol Biochem Pharmacol* 1984;**99**:111–81.
81. Gössner W, Luz A. Tumours of the jaws. In: Turusov V, Mohr U, editors. *Pathology of tumours in laboratory animals. Tumours of the mouse*, Vol. 2. Lyon: International Agency for Research on Cancer; 1994.
82. Ernst E, Long PH, Wadsworth PF, Leininger JR, Reiland S, Konishi Y. Skeletal system and teeth. In: Mohr U, editor. *International classification of rodent tumors. The mouse*. Berlin: Springer-Verlag; 2001. p. 389–415.
83. Cullen JM, Ruebner BH, Hsieh DPH, Burkes EJ. Odontogenic tumours in Fischer rats. *J Oral Pathol Med* 1987;**16**:469–73.
84. Sonis ST, Fey EG. Oral complications of cancer therapy. *Oncology-NY* 2002;**16**:680–6.
85. Levine MJ, Reddy MS, Tabak LA, Loomis RE, Bergey EJ, Jones PC, et al. Structural aspects of salivary glycoproteins. *J Dent Res* 1987;**66**:436–41.
86. Schulte BA. Genetic and sex-related differences in the structure of submandibular glycoconjugates. *J Dent Res* 1987;**62**:442–50.
87. Nair PNR, Schroeder HE. Duct-associated lymphoid tissue (DALT) of minor salivary glands and mucosal immunity. *Immunology* 1986;**57**:171–80.
88. Nair PNR, Zimmerli I, Schroeder HE. Minor salivary gland duct-associated lymphoid tissues (DALT) in monkeys, changes with age. *J Dent Res* 1987;**66**:407–11.
89. Junqueira LCU, Toledo AMS, Doine AI. Digestive enzymes in the parotid and submandibular glands of mammals. *An Acad Bras Cienc* 1973;**45**:629–33.
90. Chabot J-G, Walker P, Pelletier G. Thyroxine accelerates the differentiation of granular convoluted tubule cells and the appearance of epidermal growth factor in the submandibular gland of the neonatal mouse. A fine structural immunocytochemical study. *Cell Tissue Res* 1987;**248**:351–8.
91. Tandler B, Pinkstaff CA, Riva A. Ultrastructure and histochemistry of human anterior lingual salivary-glands (glands of Blandin and Nuhn). *Anat Rec* 1994;**240**:167–77.
92. Pinkstaff CA. The cytology of salivary glands. *Int Rev Cytol* 1980;**63**:141–261.
93. Munhoz COG. Histochemical classification of acini and ducts of parotid glands from artiodactyles, carnivores and rodents. *Acta Histochem* 1971;**39**:302–17.
94. Pinkstaff CA. Salivary gland sexual dimorphism: a brief review. *Eur J Morphol* 1998;**36**(Suppl.):31–4.
95. Pinkstaff CA. Serous, seromucous, and special serous cells in salivary-glands. *Microsc Res Tech* 1993;**26**: 21–31.
96. de Rijk E, Ravesloot WTM, Hafmans TGM, van Esch E. Multifocal ductal cell hyperplasia in the submandibular salivary glands of Wistar rats chronically treated with a novel steroidal compound. *Toxicol Pathol* 2003;**31**:1–9.
97. Barka T. Biologically active peptides in submandibular glands. *J Histochem Cytochem* 1980;**28**:836–59.
98. Mori M, Hamada K, Naito R, Tsukitan K, Asano K. Immuno-histochemical localization of epidermal growth factor in rodent submandibular glands. *Acta Histochem Cytochem* 1983;**16**:536–48.
99. Hiramatsu M, Kashimata M, Takayama F, Minami N. Developmental changes in and hormonal modulation of epidermal growth-factor concentration in the rat submandibular-gland. *J Endocrinol* 1994;**140**:357–63.
100. Cohen S. Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the newborn animal. *J Biol Chem* 1962;**237**:1555–62.
101. Cohen, S. Origins of growth factors: NGF and EGF. In *Understanding and optimizing human development: From cells to patients to populations*, Vol. 1038. 98–102; (2004).
102. Gresik EW. The granular convoluted tubule (GCT) cell of rodent submandibular glands. *Microsc Res Tech* 1994;**27**:1–24.
103. Lantini MS, Piludu M, Cossu M. Subcellular localization of epidermal growth factor in human parotid gland. *Histochem J* 2001;**33**:427–31.

104. Murphy RA, Watson AY, Metz J, Forssmann WG. The mouse submandibular gland: an exocrine organ for growth factors. *J Histochem Cytochem* 1980;**28**:890–902.
105. Tsukitani K, Mori M. Immunohistochemistry and radioimmunoassay of EGF in submandibular glands of mice treated with secretagogues. *Cell Mol Biol* 1986;**32**:677–83.
106. Noda Y, Taki Y, Hikosaka N, Meenaghan MA, Mori M. Immunohistochemical localization of carbonic anhydrase in submandibular salivary glands of mice and hamsters treated with phenylephrine, testosterone or duct ligation. *Arch Oral Biol* 1986;**31**:441–7.
107. Schulte BA, Spicer SS. Light microscopic detection of sugar residues in glycoconjugates of salivary glands and the pancreas with lectin-horseradish peroxidase conjugates. I. Mouse. *Histochem J* 1983;**15**:1217–38.
108. Schulte BA, Spicer SS. Light microscopic detection of sugar residues in glycoconjugates of salivary glands and the pancreas with lectin-horseradish peroxidase conjugates. II. Rat. *Histochem J* 1984;**16**:3–20.
109. Reifel CW, Travill AA. Structure and carbohydrate histochemistry of postnatal canine salivary glands. *Am J Anat* 1972;**134**:377–94.
110. Nagayo T, Tandler B. Ultrastructure of dog parotid gland. *J Submicrosc Cytol* 1986;**18**:67–74.
111. Pedini V, Ceccarelli P, Gargiulo AM. Glycoconjugates in the mandibular salivary gland of adult dogs revealed by lectin histochemistry. *Res Vet Sci* 1994;**57**:353–7.
112. Stephens LC, King GK, Peters LJ, Ang KK, Schultheiss TE, Jardine JH. Acute and late radiation injury in rhesus monkey parotid glands. Evidence of interphase death. *Am J Pathol* 1986;**124**:469–78.
113. Stephens LC, King GK, Peters LJ, Ang KK, Schultheiss TE, Jardine JH. Unique radiosensitivity of serous cells in rhesus monkey submandibular glands. *Am J Pathol* 1986;**124**:479–87.
114. Losco PE, Evans EW, Barat SA, Blackshear PE, Reyderman L, Fine JS, et al. The toxicity of SCH 351591, a novel phosphodiesterase-4 inhibitor, in cynomolgus monkeys. *Toxicol Pathol* 2004;**32**:295–308.
115. Innes JRM, Stanton MF. Acute disease of the submaxillary and harderian glands (sialodacryoadenitis) of rats with cytomegaly and no inclusion bodies. *Am J Pathol* 1961;**38**:455–68.
116. Carthew P. & Slinger, R.P. Diagnosis of sialodacryoadenitis virus infection of rats in a virulent enzootic outbreak. *Lab Anim* 1981;**15**:339–42.
117. Percy DH, Wojcinski ZW. Diagnostic exercise: inter-mandibular swelling in rats. *Lab Anim Sci* 1986;**36**:665–6.
118. Percy DH, Hayes MA, Kocal TE, Wojcinski ZW. Depletion of salivary gland epidermal growth factor by sialodacryoadenitis virus infection in the Wistar rat. *Vet Pathol* 1988;**25**:183–92.
119. Hanna PE, Percy DH, Paturzo F, Bhatt PN. Sialodacryoadenitis in the rat: effects of immunosuppression on the course of the disease. *Am J Vet Res* 1984;**45**:2077–83.
120. Arseculeratne SN, Panabokke RG, Navaratnam C, Weljange LV. An epizootic of *Klebsiella aerogenes* infection in laboratory rats. *Lab Anim* 1981;**15**:333–7.
121. Hayashi Y, Kurashima C, Utsuyama M, Hirokawa K. Spontaneous development of auto-immune sialadenitis in aging BDF1 mice. *Am J Pathol* 1988;**132**:173–9.
122. Fugino-Kurihara H, Fujita H, Hakura A, Nonaka K, Tarui S. Morphological aspects on pancreatic islets of non-obese diabetic (NOD) mice. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1985;**49**:107–20.
123. Törnwall J, Lane TE, Fox RI, Fox HS. T cell attractant chemokine expression initiates lacrimal gland destruction in nonobese diabetic mice. *Lab Invest* 1999;**79**:1719–26.
124. Hayashi Y, Sato M, Hirokawa K. Induction of experimental allergic sialadenitis in mice. *Am J Pathol* 1985;**118**:476–83.
125. Fox RI. Sjögren's syndrome. *Lancet* 2005;**366**:321–31.
126. Scofield RH, Asfa S, Obeso D, Jonsson R, Kurien BT. Immunization with short peptides from the 60-kDa Ro antigen recapitulates the serological and pathological findings as well as the salivary gland dysfunction of Sjögren's syndrome. *J Immunol* 2005;**175**:8409–14.
127. McMartin DN. Morphologic lesions in ageing Syrian hamsters. *J Gerontol* 1979;**34**:502–11.
128. Kelly DF, Lucke VM, Denney HR, Lane JG. Histology of salivary gland infarction in the dog. *Vet Pathol* 1979;**16**:438–43.
129. Chamanza R, Marxfeld HA, Blanco AI, Naylor SW, Bradley AE. Incidences and range of spontaneous findings in control cynomolgus monkeys (*Macaca fascicularis*) used in toxicity studies. *Toxicol Pathol* 2010;**38**:642–57.

130. Waterhouse JP, Chisolm DM, Winter RB, Patel M, Yale RS. Replacement of functional parenchymal cells by fat and connective tissue in human submandibular salivary glands. An age related change. *J Oral Pathol Med* 1973;**2**:16–27.
131. Scott J. Quantitative age changes in the histological structure of human submandibular salivary glands. *Arch Oral Biol* 1977;**22**:221–7.
132. Sashima M. Age-related changes of rat submandibular gland: a morphometric and ultrastructural study. *J Oral Pathol Med* 1986;**15**:507–12.
133. Boyd EM, Cehn CP, Muis LF. Resistance to starvation in albino rats fed from weaning on diets containing from 0 to 81% of protein as casein. *Growth* 1970;**24**:99–112.
134. McBride RK, Harper C, Siegel IA. Methotrexate-induced changes in rat parotid and submandibular gland function. *J Dent Res* 1987;**66**:1445–8.
135. Smith B, Butler M. The effects of long-term propranolol on the salivary glands and intestinal mucosa of the mouse. *J Pathol* 1978;**124**:185–7.
136. Kajikawa S, Takeuchi A, Nii A, Nakayama H, Doi K. Temporal reduction in size of salivary acinus in rats induced by theophylline. *Toxicol Pathol* 2005;**33**:218–24.
137. Denny PC, Ball WD, Redman RS. Salivary glands: a paradigm for diversity of gland development. *Crit Rev Oral Biol Med* 1997;**8**:51–75.
138. Price LH, Heninger GR. Lithium in the treatment of mood disorders. *N Engl J Med* 1994;**331**:591–8.
139. Brandenburg AH, Smits MG, Voorbrood BS, Hemmes AM, Ros JJW. Submandibular salivary-gland hypertrophy induced by phenytoin. *Epilepsia* 1993;**34**:151–2.
140. Riddell RH. The gastrointestinal tract. In: Riddell RH, editor. *Pathology of drug-induced and toxic diseases*. New York: Churchill Livingstone; 1982. p. 515–606.
141. Manetti L, Bogazzi F, Brogioni S, Grasso L, Lupi I, Genovesi M, et al. Submandibular salivary gland volume is increased in patients with acromegaly. *Clin Endocrinol* 2002;**57**:97–100.
142. Brenner GM, Stanton HC. Adrenergic mechanisms responsible for submandibular salivary glandular hypertrophy in the rat. *J Pharmacol Exp Ther* 1970;**173**:166–75.
143. Simson, JAV, Spicer, SS & Hall, BJ. Morphology and cyto-chemistry of rat salivary gland acinar secretory granules and their alteration by isoproterenol. I. Parotid gland. *J Ultrastruct Res* **48**: 465–82.
144. Ten Hagen KG, Balys MM, Tabak LA, Melvin JE. Analysis of isoproterenol-induced changes in parotid gland gene expression. *Physiol Genomics* 2002;**8**:107–14.
145. Barka T, Chang WWL, Van Der Noen H. The effect of 6-hydroxydopamine on rat salivary glands and on their response to isoproterenol. *Lab Invest* 1972;**27**:594–9.
146. Wells DJ, Humphreys-Beher MG. Analysis of protein synthesis in rat salivary glands after chronic treatment with β -receptor agonists and phosphodiesterase inhibitors. *Biochem Pharmacol* 1985;**34**:4229–37.
147. Rogers S, Barsoum N, DiFonzo C, Gracon S, Houston B, Martin R, et al. Intravenous toxicology of a new cardiotoxic agent. *Toxicologist* 1985;**5**:111.
148. Jayasekara MU, Dewit RH, Peter GK, Fitzgerald JE. Subchronic toxicity of C1-930, a novel cardiotoxic agent in rats and dogs. *Toxicologist* 1986;**6**:203.
149. Westwood FR, Iswaran TJ, Greaves P. Long-term effects of an inotropic phosphodiesterase inhibitor (ICI 153,110) on the rat salivary gland, Harderian gland, and intestinal mucosa. *Toxicol Pathol* 1991;**19**:214–23.
150. Larson JL, Pino MV, Geiger LE, Simeone CR. The toxicity of repeated exposures to rolipram, a type IV phosphodiesterase inhibitor, in rats. *Pharmacol Toxicol* 1996;**78**:44–9.
151. Jackson CD, Blackwell B-N. Subchronic studies of doxylamine in Fischer 344 rats. *Fundam Appl Toxicol* 1988;**10**:243–53.
152. Hamperl H. Onkocytes and so-called Hürthle cell tumor. *Arch Pathol* 1950;**49**:563–7.
153. Ghadially, FN. Mitochondria. In: *Ultrastructural Pathology of the Cell and Matrix*. London: Butterworths; 1982.
154. Bogart BI. The effect of aging on the rat submandibular gland. An ultrastructural, cytochemical and biochemical study. *J Morphol* 1970;**130**:337–52.
155. Takeda Y, Suzuki A, Ishikawa G. Nodular hyperplasia of oncocytes in mouse submandibular glands. *J Oral Pathol Med* 1985;**14**:182–9.
156. Chiu T, Chen HC. Spontaneous basophilic hypertrophic foci of the parotid glands in rats and mice. *Vet Pathol* 1986;**23**:606–9.

157. Colerangle, J. Byetta® (exenatide). *Pharmacology/toxicology review and evaluation*. Application No: 021773. Maryland, USA: Center for Drug Evaluation and Research. Food and Drug Administration; 2005.
158. Dardick I, Jeans MTD, Sinnott NM, Wittkuhn JF, Kahn HJ, Bauml R. Salivary gland components involved in the formation of squamous metaplasia. *Am J Pathol* 1985;**119**:33–43.
159. Haseman JK, Hailey JR, Morris RW. Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: a National Toxicology Program update. *Toxicol Pathol* 1998;**26**:428–41.
160. Bonavina L, Demeester TR, Mcchesney L, Schwizer W, Albertucci M, Bailey RT. Drug-induced esophageal strictures. *Ann Surg* 1987;**206**:173–83.
161. Geagea A, Cellier C. Scope of drug-induced, infectious and allergic esophageal injury. *Curr Opin Gastroenterol* 2008;**24**:496–501.
162. Pace F, Antinori S, Repici A. What is new in esophageal injury (infection, drug-induced, caustic, stricture, perforation)? *Curr Opin Gastroenterol* 2009;**25**:372–9.
163. Higuchi D, Sugawa C, Shah SH, Tokioka S, Lucas CE. Etiology, treatment, and outcome of esophageal ulcers: a 10-year experience in an urban emergency hospital. *J Gastrointest Surg* 2003;**7**:836–42.
164. Bott SJ, McCallum RW. Medication-induced oesophageal injury. Survey of the literature. *Med Toxicol* 1986;**1**:449–57.
165. Brors O. Gastrointestinal mucosal lesions: a drug formulation problem. *Med Toxicol* 1987;**2**:105–11.
166. Kikendall JW. Pill esophagitis. *J Clin Gastroenterol* 1999;**28**:298–305.
167. Levine MS. Drug-induced disorders of the esophagus. *Abdom Imaging* 1999;**24**:3–8.
168. Marvola M, Jajaniemi M, Marttila E, Vahervuo K, Sothmann A. Effect of dosage form and formulation factors on the adherence of drugs to the esophagus. *J Pharm Sci* 1983;**72**:1034–6.
169. Kadayifci A, Gulsen MT, Koruk M, Savas MC. Doxycycline-induced pill esophagitis. *Dis Esophagus* 2004;**17**:168–71.
170. Parfitt JR, Driman DK. Pathological effects of drugs on the gastrointestinal tract: a review. *Hum Organ* 2007;**38**:527–36.
171. Sharpe M, Noble S, Spencer CM. Alendronate – an update of its use in osteoporosis. *Drugs* 2001;**61**:999–1039.
172. Ruben Z, Rohrbacher E, Miller JE. Esophageal impaction in the BHE rats. *Lab Anim Sci* 1983;**33**:63–5.
173. Harkness JE, Ferguson FG. Idiopathic megaesophagus in rat. *Lab Anim Sci* 1979;**29**:495–8.
174. Randelia HP, Lalitha VS. Megaesophagus in ICRC mice. *Lab Anim* 1988;**22**:23–6.
175. Maeda H, Gleiser CA, Masoro EJ, Murata I, McMahan CA, Yu BP. Nutritional influences on aging of Fischer 344 rats: II. Pathology. *J Gerontol* 1985;**40**:671–88.
176. Tucker Jr WE, Macklin AW, Szot RJ, Johnson RE, Elion GB, Demiranda P, et al. Preclinical toxicology studies with acyclovir: acute and sub-chronic tests. *Fundam Appl Toxicol* 1983;**3**:573–8.
177. Mascrès C, Ming-Wen F, Joly JC. Morphologic changes of esophageal mucosa in the rat after chronic alcohol ingestion. *Exp Pathol* 1984;**25**:147–53.
178. Nelson LW, Kelly WA, Weikel JH. Mesovarial leiomyomas in rats in a chronic toxicity study of musuprine hydrochloride. *Toxicol Appl Pharmacol* 1972;**23**:731–7.
179. Vinter-Jensen L. *Pharmacological effects of epidermal growth factor (EGF) with focus on the urinary and gastrointestinal tracts*. APMIS, **107**(Suppl. 93).1999
180. Carlborg B, Densert O. Esophageal lesions caused by orally administered drugs. An experimental study in the cat. *Eur Surg Res* 1980;**12**:270–82.
181. Carlborg B, Densert O, Lindqvist C. Tetracycline induced esophageal ulcers. A clinical and experimental study. *Laryngoscope* 1983;**93**:184–7.
182. Olovson SG, Björkman JA, Ek L, Havu N. The ulcerogenic effect on the oesophagus of three b-adrenoceptor antagonists, investigated in a new porcine oesophagus test model. *Acta Pharmacol Toxicol* 1983;**53**:385–91.
183. Smith SM, Handt LK, Peter CP, Klein HJ. Novel techniques for testing of esophageal irritancy of liquids and tablets in dogs. *Contemp Top Lab Anim Sci* 1998;**37**:66–9.
184. Gärtner K, Pfaff J. The forestomach in rats and mice, a food store without bacterial protein digestion. *Zentralblatt für Veterinärmedizin. Reihe A* 1979;**26**:530–41.
185. Sawrey JM, Sawrey WL. Age, weight and social effects on ulceration in rats. *J Comp Psychol* 1986;**61**:464–6.
186. Boyd EM, Cehn CP, Muis LF. Resistance to starvation in albino rats fed from weaning on diets containing from 0 to 81% of protein as casein. *Growth Factors* 1970;**34**:99–112.

187. Sano T, Ozaki K, Kodama Y, Matsuura T, Narama I. Prevention of proliferative changes of forestomach mucosa by blood glucose control with insulin in alloxan-induced diabetic rats. *Cancer Sci* 2009;**100**:595–600.
188. Sano T, Ozaki K, Kodama Y, Matsuura T, Narama I. Effects of the antifungal agent itraconazole on proliferative changes of the forestomach mucosa in alloxan-induced diabetic rats. *Toxicol Pathol* 2009;**37**:790–8.
189. Williams TM, Donnelly KB. Gastric mucosal damage following repeat administration of melanocortin subtype-4 receptor ligands to Fischer 344 rats. *Toxicol Pathol* 2006;**34**:738–43.
190. Greaves, P & Faccini, JM. Digestive system. In: *Rat histopathology. A glossary for use in toxicity and carcinogenicity studies*. Amsterdam: Elsevier; 1992. p. 105–169.
191. Yoshitomi K, Maronpot RR, Solleveld HA, Boorman GA, Eustis SL. Forestomach ulcers in Crj:B6C3 (C57BL/6NCrj x C3H/HeNCrj) F1 mice. *Lab Anim Sci* 1986;**36**:501–3.
192. Altmann H-J, Wester PW, Matthiaschek G, Grunow W, Van Der Heijden CA. Induction of early lesions in the forestomach of rats by 3-tert-butyl-4-hydroxy-anisole (BHA). *Food Chem Toxicol* 1985;**23**:723–31.
193. Klein-Szanto AJP, Martin D, Segal M. Hyperkeratinization and hyperplasia of the forestomach epithelium in vitamin A deficient rats. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1982;**40**:387–94.
194. Anon. Toxicology and carcinogenesis studies of ampicillin trihydrate in F344/N rats and B6C3F1 mice. NIH Publication No. 87-2574. In *National toxicology program technical report* 9–10 (1987).
195. Hibino T, Hirasawa Y, Arai M. Morphologic changes in the urinary bladder and stomach after long-term administration of sodium saccharin in F344 rats. *Cancer Lett* 1985;**29**:255–63.
196. Levin S. Structural changes of the gastrointestinal mucosa induced by prostaglandins. *Toxicol Pathol* 1988;**16**:237–44.
197. Kotsonis FN, Dodd DC, Regnier B, Kohn FE. Preclinical toxicology profile of misoprostol. *Dig Dis Sci* 1985;**30**:1425–65.
198. Kramer AW, Dougherty WJ, Belson AR, Iatropoulos MJ. Morphologic changes in the gastric mucosa of rats and dogs treated with an analog of prostaglandin E1. *Toxicol Pathol* 1985;**13**:26–35.
199. Reinhart WH, Müller O, Halter F. Influence of long-term 16,16-dimethyl prostaglandin E2 treatment on the rat gastrointestinal mucosa. *Gastroenterology* 1983;**85**:1003–10.
200. Ghanayem BI, Matthews HB, Maronpot RR. Sustainability of forestomach hyperplasia treated with ethyl acrylate for 13 weeks and regression after cessation of dosing. *Toxicol Pathol* 1991;**19**:273–9.
201. Fukishima S, Ito N. Squamous cell carcinoma, forestomach, rat. In: Jones TC, Mohr U, Hunt RD, editors. *Digestive system. Monographs on pathology of laboratory animals*. Berlin: Springer-Verlag; 1985. p. 292–5.
202. Leininger JR, Jokinen MP. Tumours of the oral cavity, pharynx, oesophagus and stomach. In: Turusov VS, Mohr U, editors. *Pathology of tumours in laboratory animals*, Vol. 3. Lyon: International Agency for Research on Cancer; 1994.
203. Tatematsu M. Neoplasia and preneoplasia of the stomach. In: Bannasch P, Gössner W, editors. *Pathology of neoplasia and preneoplasia in rodents*. Stuttgart: Schattauer; 1997. p. 55–73..
204. Kroes R, Wester PW. Forestomach carcinogens: possible mechanisms of action. *Food Chem Toxicol* 1986;**24**:1083–9.
205. Gold LS, Slone TH, Stern BR, Bernstein L. Comparison of target organs of carcinogenicity for mutagenic and nonmutagenic chemicals. *Mutat Res* 1993;**286**:75–100.
206. Chandra SA, Nolan MW, Malarkey DE. Chemical carcinogenesis of the gastrointestinal tract in rodents: an overview with emphasis on NTP carcinogenesis bioassays. *Toxicol Pathol* 2010;**38**:188–97.
207. Whysner J, Williams GM. Butylated hydroxyanisole mechanistic data and risk assessment: conditional species-specific cytotoxicity, enhanced cell proliferation, and tumor promotion. *Pharmacol Ther* 1996;**71**:137–51.
208. Rodrigues C, Lok E, Nera E, Iverson F, Page D, Karpinski K, et al. Short-term effects of various phenols and acids on the Fischer 344 male forestomach epithelium. *Toxicology* 1986;**38**:103–17.
209. Betton GR, Salmon GK. Pathology of the forestomach in rats treated for 1 year with a new histamine H2-receptor antagonist, SK&F 93479 trihydrochloride. *Scand J Gastroenterol* 1984;**19**(Suppl. 101):103–8.
210. Kloss MW, Patrick DH, Macdonald JS. Studies on the effects of 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors on the rodent forestomach. *Food Chem Toxicol* 1991;**29**:621–8.
211. Buelde JE, Bannenberg G, Netter KJ. Effects of propionic acid and pravastatin on HMG-CoA reductase activity in relation to forestomach lesions in the rat. *Pharmacol Toxicol* 1996;**78**:229–34.
212. Akiba T, Shibuta T, Amano Y, Asanuma A, Okubo M, Nishigaki K, et al. Six-month repeated oral toxicity study of NK-104 in rats. *J Toxicol Sci* 1998;**23**(Suppl. 5):713–20.

213. Göggelmann W, Robisch G, Schimmer O. Aristolochic acid is a direct mutagen in *S. typhimurim*. *Mutat Res* 1982;**105**:201–4.
214. Schmeiser HH, Pool BL, Wiessler M. Identification and mutagenicity of metabolites of aristolochic acid formed by rat liver. *Carcinogenesis* 1986;**7**:59–63.
215. Ito N, Fukushima S, Hagiwara A, Shibata M, Ogiso T. Carcinogenicity of butylated hydroxyanisole in F344 rats. *J Natl Cancer Inst* 1983;**70**:343–52.
216. Iverson F, Lok E, Nera E, Karpinski K, Clayson DB. A 13 week feeding study of butylated hydroxyanisole: the subsequent regression of the induced lesions in male Fischer 344 rat forestomach epithelium. *Toxicology* 1985;**35**:1–11.
217. Tamano S, Hirose M, Tanaka H, Hagiwara A, Shirai T. Variation in susceptibility to the induction of forestomach tumours by butylated hydroxyanisole among rats of different strains. *Food Chem Toxicol* 1998;**36**:299–304.
218. Iverson F, Truelove J, Nera E, Wong J, Clayson DB. An 85-day study of butylated hydroxyanisole in the cynomolgus monkey. *Cancer Lett* 1985;**26**:43–50.
219. Moch RW. Forestomach lesions induced by butylated hydroxyanisole and ethylene dibromide: a scientific and regulatory perspective. *Toxicol Pathol* 1988;**16**:172–83.
220. Funk J, Landes C. Histopathologic findings after treatment with different oxidosqualene cyclase (OSC) inhibitors in hamsters and dogs. *Exp Toxicol Pathol* 2005;**57**:29–38.
221. Pyrah IT, Kalinowski A, Jackson D, Davies W, Davis S, Aldridge A, et al. Toxicologic lesions associated with two related inhibitors of oxidosqualene cyclase in the dog and mouse. *Toxicol Pathol* 2001;**29**:174–9.
222. Anon. *LESCOL[®] (fluvastatin sodium) prescribing information*. East Hanover, New Jersey: Novartis Pharmaceuticals Corporation; 2003.
223. von Keutz E, Schluter G. Preclinical safety evaluation of cerivastatin, a novel HMG-CoA reductase inhibitor. *Am J Cardiol* 1998;**82**:11J–7J.
224. Singer I, Kawka DW, Scott S, Bailey P, Kloss MW, Majka J , et al. Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme-A reductase induce reductase accumulation and altered lamellar bodies in rat forestomach keratinocytes. *Arterioscler Thromb* 1991;**11**:1156–65.
225. Mengs U, Lang W, Poch J-A. The carcinogenic action of aristolochic acid in rats. *Arch Toxicol* 1982;**51**:107–19.
226. Schmeiser HH, Stiborova M, Arlt VM. Chemical and molecular basis of the carcinogenicity of Aristolochia plants. *Curr Opin Drug Discov Devel* 2009;**12**:141–8.
227. Proctor DM, Gatto NM, Hong SJ, Allamneni KP. Mode-of-action framework for evaluating the relevance of rodent forestomach tumors in cancer risk assessment. *Toxicol Sci* 2007;**98**:313–26.
228. Greaves P, Williams A, Eve M. First dose of potential new medicines to humans: how animals help. *Nat Rev Drug Discov* 2004;**3**:226–36.
229. Igarashi T, Nakane S, Kitagawa T. Predictability of clinical adverse reactions of drugs by general pharmacology studies. *J Toxicol Sci* 1995;**20**:77–92.
230. Freireich EJ, Gehen EA, Rall DP, Schmidt LH, Skipper HE. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. *Cancer Chemoth Rep* 1966;**50**:219–44.
231. Owens AH. Predicting anticancer drug effects in man from laboratory animal studies. *J Chronic Dis* 1962;**15**: 223–8.
232. Sarna SK. Cyclic motor activity – migrating motor complex – 1985. *Gastroenterology* 1985;**89**:894–913.
233. Dressman JB. Comparison of canine and human gastrointestinal physiology. *Pharm Res* 1986;**3**:123–31.
234. Ward FW, Coates ME. Gastrointestinal pH measurement in rats: influence of microbial flora, diet and fasting. *Lab Anim* 1987;**21**:216–22.
235. Vidal JD, Mirabile RC, Thomas HC. Evaluation of the cynomolgus monkey stomach: recommendations for standard sampling procedures in nonclinical safety studies. *Toxicol Pathol* 2008;**36**:250–5.
236. Canfield V, West AB, Goldenring JR, Levenson R. Genetic ablation of parietal cells in transgenic mice: a new model for analyzing cell lineage relationships in the gastric mucosa. *Proc Natl Acad Sci U S A* 1996;**93**:2431–5.
237. Ueyama T, Shirasawa N, Numazawa M, Yamada K, Shelangouski M, Ito T , et al. Gastric parietal cells: potent endocrine role in secreting estrogen as a possible regulator of gastro-hepatic axis. *Endocrinology* 2002;**143**:3162–70.
238. Håkanson R, Oscarson J, Sundler F. Gastrin and the trophic control of gastric mucosa. In: Borg KO, Olbe L, Rune SJ, Walan A, editors. *Proceedings of the first international symposium on omeprazole*. Molndal: A.B. Hassle; 1986. p. 18–30..

239. Grimelius L. A silver nitrate stain for alpha-2 cells in human pancreatic islets. *Acta Soc Med Ups* 1968;**73**:243–70.
240. Grimelius L, Wilander E. Silver stains in the study of endocrine cells of the gut and pancreas. *Invest Cell Pathol* 1980;**3**:3–12.
241. Masson P. La glande endocrine de l'intestin chez l'homme. *Comptes Rendus des Séances de l'Académie des Sciences* 1914;**158**:59–61.
242. Betton GR, Dormer CS, Wells T, Pert P, Price CA, Buckley P. Gastric ECL-cell hyperplasia and carcinoids in rodents following chronic administration of the H2 antagonist SK&F 93479 and omeprazole. *Toxicol Pathol* 1988;**16**:288–98.
243. Sundler F, Håkanson R, Carlsson E, Larsson H, Mattsson H. Hypergastrinemia after blockade of acid secretion in the rat. Trophic effects. *Digestion* 1986;**35**(Suppl. 1):56–69.
244. Norlen P, Curry WJ, Björkqvist M, Maule A, Cunningham RT, Hogg RB, et al. Cell-specific processing of chromogranin A in endocrine cells of the rat stomach. *J Histochem Cytochem* 2001;**49**:9–18.
245. Bishop AE, Allen JM, Daly MJ, Larsson H, Carlsson E, Bloom SR, et al. Gastric regulatory peptides in rats with reduced acid secretion. *Digestion* 1986;**35**(Suppl. 1):70–83.
246. Dockray GJ. Gastric and gastric epithelial physiology. *J Physiol* 1999;**518**:315–24.
247. Hinkle KL, Samuelson LC. Lessons from genetically engineered animal models III. Lessons learned from gastrin gene deletion in mice. *Am J Physiol* 1999;**277**:G500–5.
248. Kopin AS, Lee MY, McBride EW, Miller LJ, Lu M, Lin YH, et al. Expression cloning and characterization of the canine parietal cell gastrin receptor. *Proc Natl Acad Sci U S A* 1992;**89**:3605–9.
249. Wank SA. Cholecystokinin receptors. *Am J Physiol* 1995;**269**:G628–46.
250. Inokuchi H, Fujimoto S, Kawai K. Cellular kinetics of gastrointestinal mucosa, with special reference to gut endocrine-cells. *Arch Histol Cytol* 1983;**46**:137–57.
251. Karam SM. New insights into the stem cells and the precursors of the gastric epithelium. *Nutrition* 1995;**11**:607–13.
252. Li S, Karam SM, Gordon JI. Diphtheria toxin-mediated ablation of parietal cells in the stomach of transgenic mice. *J Biol Chem* 1996;**271**:3671–6.
253. Hattori T. On cell proliferation and differentiation of the fundic mucosa of the golden hamster. Fractographic study combined microscopy and 3H-thymidine autoradiography. *Cell Tissue Res* 1974;**148**:213–26.
254. Hattori T, Fujita S. Tritiated thymidine autoradiographic study on cellular migration in the gastric gland of the golden hamster. *Cell Tissue Res* 1976;**172**:171–84.
255. Tulassay Z, Herszenyi L. Gastric mucosal defense and cytoprotection. *Best Practice Res Clin Gastroenterol* 2010;**24**:99–108.
256. Sheahan DG, Jarvis HR. Comparative histochemistry of gastrointestinal mucosubstances. *Am J Anat* 1976;**146**:103–32.
257. Filipe MI. Mucins in the human gastrointestinal epithelium: a review. *Invest Cell Pathol* 1979;**2**:195–216.
258. Tsiftsis D, Jass JR, Filipe MI, Wastell C. Altered patterns of mucin secretion in the precancerous lesions induced in the glandular part of the rat stomach by the carcinogen N-methyl-N'-nitro-N-nitrosoguanidine. *Invest Cell Pathol* 1980;**3**:399–408.
259. Berger EG, Buddecke E, Kamerling JP, Kobata A, Paulson JC, Vlie-Genthart JFG. Structure, biosynthesis and functions of glycoprotein glycans. *Experientia* 1982;**38**:1129–258.
260. Van Klinken B, Willem J, Dekker J, Büller HA, Einerhand AWC. Mucin gene structure and expression: protection vs. adhesion. *Am J Physiol* 1995;**269**:G613–27.
261. Ishihara K, Ohara S, Azuumi Y, Goso K, Hotta K. Changes of gastric mucus glycoproteins with aspirin administration in rats. *Digestion* 1984;**29**:98–102.
262. Nicholson GL. The interactions of lectins with animal cell surfaces. *Int Rev Cytol* 1974;**39**:89–190.
263. Goldstein IJ, Hayes CE. The lectins: carbohydrate-binding proteins of plants and animals. *Adv Carbohydr Chem Biochem* 1978;**35**:127–340.
264. Debray H, Decout D, Strecker G, Spik G, Montreuil J. Specificity of twelve lectins towards oligosaccharides and glycopeptides related to N-glycosylproteins. *Eur J Biochem* 1981;**117**:41–55.
265. Giannasca PJ, Giannasca KT, Falk P, Gordon JI, Neutra MR. Regional differences in glycoconjugates of intestinal M cells in mice: potential targets for mucosa vaccines. *Am J Physiol* 1994;**267**:G1108–21.

266. Jass JR. Role of intestinal metaplasia in the histogenesis of gastric carcinoma. *J Clin Pathol* 1980;**33**:801–10.
267. Greaves P, Boiziau JL. Mucin histochemistry of spontaneous mouse proliferative gastritis. *Zeitschrift für Versuchstierkunde* 1982;**24** 35–35
268. Kuhlmann WD, Preschke P, Wurster K. Lectin-peroxidase conjugates in histopathology of gastrointestinal mucosa. *Virchows Arch A Pathol Anat Histopathol* 1983;**398**:319–28.
269. Suganuma T, Tsuyama S, Suzuki S, Murata F. Lectin-peroxidase reactivity in rat gastric mucosa. *Arch Histol Cytol* 1984;**47**:197–207.
270. Chen Z, Scudiere JR, Montgomery E. Medication-induced upper gastrointestinal tract injury. *J Clin Pathol* 2009;**62**:113–9.
271. Leung FW. Risk factors for gastrointestinal complications in aspirin users: review of clinical and experimental data. *Dig Dis Sci* 2008;**53**:2604–15.
272. Lai LH, Chan FKL. Nonsteroid anti-inflammatory drug-induced gastroduodenal injury. *Curr Opin Gastroenterol* 2009;**25**:544–8.
273. Becker JC, Domschke W, Pohle T. Current approaches to prevent NSAID-induced gastropathy – COX selectivity and beyond. *Br J Clin Pharmacol* 2004;**58**:587–600.
274. Huang JQ, Sridhar S, Hunt RH. Role of *Helicobacter pylori* infection and non-steroidal antiinflammatory drugs in peptic-ulcer disease: a meta-analysis. *Lancet* 2002;**359**:14–22.
275. Ferner RE, Whittington RM. Coroner's cases of death due to errors in prescribing or giving medicines or to adverse drug reactions: Birmingham 1986–1991. *J R Soc Med* 1994;**87**:145–8.
276. Fradet G, Legac X, Charlois T, Ponge T, Cottin S. Iatrogenic pathology in elderly, inducing hospitalisation. A one year retrospective study in an internal medicine department. *Revue de Médecine Interne* 1996;**17**:456–60.
277. Lagnaoui R, Moore N, Fach J, Longy-Boursier M, Bégau B. Adverse drug reactions in a department of systemic diseases-oriented internal medicine: prevalence, incidence, direct costs and avoidability. *Eur J Clin Pharmacol* 2000;**55**:181–6.
278. Pouyanne P, Haramburu F, Imbs JL, Bégau B. Admissions to hospital caused by adverse drug reactions: cross sectional incidence study. *Br Med J* 2000;**320** 1036–1036
279. Mjörndal T, Boman MD, Hagg S, Backstrom M, Wiholm BE, Wahlin A, et al. Adverse drug reactions as a cause for admissions to a department of internal medicine. *Pharmacoepidemiol Drug Saf* 2002;**11**:65–72.
280. Capuano A, Motola G, Russo F, Avolio A, Filippelli A, Rossi F, et al. Adverse drug events in two emergency departments in Naples, Italy: an observational study. *Pharmacol Res* 2004;**50**:631–6.
281. Soll AH. Pathogenesis of peptic ulcer and implications for therapy. *N Engl J Med* 1990;**322**:909–16.
282. Richter-Dahlfors A, Heczko U, Meloche RM, Finlay BB, Buchan AMJ. *Helicobacter pylori*-infected human antral primary cell cultures: effect on gastrin cell function. *Am J Physiol* 1998;**275**:G393–401.
283. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. *Nature* 1971;**231**:232–5.
284. Wallace JL. Pathogenesis of NSAID-induced gastroduodenal mucosal injury. *Best Practice Res Clin Gastroenterol* 2001;**15**:691–703.
285. Wallace JL. Recent advances in gastric ulcer therapeutics. *Curr Opin Pharmacol* 2005;**5**:573–7.
286. Drazen JM. COX-2 inhibitors – a lesson in unexpected problems. *N Engl J Med* 2005;**352**:1131–2.
287. Psaty BM, Furberg CD. COX-2 inhibitors – lessons in drug safety. *N Engl J Med* 2005;**352**:1133–5.
288. Neiger R, Simpson KW. *Helicobacter* infection in dogs and cats: facts and fiction. *J Vet Intern Med* 2000;**14**:125–33.
289. Reed KD, Berridge BR. *Campylobacter*-like organisms in the gastric mucosa of rhesus monkeys. *Lab Anim Sci* 1988;**38**:329–33.
290. Nedrud JG. Animal models for gastric *Helicobacter* immunology and vaccine studies. *FEMS Immunol Med Microbiol* 1999;**24**:243–50.
291. Elfvin A, Bolin I, Von Bothmer C, Stolte M, Watanabe H, Fandriks L, et al. *Helicobacter pylori* induces gastritis and intestinal metaplasia but no gastric adenocarcinoma in Mongolian gerbils. *Scand J Gastroenterol* 2005;**40**:1313–20.
292. Rogers AB, Fox JG. Inflammation and cancer – I. Rodent models of infectious gastrointestinal and liver cancer. *Am J Physiol Gastrointest Liver Physiol* 2004;**286**:G361–6.
293. Madsen LW, Jensen AL, Larsen S. Spontaneous lesions in clinically healthy, microbiologically defined Gottingen minipigs. *Scand J Lab Anim Sci* 1998;**25**:159–66.

294. Goldenberg MM. Study of cold plus restraint stress gastric lesions in spontaneously hypertensive, Wistar and Sprague-Dawley rats. *Life Sci* 1973;12:519–27.
295. Fowler PD. Aspirin, paracetamol and non-steroidal anti-inflammatory drugs. A comparative review of side effects. *Med Toxicol* 1987;2:338–66.
296. Puurunen J, Huettunen P, Hirvonen J. Is ethanol-induced damage of the gastric muosa a hyperosmotic effect? Comparative studies on the effects of ethanol, some other hyperosmotic solutions and acetyl-salicylic acid on rat gastric mucosa. *Acta Pharmacol Toxicol* 1980;47:321–7.
297. Vences-Mejia A, Caballero-Ortega H, Dorado-Gonzalez V, Gamboa-Dominguez A, Gomez-Ruiz C, Camacho-Carranza R, et al. Cytochrome P450 expression in rat gastric epithelium with intestinal metaplasia induced by high dietary NaCl levels. *Environ Toxicol Pharmacol* 2005;20:57–64.
298. Beattie D. Effect of drugs on rats exposed to cold-restraint stress. *J Pharm Pharmacol* 1977;29:748–51.
299. Rainsford KD. Synergistic interaction between aspirin, or other non-steroidal anti-inflammatory drugs, and stress which produces severe gastric mucosal damage in rats and pigs. *Agents Actions* 1975;5:553–8.
300. Boyd EM, Cehn CP, Muis LF. Resistance to starvation in albino rats fed from weaning on diets containing from 0 to 81% of protein as casein. *Growth* 1970;34:99–112.
301. Shriver DA, White CB, Sandor A, Rosenthale ME. A profile of the gastrointestinal toxicity of drugs used to treat inflammatory diseases. *Toxicol Appl Pharmacol* 1975;32:73–83.
302. Ramiro-Ibanez F, Trajkovic D, Jessen B. Gastric and pancreatic lesions in rats treated with a pan-CDK inhibitor. *Toxicol Pathol* 2005;33:784–91.
303. Suwa T, Urano H, Kohno Y, Suzuki A, Amano T. Comparative studies on the gastrointestinal lesions caused by several non-steroidal anti-inflammatory agents in the rats. *Agents Actions* 1987;21:167–72.
304. McCormack K, Brune K. Classical absorption theory and the development of gastric mucosal damage associated with non-steroidal anti-inflammatory drugs. *Arch Toxicol* 1987;60:261–9.
305. Satoh H, Inada H, Hirata T, Mak Y. Indomethacin produces gastric antral ulcers in the re-fed rat. *Gastroenterology* 1981;81:719–25.
306. Eastwood GL, Quimby GF. Effect of chronic aspirin ingestion on epithelial proliferation in rat fundus, antrum and duodenum. *Gastroenterology* 1982;82:852–6.
307. Rainsford KD, Willis CM, Walker SA, Robins PG. Electron microscopic observations comparing the gastric mucosal damage induced in rats and pigs by benoxaprofen and aspirin, reflecting their differing actions as prostaglandin-synthesis-inhibitors. *Br J Exp Pathol* 1982;63:25–34.
308. Tibble JA, Sigthorsson G, Foster R, Bjarnason I. Comparison of the intestinal toxicity of celecoxib, a selective COX-2 inhibitor, and indomethacin in the experimental rat. *Scand J Gastroenterol* 2000;35:802–7.
309. Esser R, Berry C, Du ZM, Dawson J, Fox A, Fujimoto RA, et al. Preclinical pharmacology of lumiracoxib: a novel selective inhibitor of cyclooxygenase-2. *Br J Pharmacol* 2005;144:538–50.
310. Whittle BJR. The COX controversy: viewpoint 2 – new dogmas or old? *Gut* 2003;52:1379–81.
311. Masferrer JL, Zweifel BS, Manning PT, Hauser SD, Leahy KM, Smith WG, et al. Selective-inhibition of inducible cyclooxygenase-2 in-vivo Is antiinflammatory and nonulcerogenic. *Proc Natl Acad Sci U S A* 1994;91:3228–32.
312. Chan CC, Boyce S, Brideau C, Charleson S, Cromlish W, Ethier D, et al. Rofecoxib [Vioxx, MK-0966; 4-(4'-methylsulfonylphenyl)-3-phenyl-2-(5H)-furanone]: a potent and orally active cyclooxygenase-2 inhibitor. Pharmacological and biochemical profiles. *J Pharmacol Exp Ther* 1999;290:551–60.
313. Bjarnason I, Takeuchi K, Simpson R. The COX controversy: viewpoint 1 – NSAIDs: the Emperor's new dogma? *Gut* 2003;52:1376–8.
314. Schmassmann A, Peskar BM, Stettler C, Netzer P, Stroff T, Flogerzi B, et al. Effects of inhibition of prostaglandin endoperoxide synthase-2 in chronic gastro-intestinal ulcer models in rats. *Br J Pharmacol* 1998;123:795–804.
315. Yang, WCJ. *Pharmacology and toxicology review*. Celecoxib (Celebrex™). NDA 20-998. Rockville: Center for Drug Evaluation and Research. US Food and Drug Administration; 1998.
316. Haworth R, Oakley K, McCormack N, Pilling A. Differential expression of COX-1 and COX-2 in the gastrointestinal tract of the rat. *Toxicol Pathol* 2005;33:239–45.
317. Imai K, Yoshimura S, Ohtaki T, Hashimoto K. Experimental toxicity studies with captopril, an inhibitor of angiotensin 1-converting enzymes 2. One month studies of chronic toxicity of captopril in rats. *J Toxicol Sci* 1981;6(Suppl. 2):189–214.

318. Barker IK, Van Dreumel AA. The alimentary system. In: Jubb KVF, Kennedy PC, Palmer N, editors. *Pathology of domestic animals*, Vol. 2. Orlando: Academic Press; 1985.
319. Anderson DD, Hayes TJ. Toxicity of human recombinant interleukin-2 in rats. Pathologic changes are characterized by marked lymphocytic and eosinophilic proliferation and multisystem involvement. *Lab Invest* 1989;**60**:331–46.
320. Lambert R, Andre C, Martin F. Incorporation of radiosulfate in the gastric mucosa of the rat subjected to restraint. *Gastroenterology* 1969;**56**:200–5.
321. Denko CW. The effect of hydrocortisone and cortisone on fixation of 35S in the stomach. *J Lab Clin Med* 1958;**51**:174–7.
322. Denko CW. The effect of phenylbutazone and its derivatives, oxyphenbutazone and sulfinpyrazole, on 35S sulfate incorporation in cartilage and stomach. *J Lab Clin Med* 1964;**63**:953–8.
323. Gerard A. Histochemie de la muqueuse gastrique fundique du chien traité par des drogues ulcérogène. *Comptes Rendue de la Société de Biologie* 1965;**159**:1473–6.
324. Lichtenberger LM, Wang ZM, Romero JJ, Ulloa C, Perez JC, Giraud MN, et al. Nonsteroidal antiinflammatory drugs (NSAIDS) associate with zwitterionic phospholipids – insight into the mechanism and reversal of NSAID-induced gastrointestinal injury. *Nat Med* 1995;**1**:154–8.
325. Yoshimura K, Delbarre SG, Kraus E, Boland CR. The effects of omeprazole and famotidine on mucin and PGE2 release in the rat stomach. *Aliment Pharmacol Ther* 1996;**10**:111–7.
326. Morson BC. Intestinal metaplasia of the gastric mucosa. *Br J Cancer* 1955;**9**:365–76.
327. Lev R. The mucin histochemistry of normal and neoplastic gastric mucosa. *Lab Invest* 1966;**14**:2080–100.
328. Goldman H, Ming SC. Mucins in normal and neoplastic gastrointestinal epithelium. *Arch Pathol* 1968;**85**:580–6.
329. Planteydt HT, Willighagen RGJ. Enzyme histochemistry of the human stomach with special reference to intestinal metaplasia. *J Pathol Bacteriol* 1960;**80**:317–22.
330. Watanabe H, Naito M, Ito A. The effect of sex difference on induction of intestinal metaplasia in rats. *Acta Pathol Jpn* 1984;**32**:305–12.
331. Ward JM. Proliferative lesions of the glandular stomach and liver in F344 rats fed diets containing aroclor 1254. *Environ Health Perspect* 1985;**60**:89–95.
332. Jass JR, Filipe MI. A variant of intestinal metaplasia associated with gastric carcinoma: a histochemical study. *Histol Histopathol* 1979;**3**:191–9.
333. Teglbjaerg PS, Nielson HO. ‘Small intestinal type’ and ‘colonic type’ intestinal metaplasia of the human stomach and their relationship to the histogenetic types of gastric adenocarcinoma. *Acta Pathol Microbiol Scand* 1978;**86**:351–5.
334. Wells M, Stewart M, Dixon MF. Mucin histochemistry of gastric intestinal metaplasia. *J Pathol* 1982;**137**:70–1.
335. Otsuka T, Tsukamoto T, Mizoshita T, Inada K, Takenaka Y, Kato S, et al. Coexistence of gastric- and intestinal-type endocrine cells in gastric and intestinal mixed intestinal metaplasia of the human stomach. *Pathol Int* 2005;**55**:170–9.
336. Steer HW. Surface morphology of the gastroduodenal mucosa in duodenal ulceration. *Gut* 1984;**25**:1203–10.
337. Ectors N, Dixon MF. The prognostic value of sulphomucin positive intestinal metaplasia in the development of gastric cancer. *Histol Histopathol* 1986;**10**:1271–7.
338. Morgan RW, Ward JM, Hartman PE. Aroclor 1254-induced intestinal metaplasia and adenocarcinoma in the glandular stomach of F344 rats. *Cancer Res* 1981;**41**:5052–9.
339. McConnell EE, Hass JR, Altman N, Moore JA. A spontaneous outbreak of polychlorinated biphenyl (PCB) toxicity in rhesus monkeys (*Macaca mulatta*): toxicopathology. *Lab Anim Sci* 1979;**29**:666–73.
340. Allen JR. Response of the non-human primate to polychlorinated biphenyl exposure. *Fed Proc* 1975;**34**:1675–9.
341. Watanabe H. Experimentally induced intestinal metaplasia in Wistar rats by X-ray irradiation. *Gastroenterology* 1978;**75**:796–9.
342. Watanabe H, Fujii I, Terada Y. Induction of intestinal metaplasia in the rat gastric mucosa by local X-irradiation. *Pathol Res Pract* 1980;**70**:104–14.
343. Watanabe K, Reddy BS, Wong CQ, Weisberger JH. Effect of dietary undegraded carrageenan on colon carcinogenesis in F344 treated with azoxymethane or methyl-nitrosourea. *Cancer Res* 1978;**38**:4427–30.

344. Shirai T, Takahashi M, Fukushima S, Ito N. Marked epithelial hyperplasia of the rat glandular stomach induced by long-term administration of iodoacetamide. *Acta Pathol Jpn* 1985;**35**:35–43.
345. Leininger JR, McDonald MM, Abbott DP. Hepatocytes in the mouse stomach. *Toxicol Pathol* 1990;**18**:678–86.
346. Mortensen JT, Brinck P, Binderup L. Toxicity of vitamin-D analogs in rats fed diets with standard or low calcium contents. *Pharmacol Toxicol* 1993;**72**:124–7.
347. Cheville NF. Uremic gastropathy in the dog. *Vet Pathol* 1979;**16**:292–309.
348. Brown AP, Courtney CL, King LM, Groom SC, Graziano MJ. Cartilage dysplasia and tissue mineralization in the rat following administration of a FGF receptor tyrosine kinase inhibitor. *Toxicol Pathol* 2005;**33**:449–55.
349. Rees J, Spencer A, Wilson S, Reid A, Harpur E. Time course of stomach mineralization, plasma, and urinary changes after a single intravenous administration of gadolinium(III) chloride in the male rat. *Toxicol Pathol* 1997;**25**:582–9.
350. Anver MR, Cohen BJ, Lattuada CP, Foster SJ. Age-associated lesions in barrier-reared male Sprague-Dawley rats: a comparison between Hap: (SD) and CrL:COBS[R] CD[R] (SD) stocks. *Exp Aging Res* 1982;**8**:3–24.
351. Gjurlidsen ST, Myren J, Fretheim B. Alterations of gastric mucosa following a graded partial gastrectomy. *Scand J Gastroenterol* 1968;**3**:465–70.
352. Neilsen JA, Hessthaysen E, Olesen H, Nielsen R. Fundal gastritis after Billroth-II type resection in patients with duodenal ulcer. *Scand J Gastroenterol* 1972;**7**:337–43.
353. Håkanson R, Larsson L-I, Liedberg G, Oscarson J, Sundler F, Vang J. Effects of antrectomy or porta-aval shunting on the histamine-storing endocrine-like cells in oxyntic mucosa of rat stomach. A fluorescence histochemical, electron microscopic and chemical study. *J Physiol* 1976;**259**:785–800.
354. Dethloff LA, Robertson DG, Tierney BM, Breider MA, Bestervelt LL. Gastric gland degeneration induced in monkeys by the CCK-B/gastrin receptor antagonist CI-988. *Toxicol Pathol* 1997;**25**:441–8.
355. Crean GP, Cunn AA, Rumsey RDE. The effects of vagotomy on the gastric mucosa of the rat. *Scand J Gastroenterol* 1969;**4**:675–80.
356. Aase S, Roland M. Light and electron microscopical studies of parietal cells before and one year after proximal vagotomy in duodenal ulcer patients. *Scand J Gastroenterol* 1977;**12**:417–20.
357. Nakamura R. Quantitative light and electron microscopic studies on the effect of vagotomy on parietal cells in rats. *Tohoku J Exp Med* 1985;**145**:269–82.
358. Håkanson R, Vallgren S, Ekelund M, Rehfeld JF, Sundler F. The vagus exerts trophic control of the stomach in the rat. *Gastroenterology* 1984;**86**:28–32.
359. Bastie MJ, Balas D, Laval J, Bertrand J, Ribet A. Comparative study of histological and kinetic variations of the digestive mucosa and pancreatic parenchyma after hypophysectomy in the rat. *Acta Anat* 1985;**124**:133–44.
360. Hansson E, Havu N, Carlsson E. Toxicology studies with omeprazole. In: Borg KO, Oble L, Rune SJ, Walan A, editors. *Proceedings of the first international symposium on omeprazole*. Mölndal: A.N. Hässle; 1986. p. 89–91.
361. Lehy T, Gres L, Bonfils S. Effect de l'administration prolongée d'un antisécrétoire gastrique, le pirenzepin, sur les populations cellulaires de l'estomac de rat. *Gastroenterol Clin Biol* 1978;**2**:1001–9.
362. Iacobuzio-Donahue CA, Lee EL, Abraham SC, Yardley JH, Wu TT. Colchicine toxicity – distinct morphologic findings in gastrointestinal biopsies. *Am J Surg Pathol* 2001;**25**:1067–73.
363. Stemmerm GN, Hayashi T. Colchicine intoxication – reappraisal of its pathology based on a study of three fatal cases. *Hum Organ* 1971;**2**:321–31.
364. Daniels JA, Gibson MK, Xu L, Sun SL, Canto MI, Heath E, et al. Gastrointestinal tract epithelial changes associated with taxanes: marker of drug toxicity versus effect. *Am J Surg Pathol* 2008;**32**:473–7.
365. Joseph, X. *Review and evaluation of new toxicology data submitted with FOSRENOL[®] NDA resubmission*. NDA 21–468. Rockville MD: Center for Drug Evaluation and Research. US Food and Drug Administration; 2004.
366. Kanda N, Seno H, Kawada M, Sawabu T, Uenoyoma Y, Nakajima T, et al. Involvement of cyclooxygenase-2 in gastric mucosal hypertrophy in gastrin transgenic mice. *Am J Physiol Gastrointest Liver Physiol* 2006;**290**:G519–27.
367. Willems G, Lehy T. Radioautographic and quantitative studies on parietal and peptic cell kinetics in the mouse: a selective effect of gastrin on parietal cell proliferation. *Gastroenterology* 1975;**69**:416–26.
368. Crean GP, Daniel D, Leslie GB, Bates C. The effect of prolonged administration of large doses of cimetidine on the gastric mucosa of rats. In: Wastell C, Lance P, editors. *Cimetidine. The westminster hospital symposium*. Edinburgh: Churchill Livingstone; 1978. p. 191–206.

369. Balas D, Senegas-Balas F, Pradayrol L, Vayssette J, Bertrand C, Ribet A. Long-term comparative effect cholecystokinin and gastrin on mouse stomach, antrum, intestine, and exocrine pancreas. *Am J Anat* 1985;174:27–43.
370. Witzel L, Halter F, Olah AJ, Häcki WH. Effect of prolonged metiamide medication on the fundic mucosa. *Gastroenterology* 1977;73:797–803.
371. Mazzacca G, Cascione F, Budillon G, D'Agostino L, Cimimo L, Femiano C. Parietal cell hyperplasia induced by long-term administration of antacids to rats. *Gut* 1978;19:798–801.
372. Kaduk B, Häuser H. Morphologische Veränderungen der Magenmukosa von Ratten nach chronischer Antazidagabe. *Zeitschrift für Gastroenterologie* 1980;18:138–47.
373. White SL, Smith WC, Fisher LF, Gatlin CL, Hanasono GK, Jordan WH. Quantitation of glandular gastric changes in rats given a proton pump inhibitor for 3 months with emphasis on sampling scheme selection. *Toxicol Pathol* 1998;26:403–10.
374. Matsuzaki J, Suzuki H, Minegishi Y, Sugai E, Tsugawa H, Yasui M, et al. Acid suppression by proton pump inhibitors enhances aquaporin-4 and KCNQ1 expression in gastric fundic parietal cells in mouse. *Dig Dis Sci* 2010;55:3339–48.
375. Burek JD, Majka JA, Bokelman DL. Famotidine. Summary of preclinical safety assessment. *Digestion* 1985;32 (Suppl. 1):7–14.
376. Fich A, Arber N, Okon E, Zajicek G, Rachmilewitz D. Effect of chronic misoprostol ingestion on rat gastric morphology and turnover. *Arch Toxicol* 1988;61:314–7.
377. Prahalada S, Stabinski LG, Chen HY, Morrissey RE, De Burlet G, Holder D, et al. Pharmacological and toxicological effects of chronic porcine growth hormone administration in dogs. *Toxicol Pathol* 1998;26:185–200.
378. Franzin G, Novelli P. Gastritis cystica profunda. *Histol Histopathol* 1981;5:535–47.
379. Thomson ABR, Sauve MD, Kassam N, Kamitakahara H. Safety of the long-term use of proton pump inhibitors. *World J Gastroenterol* 2010;16:2323–30.
380. McCarthy DM. Adverse effects of proton pump inhibitor drugs: clues and conclusions. *Curr Opin Gastroenterol* 2010;26:624–31.
381. Berenson MM, Sennella J, Freston JW. Ménétrier's disease. Serial morphological, secretory, and serological observations. *Gastroenterology* 1976;70:257–63.
382. Wilkerson ML, Meschter SC, Brown RE. Menetrier's disease presenting as iron deficiency anaemia. *Ann Clin Lab Sci* 1998;28:14–8.
383. Burdick JS, Chung EK, Tanner G, Sun M, Paciga JE, Cheng JQ, et al. Treatment of Ménétrier's disease with a monoclonal antibody against the epidermal growth factor receptor. *N Engl J Med* 2000;343:1697–701.
384. Demsey PJ, Goldenring JR, Soroka CJ, Modlin IM, McClure RW, Lind CD, et al. Possible role of transforming growth factor alpha in the pathogenesis of Menetrier's disease: supporting evidence from humans and transgenic mice. *Gastroenterology* 1992;103:1950–63.
385. Ogawa T, Maeda K, Tonai S, Kobayashi T, Watanabe T, Okabe S. Utilization of knockout mice to examine the potential role of gastric histamine H₂-receptors in Menetrier's disease. *J Pharmacol Sci* 2003;91:61–70.
386. Jubb KVF, Kennedy PC. *Pathology of domestic animals*. New York: Academic Press; 1970.
387. Cook RW, Williams JF, Lichtenberger LM. Hyperplastic gastropathy in the rat due to *Taenia taeniaeformis* infection: parabiotic transfer and hypergastrinaemia. *Gastroenterology* 1981;80:728–34.
388. Kuhn N, Grone A, Pagan O, Bacciarini LN. Metastatic gastric adenocarcinoma and diffuse hyperplastic gastritis resembling human Menetrier's disease in a camel (*Camelus ferus bactrianus*). *J Vet Med A Physiol Pathol Clin Med* 2003;50:359–62.
389. Stewart HL, Andervont HB. Pathologic observations on the adenomatous lesions of the stomach in mice of strain I. *Arch Pathol* 1938;26:1009–22.
390. Rowlatt C, Franks LM, Sheriff MU, Chesterman FC. Naturally occurring tumors and other lesions of the digestive tract in untreated C57BL mice. *J Natl Cancer Inst* 1969;43:1353–68.
391. Chvédoff M, Clarke MR, Irisarri E, Faccini JM, Monro AM. Effects of housing conditions on food intakes, body weight and spontaneous lesions in mice. A review of the literature and results of an 18-month study. *Food Chem Toxicol* 1980;18:517–22.
392. Poynter D, Selway SAM, Papworth SA, Riches SR. Changes in the gastric mucosa of the mouse associated with long lasting unsurmountable histamine H₂ blockade. *Gut* 1986;27:1338–46.

393. Betton GR, Dormer C, Wells T, Pert P, Price CA, Buckley P. Fundic mucosal ECL cell hyperplasia and carcinoids in rodents following chronic administration of the histamine H₂-receptor antagonist SK&F 93479 and other antisecretory agents. *Toxicol Pathol* 1987;**15**:365.
394. Rehm S, Sommer R, Deerberg F. Spontaneous non-neoplastic gastric lesions in female Han: NMRI mice, and influence of food restriction throughout life. *Vet Pathol* 1987;**24**:216–25.
395. Takagi H, Jhappan C, Sharp R, Merlino G. Hypertrophic gastropathy resembling Menetrier's disease in transgenic mice overexpressing transforming growth factor α in the stomach. *J Clin Invest* 1992;**90**:1161–7.
396. Suzuki Y, Taguchi O, Kojima A, Matsuyama M, Nishizuka Y. Fine structure of giant hypertrophic gastritis developed in thymectomized mice. *Lab Invest* 1981;**45**:209–17.
397. Greaves P, Boiziau JL. Altered patterns of mucin secretion in gastric hyperplasia in mice. *Vet Pathol* 1984;**21**:224–8.
398. Kojima A, Taguchi O, Nishizuka Y. Experimental production of possible autoimmune gastritis followed by macrocytic anemia in athymic mice. *Lab Invest* 1980;**42**:387–95.
399. Tucker MJ, Jones DV. Effects of cyproterone acetate in C57Bl/10J mice. *Hudson Rev* 1996;**15**:64–6.
400. Oshima CT, Wonraht DR, Catarino RM, Mattos D, Forones NM. Estrogen and progesterone receptors in gastric and colorectal cancer. *Curr Top Microbiol* 1999;**46**:3155–8.
401. Anon. FOSRENOL[®] (Lanthanum carbonate) prescribing information. Wayne PA: Shire US Inc; 2005.
402. Joseph, X. FOSRENOL[™] (lanthanum carbonate hydrate). Review and evaluation of pharmacology and toxicology data. NDA 21-468. Rockville MD: Food and Drug Administration Center for Drug Evaluation and Review; 2003.
403. Kobayasi S, Tatematsu M, Ogawa K, De Camargo JLV, Rodrigues MAM, Ito N. Reversibility of adenomatous hyperplasia in the gastric stump after diversion of bile reflux in rats. *Carcinogenesis* 1991;**12**:1437–43.
404. St John DJB, Yeomans ND, Bourne CAJ, De Boer WGRM. Aspirin-induced glandular dysplasia of the stomach. Histologic and histochemical studies in rats. *Arch Pathol Lab Med* 1977;**101**:44–8.
405. Nagayo T. Dysplasia of the gastric mucosa and its relation to the precancerous state. *Jpn J Cancer Res* 1981;**72**:813–23.
406. Morson BC, Sobin LH, Grundmann E, Johansen A, Nagayo T, Serck-Hanssen A. Precancerous conditions and epithelial dysplasia in the stomach. *J Clin Pathol* 1980;**33**:711–21.
407. Streett CS, Cimprich RE, Robertson JL. Pathologic findings in the stomach of rats treated with the H₂-receptor antagonist tiotidine. *Scand J Gastroenterol* 1984;**19**(Suppl. 101):109–17.
408. Streett CS, Robertson JL, Crissman RE. Morphologic stomach findings in rats and mice treated with the H₂-receptor antagonists, ICI 125211 and ICI 162846. *Toxicol Pathol* 1988;**16**:299–304.
409. Anon. *Clinical brochure: Taxol* (NSC 125973). 6–12. Bethesda, MD: National Cancer Institute. Division of Cancer Treatment; 1983.
410. Hruban RH, Yardley JH, Donehower RC, Boitnott JK. Taxol toxicity – epithelial necrosis in the gastrointestinal tract associated with polymerized microtubule accumulation and mitotic arrest. *Cancer* 1989;**63**:1944–50.
411. Havu N. Enterochromaffin-like cell carcinoids of gastric mucosa in rats after life long inhibition of gastric secretion. *Digestion* 1986;**35**(Suppl. 1):42–55.
412. Ekman L, Hansson E, Havu N, Carlsson E, Lundberg C. Toxicological studies on omeprazole. *Scand J Gastroenterol* 1985;**20**(Suppl. 108):53–69.
413. Fellenius E, Berglindh T, Sachs G, Olbe L, Elander B, Sjöstrand S-E, et al. Substituted benzimidazoles inhibit acid secretion by blocking (H + + K +) ATPase. *Nature* 1981;**290**:159–61.
414. Anon. NEXIUM[®] (esomeprazole magnesium) prescribing information. Wilmington: AstraZeneca; 2005.
415. Anon. PROTONIX[®] (pantoprazole sodium) prescribing information. Philadelphia: Wyeth Pharmaceuticals Inc; 2005.
416. Anon. PREVACID[®] (lansoprazole) prescribing information. Lake Forest IL: TAP Pharmaceutical Products Inc; 2004.
417. Sundler F, Håkanson R, Carlsson E, Larsson H, Mattsson H. Hypergastrinemia after blockade of acid secretion in the rat. Trophic effects. *Digestion* 1986;**35**(Suppl. 1):56–69.
418. Creutzfeldt W, Stöckmann F, Conlon JM, Fölsch UR, Bonatz G, Wülfrath M. Effect of short- and long-term feeding of omeprazole on rat gastric endocrine cells. *Digestion* 1986;**35**(Suppl. 1):84–97.
419. Blom H. Alterations in gastric mucosal morphology induced by long-term treatment with omeprazole in rats. *Digestion* 1986;**35**(Suppl. 1):98–105.

420. Solcia E, Capella C, Sessa F, Rindi G, Cornaggia M, Riva C, et al. Gastric carcinoids and related endocrine growths. *Digestion* 1986;**35**(Suppl. 1):3–22.
421. Müller J, Kirchner T, Müller-Hermelink JK. Gastric endocrine cell hyperplasia and carcinoid tumors in atrophic gastritis type A. *Am J Surg Pathol* 1987;**11**:909–17.
422. Larsson H, Carlsson E, Mattsson H, Lundell L, Sundler F, Sundell G, et al. Plasma gastrin and gastric enterochromaffin-like cell activation and proliferation. Studies with omeprazole and ranitidine in intact and antrectomized rats. *Gastroenterol Clin North Am* 1986;**90**:391–9.
423. Thoolen B, Koster H, van Kolschoten A, de Haan M. Gastric neuroendocrine tumors in a 2-year oncogenicity study with CD-1 mice. *Toxicol Pathol* 2002;**30**:322–7.
424. Leslie GB, Walker TF. A toxicological profile of cimetidine. In: Burland WL, Alison-Simkins M, editors. *Cimetidine. proceedings of the second international symposium on histamine H2-receptor antagonists*. Amsterdam: Excerpta Medica; 1977. p. 24–33.
425. Hirth RS, Evans LD, Buroker RA, Oleson FB. Gastric enterochromaffin-like hyperplasia and neoplasia in the rat: an indirect effect of the histamine H2-receptor antagonist BL-6341. *Toxicol Pathol* 1988;**16**:273–87.
426. Poynter D, Pick CR, Harcourt RA, Selway SAM, Ainge G, Harman IW, et al. Association of long lasting unsurmountable histamine H2 blockade and gastric carcinoid tumours in the rat. *Gut* 1985;**26**:1284–95.
427. Delaney JP, Michel HM, Bonsack ME, Eisenberg MM, Dunn DH. Adrenal corticosteroids cause gastrin cell hyperplasia. *Gastroenterology* 1979;**76**:913–6.
428. Sugimura T, Fujimura S. Tumour production in glandular stomach of rat by N-methyl-N'-nitro-N-nitrosoguanidine. *Nature* 1967;**216**:943–4.
429. Correa P, Haenszel W, Cuello C, Tannenbaum SR, Archer M. A model for gastric cancer epidemiology. *Lancet* 1975;**2**:58–60.
430. Pocock SJ. Nitrates and gastric cancer. *Hum Toxicol* 1985;**4**.
431. Gillatt PN, Palmer RC, Smith PLR, Walters CL, Reed PI. Susceptibilities of drug to nitrosation under simulated gastric conditions. *Food Chem Toxicol* 1985;**23**:849–55.
432. Martelli A, Robbiano L, Grossi S, Mattioli F, Brambilla G. Formation of DNA-damaging N-nitroso compounds from the interaction of calcium-channel blockers with nitrite. *Toxicology* 2007;**238**:211–5.
433. Brambilla G, Martelli A. Genotoxic and carcinogenic risk to humans of drug-nitrite interaction products. *Mutat Res-Rev Mutat Res* 2007;**635**:17–52.
434. Bellander T, Österdahl B-G, Hagmar L. Formation of N-mono-nitrosopiperazine in the stomach and its secretion in the urine after oral intake of piperazine. *Toxicol Appl Pharmacol* 1985;**80**:193–8.
435. Elder JN, Ganguli PC, Gillespie I. Cimetidine and gastric cancer. *Lancet* 1979;**1**:1005–6.
436. Reed PI, Smith PLR, Haines K, House FR, Walters CL. Effect of cimetidine on gastric juice N-nitrosamine concentration. *Lancet* 1981;**2**:553–6.
437. Hawker RC, Muscroft TJ, Keighley MRB. Gastric cancer after cimetidine in a patient with two negative pre-treatment biopsies. *Lancet* 1980;**1**:709–10.
438. Penston J, Wormsley KG. H2-receptor antagonists and gastric cancer. *Med Toxicol* 1986;**1**:163–8.
439. Colin-Jones DG, Langman MJS, Lawson DH, Vessey MP. Post marketing surveillance of the safety of cimetidine: mortality during second, third, and fourth years of follow-up. *Br Med J* 1985;**291**:1084–8.
440. Langman MJS. Antisecretory drugs and gastric cancer. *Br Med J* 1985;**290**:1850–2.
441. Anderson LM, Giner-Sorolla A, Haller IM, Budinger JM. Effects of cimetidine, cimetidine plus nitrite, and nitrosocimetidine on tumors in mice following transplacental chronic lifetime exposure. *Cancer Res* 1985;**45**:3561–6.
442. Walker TF, Whitehead SM, Leslie GB, Crean GP, Roe FJC. Safety evaluation of cimetidine: report at the termination of a seven-year study in dogs. *Hum Toxicol* 1987;**6**:159–64.
443. Anon. Nitrosatable drugs: an assessment of the risks. In *Drug Information Report, PD/D1/78.2* 4–8. World Health Organization; 1978.
444. Szentirmay Z, Sugar J. Adenocarcinoma, glandular stomach, rat. In: Jones TC, Mohr U, Hunt RD, editors. *Digestive system. Monographs on pathology of laboratory animals*. Berlin: Springer-Verlag; 1985. p. 301–9.
445. Fujita M, Taguchi T, Takami M, Usugane M, Takahashi A, Shiba S. Carcinoma and related lesions in dog stomach induced by oral administration of N-methyl-N'-nitro-N-nitrosoguanidine. *Jpn J Cancer Res* 1974;**65**:207–14.
446. Stewart HL, Snell KC, Morris HP, Wagner BP, Ray FE. Carcinoma of the glandular stomach of rats ingesting N,N',2,7-fluorenyl-bisacetamine. *NCI Monogr* 1961;**5**:105–39.

447. Bode G, Clausning P, Gervais F, Loegsted J, Luft J, Nogues V, et al. The utility of the minipig as an animal model in regulatory toxicology. *J Pharmacol Toxicol Methods* 2010;**62**:196–220.
448. Breckenridge A. Enzyme induction in humans. Clinical aspects: an overview. *Pharmacol Ther* 1987;**33**:95–9.
449. Hänninen O, Linstrom-Seppä P, Pelkonen K. Role of gut in xenobiotic metabolism. *Arch Toxicol* 1987;**60**:34–6.
450. Bonkovsky HL, Hauri H-P, Marti U, Gasser R, Meyer UA. Cytochrome P450 of small intestinal epithelial cells. Immunocytochemical characterization of the increase in cytochrome P450 caused by phenobarbital. *Gastroenterology* 1985;**88**:458–67.
451. Perloff MD, Von Moltke LL, Greenblatt DJ. Differential metabolism of midazolam in mouse liver and intestine microsomes: a comparison of cytochrome P450 activity and expression. *Xenobiotica* 2003;**33**:365–77.
452. Kyokawa Y, Nishibe Y, Wakabayashi M, Harauchi T, Maruyama T, Baba T, et al. Induction of intestinal cytochrome P450 (CYP3A) by rifampicin in beagle dogs. *Chem Biol Interact* 2001;**134**:291–305.
453. Lindell M, Lang M, Lennernäs H. Expression of genes encoding for drug metabolising cytochrome P450 enzymes and P-glycoprotein in the rat small intestine; comparison to the liver. *Eur J Drug Metab Pharmacokinet* 2003;**28**:41–8.
454. Hoensch H, Woo CH, Raffin SB, Schmid R. Oxidative metabolism of foreign compounds in rats small intestine: cellular localization and dependence on dietary iron. *Gastroenterology* 1976;**70**:1063–70.
455. Ogasawara T, Hoensch H, Ohnhaus EE. Distribution of glutathione and its related enzymes in small intestinal mucosa of rats. *Arch Toxicol* 1985;(Suppl. 8):110–3.
456. Williamson RCN. Intestinal adaptation. Mechanisms of control. *N Engl J Med* 1978;**298**:1444–50.
457. Williamson RCN. Intestinal adaptation. Structural, functional and cytochemical changes. *N Engl J Med* 1978;**298**:1393–402.
458. Cheng H, Leblond CP. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. III. Entero-endocrine cells. *Am J Anat* 1974;**141**:521–36.
459. Imondi AR, Balis ME, Lipkin M. Changes in enzyme levels accompanying differentiation of intestinal epithelial cells. *Exp Cell Res* 1969;**58**:323–30.
460. Altmann GG, Enesco M. Cell number as a measure of distribution and renewal of epithelial cells in the small intestine of growing and adult rats. *Am J Anat* 1967;**121**:319–36.
461. Parker FG, Barnes EN, Kaye GI. The pericryptal fibroblast sheath. IV. Replication, migration and differentiation of the subepithelial fibroblasts of the crypts and villus of the rabbit jejunum. *Gastroenterology* 1974;**67**:607–21.
462. Hasegawa J, Watanabe K, Nakamura T, Nagura H. Immunocytochemical localization of alkaline phosphatase in absorptive cells of rat small intestine after colchicine treatment. *Cell Tissue Res* 1987;**250**:521–9.
463. Herman-Taylor J, Perrin J, Grant DAW, Appleyard A, Bubel M, Magee AI. Immunofluorescent localization of enterokinase in human small intestine. *Gut* 1977;**18**:259–65.
464. Sandow JM, Whitehead R. The Paneth cell. *Gut* 1979;**20**:420–31.
465. Satoh Y, Ishikawa K, Tanaka H, Ono K. Immunohistochemical observations of immunoglobulin A in the Paneth cells of germ-free and formerly-germ-free rats. *Histochemistry* 1986;**85**:197–201.
466. Rhodin, JAG. Digestive system: intestines. In: *Histology. A Text and Atlas*, 554–577. New York: Oxford University Press; 1974.
467. Lewin K. Histochemical observations on Paneth cells. *J Anat* 1969;**105**:171–716.
468. Rieken EO, Pearse AGE. Histochemical study on the Paneth cell in the rat. *Gut* 1966;**7**:86–93.
469. Speece AJ. Histochemical distribution of lysozyme activity in organs of normal mice and radiation chimeras. *J Histochem Cytochem* 1964;**12**:384–91.
470. Ghos Y, Vantrappen G. The cytochemical localization of lysozyme in Paneth cell granules. *Histochem J* 1971;**3**:175–8.
471. Ouellette AJ. Mucosal immunity and inflammation IV. Paneth cell antimicrobial peptides and the biology of the mucosal barrier. *Am J Physiol* 1999;**277**:G257–61.
472. Ouellette AJ. Paneth cells and innate immunity in the crypt microenvironment. *Gastroenterology* 1997;**113**:1779–84.
473. Porter EM, Bevins CL, Ghosh D, Ganz T. The multifaceted Paneth cell. *Cell Mol Life Sci* 2002;**59**:156–70.
474. Bevins CL. The Paneth cell and the innate immune response. *Curr Opin Gastroenterol* 2004;**20**:572–80.
475. Inokuchi Fujimoto, Kawai S. K. Cellular kinetics of gastrointestinal mucosa, with special reference of gut endocrine cells. *Arch Histol Cytol* 1983;**46**:137–57.

476. Pabst R. The anatomical basis for the immune function of the gut. *Anat Embryol* 1987;**176**:135–44.
477. Selby WS, Janossy G, Jewell DP. Immunohistological characterization of intra-epithelial lymphocytes of the human gastrointestinal tract. *Gut* 1981;**22**:169–76.
478. Hirata I, Berribi G, Austin LL, Keren DF, Dobbins WO. Immunohistological characterization of intra-epithelial and lamina propria lymphocytes in control ileum and colon and inflammatory bowel disease. *Dig Dis Sci* 1986;**31**:593–603.
479. Bruder MC, Spanhaak S, Bruijntjes JP, Michielsen CPPC, Vos JG, Kuper CF. Intestinal T lymphocytes of different rats strains in immunotoxicity. *Toxicol Pathol* 1999;**27**:171–9.
480. Michalek SM, Rahman AFR, McGhee JR. Rat immunoglobulins in serum and secretions: comparison of IgA and IgG in serum, colostrum, milk and saliva of protein malnourished and normal rats. *Proc Soc Exp Biol Med* 1975;**148**:1114–8.
481. Brandtzaeg P, Valnes K, Scott H, Rognum TO, Bjerke K, Bakken K. The human gastrointestinal secretory immune system in health and disease. *Scand J Gastroenterol* 1985;**20**(Suppl. 114):17–38.
482. Rodning CB, Erlansen SL, Wilson ID, Carpenter A-M. Light microscopic morphometric analysis of rat ileal mucosa. I. Component quantitation of IgA-containing immunocytes. *Dig Dis Sci* 1983;**28**:742–50.
483. Cesta MF. Normal structure, function, and histology of mucosa-associated lymphoid tissue. *Toxicol Pathol* 2006;**34**:599–608.
484. Owen RL, Nemanic P. Antigen processing structures of the mammalian intestinal tract: an SEM study of lymphoepithelial organs. In: Becker RP, Johari O, editors. *Scanning electron microscopy. Part II*. O'Hare: Scanning Electron Microscopy Inc.; 1978. p. 367–78.
485. Cornes JS. Number, size and distribution of Peyer's patches in the human small intestine. *Gut* 1965;**6**:225–33.
486. Martin MS, Hamann A, Martin F. Gut-associated lymphoid tissue and 1,2-demethylhydrazine intestinal tumors in the rat: a histological and immunoenzymatic study. *Int J Cancer* 1986;**38**:75–80.
487. Elmore SA. Enhanced histopathology of mucosa-associated lymphoid tissue. *Toxicol Pathol* 2006;**34**:687–96.
488. Yamaguchi K, Schoefl GI. Blood vessels of the Peyer's patch in the mouse. III High endothelial venules. *Anat Rec* 1983;**206**:419–38.
489. Bland PW, Warren LG. Immunohistologic analysis of the T-cell and macrophage infiltrate in 1,2-dimethylhydrazine-induced colon tumors in the rat. *J Natl Cancer Inst* 1985;**75**:757–64.
490. Owen RL, Bhalla DK. Cytochemical analysis of alkaline phosphatase and esterase activities and of lectin-binding and anionic sites in rat and mouse Peyer's patch M cells. *Am J Anat* 1983;**168**:199–212.
491. Wolf JL, Bye WA. The membraneous epithelial (M) cell and the mucosal immune system. *Annu Rev Med* 1984;**35**:95–112.
492. Owen RL. Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: an ultrastructural study. *Gastroenterology* 1977;**72**:440–50.
493. Jeurissen SHM, David S, Smimia T. Uptake of particulate and soluble antigens in the small intestines of the rat. *Cell Biol Int Rep* 1985;**9**:523.
494. Smith MW, James PS, Tivey DR. M cell numbers increase after transfer of SPF mice to a normal animal house environment. *Am J Pathol* 1987;**128**:385–9.
495. Neutra MR. Current concepts in mucosal immunity V. Role of M cells in transepithelial transport of antigens and pathogens to the mucosal immune system. *Am J Physiol* 1998;**274**:G785–91.
496. Miller HRP. The structure, origin and function of mucosal mast cells. A brief review. *Biol Cellulaire* 1980;**39**:229–32.
497. Wingren U, Enerback L. Mucosal mast cells of the rat intestine: a re-evaluation of fixation and staining properties with special reference to protein blocking and solubility of the granular glycosaminoglycan. *Histochem J* 1983;**15**:571–82.
498. Schuurman HJ, Kuper CF, Vos JG. Histopathology of the immune system as a tool to assess immunotoxicity. *Toxicology* 1994;**86**:187–212.
499. Moolenbeck C, Ruitenber EJ. The 'Swiss Roll'. A simple technique for histological studies of the rodent intestine. *Lab Anim* 1981;**15**:57–9.
500. Filipe MI, Branfoot AC. Abnormal patterns of mucous secretion in apparently normal mucosa of large intestine with carcinoma. *Cancer* 1974;**34**:282–90.
501. Tiwari A, Moghal M, Meleagros L. Life threatening abdominal complications following cocaine abuse. *J R Soc Med* 2006;**99**:51–2.

502. Muniz AE, Evans T. Acute gastrointestinal manifestations associated with use of crack. *Am J Emerg Med* 2001;**19**:61–3.
503. Holmberg CA, Leiniger R, Wheeldon E, Slater D, Henrickson R, Anderson J. Clinicopathological studies of gastrointestinal disease in macaques. *Vet Pathol* 1982;**19**(Suppl. 7):163–70.
504. Toft JD. The pathoparasitology of the alimentary tract and pancreas of non-human primates: a review. *Vet Pathol* 1982;**19**(Suppl. 7):44–92.
505. Chitwood M, Lichtenfels JR. Parasitological review. Identification of parasitic metazoa in tissue section. *Exp Parasitol* 1973;**32**:407–519.
506. Waggie KS, Thornburg LP, Grove KJ, Wagner JE. Lesions of experimentally induced Tyzzer's disease in Syrian hamsters, guinea pigs, mice and rats. *Lab Anim* 1987;**21**:155–60.
507. Ganaway JR. Tyzzer's disease, intestine, mouse, rat, hamster. In: Jones TC, Mohr U, Hunt RD, editors. *Digestive system. monographs on pathology of laboratory animals*. Berlin: Springer-Verlag; 1985. p. 330–3.
508. Ganaway JR. Salmonellosis, intestine, mouse, rat, hamster. In: Jones TC, Mohr U, Hunt RD, editors. *Digestive system. Monographs on pathology of laboratory animals*. Berlin: Springer-Verlag; 1985. p. 333–7.
509. McOrist S, Gebhart CJ, Lawson GHK. Enterocyte proliferation and intracellular bacteria in animals. *Gut* 1994;**35**:1483–6.
510. Jacoby RO. Transmissible ileal hyperplasia, hamster. In: Jones TC, Mohr U, Hunt RD, editors. *Digestive system. Monographs of pathology of laboratory animals*. Berlin: Springer-Verlag; 1985. p. 346–55.
511. Fox JG, Stills HF, Paster BJ, Dewhirst FE, Yan L, Palley L, et al. Antigen specificity and morphological characteristics of *Chlamydia trachomatis*, strain SFPD, isolated from hamsters with proliferative ileitis. *Lab Anim Sci* 1993;**43**:405–10.
512. Peace TA, Brock KV, Stills HFJ. Comparative analysis of the 16S RNA gene sequence of the putative agent of proliferative ileitis of hamsters. *Int J Syst Bacteriol* 1994;**44**:832–5.
513. Fox JG, Claps MC, Taylor NS, Maxwell KO, Ackerman JI, Hoffman SB. *Campylobacter jejuni/coli* in commercially reared beagles. Prevalance and serotypes. *Lab Anim Sci* 1988;**38**:262–5.
514. Prescott JF, Munroe DL. *Campylobacter jejuni* enteritis in man and domestic animals. *J Am Vet Med Assoc* 1982;**181**:1524–30.
515. Gimenez DF. Staining Rickettsiae in yolk sac cultures. *Stain Technol* 1964;**39**:135–40.
516. Burnett RA, Brown IL, Findlay J. Cresyl fast violet staining method for *Campylobacter*-like organisms. *J Clin Pathol* 1987;**40**:353.
517. McMullen L, Walker MM, Bain LA, Karim QN, Baron JH. Histological identification of *Campylobacter* using Gimenez technique in gastric antral mucosal. *J Clin Pathol* 1987;**40**:464–5.
518. Boorman GA, Van Hooft JIM, Van Der Waaij D, Van Noord MJ. Synergistic role of intestinal flagellates and normal intestinal bacteria in a post-weaning mortality of mice. *Lab Anim Sci* 1973;**23**:187–93.
519. Wagner JE, Doyle RE, Ronald NC, Garrison RC, Schmitz JA. Hexamitis in laboratory mice, hamsters, and rats. *Lab Anim Sci* 1974;**24**:249–354.
520. Gillon J, Althamery D, Ferguson A. Features of small intestinal pathology (epithelial cell kinetics, intra-epithelial lymphocytes, disaccharidases) in a primary *Giardia muris* infection. *Gut* 1982;**23**:498–506.
521. Nair KV, Gillon J, Ferguson A. Corticosteroid treatment increases parasite numbers in murine giardiasis. *Gut* 1981;**22**:475–80.
522. Casemore DP, Sands RL, Curry A. *Cryptosporidium* species a 'new' human pathogen. *J Clin Pathol* 1985;**38**:1321–36.
523. Cockrell BY, Valerio MG, Garner FM. Cryptosporidiosis in the intestines of rhesus monkeys (*Macaca mulatta*). *Lab Anim Sci* 1974;**24**:881–7.
524. Rehg JE, Lawton GW, Pakes SP. *Cryptosporidium cuniculus* in the rabbit (*Oryctolagus cuniculus*). *Lab Anim Sci* 1979;**29**:656–60.
525. Davis AJ, Jenkins SJ. Cryptosporidiosis and proliferative ileitis in a hamster. *Vet Pathol* 1986;**23**:632–3.
526. Fukishima K, Helman RG. Cryptosporidiosis in a pup with distemper. *Vet Pathol* 1984;**21**:247–8.
527. Hsu C-K. Parasitic diseases. In: Baker HJ, Lindsey JR, Weisbroth SH, editors. *The laboratory rat. Biology, diseases*. Academic Press. Vol. 1. p. 305–31.
528. Barthold SW. Mouse hepatitis virus infection, intestine, mouse. In: Jones TC, Mohr U, Hunt RD, editors. *Digestive system. Monographs on pathology of laboratory animals*. Berlin: Springer-Verlag; 1985. p. 317–21.
529. Barthold SW. Murine rotavirus infection, intestine, mouse. In: Jones TC, Mohr U, Hunt RD, editors. *Digestive system. Monographs on pathology of laboratory animals*. Berlin: Springer-Verlag; 1985. p. 321–5.

530. Kalter SS. Enteric viruses of non human primates. *Vet Pathol* 1982;**19**(Suppl. 7):33–43.
531. Lerche NW, Osborn KG. Simian retrovirus infections: potential confounding variables in primate toxicology studies. *Toxicol Pathol* 2003;**31**(Suppl.):103–10.
532. Bjarnason I, Zanelli G, Smith T, Prouse P, Williams P, Smethurst P, et al. Non-steroidal anti-inflammation in humans. *Gastroenterology* 1987;**93**:480–9.
533. Tabata K, Okabe S. Effects of 16,16-dimethyl prostaglandin E2-methyl ester on aspirin- and indomethacin-induced gastrointestinal lesions in dogs. *Dig Dis Sci* 1980;**25**:439–48.
534. Whittle BJR. Temporal relationship between cyclooxygenase inhibition, as measured by prostacyclin biosynthesis, and the gastro-intestinal damage induced by indomethacin in the rat. *Gastroenterology* 1981;**80**:94–8.
535. Rainsford KD. The effects of aspirin and other non-steroid anti-inflammatory/analgesic drugs on gastrointestinal mucus glycoprotein biosynthesis in vivo: relationship to ulcerogenic actions. *Biochem Pharmacol* 1978;**27**:877–85.
536. Stewart THM, Hetenyi C, Rowsell H, Orizaga M. Ulcerative enterocolitis in dogs induced by drugs. *J Pathol* 1980;**131**:363–78.
537. Brodie DA, Tate CL, Hooke KF. Aspirin: intestinal damage in rats. *Science* 1970;**170**:183–5.
538. Djaldetti M, Fishman P. The effect of aspirin on small intestinal mucosa. *Arch Pathol Lab Med* 1981;**105**:144–7.
539. Ettarh RR, Carr KE. Morphometric analysis of the small intestinal epithelium in the indomethacin-treated mouse. *J Anat* 1996;**189**:51–6.
540. Donald S, Verschoyle RD, Edwards R, Judah DJ, Davies R, Riley J, et al. Hepatobiliary damage and changes in hepatic gene expression caused by the antitumor drug ecteinascidin-743 (ET-743) in the female rat. *Cancer Res* 2002;**62**:4256–62.
541. Bregman CL, Comerkeski CR, Buroker RA, Hirth RS, Madisoo H, Hottendorf GH. Single-dose and multiple-dose intravenous toxicity studies of BMY-25282 in rats. *Fundam Appl Toxicol* 1987;**9**:90–109.
542. Nolte, T & Harleman, JH. Alkylating cytostatics. In: *Classic examples in toxicologic pathology*. Hannover: European Society of Toxicologic Pathology; 2005.
543. Schaffner, J-C, Ernst, R, Junker, U, Thomas, H & Germann, P-G. Vascular endothelial growth factor inhibitors (VEGF inhibitors). In: *Classic examples in toxicologic pathology*. Hannover: European Society of Toxicologic Pathology; 2005.
544. Schaffner, J-C, Müller, L, Wartmann, M & Germann, P-G. Microtubule-stabilizing (epothilone-like) agents. In: *Classic examples in toxicologic pathology*. Hannover: European Society of Toxicologic Pathology; 2005.
545. Schein PS, Davis RD, Carter S, Newman J, Schein DR, Rall DP. The evaluation of anticancer drugs in dogs and monkeys for the prediction of qualitative toxicities in man. *Clin Pharmacol Ther* 1970;**11**:3–40.
546. Hesketh PJ. Drug therapy: chemotherapy-induced nausea and vomiting. *N Engl J Med* 2008;**358**:2482–94.
547. Taminiau J, Gall DG, Hamilton JR. Response of the rat small-intestine epithelium to methotrexate. *Gut* 1980;**21**:486–92.
548. Pinkerton CR, Cameron CHS, Sloan JM, Glasgow JFT, Gwevava NJT. Jejunal crypt cell abnormalities associated with methotrexate treatment in children with acute lymphoblastic-leukaemia. *J Clin Pathol* 1982;**35**:1272–7.
549. Renes IB, Verburg M, Bulsing NP, Ferdinandusse S, Buller HA, Dekker J, et al. Protection of the Peyer's patch-associated crypt and villus epithelium against methotrexate-induced damage is based on its distinct regulation of proliferation. *J Pathol* 2002;**198**:60–8.
550. Martin RA, Barsoum NJ, Sturgess JM, de la Iglesia FA. Leucocyte and bone marrow effects of a thiomorpholine quinazolinone antihypertensive agent. *Toxicol Appl Pharmacol* 1985;**81**:166–73.
551. Capps GW, Fulcher AS, Szucs RA, Turner MA. Imaging features of radiation-induced changes in the abdomen. *Radiographics* 1997;**17**:1455–73.
552. Coia LR, Myerson RJ, Tepper JE. Late effects of radiation-therapy on the gastrointestinal-tract. *Int J Radiat Oncol Biol Phys* 1995;**31**:1213–36.
553. Dubrow RA. Radiation changes in the hollow viscera. *Semin Roentgenol* 1994;**29**:38–52.
554. Hauer-Jensen M. Late radiation-injury of the small-intestine – clinical, pathophysiological and radiobiological aspects – a review. *Acta Oncol* 1990;**29**:401–15.
555. Szabo S, Cho CH. From cysteamine to MPTP structure–activity studies with duodenal ulcerogens. *Toxicol Pathol* 1988;**16**:205–12.
556. Szabo S. Dopamine disorder in duodenal ulceration. *Lancet* 1979;**2**:880–2.

557. Szabo S. Pathogenesis of duodenal-ulcer disease. *Lab Invest* 1984;**51**:121–47.
558. Visscher GE, Robinson RL, Hartmen HA. Chemically induced lipidosis of the small intestinal villi in the rat. *Toxicol Appl Pharmacol* 1980;**55**:535–44.
559. Dobbins WO. Morphologic aspects of lipid absorption. *Am J Clin Nutr* 1969;**22**:257–65.
560. Gray JE, Weaver RN, Sinkula AA, Schurr PE, Moran J. Drug induced enteropathy characterized by lipid in macrophages. *Toxicol Appl Pharmacol* 1974;**27**:145–57.
561. Friedman HI, Cardell RR. Effects of puromycin on the structure of rat intestinal epithelial cells during fat absorption. *J Cell Biol* 1972;**52**:15–40.
562. Hyams DE, Sabesin SM, Greenberger NJ, Isselbacher KJ. Inhibition of intestinal protein synthesis and lipid transport by ethionine. *Biochim Biophys Acta* 1966;**125**:166–73.
563. Murgatroyd LN. A morphological and histochemical study of a drug-induced enteropathy in the Alderley Park rat. *Br J Exp Pathol* 1980;**61**:567–78.
564. Nelson AA, Fitzhugh OG. Chloroquine: pathological changes observed in rats which for two years had been fed various proportions. *Arch Pathol* 1948;**45**:454–62.
565. Mazué G, Vic P, Gouy D, Remandet B, Lacheretz F, Berthe J, et al. Recovery from amiodarone-induced lipidosis in laboratory animals. A toxicological study. *Fundam Appl Toxicol* 1984;**4**:992–9.
566. Vic P, Gouy D, Lacheretz F, Verschuere B, Cros M, Remandet J, et al. Intestinal pathology in the dog induced by sublethal doses of amiodarone. *Arch Toxicol* 1985;(Suppl. 8):104–9.
567. Kennedy MFG, Tutton PJM, Barkla DH. Adrenergic factors involved in the control of crypt cell proliferation in jejunum and descending colon of mouse. *Clin Exp Pharmacol Physiol* 1983;**10**:577–86.
568. Botsios DS, Vasilidiadis KD. Factors enhancing intestinal adaptation after bowel compensation. *Dig Dis* 2003;**21**:228–36.
569. Dowling RH. Glucagon-like peptide-2 and intestinal adaptation: an historical and clinical perspective. *J Nutr* 2003;**133**:3703–7.
570. Tappenden KA. Mechanisms of enteral nutrient-enhanced intestinal adaptation. *Gastroenterology* 2006;**130**:S93–9.
571. Weale AR, Edwards AG, Bailey M, Lear PA. Intestinal adaptation after massive intestinal resection. *Postgrad Med J* 2005;**81**:178–84.
572. Cisler JJ, Buchman AL. Intestinal adaptation in short bowel syndrome. *J Investig Med* 2005;**53**:402–13.
573. MacKay EM, Callaway JW, Barnes RH. Hyperalimentation in normal animals produced by protamine insulin. *J Nutr* 1940;**20**:59–66.
574. Levin RJ, Smyth DH. The effect of the thyroid gland on intestinal absorption of hexoses. *J Physiol* 1963;**169**:755–69.
575. Jarvis EL, Levin RJ. Anatomic adaption of the alimentary tract of the rat to the hyperphagia of chronic alloxan-diabetes. *Nature* 1966;**210**:391–3.
576. Forrester JM. The number of villi in rat's jejunum and ileum: effect of normal growth, partial enterectomy and tube feeding. *J Anat* 1972;**3**:283–91.
577. Hammond KA. Adaptation of the maternal intestine during lactation. *J Mammary Gland Biol Neoplasia* 1997;**2**:243–52.
578. Hanson WR, Osborne JW. Epithelial cell kinetics in the small intestine of the rat 60 days after resection of 70 percent of the ileum and jejunum. *Gastroenterol Clin North Am* 1971;**60**:1087–97.
579. Hanson WR, Osborne JW, Sharp JG. Compensation by the residual intestine after intestinal resection in the rat. *Gastroenterology* 1977;**73**:692–700.
580. Olubuyide IO, Williamson RCN, Bristol JB, Read AE. Goblet cell hyperplasia is a feature of the adaptive response to jejunoileal bypass in rats. *Gut* 1984;**25**:62–8.
581. Rodriguez MB, Tomas FJD, Vaquero C. Morphologic changes in the proximal jejunum after partial hepatectomy in rats. *Rev Esp Enferm Dig* 2008;**100**:615–8.
582. Burkhardt JE, Biehl ML, Kowsz KP, Kadyszewski E, Fisher DO, Ochoa R. Effects of cholestyramine and diet on small intestinal histomorphology in rats. *Toxicol Pathol* 1998;**26**:271–5.
583. Smith JH, Kistic A, Diaz-Arrastia R, Pelly RP, Schroepfer GJ. Inhibitors of sterol synthesis. Morphological studies in rats after dietary administration, administration of 5 a-cholest-8(14)-en-3 β -ol-15-one, a potent hypocholesterolemic compound. *Toxicol Pathol* 1989;**17**:506–15.
584. Gona O. Prolactin and ergocryptine effects mucus glycoproteins of the rat ileum. *Histochem J* 1981;**13**:101–7.

585. Hopfner M, Berger A, Folsch UR, Loser C. Effects of insulin-like growth factor I on growth and polyamine metabolism in various organs in rats. *Digestion* 2002;**65**:103–11.
586. Park CM, Reid PE, Owen DA, Sanker JM, Applegrath DA. Morphological and histochemical changes in intestinal mucosa in the reserpine-treated rat model of cystic fibrosis. *Exp Mol Pathol* 1987;**47**:1–12.
587. Tutton PJM, Helme RD. The influence of adrenoreceptor activity on crypt cell proliferation in rat jejunum. *Cell Tissue Kinet* 1974;**7**:125–36.
588. Hare WV, Stewart HL. Chronic gastritis of the glandular stomach, adenomatous polyps of the duodenum, and calcareous pericarditis in strain DBA mice. *J Natl Cancer Inst* 1956;**16**:889–911.
589. Seronde J. Chronic duodenal ulcers in pantothenate deficient mice. *Gastroenterology* 1965;**48**:612–5.
590. Seronde J. Focal avillous hyperplasia of the mouse duodenum. *J Pathol* 1970;**100**:245–8.
591. Ito A, Watanabe H, Naito M, Naito Y. Induction of duodenal tumors in mice by oral administration of hydrogen peroxide. *Jpn J Cancer Res* 1981;**72**:174–5.
592. Port CD, Dodd D, Deslex P, Regnier B, Sanders P, Indacochea-Redmond N. Twenty-one month evaluation of misoprostol for carcinogenicity in CD-1 mice. *Toxicol Pathol* 1987;**15**:134–42.
593. Dodd DC, Port CD, Deslex P, Regnier B, Sanders P, Indacochea-Redmond N. Two-year evaluation of misoprostol for carcinogenicity in CD Sprague-Dawley rats. *Toxicol Pathol* 1987;**15**:125–33.
594. Ito A, Watanabe H, Naito M, Naito Y. Induction of duodenal tumors in mice by oral administration of hydrogen peroxide. *Gann* 1981;**72**:174–5.
595. Desesso JM, Lavin AL, Hsia SM, Mavis RD. Assessment of the carcinogenicity associated with oral exposures to hydrogen peroxide. *Food Chem Toxicol* 2000;**38**:1021–41.
596. Rerat A. Digestion and absorption of carbohydrate and nitrogenous matter in hindgut of the omnivorous non-ruminant animal. *J Anim Sci* 1978;**46**:1808–37.
597. Snipes RL. Anatomy of the cecum of the laboratory mouse and rat. *Anat Embryol* 1981;**162**:455–74.
598. Ambuhl S, Williams VJ, Senior W. Effects of caecotomy in the young adult female rat on digestibility of food offered *ad libitum* and in restricted amounts. *Aust J Biol Sci* 1979;**32**:205–13.
599. Rowland IR, Mallett AK, Wise A. The effect of diet on the mammalian gut flora and its metabolic activities. *CRC Crit Rev Toxicol* 1986;**16**:31–103.
600. Rowland IR. Interactions of the gut microflora and the host in toxicology. *Toxicol Pathol* 1988;**16**:147–53.
601. Wise A, Mallett AK, Rowland IR. Effect of mixtures of dietary fibres on the enzyme activity of the rat caecal microflora. *Toxicology* 1986;**38**:241–8.
602. Midtvedt T. Influence of ofloxacin on the faecal flora. *Drugs* 1987;**34**(Suppl. 1):154–8.
603. Chang WWL, Leblond CP. Renewal of the epithelium in the descending colon of the mouse. I. Presence of three cell populations: vacuolated-columnar, mucous and argentaffin. *Am J Anat* 1971;**131**:73–100.
604. Ponder BAJ, Schmidt GH, Wilkinson MM, Wood MJ, Monk M, Reid A. Derivation of mouse intestinal crypts from single progenitor cells. *Nature* 1985;**313**:689–91.
605. Sigthorsson G, Simpson RJ, Walley M, Anthony A, Foster R, Hotz-Behoftsitz C, et al. COX-1 and 2, intestinal integrity, and pathogenesis of nonsteroidal anti-inflammatory drug enteropathy in mice. *Gastroenterology* 2002;**122**:1913–23.
606. McKinnon RA, Burgess WM, Hall P, Roberts-Thomson SJ, Gonzalez FJ, McManus ME. Characterization of CYP3A gene subfamily expression in human gastrointestinal tissues. *Gut* 1995;**36**:259–67.
607. Thörn M, Finnström N, Lundgren S, Rane A, Lööf L. Cytochromes P450 and MDR1 mRNA expression along the human gastrointestinal tract. *Br J Clin Pharmacol* 2005;**60**:54–60.
608. Sun J, Strobel HW. Ageing affects the drug metabolism systems of rat liver, kidney, colon and lung in a differential fashion. *Exp Geront* 1986;**21**:523–34.
609. Scott GBD. Mucosal microhernias in the nonhuman primate colon: their role in the pathogenesis of colonic diseases. *Vet Pathol* 1982;**19**(Suppl. 7):134–40.
610. Kealy WF. Colonic lymphoid-glandular complex (microbursa): nature and morphology. *J Clin Pathol* 1976;**29**:241–4.
611. Klohs WD, Steinkampf RW. Possible link between the intrinsic drug-resistance of colon tumors and a detoxification mechanism of intestinal-cells. *Cancer Res* 1988;**48**:3025–30.
612. Beaugerie L, Petit J-C. Antibiotic-associated diarrhoea. *Best Pract Res Clin Gastroenterol* 2004;**18**:337–52.
613. Bartlett JG, Onderdonk AB, Cisneros RL, Kasper DL. Clindamycin-associated colitis due to a toxin-producing species of *Clostridium* in hamsters. *J Infect Dis* 1977;**136**:701–5.

614. Bartlett JG, Chang TW, Gurwith M, Gorbach SL, Onderdonk AB. Antibiotic-associated pseudomembranous colitis due to toxin-producing clostridia. *N Engl J Med* 1978;**298**:531–4.
615. Milligan DW, Kelly JK. Pseudomembranous colitis in a leukaemia unit: a report of five fatal cases. *J Clin Pathol* 1979;**32**:1237–43.
616. Rehg JE, Lu Y-S. Clostridium difficile colitis in a rabbit following antibiotic therapy for pasteurellosis. *J Am Vet Med Assoc* 1981;**179**:1296–7.
617. Rehg JE, Lu Y-S. Clostridium difficile typhlitis in hamsters not associated with antibiotic therapy. *J Am Vet Med Assoc* 1982;**181**:1422–3.
618. Rehg JE, Pakes SP. Clostridium difficile antitoxin neutralization of cecal toxin(s) from guinea pigs with penicillin-associated colitis. *Lab Anim Sci* 1981;**31**:156–60.
619. Rehg JE. Clostridial enteropathies, hamster. In: Jones TC, Mohr U, Hunt RD, editors. *Digestive system. Monographs on pathology of laboratory animals*. Berlin: Springer-Verlag; 1985. p. 340–6.
620. Cudmore M, Silva J, Fekety R. Clostridial enterocolitis produced by methotrexate in hamsters. *Clin Res* 1979;**27**:A383–A383.
621. Barthold SW, Coleman GL, Bhatt PN, Osbaldiston GW, Jonas AM. The etiology of transmissible murine colonic hyperplasia. *Lab Anim Sci* 1976;**26**:889–94.
622. Barthold SW, Coleman GL, Jacoby RO, Livstone EM, Jonas AM. Transmissible murine colonic hyperplasia. *Vet Pathol* 1978;**15**:223–36.
623. Barthold SW, Osbaldiston GW, Jonas AM. Dietary, bacterial, and host genetic interactions in the pathogenesis of transmissible murine colonic hyperplasia. *Lab Anim Sci* 1977;**27**:938–45.
624. Ediger RD, Kovatch RM, Rabstein MM. Colitis in mice with high incidence of rectal prolapse. *Lab Anim Sci* 1974;**24**:488–94.
625. Takeuchi A. Early colonic lesions in experimental shigella infections in rhesus monkeys: revisited. *Vet Pathol* 1982;**19**(Suppl. 7):1–8.
626. Holmberg CA, Henrickson RV, Malaga R, Schneider R, & Gribble D. Non-tuberculous myobacterial disease in rhesus monkeys. *Vet Pathol* 1982;**19**(Suppl. 7):9–16.
627. Polderman AM, Blotkamp J. Oesophagostomum infections in humans. *Parasitol Today* 1995;**11**:451–6.
628. Bogers JJ, Storey PA, Faile G, Hewitt E, Yelifari L, Polderman A, et al. Human oesophagostomiasis: a histomorphometric study of 13 new cases in northern Ghana. *Virchows Arch A Pathol Anat Histopathol* 2001;**439**:21–6.
629. Storey PA, Faile G, Hewitt E, Yelifari L, Polderman AM, Magnussen P. Clinical epidemiology and classification of human oesophagostomiasis. *Trans R Soc Trop Med Hyg* 2000;**94**:177–82.
630. Lumb GD, Beamer PR, Rust JH. Oesophagostomiasis in feral monkeys (*Macaca mulatta*). *Toxicol Pathol* 1985;**13**:209–14.
631. Fellows IW, Clarke JMF, Robberts PF. Nonsteroidal anti-inflammatory drug induced jejunal and colonic diaphragm disease: a report of two cases. *Gut* 1992;**33**:1424–6.
632. Haque S, Haswell DHE, Dreznick JT, West AN. A cecal diaphragm associated with the use of nonsteroidal anti-inflammatory drugs. *J Clin Gastroenterol* 1992;**15**:332–5.
633. Wolfe MM, Lichtenstein DR, Singh G. Medical progress: gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. *N Engl J Med* 1999;**340**:1888–99.
634. Davies NM. Toxicity of nonsteroidal antiinflammatory drugs in the large-intestine. *Dis Colon Rectum* 1995;**38**:1311–21.
635. Mulcahy HE, O'Donoghue D. Nonsteroidal anti-inflammatory drugs and their colonic effects: more interesting than irritating? *Eur J Gastroenterol Hepatol* 2002;**14**:1177–8.
636. Van Velzen D, Ball LM, Dezfulian AR, Southgate A, Howard CV. Comparative and experimental pathology of fibrosing colonopathy. *Postgrad Med J* 1996;**72**:S39–48.
637. Smyth RL, Van Velzen D, Smyth AR, Lloyd DA, Heaf DP. Strictures of ascending colon in cystic fibrosis and high-strength pancreatic enzymes. *Lancet* 1994;**343**:85–6.
638. FitzSimmons SC, Burkhart GA, Borowitz D, Grand RJ, Hammerstrom T, Durie PR, et al. High-dose pancreatic-enzyme supplements and fibrosing colonopathy in children with cystic fibrosis. *N Engl J Med* 1997;**336**:1283–9.
639. Linder JD, Monkemuller KE, Raijman I, Johnson L, Lazenby AJ, Wilcox CM. Cocaine-associated ischemic colitis. *South Med J* 2000;**93**:909–13.

640. Brown DN, Rosenholtz MJ, Marshall JB. Ischemic colitis related to cocaine abuse. *Am J Gastroenterol* 1994;**89**:1558–61.
641. Jonas G, Mahoney A, Murray J, Gertler S. Chemical colitis due to endoscopic cleaning solutions: a mimic of pseudomembranous colitis. *Gastroenterology* 1988;**95**:1403–8.
642. Ishioka T, Kuwabara N, Oohashi Y, Wakabayashi K. Induction of colorectal tumours in rats by sulphated polysaccharides. *CRC Crit Rev Toxicol* 1987;**17**:215–44.
643. Sharratt M, Grasso P, Carpanini F, Gangolli SD. Carrageenan ulceration as a model for human ulcerative colitis. *Lancet* 1970;**2**:932.
644. Fath RB, Deschner EE, Winawer SJ, Dworkin BM. Degraded carrageenan-induced colitis in CF1 mice. A clinical, histopathological and kinetic analysis. *Digestion* 1984;**29**:197–203.
645. Marcus R, Watt J. Colonic ulceration in young rats fed degraded carrageenan. *Lancet* 1971;**2**:765–6.
646. Benitz KF, Goldberg L, Coulston F. Intestinal effects of carrageenans in the rhesus monkey (*Macaca mulatta*). *Food Cosmet Toxicol* 1973;**11**:565–75.
647. Kitano A, Matsumoto T, Hiki M, Hashimura H, Yoshiyasu K, Okawa K, et al. Epithelial dysplasia of the rabbit colon induced by degraded carrageenan. *Cancer Res* 1986;**46**:1374–6.
648. Hirono I, Kuhara K, Hosaka S, Tomizawa S, Goldberg L. Induction of intestinal tumors in rats by dextran sulphate sodium. *J Natl Cancer Inst* 1981;**66**:579–83.
649. Oohashi Y, Ishioka T, Wakabayashi K, Kuwabara N. A study on carcinogenesis induced by degraded carrageenan arising from squamous metaplasia of the rat colorectum. *Cancer Lett* 1981;**14**:267–72.
650. Delahunty T, Recher L, Hollander D. Intestinal permeability changes in rodents. A possible mechanism for degraded carrageenan-induced colitis. *Food Chem Toxicol* 1987;**25**:113–8.
651. Ahmed S, Gunaratnam NT. Melanosis coli. *N Engl J Med* 2003;**349**:1349.
652. Mennecier D, Nizou C, Moulin O, Ciribilli JM, Vergeau B. Color nigricans. *Presse Med* 1999;**28**:106–106.
653. Mennecier D, Vergeau B. Melanosis coli? *N Engl J Med* 2004;**350**:197–197.
654. Schrodt GR. Melanosis coli: a study with the electron microscope. *Dis Colon Rectum* 1963;**6**:277–83.
655. Ghadially FN, Parry EW. An electron microscope and histochemical study of melanosis coli. *J Pathol Bacteriol* 1966;**92**:313–7.
656. Steer HW, Colin-Jones DG. Melanosis coli: studies of the toxic effects of irritant purgatives. *J Pathol* 1975;**115**:199–205.
657. Walker NI, Bennett RE, Axelsen RA. Melanosis coli: a consequence of anthraquinone-induced apoptosis of colonic epithelial cells. *Am J Pathol* 1988;**131**:465–76.
658. Mengs U. Toxic effects of sennosides in laboratory animals and *in vitro*. *Pharmacology* 1988;**36**(Suppl. 1):180–7.
659. Lyden-Sokolowski A, Nilsson A, Sjoberg P. Two-year carcinogenicity study with sennosides in the rat: emphasis on gastrointestinal alterations. *Pharmacology* 1993;**47**(Suppl. 1):209–15.
660. Mitchell JM, Mengs U, McPherson S, Zijlstra J, Dettmar P, Gregson R, et al. An oral carcinogenicity and toxicity study of senna (Tinnevelly senna fruits) in the rat. *Arch Toxicol* 2006;**80**:34–44.
661. Nusko G, Schneider B, Schneider I, Wittekind C, Hahn EG. Anthranoid laxative use is not a risk factor for colorectal neoplasia: results of a prospective case control study. *Gut* 2000;**46**:651–5.
662. Dowling RH, Riecken EO, Laws JW, Booth CC. The intestinal response to high bulk feeding in the rat. *Clin Sci* 1967;**32**:1–9.
663. Stragand JJ, Hagemann RF. Effect of luminal contents on colonic cell replacement. *Am J Physiol* 1977;**233**:E208–11.
664. Barkla DH, Tutton PJM. Proliferative and morphologic changes in rat colon following bypass surgery. *Am J Pathol* 1985;**119**:402–11.
665. Leegwater DC, De Groot AP, Van Kalmthout-Kuper M. The aetiology of caecal enlargement in the rat. *Food and Cosmet Toxicol* 1974;**12**:687–97.
666. Roe FJC, Bär A. Enzootic and epizootic adrenal medullary proliferative diseases of rats: influence of dietary factors which affect calcium absorption. *Hum Toxicol* 1985;**4**:27–52.
667. Newberne PM, Conner MW, Estes PC. The influence of food additives and related materials on lower bowel structure and function. *Toxicol Pathol* 1988;**16**:184–97.
668. Stark A, Nyska A, Madar Z. Metabolic and morphometric changes in small and large intestine in rats fed high-fiber diets. *Toxicol Pathol* 1996;**24**:166–71.

669. Whiteley LO, Purdon MP, Ridder GM, Bertram TA. The interactions of diet and colonic microflora regulating colonic mucosal growth. *Toxicol Pathol* 1996;**24**:305–14.
670. Mengs U, Mitchell J, McPherson S, Gregson R, Tigner JA. 13-week oral toxicity study of senna in the rat with an 8-week recovery period. *Arch Toxicol* 2004;**78**:269–75.
671. Juhr N-C, Ladeburg M. Intestinal accumulation of urea in germ-free animals: a factor in caecal enlargement. *Lab Anim* 1986;**20**:238–41.
672. Van Leeuwen PAM, Drukker J, Van Der Kleyn NM, Van Den Boogaard AEJM, Soeters PN. Morphological effects of high dose neomycin sulphate on the small and large intestine. *Acta Morphol Neerl Scand* 1986;**24**:223–34.
673. Shamsuddin AKM, Trump BF. Colon epithelium. 2. In vivo studies of colon carcinogenesis – light microscopic, histochemical, and ultrastructural studies of histogenesis of azoxymethane-induced colon carcinomas in Fischer-344 rats. *J Natl Cancer Inst* 1981;**66**:389–401.
674. Kozuka S. Premalignancy of the mucosal polyp in the large intestine: I. Histologic gradation of the polyp on the basis of epithelial pseudostratification and glandular branching. *Dis Colon Rectum* 1975;**18**:483–93.
675. Tanaka T. Colorectal carcinogenesis: review of human and experimental animal studies. *J Carcinog* 2009;**8**:5.
676. Riddell RH, Goldman H, Ransohoff DF, Appelman HD, Fenoglio CM, Haggitt RC, et al. Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. *Hum Organ* 1983;**14**:931–68.
677. Kullmann F, Messmann H, Alt M, Gross V, Bocker T, Scholmerich J, et al. Clinical and histopathological features of dextran sulfate sodium induced acute and chronic colitis associated with dysplasia in rats. *Int J Colorectal Dis* 2001;**16**:238–46.
678. Clapper ML, Cooper HS, Chang WCL. Dextran sulfate sodium-induced colitis-associated neoplasia: a promising model for the development of chemopreventive interventions. *Acta Pharmacol Sin* 2007;**28**:1450–9.
679. Lingeman CH, Garner FM. Comparative study of intestinal adenocarcinoma of animals and man. *J Natl Cancer Inst* 1972;**48**:325–46.
680. DePaoli A, McClure HM. Gastrointestinal neoplasms in non-human primates: a review and report of new cases. *Vet Pathol* 1982;**19**(Suppl. 7):104–25.
681. Burn JI, Sellwood RA, Bishop M. Spontaneous carcinoma of the colon of the rat. *J Pathol Bacteriol* 1966;**91**:253–4.
682. Wells GAH. Mucinous carcinoma of the ileum in the rat. *J Pathol* 1971;**103**:271–5.
683. Zwicker GM, Eyster RC, Sells DM, Gass JH. Naturally occurring intestinal neoplasms in aged CRL:CD BR rats. *Toxicol Pathol* 1992;**20**:253–9.
684. Vanderberghe J, Verheyen A, Lauwers S, Geboes K. Spontaneous adenocarcinoma of the ascending colon in Wistar rats: the intracytoplasmic presence of a Campylobacter-like bacterium. *J Comp Pathol* 1985;**95**:45–55.
685. Fortner JG. Spontaneous tumors including gastrointestinal neoplasms and malignant melanoma, in Syrian hamster. *Cancer* 1957;**10**:1153–6.
686. McMaster, O. Sporanox (itraconazole) injection. *Pharmacology/toxicology review and evaluation*. Application. No: 020996. Maryland, USA: Center for Drug Evaluation and Research. Food and Drug Administration; 1999.
687. Ward JM. Morphogenesis of chemically induced neoplasms of the colon and small intestine in rats. *Lab Invest* 1974;**30**:505–13.
688. Heyer J, Yang K, Lipkin M, Edelmann W, Kucherlapati R. Mouse models for colorectal cancer. *Oncogene* 1999;**18**:5325–33.
689. Cai H, Al-Fayez M, Tunstall RG, Platton S, Greaves P, Steward WP, et al. The rice bran constituent triclin potently inhibits cyclooxygenase enzymes and interferes with intestinal carcinogenesis in Apc(Min) mice. *Mol Cancer Ther* 2005;**4**:1287–92.
690. Newman JV, Kosaka T, Sheppard BJ, Fox JG, Schauer DB. Bacterial infection promotes colon tumorigenesis in Apc(Min/+) mice. *J Infect Dis* 2001;**184**:227–30.
691. Corpet DE, Pierre F. How good are rodent models of carcinogenesis in predicting efficacy in humans? A systematic review and meta-analysis of colon chemoprevention in rats, mice and men. *Eur J Cancer* 2005;**41**:1911–22.
692. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. (Report). *Nature* 2008;**454** (436):439.
693. Kraus S, Arber N. Inflammation and colorectal cancer. *Curr Opin Pharmacol* 2009;**9**:405–10.
694. Tache S, Peiffer G, Millet AS, Corpet DE. Carrageenan gel and aberrant crypt foci in the colon of conventional and human flora-associated rats. *Nutr Cancer* 2000;**37**:193–8.