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# VII. Digestive System 1

## MOUTH AND OROPHARYNX

The oral mucosa can be damaged by excessive local trauma from foreign materials, hard fragments in food and damaged teeth may produce ulceration of the mucosa with subsequent infection. However, the oral mucosa may show manifestations of local or systemic disease or derangement produced by therapeutic agents. Excessive contact by therapeutic agents such as aspirin, potassium supplements and corticosteroids have been reported to produce local ulceration in the mouth (Zentler-Monro and Northfield, 1979). The increased use of mouthwashes over the last 20 years has resulted in a number of reported adverse effects to the buccal mucosa in people (Gargari and Kabani, 1995). Systemic disorders produced by anticoagulants or chemotherapeutic drugs may also be evident by bleeding or ulceration in the oral cavity (Goepp, 1982). Buccal ulceration is also described as part of a generalised hypersensitivity reaction to drugs (Zentler-Monro and Northfield, 1979).

The major and minor salivary glands and their secretions also represent and 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.

Drugs that effect motor co-ordination can give rise to drooling and disruption of cricopharyngeal co-ordination (Wyllie et al., 1986). Drug-induced abnormalities of taste sensation are also well-described phenomena occurring in man although human studies are necessary for the detection of these effects. Indeed, many alterations in the oral mucosa are those that are more readily detected by careful clinical and macroscopic observation rather than exhaustive histopathological examination of the buccal mucosa in laboratory animals – provided the basic toxicity profile of a novel agent is adequately assessed in the usual preclinical studies.

*Oral mucosa irritation studies*

Oral irritation studies are used in the testing of products for use in the oral cavity, mainly for surgical, dental and hygiene purposes but also therapeutic agents administered by the sublingual route. This route 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 route, conventional oral or parenteral routes are preferred 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 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*

Inflammation of the oral cavity (*stomatitis*) may involve the buccal mucosa, the gingiva (*gingivitis*), the tongue (*glossitis*) and the peridontal tissues (*peridontitis*). Although inflammatory lesions are found sporadically in untreated laboratory rodents, dogs and primates, stomatitis can be induced by systemic administration of high doses of therapeutic agents. Anticancer and antimitotic agents are particularly liable to induce stomatitis. A notable example is bleomycin that is capable of producing stomatitis as part of its general effect on squamous cells (Thompson et al., 1972). In humans, the adverse effects on therapeutic ionising radiation on the salivary glands may also give rise to inflammatory changes in the oral cavity (Fox, 1998)

Diuretics and other agents, which are capable of producing severe electrolyte disturbances and uraemia at excessive doses, can also produce stomatitis when then are administered in high doses to laboratory animals (Garthoff et al., 1982). These lesions may be analogous to the well-described association of ulcerative stomatitis and uraemia in man and laboratory animals (Boyd, 1978; Barker and van Dreumel, 1985). The dog appears very sensitive to the ulcerogenic effects of uraemia in the oral cavity, although as there is a poor correlation between actual levels of blood urea and stomatitis, other biochemical factors are undoubtedly involved.

*Pigmentation*

Compounds, which effect pigmentation of the skin, can produce similar changes in pigmented oral mucosa. A number of drugs including chlorpromazine, quinacrine, chloroquine, amodiaquine and pyrimethamine cause pigmentation of the oral mucosa in man notably over the hard palate. Chloroquine and pyrimethamine have also been shown to significantly increase numbers of active melano-

cytes within the palatal mucosa of pigmented DA rats when treated orally for 12 weeks (Savage et al., 1986). Melanocytes in treated rats were shown to be enlarged and packed with melanin pigment and to possess extensive arborisation 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 (Gracon et al., 1982; Walsh and Gough, 1989). 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 routine 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 (Dunn, 1967). Mice, especially DBA and DBA/2NCrj strains, are liable to develop calcification in the lingual muscle spontaneously, even at a young age (Imaoka et al., 1986). 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. In the DBA/2NCrj mice, mineralisation of the tongue is associated with myocardial and aortic mineralisation (Doi et al., 1985).

Therapeutic agents 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-derived rats, this agent produced myocyte necrosis and inflammation specifically located below the ventral subepithelial surface of the tongue (Westwood et al., 1996). 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 characterised by a uniform increase in the thickness of the squamous epithelium in both species (Breider et al., 1996; Reindel et al., 1996). At high doses, the squamous epithelium of the tongue of the primates was twice the thickness of control mucosa associated with elongation of rete pegs.

## 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 visualised 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 (Losco, 1995).

The rat incisor and its pathology has been the subject of an excellent review (Kuijpers et al., 1996). Unlike in humans, the rodent incisor continues to grow and differentiate throughout life and is renewed every 40–50 days. Located at the centre of the tooth is the vascular pulp. This is surrounded by proliferating ondotoblasts 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 xenobiotics, vitamin deficiencies, calcium, phosphate or magnesium deficiency, parathyroidectomy, hypophysectomy, hyperparathyroidism, adrenal insufficiency and fluorosis (Kuijpers et al., 1996).

Although in humans the mature dentition is no longer growing, 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 enamel hypoplasia and missing teeth (Welbury et al., 1984). 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 (MacLeod et al., 1987). It has also been shown that vincristine, a drug which interferes with the assembly of microtubules and reduces secretory activity in a number of cells including osteoblasts and chondroblasts, also effects dentine formation in the rat incisor (Stene and Koppang, 1976). Two weeks following a single intravenous dose of vincristine to young adult rats, a faint incremental line in the dentine was observed, probably a reflection of a direct effect of the drug on the dentinogenic tissue at the time of injection. At higher doses, focal niche-like or punched out defects in dentine were observed, expression of more severe injury to highly sensitive dentinogenic populations at the time of injection (Stene and Koppang, 1976). The precise mechanism of damage is not fully understood although decreased secretion of dentine matrix by odontoblasts has been demonstrated. Calcification appears unaltered (Stene and Koppang, 1980).

Administration of the alkylating anticancer agent cyclophosphamide or a sin-

gle exposure to ionising radiation, produces localised niche-like or punched out defects in the rat incisor, rather than the more diffuse changes induced by vincristine. This presumably reflects more localised injury to a sensitive subpopulation of dentinogenic cells (Koppang, 1973; Vahlsing et al., 1997).

Anticonvulsant drugs also produce changes in the dentition of man and experimental animals. In humans, 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 (Robinson et al., 1983). Tooth root alterations were also reported in a study in which young male Wistar rats were treated with diphenylhyantoin for 1 month. 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. Robinson and Harvey (1989) showed that the changes were similar to those occurring in parathyroidectomized rats but not those in rats made hypocalcaemic with a calcium deficient diet. They suggested that the changes induced by diphenylhydantoin in rats were similar to those in pseudohypoparathyroidism in which resistance of tooth roots to resorption is reduced.

Discoloration of teeth and bone is a well-described side effect of tetracycline administration and it has also been reported in patients treated with the semisynthetic derivative, minocycline (Cale et al., 1988).

Interestingly the ameloblastic epithelium of the enamel forming tissues of growing incisors in Wistar rats treated with high doses of human recombinant epidermal growth factor showed hyperplasia characterised by pseudostratification, increased nuclear-cytoplasmic ratio and increased cytoplasmic eosinophilia (Breider et al., 1996). This finding is consistent with the presence of epidermal growth factor receptors in the cells of the enamel organ (Martineau et al., 1991).

## Periodontitis

Periodontitis is a common and important disease in man and animals although overt cases are not usually seen in toxicity studies. However, periodontitis of a degree sufficient to disrupt chronic rat toxicity and carcinogenicity studies has been reported. Robinson (1985) described periodontitis in Alpk/AP rats in which there were erosive granulomatous cavities adjacent to molar teeth with fistulas opening into the nasal cavity. These changes were associated with penetrating food fibres in the gingival sulcus and it was suggested that the presence of long pointed food fibres in the powdered diet was the main reason for occurrence of periodontitis. Peridontitis in rodents also results from the effects of dental pathology such as fractures, malformation or malposition of incisors (Losco, 1995).

## 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 man, these changes have been associated with diphenylhydantoin (phenytoin) (Beghi et al., 1986) nifedipine, calcium channel blockers (Ledermann et al., 1984), cyclosporin A (Barthold, 1987) and valproic acid (Syrjamen and Syrjamen, 1979). Cyclosporin A, diphenylhydantoin and calcium channel blockers have been associated with similar changes in laboratory animals (Do'Nascimento et al., 1985; Latimer et al., 1986; Waner et al., 1988). In most instances there is swelling of the gingiva by firm nodular overgrowths around the teeth. Histologically, these overgrowths are characterised by marked acanthosis of the squamous epithelium overlying connective tissue that is infiltrated by large numbers of chronic inflammatory cells. Fibrovascular proliferation may be marked. In patients treated with cyclosporin, myxomatous degeneration is described in association with dense infiltration of plasma cells and lymphocytes (Barthold, 1987). Secondary acute inflammation in association with food debris and hair shafts is described in dogs treated with oxodipine (Waner et al., 1988).

The forces behind these changes are unclear. Studies of changed induced by nifedipine and hydantoin have shown increases in extracellular ground substance and increased numbers of fibroblasts containing sulphated acid mucopolysaccharides (Kantor and Hassel, 1983; Lucas et al., 1985). These drugs may alter fibroblastic proliferative and synthetic activity, possibly by selection of a subpopulation of fibroblasts (Hassel et al., 1976). 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 (Beghi et al., 1986). 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 (Barthold, 1987).

A spontaneous form of gingival hyperplasia has been described in non-human primates (*Macaca mulata*). This is characterised by an enlargement of the marginal and alveolar gingiva by connective tissue consisting of relatively poorly cellular bundles of collagen fibres. The lesions show little inflammatory alterations and the overlying squamous epithelium shows mild hyperkeratosis only (Schiødt et al., 1994). 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 (Odashima, 1979; Emminger and Mohr, 1982; Leiniger and Jokinen,

1994; Takahashi and Okamiya, 1996; Mohr, 1997), rabbits (Sundberg et al., 1985; Sundberg and Everitt, 1986), and beagle dogs (Watrach et al., 1970).

The microscopic structure of these neoplasms in rodents resembles those occurring in squamous epithelium in other sites. Although a number of agents induce squamous neoplasms in the oral cavity, spontaneous squamous carcinomas are generally uncommon spontaneous lesions in laboratory animals. However, some strains of rodent may develop squamous neoplasms more commonly. For instance, in life time 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 (Thurman et al., 1997). It was suggested that certain pedigrees possessed a genetic predisposition to these neoplasms.

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 (Howley et al., 1986).

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 a distribution limited to the ventral surface of the tongue (Sundberg et al., 1985). 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 (Watrach et al., 1970). Histologically, they are composed of proliferative masses of epithelial cells, keratinised on the surface and resting on an irregular connective tissue stroma or pedicule. Large vesicular cells with basophilic intranuclear inclusions are also found in the granular cell layer, identifiable as virus arrays by electron microscopy (Cheville and Olson, 1964). Malignant change has been described in these canine lesions and this can occur in young beagle dogs (Watrach et al., 1970).

Although many types of papilloma viruses have been identified in both man and animals (Pfister, 1984), common antigenic determinants exist between viruses in different species. This immunological cross-reactivity can be exploited in the immunocytochemical localisation 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 (Sundberg et al., 1984). 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 tumours are rare in rodents but they have been induced in laboratory animals given carcinogens such as nitrosoureas or exposed to ionising radiation (Gössner and Luz, 1994). A range of tumours originating from dental tissues with epithelial, mesenchymal or mixed appearances has been reported in rodents (Kuijpers et al., 1996). The classification of odontogenic tumours 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 ondontogenic epithelial cells within a fibrous stroma. The tumour 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 tumours 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. In the rat these have been classified as *odontoma* characterised by the presence of all dental hard tissues and *odontogenic fibroma* composed of undifferentiated or primitive mesenchymal cells of developing dental tissue (Mohr, 1997).

Odontogenic tumours 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 (Cullen et al., 1987). Occasional inclusions of solid epithelial nests were also seen. No metastatic deposits were found although the neoplasms were locally aggressive.

In addition, squamous tumours and neoplasms of mesenchymal origin typical of other organs, bones 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. Depending on the particular glands and cells affected, dry mouth, mucositis, and dental caries may develop (Stephens et al., 1986). The severe oral complications of irreversible salivary damage and dysfunction, which can occur 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 (Fox, 1998).

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 (Levine et al., 1987). The heterogeneity of salivary glycoproteins suggests that they act as a defence against pathogenic microorganisms by competing with microbial binding sites of similar structure on the surface of cells lining the digestive tract (Schulte, 1987). Minor salivary glands may also play an important part in the local immunosurveillance of the oral cavity for their ducts are anatomically closely associated with lymphoid tissue (Nair and Schroeder, 1986; Nair et al., 1987). 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 (Junqueira et al., 1973).

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 (Ingbar, 1985). It has been shown that thyroxine accelerates the differentiation of the granular convoluted tubule cells and the appearance of epidermal growth factor in the submandibular gland of the neonatal mouse (Chabot et al., 1987).

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. In dogs and other carnivores, the zygomatic (infra-orbital) 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 (see detailed discussion of this problem by Pinkstaff, 1980). 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

(Munhoz, 1971; Glucksmann and Cherry, 1973; Dawe, 1979; Pinkstaff, 1980; Emmiger and Mohr, 1982). Moreover, it has been demonstrated that salivary glands in rodents as well as a number of other species show morphological and histochemical sexual dimorphism (Pinkstaff, 1998). 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*. 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 kallikrinins (Barka, 1980; Mori et al., 1983). The precise physiological role of many of these peptides in salivary gland remains uncertain. Epidermal growth factor was originally isolated from the mouse salivary gland. It initiates premature eyelid opening and incisor eruption when injected into the neonatal mouse (Cohen, 1962).

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 $\alpha$-receptors and isoprenaline (isoproterenol), a $\beta$-adrenergic agent (Murphy et al., 1980). Immunohistochemical study also demonstrates that epidermal growth factor becomes depleted in mouse salivary tissue following administration of phenylephrine and similar agents (Tsukitani and Mori, 1987). 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 which participates both in membrane transport of bicarbonate ions into saliva and glandular secretion (Noda, 1986). Morphological studies have shown that both acinar and granular tubular cells participate in this response to adrenergic agents (Murphy et al., 1980). 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.

Glycoprotein secretion of rodent salivary glands has stimulated histochemical study using both conventional mucin histochemical techniques and labelled lectins which possess affinity for specific sugars or sugar sequences (Tables 1 and 2, pages 340 and 342). Studies of rat, mouse and hamster salivary glands using batteries of labelled lectins have shown a greater heterogeneity of oligosaccharides in salivary glands than seen by classical histochemical techniques.

There are considerable species differences and variations between murine strains and sexes of the same strain as well as heterogeneity among morphologically similar cells within one gland (Schulte and Spicer, 1983, 1984; Schulte, 1987). The results of histochemical studies are in excellent agreement with studies using biochemical methods but suggest a significant influence of genetic and hormonal factors on the synthesis of salivary glycoproteins.

## Dog salivary gland

Less attention has been paid to the structure and cytochemistry of the dog salivary glands. There appears to be little variation between the structure of salivary tissues between beagles and other strains although variation with age has been reported (Reifel and Travill, 1972; Nagoyo and Tandler, 1986). Munhoz (1971) has described the histochemical features of the dog parotid gland. The dog parotid is of seromucinous type secreting both acidic and neutral mucosubstances, in contrast to the more neutral mucosubstances secreted by rodent glands.

## Primate salivary glands

The salivary glands of non-human primates are similar to those in man. 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 ionising radiation in a similar manner to human salivary tissue (Stephens et al., 1986).

## Non-neoplastic lesions

### *Inflammation and necrosis*

Focal chronic inflammation of the salivary glands occurs sporadically in untreated rats, mice, hamsters, dogs or primates employed in toxicology although severity and prevalence is variable. Sialoadenitis as a result of a corona virus, the sialodacryoadenitis virus, is a well-known and fairly ubiquitous condition in rats, first described by Innes and Stanton (1961). The condition is characterised histologically by oedema and congestion of submandibular and parotid salivary glands as well as extra-orbital lachrymal and harderian glands. 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 (Fig. 44). The regenerative hyperplasia of the duct epithelium may be quite intense about a week after infection but all changes regress after about 2 weeks and glands are essentially normal after 3 or 4 weeks (Carthew and Slinger, 1981;

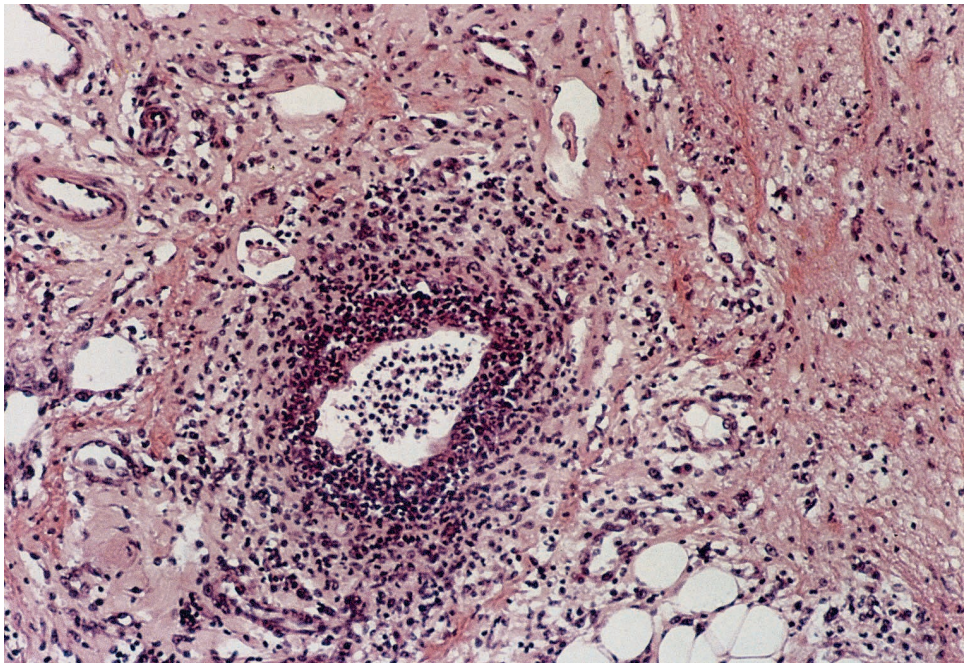

Fig. 44. Section from salivary tissue from a Sprague-Dawley rat during an infection with the sialodacryoadenitis virus showing intense ductular inflammation. (HE, ×25.)

Percy and Wojcinski, 1986). There may be a delay in the appearance of inflammatory cells and the onset of repair in rats immunosuppressed with cyclophosphamide (Hanna et al., 1984). Depletion of salivary gland epidermal growth factor also occurs during the infection (Percy et al., 1988).

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 (Arseculeratne et al., 1981).

Sialadenitis occurs spontaneously in autoimmune-prone strains of mice such as the NZB/NZW and SL/Ni strains and it has been reported in ageing female, but not male BDF1 mice (Hayashi et al., 1988). The non-obese diabetic mouse known for its spontaneous insulin-dependent diabetes mellitus also develops immune mediated damage to submandibular glands (Fujino-Kurihara et al., 1985; Törnwall et al., 1999). In ageing BDF1 females the submandibular gland was shown to be involved by a destructive inflammatory process characterised 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 (Thy-1.2 and Lyt-1 positive) of the helper/inducer subset (L3T4 or CD4 positive) and less than 10% were of suppresser/cytotoxic (Lyt-2 or CD8

positive) type (see Haemopoietic and Lymphatic Systems, Chapter III). Circulating anti-salivary 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 cytoxic T-cell subsets may directly destroy glandular tissue. It has been suggested that this process in ageing females is related to the decline in the number of splenic Lyt-2 cells in mice with advancing age (Hayashi et al., 1988). These cells are believe to be the most susceptible to ageing (see Haemopoietic and Lymphatic Systems, Chapter III)

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 insulitis is most marked. This suggests that there is an extension of the autoimmune process to salivary tissue (Fujino-Kurihara et al., 1985). 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-$\gamma$ inducible protein 10) and RANTES (regulated upon activation normal T cell expressed and secreted) may have an important role (Törnwall et al., 1999).

An autoimmune type of sialoadenitis can also be experimentally induced certain strains of mice. CRJ:CD1 mice, thymectomized at 3 days, a time point at which Lyt-2 positive cells (CD8 suppresser T lymphocytes) can be maximally reduced, followed by immunisation at 28 and 42 days after birth with homogenates of salivary gland and complete Freund's adjuvent, develop a distinctive sialoadenitis in the submandibular and to some extent the parotid glands (Hayashi et al., 1985). This sialoadenitis is characterised by degenerative changes in salivary glandular tissue associated with an extensive and intense infiltrate of small and medium sized lymphocytes. These cells appear shortly after immunisation but increase in number with time. Immunocytochemical study has shown that many of these cells are reactive to antisera to Thy-1.2 and Lyt-2 (CD8) features of suppresser/cytotoxic T lymphocytes. Later appearing cells demonstrate features of plasmacytoid lymphocytes and contain immunoglobulin of mainly IgG class (Hayashi et al., 1985). These authors therefore suggested the sialoadenitis appeared as both a result of cytotoxic/suppresser T-cell activity and an antibody-dependent cell-mediated cytotoxicity.

In the hamster salivary glands, interstitial infiltrates of lymphocytes and plasma cell are quite common and may become more marked with advancing age (McMartin, 1979).

Whereas necrosis of the parotid gland of uncertain aetiology sometimes occurs in the dog, mild focal chronic inflammation is quite a common incidental finding in canine salivary glands and has been reported in about 5% of normal beagle dogs (Kelly et al., 1982).

Although the inflammation in salivary tissue which results from ionising radiation is only indirectly relevant to drug safety evaluation, it is of interest in view of the notable species differences in sensitivity to this form of insult. Serous acinar cells in man and rhesus monkey appear least resistant to the effects

of ionising radiation, where damage is characterised by widespread degranulation and degeneration of acini, infiltration by polymorphonuclear cells followed by lymphocytes, plasma cells and subsequent atrophy and fibrosis (Stephens et al., 1986). These changes contrast with the lesser effects of ionisation radiation on the rodent salivary glands in which there is little or no acute inflammatory response.

*Lymphoid bodies*

Lymphoid bodies are sharply circumscribed collections of lymphoid cells generally located between the parotid and sublingual glands close to a cervical lymph node in mice. They are apparently normal aggregates of lymphoid tissue.

*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 man, the gland parenchyma frequently becomes atrophic and replaced by connective tissue or fat with advancing age, possibly partly related to vascular changes (Waterhouse et al., 1973; Scott, 1977). In ageing rats, the extent and height of granular ducts and their content of mature secretory granules has also been shown to decrease with age (Sashima, 1986).

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 (Boyd et al., 1970, McBride et al., 1987).

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 $\beta$-adrenergic blocking agent, propranolol (Smith and Butler, 1977). 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.

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 (Reuterving et al., 1987). It is probable that alloxan exerts a cytotoxic effect on the acinar cells of the rat submandibular gland (Sagström et al., 1987). 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 (McBride et al., 1987).

Ligation of the main excretory ducts has frequently been used as an ex-

perimental model for study of salivary gland atrophy as well as the regeneration that follows removal of the ligature. There is marked atrophy of all cell types but most markedly the acinar cells through 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 are relatively intolerant to a decrease in oxygen and nutrient (Denny et al., 1997).

*Weight increase, diffuse hypertrophy and hyperplasia*

A number of therapeutic agents increase salivary gland size in man, although the scarcity of biopsy data precludes a critical assessment of the precise mechanism in many cases. Drugs reported to produce salivary gland enlargement in man include iodide-containing radiological contrast media, isoprenaline and anti-inflammatory agents phenylbutazone and oxyphenbutazone. Enlargement may also occur after endotracheal anaesthesia and upper gastrointestinal tract endoscopy in man (Riddell, 1982). Some of these agents and procedures may produce spasm of large salivary ducts and retention of secretions.

Several pharmacological agents, particularly sympathomimetic amines, have been shown to produce increases in salivary gland size in rodents following repeated dosing (Brenner and Stanton, 1970). There is an intimate relationship of sympathomimetic amines with the control of the secretory process in salivary tissue. Whereas a single injection of isoprenaline (isoprotorenol) in the range of 20–200 mg/kg induces discharge of preformed secretory granules followed by gradual re-synthesis and reconstitution, repeated injections produces an increase in the size of salivary glands (Simson et al., 1974). Histologically, the enlarged glands are composed of secretory cells of increased size that contain increased amounts of secretory substances in the cytoplasm (Simson et al., 1974). Although these histological features are principally those of diffuse cellular hypertrophy, the increase in DNA content and radioactive thymidine uptake described in the salivary tissue following repeated administration of isoprenaline suggests that a degree of hyperplasia also occurs (Barka et al., 1972).

These effects do not depend on the integrity of the autonomic nerves because they occur after ablation of the autonomic ganglia (Barka et al., 1972). They appear to be mediated by an effect on adrenergic $\beta$-receptors. The effects can be blocked by propranalol, a $\beta$-receptor antagonist but not by phenoxybenzamine, an $\alpha$-receptor antagonist (Brenner and Stanton, 1970). As theophylline and caffeine also elicit salivary gland enlargement in rats, a role for cyclic 3',5-adenosine monophosphate (cAMP) in salivary gland enlargement has been postulated (Brenner and Stanton, 1970).

Detailed study of hypertrophy, protein synthesis, and intracellular cAMP activity in the salivary glands of rats treated for 10 days with isoprenaline (isoproterenol), a series of $\beta$-adrenergic receptor agonists and the phosphodiesterase inhibitors, theophylline and caffeine, showed that similar effects occurred with all agents although differences in the degree of hypertrophy, the nature of pro-

tein and glycoprotein synthesis and Golgi membrane enzyme activity were recorded (Wells and Humphreys-Beher, 1985). The parotoid gland showed the most pronounced hypertrophy followed by the submandibular gland but the sublingual gland appeared to be unaffected by treatment.

The degree and nature of the changes induced by the various $\beta 1/\beta 2$ receptor agonists suggested that most of these effects were mediated through $\beta 1$ receptors which are present in greatest numbers on the parotid and salivary cells. It was suggested that the effects of $\beta$-adrenergic agonists on salivary gland are produced by a receptor-mediated stimulation of adenylate cyclase activity causing an increase in levels of intracellular cAMP. However, other factors may be important for Wells and Humphreys-Beher (1985) also showed that although isoproterenol and caffeine increased salivary cell cAMP to comparable levels, the hypertrophy was greater with isoproterenol.

Cardioactive phosphodiesterase inhibitors were shown to produce submaxillary hypertrophy in rat subacute toxicity studies (Rogers et al., 1985; Jayasekara et al., 1986; Smith et al., 1988). Parotid and submaxillary glands were those most affected by the inotropic phosphodiesterase inhibitor ICI 153,110 (Westwood et al., 1991). As the agents produced their positive inotropic action via selective inhibition of the cardiac phosphodiesterase subfraction III specifically requiring cAMP as its substrate, it was suggested that the salivary gland hypertrophy was a result of phosphodiesterase inhibition (Smith et al., 1988).

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 characterised by a basophilic and coarsely granular or vacuolated cytoplasm (Jackson and Blackwell, 1988). The B6C3F1 mouse did not develop these changes after a similar treatment schedule.

In view of the presence of 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 (Breider et al., 1996; Reindel et al., 1996). However histological features seen are primarily those of ductular epithelial hyperplasia (see below under hyperplasia).

*Eosinophilic (oncocytic, oxyphil) cells, oncocytes*

Epithelial cells characterised by abundant granular eosinophilic cytoplasm as a result of the accumulation of mitochondria are often referred to as oncocytes, a term used by Hamperl (1950) to describe similar cells in Hürthle tumours of the thyroid gland. They may be found in various focal nodular and neoplastic states of the salivary glands in both man 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 mito-

chondrial changes represent an adaptive phenomenon or compensatory hyperplasia (Ghadially, 1982). 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 (Bogart, 1970) and in mice with experimentally induced autoallergic sialoadenitis (Takeda et al., 1985). In the study of Takeda et al. (1985) the eosinophilic cells appeared to arise predominantly in the secretory (glandular) ducts of the submandibular glands, although eosinophilic cells can apparently develop from either duct or acinar cells.

*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 (Chiu and Chen, 1986), and hamsters although their reported incidence varies between laboratory. 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 haematoxylin 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 characterised 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 (Chiu and Chen, 1986). Although they increase in prevalence with increasing age in certain strains of rat, there is no evidence to suggest that they represent pre-neoplastic lesions or possess any relationship with development of neoplasia in salivary tissue (Dawe, 1979).

*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, non-human primates and man 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 (Carthew and Slinger, 1981). It is also described specifically 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 (Kelly et al., 1979).

Detailed morphological examination with immunocytochemical study of epi-

dermal cytokeratins of the rat salivary gland after arterial ligation has shown that the acinar units can also undergo squamous metaplasia (Dardick et al., 1985). It appears that the acinar-intercalated duct complexes can rapidly reprogram to produce epidermal cytokeratin filaments in ischaemic or inflammatory states.

Hyperplasia of the ductular epithelium appears to be the principle 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 (Breider et al., 1996; Reindel et al., 1996). 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 and acinar cells showed hypertrophy with depletion secretory granules and the presence of large vesicular nuclei (Reindel et al., 1996).

Focal duct and acinar hyperplasia, showing minimal compression of the surrounding parenchyma and distinct from focal hypertrophy is also described in the classification of rat salivary lesions (Mohr, 1997).

### Neoplasia

Primary neoplasms of salivary glands are uncommon in the usual strain of rats and mice employed in carcinogenicity bioassays (Haseman et al., 1998). Acinar and tubular adenomas and adenocarcinomas as well as squamous carcinomas are reported in rats (Mohr, 1997), mice (Frith and Heath, 1994) and hamsters (Takahashi and Okamiya, 1996). Some carcinomas showing squamous or glandular differentiation may be observed infiltrating the salivary gland that originate in other local structures of the head and neck region. Occasionally, salivary gland neoplasms show adenomyomatous differentiation. Mixed glandular and lymphoid tissue patterns resembling Wartin's tumour in man are also sometimes seen. Neoplasms of soft tissues also develop in and around the major salivary gland in rodents (see Integumentary System Chapter I).

## OESOPHAGUS

In *humans* the oesophagus 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 (Bonavina et al., 1987). Severe damage can occur following prolonged contact between mucosa and ingested tablets or capsules which results in local high concentrations of potentially irritant substances (Bott and McCallum, 1986; Brors, 1987; Kikendall, 1999; Levine, 1999). Damage as a result of local contact may be more common in elderly subjects as the amplitude of oesophageal contractions decrease with age and capsules more liable to lodge in the lumen of the oesophagus (Bonavina et al.,

1987). 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 (Kikendall, 1999). The shape and surface coating of tablets may influence their tendency to adhere to the mucosa and lodge in the oesophagus (Marvola et al., 1983). A wide variety of drugs have been implicated. In the United States the majority of cases appear to be caused by ingestion of tetracycline or doxacycline (Levine, 1999). 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. Over recent years the bisphosphonate, alendronate has been one of the most commonly reported causes of adverse effects in the oesophagus with severe injury being reported. Although injury is linked to ingestion without water or failing to remain upright after swallowing the medication, alendronate is particularly caustic (Kikendall, 1999).

Oesophagitis due to *Candida albicans* is a well-described complication of antibiotic therapy. Administration of immunosuppressive drugs may predispose to viral infections in the oesophagus. A number of agents affecting neuromuscular co-ordination may also predispose to gastro-oesophageal regurgitation and reflux oesophagitis (Bott and McCallum, 1986).

In laboratory *rodents* spontaneous lesions of the oesophagus are occasionally seen. Oesophageal impaction has been described in untreated SrL:BHE rats. This is characterised by massive dilatation of the oesophagus with food or bedding (Ruben et al., 1983). Histologically, the muscle fibres in the wall of the oesophagus show varying degrees of degeneration including swelling or shrinking of fibres, myofibrillar fragmentation, cytoplasmic vacuolation and mineralisation. So-called megaoesophagus, characterised by enlargement of the oesophagus, degeneration of muscle fibres and ganglion cells in the myenteric plexus has also been described in certain strains of rats and mice (Harkness and Ferguson, 1979; Randelia and Lalitha, 1988). Its cause is unknown. A commonly occurring lesion reported in Fischer 344 rats is oesophageal hyperkeratosis, which occurs at all ages (Maeda et al., 1985). In the study by Maeda et al. (1985), it occurred more commonly in rats fed a protein-restricted, calorie unrestricted diet than in rats fed ad libitum with normal diet. It was suggested that the particular high prevalence of oesophageal hyperkeratosis observed in all groups in this particular study was related to acidification of drinking water (Maeda et al., 1985).

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

Spontaneous oesophageal lesions are uncommon in laboratory beagles, even though emesis and vomiting are frequent responses of this species following dosing in toxicity studies.

Local oesophageal irritancy potential of drugs has been assessed in a number of animal models, notably the cat and pig (Carlborg and Densert, 1980; Olovson et al., 1983). In these models, the test drugs are placed in the upper oesophagus using endoscopic techniques for 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 oesophagus. 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 oesophagus (Carlborg et al., 1983).

Systemic administration of drugs with radiomimetic or antimitotic activity can cause hypoplastic changes in the oesophageal mucosa as well as the remaining gastrointestinal tract mucosa (Tucker et al., 1983).

Conversely, hyperplasia with increased keratinization has been reported in the oesophagus of the rat following chronic high dose administration of alcohol (Mascrès et al., 1984). Acanthosis with hyperkeratosis and parakeratosis has been reported in the oesophagus but not stomach of rats treated for up to 18 months with mesuprine hydrochloride, a $\beta$-adrenergic receptor stimulator (Nelson et al., 1972). As part of its effects on the gastrointestinal tract, the oesophagus in rats and primates has been reported to develop uniform hyperplasia of the squamous epithelium following infusion of recombinant epidermal growth factor (Breider et al., 1996; Reindel et al., 1996; Vinter-Jensen, 1999).

## FORESTOMACH

In the rat, mouse and hamster the forestomach occupies about two-thirds of the proximal stomach area 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. 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 (Gärtner and Pfaff, 1979). Hence, the forestomach mucosa may be exposed to xenobiotics mixed in undigested food for far 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 oesophagus. However the squamous mucosa lining the oesophagus in species without a forestomach may react 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, strains of laboratory rodents as well as between different laboratories. The precise causes of forestomach ulceration remain 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 (Sawrey and Sawrey, 1966). In rats and mice dying of spontaneous disease, ulceration of the forestomach is also quite frequently observed. Protein restriction or starvation has also been shown to produce forestomach ulceration in rats (Boyd et al., 1970).

Ulceration of the forestomach in rodent toxicology studies may be incidental, particularly if the lesions are few and show no clear relationship to dose. If limited to high dose groups, ulceration may be a result of non-specific toxicity and stress-related. However, administered chemicals may have direct local effects of sufficient severity to cause focal damage to the forestomach mucosa.

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 characterised by loss of squamous epithelium with a variable accumulation of neutrophils, mononuclear cells, cellular debris, fibrin and hair fragments in the ulcer crater. The inflammatory process may extend deeply into the stomach wall and be associated with intramural inflammation, oedema, endarteritis and fibrosis. Haemosiderin pigment is also found in the ulcer margins. Profuse haemorrhage may follow erosion of large blood vessels and complete perforation of the stomach wall with peritoneal involvement also occurs (Greaves and Faccini, 1992). In long-standing cases of ulceration, hyperplasia of the adjoining squamous epithelium occurs, characterised by irregular acanthosis and down-growths of squamous epithelium into the submucosa (Yoshitomi et al., 1986).

Xenobiotics may produce inflammatory changes in the forestomach mucosa following initial dosing but subsequently, repair occurs even though treatment continues. An example of this phenomenon is illustrated by butylated hydroxyanisole. After 1 week of administration of this agent in a 2% mixture in diet to rats, a vesicular inflammatory reaction characterised histologically by the presence of subepithelial vesicles containing inflammatory cells and exudate was seen (Altmann et al., 1985). After further treatment, only hyperplasia of the squamous epithelium was evident, presumably as 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 localised 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 (Fig. 45). Such changes may be diffuse or focal, but they are often localised to the zone adjoining the glandular stomach mucosa. There may be evidence of basal cell proliferation and downgrowth of the epithelium into the underlying stroma.

Dietary factors also influence the thickness of the forestomach mucosa. Vitamin A deficiency, known to produce 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 3 months, hyperplasia with hyperkeratosis, not unlike that produced by known carcinogens was reported (Klein-Szanto et al., 1982).

Administration of a wide range of both industrial chemicals, therapeutic agents including both genotoxic and non-genotoxic carcinogens produces hyperkeratosis and hyperplasia of the forestomach epithelium which may be followed by preneoplastic lesions and squamous carcinoma (see below).

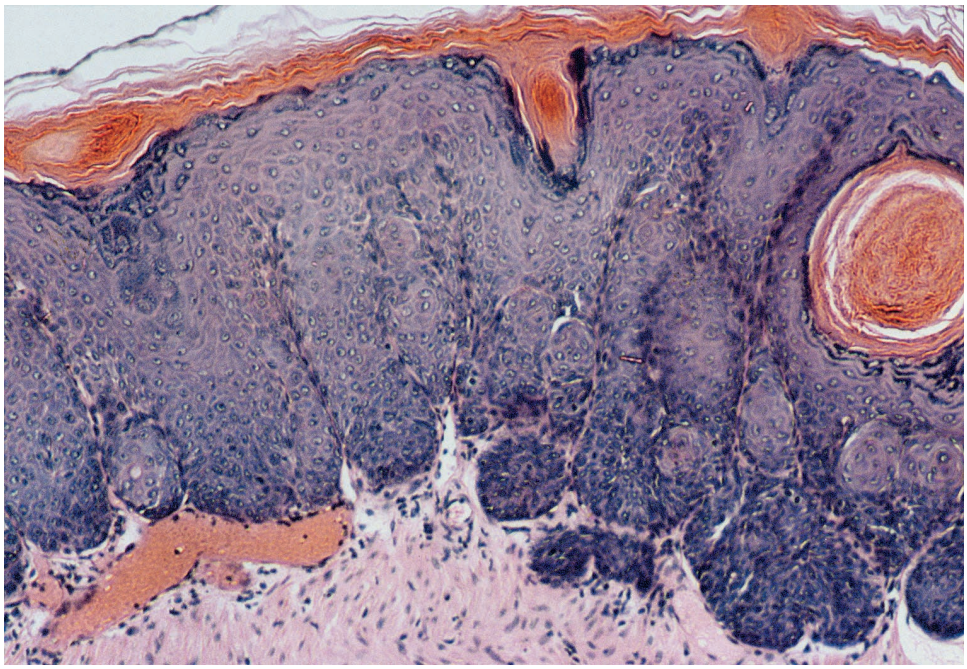

Fig. 45. A focal zone of hyperplasia with considerable acanthosis and hyperkeratosis with mild basal cell proliferation in the forestomach of an aged female Sprague-Dawley rat. (HE, ×25.)

Histologically, the changes are characterised by hyperkeratosis, parakeratosis with varying degrees of acanthosis and papillomatosis (Greaves and Faccini, 1992). 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 (Ghanayem et al., 1991). Hence, a critical feature may be the presence of cellular atypia in view of its association with agents with potent (genotoxic) carcinogenic activity.

## Neoplasia

Neoplasms arising in the forestomach of rodents are usually *squamous carcinomas* although basaloid features are also seen (Fukushima and Ito, 1985; Leiniger and Jokinen, 1994; Mohr, 1997; Tatematsu, 1997). 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 (Tatematsu 1997) as well as a range of non-genotoxic agents (see below). Some authors report basal cell carcinoma when basaloid features are pronounced (Tatematsu, 1997).

### *Extrapolation to man*

A wide range of agents is capable of producing squamous hyperplasia of the rodent forestomach and a number of these also induce squamous carcinomas. In 1986 Kroes and Wester reviewed over 60 genotoxic and non-genotoxic compounds that were reported to produce hyperplasia and carcinoma in the forestomach of rats, mice or hamsters.

A well-studied example is butylated hydroxyanisole (BHA) an important food antioxidant (reviewed by Whysner and Williams, 1996). Structurally related phenols and acids produce similar changes (Rodrigues et al., 1986). Ethyl acrylate, used in the production of materials for dental and medical devices is also capable of inducing marked squamous hyperplasia, papillomas and carcinomas after long-term treatment of F344 rats and B6C3F1 mice (NTP, 1986; Ghanayem et al., 1991). SK&F 93479, an experimental histamine $H_2$ receptor antagonist produced atypical forestomach hyperplasia in rats following administration by gavage for 1 year by a mechanism which appeared unrelated to the inhibition of the $H_2$ receptor (Betton and Salmon, 1984). Other therapeutic agents associated with squamous hyperplasia and neoplasia include the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (Kloss et al., 1991; Bueld et al., 1996; Akiba et al., 1998; Physicians' Desk Reference, 1999) and aristolochic acid (Göggelmann et al., 1982; Schmeiser et al., 1988).

Some cytoprotective prostaglandins appear capable of inducing hyperkeratosis and hyperplasia without neoplasia presumably through a mechanism related to their pharmacological activity (Levin, 1988). This occurs in rats treated with misprostol, a synthetic prostaglandin E1 methyl ester analogue with gastric anti-secretory and anti-ulcer activity (Kotsonis et al., 1985), CL115,574, a synthetic analogue of prostaglandin E1 type (Kramer et al., 1985) and 16,16-dimethyl prostaglandin E2 (Reinhart et al., 1983). Even the extensively used antibiotic, ampicillin has been associated inflammation, ulceration with acanthosis and hyperkeratosis in mice but not rats treated for 2 years (National Toxicology Program Technical Report, 1987). Sodium saccharin is also reported to produce hyperplasia without neoplasia of the forestomach in F344 rats (Hibino et al., 1985).

Among its wide range of pharmacological effects on the gastrointestinal tract, the forestomach has also responds to recombinant epidermal growth factor when infused into rats (Breider et al., 1996; Vinter-Jensen, 1999). Histological examination showed hyperkeratosis and hyperplasia of the squamous epithelium.

The large body of studies performed with butylated hydroxyanisole illustrates the various factors that can influence the development of treatment-induced hyperplasia and neoplasia of the rodent forestomach and subsequent assessment of human risk. Butylated hydroxyanisole possesses little or no mutagenic activity in vitro but when administered to rats for 2 years as a 2% mixture in the diet, it produced squamous hyperplasia, squamous papillomas and squamous carcinomas of the forestomach. At 0.5% in the diet butylated hydroxyanisole induced only hyperplasia (Ito et al., 1983). It also produces proliferative lesions in the forestomach of both mouse and hamster (Ito et al., 1986).

Studies in which butylated hydroxyanisole was fed in the diet to rats for shorter periods have shown that squamous epithelial hyperplasia occurs after only 1 week of treatment preferentially over the lesser curvature, the site at which carcinomas developed in the 2-year studies (Altmann et al., 1985). After 13 weeks' treatment, mucosal hyperplasia characterised 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 (Iverson et al., 1985). Abundant mitoses were found in the basal cells layers and tritiated-thymidine labelling confirmed that the changes were accompanied by a high rate of cell proliferation. Following cessation of administration of butylated hydroxyanisole after 13 weeks, the tritiated-thymidine labelling index rapidly reverted to control levels within about 1 week although hyperplasia took longer to regress. Nearly complete regression of the hyperplasia occurred after about 9 weeks of normal diet (Iverson et al., 1985).

The distribution of squamous hyperplasia induced in the rodent stomach by butylated hydroxyanisole is influenced by the mode of administration. Whereas following feeding of rats with butylated hydroxyanisole mixed in the diet lesions tended to be located near the limiting ridge, Altmann et al. (1985) showed that gavage of butylated hydroxyanisole in corn oil produced similar changes at the apex of the forestomach. It was suggested that this difference was 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 (Altmann et al., 1985). More recently, it was 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 was the SHR strain followed by the F334 rats and the differences correlated with the cytotoxic effects of butylated hydroxyanisole in the different strains (Tamano et al., 1998). It was suggested that the presence of vascular damage in the stomachs of the SHR rats might have contributed 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 oesophagus of animals without a forestomach, Iverson et al. (1985) have shown that high-doses given to primates are capable of producing an increase in mitotic activity in the lower end of the oesophagus similar to that occurring at equivalent exposure levels in the rat. 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 man need to be carefully assessed when hyperplastic changes are induced in the forestomach mucosa of rodents. Such information can be helpful in facilitating regulatory decisions in this area (Moch, 1988).

On balance, the evidence suggests that the tumour development by butylated hydroxyanisole in rodents represents an epigenetic phenomenon related to largely reversible cytotoxicity and increased cell proliferation (Whysner and Williams, 1996). 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 probably very small.

Similar phenomena have also been reported in studies of phenols and acids that are structurally related to butylated hydroxyanisole (Rodrigues et al., 1986). 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 (Rodrigues et al., 1986).

A number of HMG-CoA reductase inhibitors 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. The hyperplasia is time and dose dependent and may be associated with oedema and some 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 (Kloss et al., 1991; Bueld et al., 1996; Akiba et al., 1998; Physicians' Desk Reference,

1999). The mechanism of action remains unclear although the degree of hyperplasia seems related to pharmacological potency. Their carcinogenic potential in rodent bioassays does not seem to relate to the degree of hyperplasia in short-term studies. Moreover, the development of hyperplasia depends on local high concentrations of drug because when administered by non-oral routes, hyperplasia does not occur (Kloss et al., 1991). 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.

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 (Göggelmann et al., 1982; Schmeiser et al., 1988). Aristolochic acid 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 3 months. This was characterised 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, 3 or 6 months later without further treatment (Mengs et al., 1982). Even at a low dose of 0.1 mg/kg/day papillomas and squamous carcinomas developed 9 months after a 3-month period of treatment.

Quite clearly the complexity of the hyperplastic response of the rodent stomach to xenobiotics, the association of hyperplasia induced by non-mutagenic compounds with the development of forestomach carcinomas, and the similarity of response in the forestomach to that of the oesophagus, dictates the need for a careful analysis of hyperplasia induced by novel drugs in the forestomach. The prelude to this assessment is careful histopathological characterisation of the changes.

## STOMACH (GLANDULAR)

Unlike the mouth and oesophagus 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. Administration of compounds in liquid or solid form, particle size, fasting and feeding all affect the gastric motility pattern. In the fasted state there is a cyclical pattern of motility consisting of three main phases. The first is a quiescent phase, 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 man and appear to be qualitatively similar in the two species (Sarna, 1985). 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 (Dressman, 1986).

Gastric acid is also important in making ingested salts soluble. Although the presence of food in the stomach is a stimulus of acid production, the pH in the forestomach of rats is highest in full stomachs and lowest when empty, presumably as a consequence of the buffering action of food (Ward and Coates, 1987).

## Epithelial morphology and physiology

The glandular stomach is conveniently divided into the fundus characterised 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.

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 haematoxylin 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 visualised by immunocytochemical staining with antibodies directed at $H^+K^+$-ATPase (Canfield et al., 1996).

The gastric glands situated near the limiting ridge in rodents, show a modified 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 characterised. Enterochromaffin cells are quite numerous in the basal parts of the gastric glands of the fundus, particularly in the rat (Håkanson et al., 1986). They are generally argyrophilic, staining with silver staining techniques such as that of Grimelius (Grimelius, 1968; Grimelius and Willander, 1980) that utilise exogenous reducing agents. These cells contain histamine and histamine-related enzymes such as histidine decarboxylase in the rat and other species (Håkanson et al., 1986). Endocrine cells which are argentaffin in type stain with silver stains such as that of Masson (1914) because of the presence of endogenous reducing substances including 5-hydroxytryptamine and catechol-

amines are also reported in the mucosa of the fundus of some species including man but apparently not in the rat (Håkanson et al., 1986). Enterochromaffin cells are characterised ultrastructurally by the presence of numerous rounded or oval, vesicular, electron-lucent granules frequently containing a small eccentric electron dense core.

Gastric enterochromaffin cells can also be stained by immunocytochemical techniques using antisera to histamine and histidine decarboxylase as well as to non-specific enolase and chromogranin A (Watanabe et al., 1984; Sundler et al., 1986; Betton et al., 1988). Immunocytochemical study of the rat fundus using a battery of antisera to a variety of gastrointestinal peptides has shown somatostatin containing (D) cells and glucagon staining cells but no cells with gastrin (G cells) or serotonin reactivity (Bishop et al., 1986). Gastrin or G cells possess apical processes reaching the stomach lumen believed to be important in stimulation of gastrin release as a result in increases in antral lumen pH or the presence of amino acids or peptides (Håkanson et al., 1986). Glucagon and serotonin containing endocrine cells have also been located in the rat antral mucosa (Bishop et al., 1986).

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 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 (Dockray, 1999). 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 (Hinkle and Samuelson, 1999). Gastrin stimulates release of histamine from enterochromaffin cells of the body mucosa, which increases acid secretion through activation of parietal cell histamine-$H_2$ 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 noradrenaline, vasoactive intestinal peptide, calcitonin gene-related peptide, and cholecystokinin. It should also be noted that gastrin has a role in kinetics and differentiation of both parietal and enterochromaffin cells (Dockray, 1999).

Gastrin acts at the gastrin/cholecystokin-B receptor that is expressed by gastric epithelial cells and by neurones in the central nervous system (Kopin et al., 1992; Wank, 1995). The gastrin receptor is simply the cholecystokinin-B receptor located in the stomach. The other cholecystokinin receptor, cholecystokin-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. In the central nervous system cholecystokinin and its receptors contribute to the regulation of satiety, anxiety, analgesia and dopamine-related behaviour (Wank, 1995).

Finally, it is worth recording that both progesterone and oestrogen receptors have been identified in both normal and pathological gastric tissues of humans (Wu et al., 1992).

## Kinetics of the gastric mucosa

Generative cells in the gastric mucosa as shown by uptake of tritiated thymidine for DNA synthesis are distributed principally in the isthmus (Inokuchi et al., 1983). Tracing of cells using thymidine labelling 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 life span of surface epithelium in the stomach of rats, mice and hamsters has been calculated to be about 3–4 days. Studies of cell cycle and DNA synthesis time in the proliferative zones in the stomach of rat, hamster and man have suggested that the generative cells in the isthmus undergo mitoses at about 30-hour intervals in rodents and 40-hour intervals in man (Inokuchi et al., 1983).

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 show that undifferentiated cells in the region of the isthmus represent a common source for surface mucous cells and mucous neck cells (Karam and Leblond, 1995). Electron microscopic and ultrastructural cytochemistry has suggested

TABLE 1 *Lections of use in histochemistry**

| Lectin | Inhibitory saccharide |
|---|---|
| *Arachis hypogaea* (peanut) PNA | β-D-Gal-(1-3)-D-GalNAc |
| *Ricinus communis* (castor bean) RCA1 | β-D-Gal > α-D-Gal |
| *Bandeirea simplicifolia* | |
| BSA 1-B | α-D-Gal |
| BSA II4 | β-D-G1cNAc = α-D-GlcNac |
| *Dolichos biflorus* (horse gram) DBA | α-D-GalNAc |
| *Glycine max* (soybean) SBA | α-D-GalNAc > β-D-GalNac >> D-gal |
| *Lotus tetragonolobus* (asparagus pea) LTA | α-L-Fuc |
| *Ulex europeus* (gorse seed) UEA 1 | α-L-Fuc |
| *Anguilla anguilla* (AAA) | α-L-Fuc |
| *Lens culinaris* (lentil) LCA | α-D-Man > α-D-Glc > α-D-GlcNAc |
| *Canavalia ensiformis* (jackbean) Con A | α-D-Man, α-D-Glc |
| *Tritium vulgaris* (wheat germ) WGA | $(D\text{-GlcNAc})_2$, sialic acid |
| *Limulus polyphemus* (horseshoe crab) LPA | sialic acid |

*Key*: Gal = galactose; Glc = D-glucose; Man = D-mannose; Fuc = L-fucose; GalNAc = *N*-acetyl-D-galactosamine; GlcNAc = *N*-acetyl-D-glucosamine; sialic acid = *N*-acetylneuraminic acid.
*Note*: Saccharide binding specifications are much more complex than the inhibition by simpler sugars outlined above suggests. See review by Nicholson (1974).
**Source*: Nicholson, 1974; Goldstein and Hayes, 1978; Schulte and Spicer, 1983; Giannasca et al., 1994.

that chief cells in the adult rat stomach develop from undifferentiated stem cells in the isthmus (Suzuki et al., 1983). Graft experiments in mice have also suggested that immature cells of the isthmus differentiate into chief cells as well as parietal cells (Matsuyama and Suzuki, 1970). 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 (Li et al., 1996; Canfield et al., 1996). Gastrin is also an important regulator of parietal cell and enterochromaffin differentiation and number (Dockray, 1999; Montgomery et al., 1999).

Labelling experiments in the hamster stomach have shown that both chief and parietal cells possess a similar but quite long life span of about 200 days (Hattori, 1974; Hattori and Fujita, 1976). 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' (Inokuchi et al., 1983).

The origin and kinetics of endocrine cells of the stomach has also been the subject of debate but the available morphological, cytochemical and kinetic evidence suggests that the majority of these cells develop from the same stem cells as the other non-endocrine cells of the gastric mucosa, although self replication also occurs (Matsuyama and Suzuki, 1970; Inokuchi et al., 1983; Solcia et al., 1986).

## Mucin histochemistry

Much of our knowledge about mucins produced by the epithelial cells lining the gastrointestinal tract has been obtained using histochemical techniques and these approaches continue to be helpful in the understanding of spontaneous and drug-induced gastrointestinal disease (Sheahan and Jarvis, 1978; Filipe, 1979; Tsiftsis et al., 1980; Jass and Roberton, 1994). For these reasons, mucin histochemical techniques represent useful tools for the characterisation and elucidation of experimentally or drug-induced changes in the glandular mucosa of gastrointestinal tract. Techniques commonly employed are presented in Table 2.

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 (Berger et al., 1982). The principle monosaccharides present are fucose, galactose, N- acetylgalactosamine, N-acetylglucosamine and sialic acid. Traces of mannose may be present and ester sulphate residues are common (Filipe, 1979). 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

TABLE 2 *Histochemical methods for the visualisation of epithelial mucins in the gastrointestinal tract*

| | | |
|---|---|---|
| Diastase-Periodic Acid Schiff D-PAS (Pearse, 1968) | *Magenta:* | All mucosubstances containing hexoses and deoxyhexoses with vicinal glycol groups. Some non-sulphated acid mucosubstances. Neutral mucosubstances. |
| Periodate-borohydride/ saponification/PAS, PB/KOH/PAS (Reid et al., 1973; Culling et al., 1974; 1976) | *Magenta:* | PAS activity following periodate borohydride/potassium hydroxide indicates presence of O-acylated sialic acids. Periodate borohydride reduces periodate generated aldehydes. Potassium hydroxide removes O-acylesters from potential vicinoldiols and sialic residues linked glycosidically to a potential vicinoldiol. |
| Alcian blue pH 2.5 (Pearse, 1968) | *Basophilia:* | Weakly sulphated mucins. Carboxyl groups of sialomucins |
| Alcian blue pH 1.0 (Lev & Spicer, 1964) | *Basophilia:* | Sulphated mucins |
| Alcian blue pH 2.5 – periodic acid Schiff, AB/PAS (Mowry & Morard, 1957) | *Magenta:*<br>*Basophilia:*<br>*Purple-blue:* | Neutral mucins<br>Acid mucins<br>Neutral and periodate reactive acid mucins |
| High iron-diamine, HID (Spicer, 1965) | *Brown-black:*<br>*Unstained:* | Sulphated mucins<br>Sialomucins |
| High iron-diamine-alcian blue pH 2.5, HID/AB (Spicer, 1965) | *Brown-black:*<br>*Basophilia:* | Sulphated mucins<br>Non-sulphated acid mucins (sialomucins) |

*Source*: Adapted from Filipe, 1979.

may become the predominant factor (van Klinken et al., 1995). Although mucins are important in the gastrointestinal tract, it should be remembered that other products secreted by goblet cells might be important in mucosal defence. It has been recently recognised that trefoil proteins, a family of small proteins secreted by goblet cells and present on the mucosal cell surface, can also protect against a variety of deleterious agents, including bacteria, toxins and drugs (Podolsky, 1999).

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 certain drugs and chemicals (Ishihara et al., 1984).

Terminal sugars or sugar sequences can be demonstrated histochemically by the use of labelled lectins, mostly plant proteins which combine non-enzymatically with particular sugar molecules, see Table 1 (Goldstein and Hayes, 1978; Debray et al., 1981; Rudiger, 1982).

## Stomach mucins

When gastrointestinal mucins were studied in several species using histochemical techniques under uniform conditions, species differences were most obvious in the stomach and duodenum (Sheahan and Jarvis, 1976). Neutral mucins generally predominate in the stomach, contrasting with acid mucins in the small intestine, and sulphated 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. Sulphated mucins, as shown by the high iron diamine technique (HID) are also found in the deep glandular mucosa of the antrum in rat, mouse and man (Filipe, 1979; Jass, 1980; Greaves and Boiziau, 1984). Extremely heterologous staining patterns are seen in the gastric mucosa with labelled lectins, each lectin staining quite different cell populations. There are considerable interspecies differences in staining patterns with the same lectins (Kuhlmann et al., 1983; Suganuma et al., 1984). The so-called paradoxical Concanavalin A stain, in which conjugated *Concanavalin A* is used to label mucins before and after periodate oxidation, has also been used to classify the alterations in mucins in proliferative and neoplastic conditions of the rat stomach mucosa (Kobayasi et al., 1991; Tatematsu 1997).

## Non-neoplastic lesions

### *Inflammation, erosions and ulceration of the gastric glandular mucosa*

Although gastric erosions and ulcers in the glandular mucosa occur quite commonly in laboratory animals in toxicity studies, it is often difficult to determine whether such lesions in treated animals indicate a real ulcerogenic risk for the test compound. There is little that is histologically specific to drug-induced ulceration of the gastric glandular mucosa. Mucosal haemorrhage, depletion of mucin, erosions and ulcers with or without inflammation may all be found. *Erosions* represent mucosal breaks superficial to the muscularis mucosa. *Ulcers* are lesions that extend through the muscularis mucosa. Whilst 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).

In humans, biopsy data suggests that drug-induced ulceration is characteristically devoid of an inflammatory component, but the most usual histological appearances are those of underlying gastric pathology (Riddell, 1982). Formation of gastric and duodenal ulcers is dependent on the presence of both acid and peptic activity in gastric juice because acid without pepsin appears to have little digestive power. Important predisposing factors in human patients with peptic ulceration include *Helicobacter pylori* (*Campylobacter pylori)* infection of the

antrum, cigarette smoking and ingestion of non-steroidal anti-inflammatory drugs (Soll, 1990). *Helicobacter pylori* is believed to infect over half the human population and its presence in the gastric mucosa is associated with chronic atrophic gastritis and peptic ulceration (Cover and Blaser, 1996). It is a microaerophilic, 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 is related to increases in gastrin release, perhaps through bacterial products or cytokines released from activated lymphocytes (Richter-Dahlfors et al., 1998). Although *Helicobacter pylori* can infect other species, apart from non-human primates, the usual laboratory animal models do not appear to develop the inflammatory disease seen in humans (Nedrud, 1999).

Erosions and ulcers also develop 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. This may produce damaging high local concentrations on the mucosa not relevant to therapeutic doses used in clinical practice. It has been demonstrated that hyperosmolar solutions of quite innocuous substances such as glucose can cause haemorrhage, erosions and ulcers of the rat gastric mucosa (Puurunen et al., 1980). The well-known association of gastric erosions and haemorrhage with uraemia may also be manifest following administration of high doses of drugs such as diuretics which severely derange fluid and electrolyte balance (Garthoff et al., 1982). Stress ulceration may be linked to temporary ischaemia of the mucosa (Dubois, 1987). Synergism between the ulcerogenic action of drugs and stress is a well-described phenomenon (Rainsford, 1975; Beattie, 1977). Protein depletion and starvation is also capable of inducing gastric ulceration in rats (Boyd et al., 1970).

Although gastric pathology represents the largest cause of morbidity and mortality in man following therapy with non-steroidal inflammatory agents (Fowler, 1987), the reasons for this are probably multifactorial. The acidic properties of some of these drugs may cause direct local damage of gastric epithelial cells, demonstrable by the fact that appropriate formulation can reduce gastric toxicity of these agents in man (Brors, 1987). Anti-inflammatory agents are capable of decreasing synthesis of glycoproteins and this may adversely influence protective mucus production of gastric mucosa (Azuumi et al., 1980; Ishihara et al., 1984).

It has also been suggested that non-steroidal anti-inflammatory agents cause cellular damage to the gastric mucosa by back-diffusion of gastric acid into mucosal tissues (Davenport, 1964) or by causing damage to the gastric capillary bed with subsequent mucosal infarction (Robins, 1980).

The theory that has gained widespread acceptance is that the ulcerogenic potential of non-steroidal anti-inflammatory drugs is related to their pharma-

cological activity. Vane (1971) proposed that the ulcerogenic potential of these agents was largely a result of their ability to inhibit prostaglandin synthetase, thereby reducing the protective effects of prostaglandins.

Pharmacokinetic factors may also be important. Lipid solubility in the low pH environment of the stomach may influence local penetration into the mucosa (McCormack and Brune, 1987). Moreover, it has been proposed that certain anti-inflammatory agents may possess lesser ulcerogenic potential in man because their inhibition of prostaglandin production is more limited to sites of inflammation, sparing gastric mucosa (Whittle et al., 1980; Whittle and Vane, 1984).

It has also been demonstrated that factors altering the enterohepatic circulation of drugs can influence the expression of gastric damage (Overvold et al., 1987). Comparative studies of the ulcerogenic activity of indomethacin in beagle dogs and domestic pigs has suggested that the dog may be an excessively sensitive species as a result of extensive enterohepatic circulation of indomethacin in this species (Hanhijarvi et al., 1986).

Prediction of ulcerogenic potential for man based on data from animal models is clouded by the lack of good comparative data on the relative ulcerogenic potential of non-steroidal anti-inflammatory agents in man because of extensive differences in side effect reporting (Fowler, 1987). Moreover proper comparisons in man require not only equivalent therapeutic doses but also comparable dosage forms (Brors, 1987).

In laboratory animals, a variety of different patterns of drug-induced gastric damage have been described. The study by Shriver et al. (1975) in which a wide variety of different anti-inflammatory drugs were 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. Immunological agents such as azathiaprine, cyclophosphamide, methotrexate and d-penicillamine produced gastric mucosal haemorrhage whereas aspirin and related agents produced gastric mucosal haemorrhage and ulcers. The powerful non-steroidal anti-inflammatory drugs indomethacin and phenybutazone produced gastric mucosal erosion and ulcers as well as small intestinal damage.

Comparative single oral dose studies of several different non-steroidal anti-inflammatory agents at three different dose levels by Suwa et al. (1987) in the rat using histology and measurement of faecal blood loss with $^{51}$Cr-labelled blood cells have also shown that different patterns of ulceration can be produced by different agents when administered under identical conditions. 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 6 hours following dosing which completely healed 2 weeks later. This damage was associated with transient faecal 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 2 weeks and were associated with prolonged or biphasic blood loss (see Small Intestine).

In addition feeding conditions can 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 whilst in conventionally fed rats they are most prominent in the small intestine. Detailed studies by Satoh et al. (1981) showed that rats fed for 1 hour after a 24 hour-fast and given a single dose of indomethacin within 2 hours of re-feeding developed erosions and ulcers in the antrum primarily along the lesser curvature. Indomethacin given to fasted rats produced erosions in the body mucosa.

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 4 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 though increasing the rate of epithelial exfoliation (Eastwood and Quimby, 1982). Such a response may be the basis for increased resistance of the gastric mucosa to the chronic affects 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.

Both interspecies variations and strain differences have been reported in the response to ulcerogenic compounds. Rainsford et al. (1982) showed that extravasation of red blood cells and greater vascular damage was observed in rats treated with aspirin or benoxprofen than in pigs given similar doses. Sprague-Dawley rats appear less susceptible to the ulcerogenic effects of cold-restraint stress than Wistar rats (Goldenberg, 1973).

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 (Fig. 46) (Imai et al., 1981; Garthoff et al., 1982). 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 haemorrhage with uraemia. Dogs appear to have a particular predisposition to this effect where it may be associated with deposition of basophilic ground substance and mineral in connective tissues and blood vessels in the mucosa (Barker and van Dreumel, 1985).

Although inflammatory conditions due to microorganisms are generally uncommon in the stomach, gastritis is reported in laboratory rhesus monkeys in association with the presence of Helicobacter organisms (Reed and Berridge, 1988). As in the analogous condition in man, the stomach of affected animals shows an infiltration of the central mucosa by small lymphocytes and plasma cells, associated with reactive or atrophic changes in the mucosa and the presence of small curved bacteria in glands, visualised best with the Warthin–Starry stain.

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 (Anderson and Hayes, 1989).

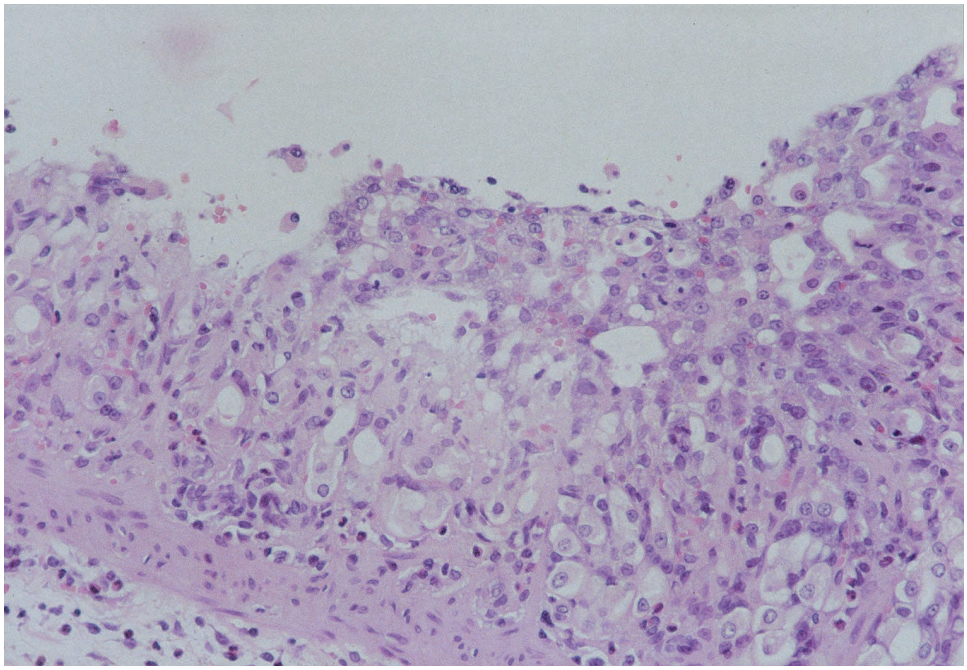

Fig. 46. Section from the glandular stomach from a rat treated with ah high dose of an angiotensin II antagonist that shows superficial degeneration and ulceration (erosion) of the mucosa. (HE, ×30.)

## Mucus depletion

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

Qualitative changes in mucus composition can also accompany mucus depletion. Gastric epithelium in man may show decreases in sulphated mucosubstances following stress, high alcohol consumption or after aspirin administration (Filipe, 1979). Similar changes occur in laboratory animals subjected to ulcerogenic regimens. Stress ulceration in the rat is accompanied by decreased sulphation of gastric glycoproteins, presumably an expression of the changes in gastric cellular activity accompanying stress (Lambert et al., 1969). Administration of aspirin and other anti-inflammatory agents including adrenocortical steroids to laboratory animals also reduces the content of sulphomucins in the gastric mucosa, probably by reducing their synthesis (Denko, 1958; 1964; Gerard, 1965; Ishihara et al., 1984).

Rather surprisingly, administration of histamine $H_2$-receptor antagonists and

proton pump inhibitors associated with reduction of 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 4 weeks was shown to inhibit prostaglandins $PGE_2$ as well as the synthesis of both total and sulphated glycoprotein synthesis along with histochemical evidence of reduction in PAS staining of the surface mucus (Yoshimura et al., 1996). Although the mechanism for this change is unclear, the reduction in mucus, particularly sulphated mucus that is believed to be particularly resistant to peptic digestion, may have implications for mucosal defence.

## Intestinal metaplasia

Intestinal metaplasia of the stomach is characterised by the presence of differentiated epithelium, which resembles small intestine on the basis of light microscopic and ultrastructural morphology, mucin patterns and enzyme histochemistry (Morson, 1955; Planteydt and Willighagen, 1960; Lev, 1966; Goldman and Ming, 1968; Watanabe et al., 1984). It develops in man in gastric mucosa altered by chronic atrophic gastritis and its significance is due to the fact that a link exists between intestinal metaplasia and gastric cancer. Although intestinal metaplasia is found much less commonly in laboratory animals, it has also been reported to occur in association with gastric cancer induced by polychlorinated biphenyls (Ward, 1985). 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 man, several forms of intestinal metaplasia have been described. These variants fall into two main groups, an incomplete type and a complete form (Teglbjaerg and Nielson, 1978; Jass and Filipe, 1979; Jass, 1980). Complete intestinal metaplasia is characterised by the presence of goblet cells, Paneth cells and absorptive cells with brush borders and variably developed intestinal villi. Incomplete forms are more heterogeneous characterised by goblet and mucous columnar cells but no absorptive cells and variable patterns of mucin. The routine alcian blue: pH 2.5, periodic acid-Schiff stain (AB/PAS) (Table 2) distinguishes between the intestinal acid mucins (blue) from the neutral mucins of gastric type. However, variable sialomucin and sulphomucin staining patterns are seen in intestinal metaplasia in man with the high iron-diamine/alcian blue stain (HID/AB) (Jass, 1980). The incomplete form of intestinal metaplasia, showing marked sulphomucin secretion, has been found more commonly in association with gastric cancer in man (Jass and Filipe, 1979; Jass, 1980; Wells et al., 1982). However, prospective studies have tended to indicate that intestinal metaplasia with sulphomucin secretion may be an age-related form of chronic atrophic gastritis and not a premalignant lesion (Ectors and Dixon, 1986). It has been suggested that intestinal metaplasia represents an adaptive response to long-

standing chronic inflammation and reduced acid secretion. It may also represent an adaptive defensive response to long-standing *Helicobacter pylori* infection because intestinal mucosa is more resistant to these organisms (Steer, 1984; Ectors and Dixon, 1986).

Intestinal metaplasia has been found in association with gastric cancer in laboratory animals. Fischer 344 rats treated with the polychlorinated biphenyl, Aroclor 1254, mixed in the diet for 2 years developed foci of intestinal metaplasia in the stomach epithelium in association with gastric adenocarcinomas (Ward, 1985). These lesions were characterised by abundant mucin-containing cells and alkaline phosphatase activity typical of the small intestine (Morgan et al., 1981; Ward, 1985). Similar, but more diffuse intestinal metaplasia was reported in the stomach of primates treated with polychlorinated biphenyls, although unassociated with gastric neoplasia (Allen, 1975; McConnell et al., 1979). Intestinal metaplasia is also found in the stomach of laboratory animals treated with powerful genotoxic gastric carcinogens. Although Tsiftsis et al. (1980) showed hyperplasia and foci of atypical changes (dysplasia) but little or no intestinal metaplasia in rats following administration of *N*-methyl-*N´*-nitro-*N*-nitroguanidine, Tatematsu et al. (1983) were able to show intestinal metaplasia in rats treated with the same agent. However, 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, localised, ionising radiation (Watanabe, 1978; Watanabe et al., 1980), injection of xenogenic stomach antigens (Watanabe et al., 1984) as well as propantheline bromide and the non-carcinogen, iodoacetamine (Watanabe et al., 1984; Shirai et al., 1985).

The characteristics of intestinal metaplasia in laboratory rodents are similar to those seen in man with early increases in intestinal enzyme activity (alkaline phosphatase, lactase, trehalase, sucrose and maltase), development of goblet cells containing neutral, sialo- , or sulphomucins, and intestinal crypts with or without Paneth cells. Both the fundus and antrum can show changes although as in man, males appear more prone to develop intestinal metaplasia than females (Watanabe et al., 1984).

Based on these experimental findings, Watanabe et al. (1984) have also 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.

On balance therefore, the evidence to date 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.

Finally, 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 2-year carcinogenicity bioassays (Leiniger et al., 1990). Histologically, 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 mineralisation with herniation into the submusosa. Whilst these foci were not believed to be related to treatment with xenobiotics, it is not clear whether represented metaplasia or congenital ectopia.

## Mineralisation

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 haematoxylin-stained sections from the stomachs of aged rats where they are associated with cystic dilatation of the gastric glands (Greaves and Faccini, 1992). 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 mineralisation 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 (Snell, 1967).

A similar phenomenon has been described in the stomach of dogs in uraemic states (Cheville, 1979). Identical changes result from the administration of drugs that induce prolonged azotemia or electrolyte disturbances. These changes are characterised by diffuse deposition of mineral in the intestinal tissue of the mucosa of the gastric body but not cardia, antrum or pylorus. Mineral deposits develop around basement membranes surrounding epithelium and blood vessels. The lamina propria becomes expanded by oedema 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 haemorrhage occurs presumably as a result of the ischaemia caused by diffuse vascular injury and altered parietal cell function.

## 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, mineralisation or vascular occlusion. These changes, characterised histologically by focal fibrosis of the mucosa, gastric glandular dilatation and atrophy variably accompanied by polymorphonuclear cells and mast cells are common in certain strains of rats when 2 years or more in age (Anver et al., 1982).

Whereas diffuse mucosal atrophy occurs following severe inflammatory insult, diffuse atrophy of the stomach glandular mucosa without inflammation can be a result of surgically or drug-induced reduction in trophic factors necessary

for the maintenance of normal gastric morphology and function. This is observed in man and experimental animals following antrectomy because this removes the peptide-producing cells of the antrum (Gjurldsen et al., 1968; Neilsen et al., 1972). In the rat, antrectomy is accompanied by hypogastrinaemia, reduced weight and height of the oxyntic mucosa and a reduced number of argyrophil cells (Håkanson et al., 1976, 1986). 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. Whilst all cell types are present, there is a most pronounced decrease in the numbers of parietal cells as well as 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 (Hinkle and Samuelson, 1999).

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 (Dethloff et al., 1997).

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 man (Crean et al., 1969; Aase and Roland, 1977). Studies in the rat have shown that diffuse atrophy of the gastric glands characterised 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 1 month after surgery (Nakamura, 1985). By contrast, unilateral vagotomy in the rat leads to marked and persistent atrophy of the oxyntic zone on the denervated side. This is characterised histologically by reduced height of the mucosa and reduced numbers and staining intensity of argyrophil cells (Hakanson et al., 1984). Håkanson and his colleagues argued that this unilateral atrophy was due to the removal of the trophic action of the vagus. The lack of lasting atrophy after bilateral but not unilateral vagotomy was explained by the subsequent rise in gastrin that occurs after bilateral vagotomy as a result of lack of acid feedback inhibition of gastrin release (Håkanson et al., 1984).

Removal or reduction in extra-gastric trophic factors or hormones may also reduce the thickness of the gastric mucosa. This is 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 (Bastie et al., 1985).

Atrophic changes in the chief cells were observed in rats treated for 6 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 (Hansson et al., 1986). Another inhibitor of gastric acid secretion, the tricyclic agent pirenzepin, also produced atrophy of the fundic mucosa of rats following 3 months but not 1 month of treatment (Lehy et al., 1978). The atrophy was characterised 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 $H_2$-receptor antagonists.

### Diffuse hypertrophy and hyperplasia of glandular mucosa

An increase in the thickness of the gastric mucosa can be the result of 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. Morphometric 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 proliferative or adenomatous overgrowth. Adenomatous hyperplasia also needs to be evaluated for atypical cytological features (dysplasia), which are linked to development of gastric carcinoma (see below).

Cells of gastric glandular mucosa undergo increases in size or number in response to the effects of gastrointestinal trophic hormones or their synthetic analogues. Similar changes also follow administration of compounds that inhibit gastric acid secretion or modify other trophic hormones or growth factors.

When gastrin or its synthetic analogue, pentagastrin is administered subcutaneously to rats and mice for several weeks, there is both an increase in the number and size of parietal cells without concomitant increase in zymogenic chief cells (Willems and Lehy, 1975; Crean et al., 1978; Balas et al., 1985). 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 (Balas et al., 1985).

Drugs which inhibit or neutralise gastric acid secretion such as histamine $H_2$-antagonists, proton pump inhibitors and antacids also induce hypertrophy or hyperplasia of the parietal cell population (Witzel et al., 1977; Crean et al., 1978; Mazzacca et al., 1978; Kaduk and Hauser, 1980; Betton et al., 1988; White et al., 1998). 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 (Witzel et al., 1977).

Not all histamine $H_2$ antagonists produce identical effects. Other cytological changes have been reported with famotidine, another $H_2$-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 (Burek et al., 1985). 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 different forms of diffuse gastric hyperplasia. Rats treated with 16,16-dimethyl prostaglandin E2 hourly for 3 weeks, not only developed 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 (Reinhart et al., 1983). Unlike treatment with gastrin and gastrin analogues there was an increase in number of surface and foveolar mucous cells associated with increase in mucus content.

Misprostal, a synthetic prostaglandin E1 methyl ester analogue also produced diffuse glandular hyperplasia, characterised by lengthening of gastric pits and increased mucous secretion in the preclinical safety studies in dogs and rats (Kotsonis et al., 1985). This glandular hyperplasia not only affected the body but also the antral mucosal. Studies with tritiated thymidine showed that the labelling index was reduced in rats treated with misprostal, 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 (Fich et al., 1988). Levin (1988) has reviewed the effects of prostaglandins of the E series on the gastrointestinal tract of dogs and rodents.

A dose-related diffuse hyperplasia of the gastric glandular mucosa has been reported in both rats and cynomolgus monkeys given human recombinant epidermal growth factor. The gastric mucosa was thickened and there was a increase in the number of undifferentiated cells particularly in the neck region and upper part of the gastric glands (Breider et al., 1996; Reindel et al., 1996). 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 (Vinter-Jensen, 1999).

Administration of recombinant growth hormone has also been reported to induce thickening of the gastric glandular mucosa in dog toxicity studies along with typical growth hormone-induced changes in other organs, body weight increases and insulin-like growth factor (Prahalada et al., 1998). 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, adenoma)

Thickening of the gastric glandular mucosa as a result of an irregular proliferation and cystic dilatation of gastric glands associated with inflammation characterises a number of non-neoplastic conditions in the stomach of man and laboratory animals. Cystic change with chronic inflammation and foveolar hyperplasia is observed in biopsies taken from the edge of chronic gastric ulcers in man (Franzin and Novelli, 1981). Ménétrier's disease ('*polydadenomes en nappes*'), a rare disease found primarily in middle-aged men is also characterised by enlarged gastric folds, foveolar hyperplasia and gastric glandular cystic dilation (Berenson et al., 1976; Wilkerson et al., 1998). Although its pathogenesis remains elusive, increased expression of transforming growth factor $\alpha$ (TGF$\alpha$) and the epidermal growth factor receptor has been described (Demsey et al., 1992). As TGF$\alpha$ is an epithelial cell mitogen that inhibits gastric acid secretion and increases gastric mucin, and transgenic mice that overexpress TGF$\alpha$ in gastric mucosa develop a similar condition (see below), it was suggested by Demsey et al. (1992) that TGF$\alpha$ might have an important role in this condition.

Similar changes have been observed in animals in association with infestation of the gastrointestinal tract by parasites (Jubb and Kennedy, 1970; Cook et al., 1981). Laboratory rodents may develop a similar pattern of changes spontaneously with advancing age, although the cause of this change remains uncertain.

The distinction between adenomatous hyperplasia and adenoma is not clear cut. Nevertheless, adenomas are usually defined as *localised* 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 but adenomas with localised downward growth are described (Mohr, 1997).

### *Mouse*

Proliferation of the gastric glandular mucosa has been well characterised in the laboratory mouse because certain strains have a particular tendency to develop this condition spontaneously with advancing age (Stewart and Andervont, 1936; Rowlatt et al., 1969). Hyperplasia also occurs spontaneously in conventional laboratory strains employed in carcinogenicity bioassays. Its prevalence can be influenced by environmental factors such as housing (Chvédoff et al., 1980), food restriction (Rehm et al., 1987) and the administration of xenobiotics (Poynter et al., 1985; Betton et al., 1987). Similar gastric changes have also been reported to occur in mice thymectomised shortly after birth (Suzuki et al., 1981).

Histologically, these changes in mice are characterised by hyperplasia of the foveolar and neck regions of the body mucosa (Fig. 47). 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 dis-

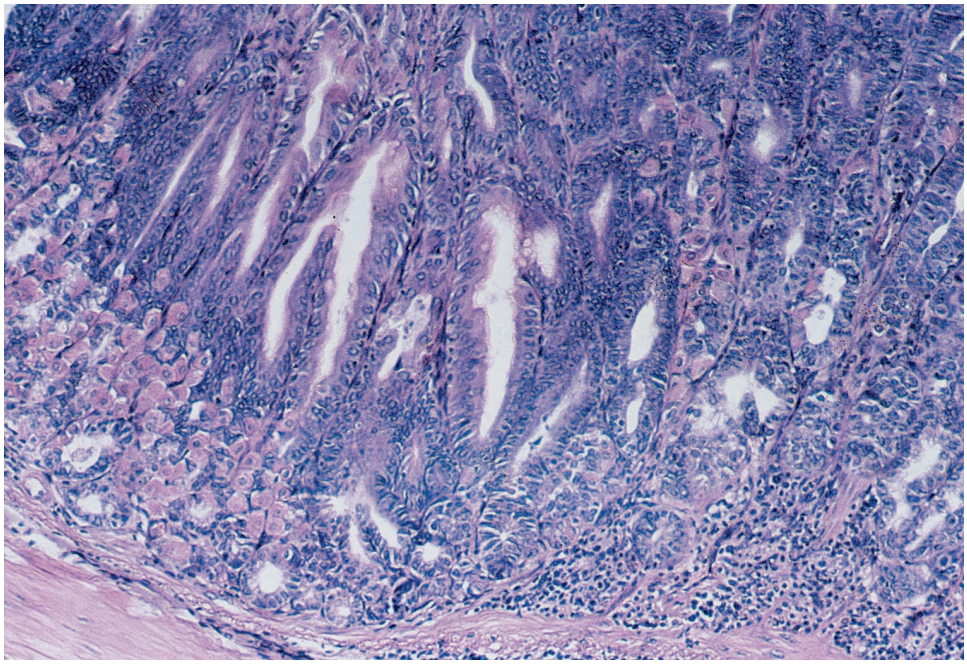

Fig. 47. Gastric glandular mucosa from an untreated 18-month CD-1 mouse showing moderate hyperplasia of the gastric glands of the body mucosa. (HE, ×25.)

place 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. Oedema 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 sulphomucin as revealed by the high-iron diamine technique (Greaves and Boiziau, 1984). It has been suggested that these features are similar to Ménétrier's disease in man and might have a similar pathogenesis (Dempsey et al., 1992; Takagi et al., 1992).

The aetiology of the spontaneous condition in the mouse is uncertain. The occurrence of similar lesions in thymectomized mice has given rise to the suggestion that autoimmune damage to the gastric mucosa may be responsible (Kojima et al., 1980). The presence of circulating anti-parietal 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 (Suzuki et al., 1981). However,

based on findings in female Han NMRI mice, Rehm et al. (1987) showed that this proliferative condition can develop in mice in the absence of antiparietal antibodies. They demonstrated that this change is associated with an increase in the number of antral gastrin cells, raising the possibility of a hormone or paracrine mechanism.

A similar proliferative form of gastropathy has been reported in mice which overexpression transforming growth factor $\alpha$ (TGF$\alpha$), a potent mitogen and member of the epidermal growth factor family of peptides. TGF$\alpha$ acts by binding to and activating the tyrosine kinase of the epidermal growth factor receptor. Transgenic mice overexpressing TGF$\alpha$ develop severe adenomatous and cystic hyperplasia of the gastric glandular mucosa starting from about 2 months of age along with loss of mature parietal cell numbers and a diminution in gastric acid production (Takagi et al., 1992). The degree of change was to some extent dependent on the genetic background on which the transgene operated.

An increased prevalence of similar changes have been reported in CD-1 mice treated with the novel histamine $H_2$-receptor antagonist SK&F 93479 for 21 months (Betton et al., 1987; 1988). 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 characterised by thickening of the mucosa by hyperplasia of the foveolar and neck regions, and downward proliferation of glandular elements into gastric glands (Betton et al., 1988). Poynter et al. (1985) have also reported similar glandular hyperplasia in the mouse stomach associated with histamine $H_2$-blockade with the agent ioxtidine. These findings were similarly associated with hyperplasia of neuroendocrine cells.

Adenomatous polyps have also been reported in the pyloric antrum of C57Bl/10J mice treated for 52 weeks with the synthetic progestin, cyproterone acetate (Tucker et al., 1996). 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 progesterone receptors have been identified in gastric tissue (Wu et al., 1992).

### *Rat*

Although usually less prevalent and less exuberant than in mice, the aged rat also develops proliferative gastric glandular changes spontaneously. These changes are characterised 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 (Greaves and Faccini, 1992). 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 Billroth II gastrectomy that allows reflux of intestinal and biliary secretions into the stomach (Kobayashi et al., 1991).

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 6 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 (St John et al., 1977). These lesions were characterised 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.

Hyperplasia of the gastric glandular mucosa also occurs in rats following the administration of powerful genotoxic carcinogens, although characteristically in association with of atypical histological changes and ultimately carcinoma. These changes have been best characterised in sequential studies with the rat using the carcinogen *N*-methyl-*N´*-nitro-*N*-nitrosoguanidine at doses low enough to avoid overt gastric ulceration and regenerative hyperplasia. It was shown that hyperplasia developing under these conditions occurs diffusely both in the body and antral mucosa. Furthermore, the changes occurred earlier in the antrum than in the body and were focal or polypoid in character (Tsiftsis et al., 1980). Involvement of the antrum in this way is quite unlike the spontaneous hyperplasia of the rat gastric mucosa. Histologically, this form of hyperplasia is characterised by lengthening of the foveolae and neck regions both in the antrum and body. Hyperplastic pits or foveolae show increased secretion of sialomucins and sulphomucins with a concomitant loss of neutral mucins.

Polychlorinated biphenyls such as Arochlor 1254, which produce intestinal metaplasia and adenocarcinoma in the stomach of rats, also induce proliferative alterations characterised by proliferative cystic lesions in the mucosa associated with inflammation and fibrosis (Morgan et al., 1981). In common with lesions induced by genotoxic agents, these changes are found primarily in the antrum and pyloric regions, zones of predilection for the development of gastric carcinoma in man and experimental animals.

## Gastric dysplasia (epithelial atypia)

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 man and laboratory animals possess features in common with lesions not associated with neoplasia. However, a key distinctive feature is the degree of *epithelial dysplasia*.

Dysplasia is considered to be the lesion common to gastric conditions in man 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 man have been characterised in laboratory animals in which precancerous gastric lesions have been studied (Tsiftsis et al., 1980). 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 man, the principle features of dysplasia are (i) cellular atypia; (ii) abnormal differentiation; and (iii) disorganised mucosal architecture (Morson et al., 1980; Nagayo, 1981). Cellular atypia is characterised 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. Disorganised 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 (Tsiftsis et al., 1980). These changes were characterised 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 (Tsiftsis et al., 1980).

These considerations were important in the safety evaluation of the histamine $H_2$ 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 (Streett et al., 1984; 1988). These changes were found mainly in the pyloric region and were characterised 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 6 months also revealed evidence of early proliferative changes

(Streett et al., 1988). 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 (Streett et al., 1988).

## Hyperplasia and neoplasia of gastric endocrine cells, carcinoid tumours

One of the most remarkable examples of drug-induced gastric alterations in rodent bioassays is the hyperplasia of enterochromaffin cells and development of carcinoid-like neoplasms in the stomach of rats treated with omeprazole (Havu, 1986). Omeprazole is a substituted benzimidazole which inhibits gastric acid secretion by blocking the enzyme $H^+$, $K^+$-ATPase, the proton pump of the parietal cells, in a specific and dose-dependent manner (Fellenius et al., 1981).

Although in rats there is a 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 (Havu, 1986). These changes were more marked in female than in male rats but 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 argyrophilic cells. These focal lesions were also associated with a dose-related increase in larger focal nodular lesions of argyrophilic cells, some of which were undoubtedly locally infiltrating carcinoid tumours. These nodular argyrophil lesions posed the usual problems of differential diagnosis of endocrine hyperplasia and neoplasia (see Endocrine System, Chapter XIII) and distinction of hyperplasia from neoplasia was uncertain.

Histologically, nodular lesions were composed of multifocal anastomosing solid or pseudoacinar cords of proliferating, regular cells with uniform nuclei and moderately abundant fine granular pale cytoplasm. These nodules showed 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 were similar to those of gastrointestinal carcinoid tumours reported in man. 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 (Ekman et al., 1985; Havu, 1986).

Electron microscopy of the altered argyophil 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 which is found normally in gastric enterochromaffin cells which produce and store histamine (Sundler et al., 1986).

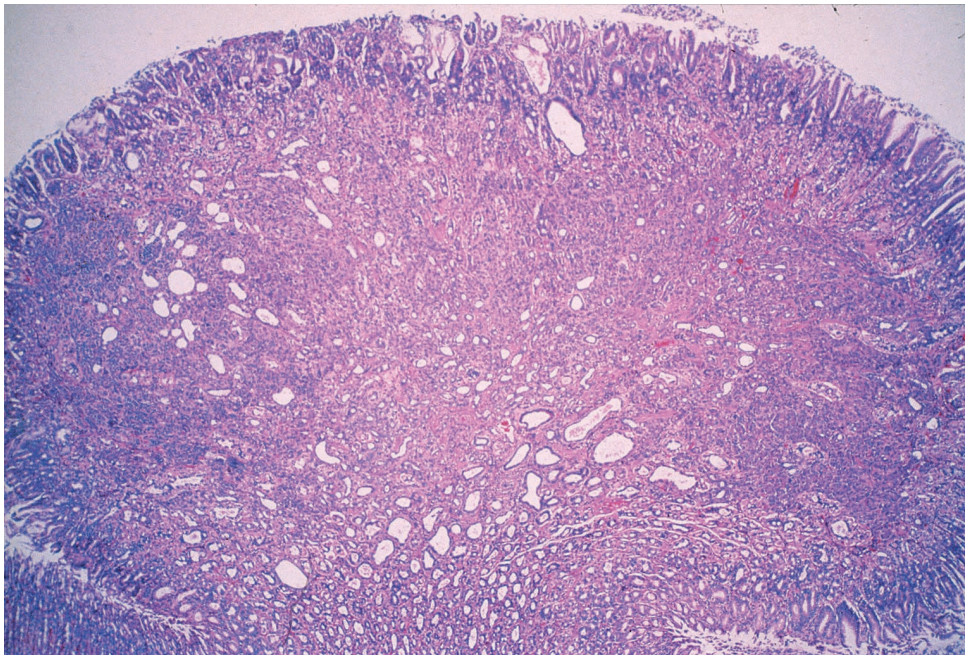

Fig. 48. Section from the gastric glandular mucosa from a rat treated with a high dose of a histamine $H_2$ antagonist for 2 years. This section shows a well-defined benign nodular zone showing both solid and glandular proliferation. Figure by courtesy of Dr Graham Betton. (HE, ×15.)

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 (Blom, 1986), an increase in the number and immunostaining properties of the antral gastrin-containing G cells and hypergastrinaemia (Bishop et al., 1986; Creutzfeldt et al., 1986). All functional and morphological changes following treatment for 60 days were fully reversible after 42 days' drug withdrawal (Creutzfeldt et al., 1986).

As a result of these 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 hypergastrinaemia. Hypergastrinaemia in turn stimulates enterochromaffin cells of the fundus, which in time results in enterochromaffin hyperplasia (Håkanson et al., 1986). This argument is supported by the fact that similar morphological findings are reported in chronic atrophic gastritis and other achlorhydric sites in man (Solchia et al., 1986; Müller et al., 1987) and that antrectomy in the rat prevents the appearance of enterochromaffin hyperplasia following treatment with omeprazole (Larsson et al., 1986).

Although mild dose-related gastric argyrophil cell hyperplasia was noted in

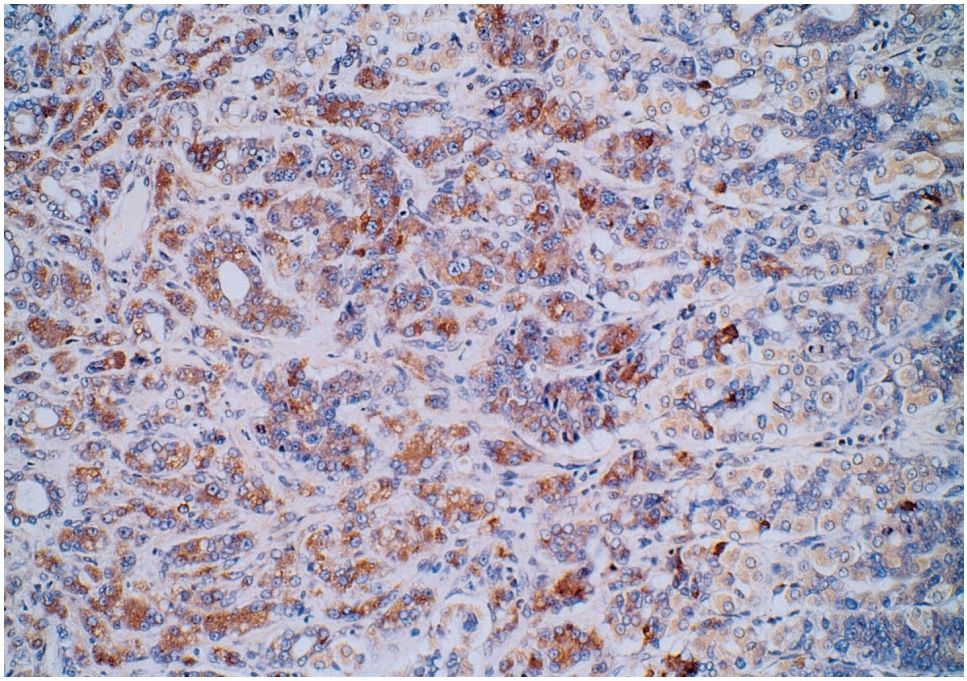

Fig. 49. Higher power view of same case as in Fig. 48 stained for chromogranin A that reveals the predominantly neuroendocrine nature of the cell proliferation. Courtesy of Dr Graham Betton. (Haematoxylin, immunoperoxidase ×80.)

dogs treated with omeprazole for 1 year, neoplasms of the stomach were not observed during this time period. Why mice neither 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 is 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 (Havu, 1986). 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 (Ekman et al., 1985).

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 $H_2$-receptor blockers cimetidine and ranitidine. Neither of these drugs completely inhibits gastric acid secretion in the rat for 24 hours (Leslie and Walker, 1977; Larsson et al., 1986), although mild gastric neuroendocrine hyperplasia has been recently described in cimetidine-treated rats (Hirth et al., 1988).

However, the long-acting $H_2$-receptor antagonist SK&F 93479 produced gastric carcinoid neoplasms when administered at a high dose (1000 mg/kg) to rats for 2 years (Figs. 48 and 49) (Betton et al., 1987; 1988). 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 3–4 times control values over this period. In a 21-month oral carcinogenicity study in CD-1 mice at the same dose level (1000 mg/kg), a diffuse neuroendocrine cell hyperplasia and multifocal glandular hyperplasia or neoplasia was also observed (Betton et al., 1987). Similarly, loxitidine, a potent, non-competitive, insurmountable histamine $H_2$-antagonist produced hyperplasia of neuroendocrine cells and carcinoid tumours in the gastric fundus of both rats and mice after 2 years' treatment in diet and drinking water, respectively (Poynter et al., 1985, 1986). 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 (Hirth et al., 1988; Streett et al., 1988).

Wormsley (1984) reviewed the considerations in the risk-benefit analysis of agents intended for long-term administration for peptic disease.

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 4 weeks and these changes were accompanied by enhanced serum and tissue gastrin levels (Delaney et al., 1979). These results suggest that adrenocorticosteroids have a trophic effect on gastrin-containing cells.

In human patients hypergastrinaemia is also produced by pharmacologically induced hypochlorhydria although this is usually only slight and hyperplasia of enterochromaffin cells has not been observed (Soll, 1990).

## Gastric carcinoma and nitrosation

A complicating factor in drug safety evaluation is the association of gastric cancer in both man 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 (1967) induced gastric adenocarcinomas in rats with *N*-methyl-*N´*-nitro-*N*-nitrosoguanidine dissolved in drinking water. Furthermore, epidemiological evidence associating *N*-nitroso compounds with human cancer is also fairly strong for the stomach (Corea et al., 1975; Pocock, 1985).

The formation of *N*-nitroso compounds is theoretically possible with a variety of compounds that contain amino groups. It has been suggested that the formation of nitrosamines occurs in vivo under the acidic conditions in the stomach following dietary ingestion of nitrite, nitrates and secondary amines (Mirvish, 1975, 1983). Low levels of preformed nitrosamines are also present in some commercial pelleted diets for laboratory animals, principally derived from fishmeal (Edwards et al., 1979). Calculations based on dietary intake and nitrosatability of precursors and carcinogenicity of derivatives have suggested that the risk that arises from endogeneous nitrosation is highly variable but highest from ureas and aromatic amines (Shephard et al., 1987).

A number of 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 (Gillatt et al., 1985). Some clinical evidence suggests that nitrosation of therapeutic agents can occur in clinical practice. For instance piperazine, a cyclic secondary amine, widely used as an antihelmintic drug, has been shown to form small quantities of *N*-mononitrosopiperazine in the human stomach as measured by gas chromatography-thermal energy analysis (Bellander et al., 1985). However, *N*-mononitrosopiperazine has not been shown to be carcinogenic in rodents (Love et al., 1977). *N*,*N*´-dinitrosopiperazine, carcinogenic to the upper gastrointestinal tract in rodents (Lijinsky and Taylor, 1975), was not detected in man after administration of piperazine under the same circumstances (Bellander et al., 1985).

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 whilst being treated with the histamine $H_2$ receptor antagonist cimetidine, were the result of treatment (Elder et al., 1979; Reed et al., 1979; Hawker et al., 1980). It now seems likely that all those observed cancers associated with cimetidine were incidental (Penston and Wormsley, 1986). However, at that time concerns were increased by the theoretical possibility that cimetidine has the potential be nitrosated in vivo (Elder et al., 1982). A further factor was the concept that the treatment-induced gastric secretory inhibition with subsequent bacterial colonisation of the stomach rendered the conditions conducive to the generation of *N*-nitroso compounds from normal dietary constituents (Reed et al., 1981; Penston and Wormsley, 1986).

These concerns appear to be unfounded. Long-term surveillance studies with cimetidine have shown no causal link between its clinical usage and gastric malignancy (Colin-Jones et al., 1965; Langman, 1985). In addition, carcinogenicity bioassays performed with cimetidine, cimetidine plus nitrite and nitrosocimetidine have not shown any tumorigenic effect in the gastric mucosa (Anderson et al., 1985). A 7-year study in dogs in which multiple gastric biopsies were taken at intervals of approximately 6 months have also shown no indication of gastric hyperplasia, dysplasia, intestinal metaplasia or neoplastic change (Walker et al., 1987). 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 (World Health Organisation, 1978). Even for gastric antisecretory agents which may be 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

Most carcinomas of the glandular mucosa are adenocarcinomas, whether induced by the potent genotoxic carcinogens or therapeutic agents. These tend to develop in the antral region in rodents (Streett et al., 1984; Szentirmay and Sugar, 1985). They range from those with well differentiated tubular or papillary features to poorly differentiated forms with trabecular, mucoid or signet ring features (Tatematsu, 1997). Squamous metaplasia within adenocarcinoma can also be observed. Stroma may be abundant with pronounced chronic inflammatory infiltration and hyalinisation. Metaplastic cartilage and bone has also been described (Szentirmay and Sugar, 1985; Mohr, 1997). Gastric adenocarcinomas induced in dogs by *N*-methyl-*N´*-nitro-*N*-nitrosoguamide show similar histological features although their reported distribution in the stomach appears more variable (Fujita et al., 1974).

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 (1961) who defined invasive cancer as a neoplastic growth reaching the *serosa*. It is now considered more appropriate to apply criteria of use in human diagnostic pathology (see Mohr, 1997). Unequivocal invasion of the *submucosa* is sufficient evidence of an invasive and therefore malignant process (Greaves and Faccini, 1992).

## 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 specialised absorptive epithelium. Furthermore, ingested substances have an extended residence time in this part of the gastrointestinal tract.

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, which influence drug absorption, have been better studied in dog and man than many other species. However, data from dog and man not only suggest that diverse factors influence drug absorption from the small intestine but that there are considerable species differences.

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 (1986) has shown using the Heidelberg capsule technique that small intestine transit time in dogs is varies from between 15 and over 200 minutes whereas in man equivalent times are between 180 and 300 minutes. These results suggest that absorption of poorly absorbable drugs is likely to be quantitatively less although more variable in

dogs than in man. 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 man.

Intestinal pH is consistently higher in dogs than in man so that drugs with half maximal absorption pH in the range pH 5–7 may also be expected to be absorbed at different rates in man and dog (Dressman, 1986). Physiological and anatomical differences in the small intestine of other test species particularly rodents and humans are also likely to have an impact on drug absorption although many of these factors are still poorly understood.

In addition to the small intestine acting as an absorptive surface, it is becoming increasingly obvious that it plays an important part in the metabolism of drugs (Breckenridge, 1987). Although monoxygenase 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 or even higher than in the liver (Hänninen et al., 1987). 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 (Hänninen et al., 1987). Gastrointestinal metabolising activity is important because that the mucosa is exposed to high concentrations of xenobiotics in toxicity studies, and this can influence their overall bioavailability (Chhabra and Eastin, 1984).

Studies in untreated rats have shown that the concentration of total cytochrome P450 in small intestinal microsomes is only about 10% of that found in liver microsomes (Bonkovsky et al., 1985). However, it exists in at least two forms and as in the liver, its activity can be induced by xenobiotics. It has been shown that in the rat, the concentration of cytochrome P450 and drug metabolising enzyme activity increases in intestinal epithelial cells as they move from crypt to villous tips and they are found in greater concentration in the proximal two-thirds of the small intestine than in the distal third (Hoensch et al., 1976; Bonkovsky et al., 1985). Bonkovsky et al. (1985) also showed that the phenobarbital-inducible form of cytochrome P450 represents less than 5% of total P450 in the small bowel, but as in the liver, phenobarbital treatment can increase this form to about 50% of total cytochrome P450 in small intestine cells. Furthermore, it has been shown that drug metabolising activity in the tips of the villi in the duodenum is greater in rats fed a conventional diet than a semisynthetic diet and that the activity depends critically on the absorption of iron from the intestine (Hoensch et al., 1976).

Glutathione is also present throughout the entire mucosa, although in rats, cells at the tips of the villi contain less than cells located more basally, whereas related enzymes $\gamma$-glutamyl transpeptidase and glutathione-S-transferase show highest activity in the villous tip region (Ogasawara et al., 1985). 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.

## Histological and histochemical characteristics

The small intestinal mucosa is constructed not only to act 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, specialised 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 (Williamson, 1978). 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. There are intrinsic controls within the mucosa itself. Exogeneous substances, intraluminal secretions, mechanical and neural factors as well as alterations in blood flow all possess potential to influence mucosal cell kinetics (Williamson, 1978).

All main epithelial cell types are believed to arise from undifferentiation columnar cells at the crypt base (Cheng and Leblond, 1974), although mucous cells may also arise by proliferation of partly differentiated mucous cells in the crypts. As cell division is limited to crypts, the cell population in the crypts have high activities of enzymes such as thymidine kinase that are involved in nucleic acid synthesis (Imondi et al., 1969).

The complete cell cycle lasts about 10–17 hours in rodents and at least 24 hours in man. Enteric epithelium is completely replaced within 2–3 days in mice and rats and within 3–6 days in man (Williamson, 1978). 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 (Imondi et al., 1969). 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 ileal mucosa (Altman and Enesco, 1967). 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 (Parker et al., 1974).

Mature absorptive cells are important in the active and passive transport of nutrients as well as in the endocytosis of macromolecules. They are characterised by the presence of a striated or brush border which is seen in haematoxylin and eosin-stained sections as a refractile bi-laminar band. The inner, wider lamina corresponds to the microvillous region that is associated with the presence of neutral mucosubstances in most species. The outer, thinner band corresponds to the glycocalyx, which is composed principally of acidic mucosubstances (Sheahan

and Jarvis, 1976). This outer band of the brush border shows histochemical staining predominantly for sulphomucins 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 $\mu$m long and 0.1 $\mu$m wide. These are considered the first site of entry of food substances into the cell. The plasma membrane of microvilli is associated with fine filamentous projections, which probably represent the polysaccharide chains of the glycocalyx (Bennett, 1969). As the glycocalyx is composed of a network of polysaccharides, it has been suggested that it may behave like an ion-exchange resin, be able to bind certain lectin-like molecules or trap substances in its matrix so providing a site for efficient intraluminal digestion (Bennett, 1969; Goldberg et al., 1969; King et al., 1986).

The plasma membrane shows a trilamina structure at ultrastructural level. Freeze fracture replicas from the microvilli which cleave this membrane through the plane of apposed non-polar groups of the lipid bilayer, demonstrate smooth complementary surfaces studded with small particles. These particles represent integral globular proteins of the plasma membrane. Some of these intramembranous particles, mostly those of 10 nm diameter show irregular outlines with a central pit and are believed to represent gap junctions or transport channels (Yamamoto, 1982).

A particularly 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, although its precise role here uncertain (Owen and Bhalla, 1983). Immunocytochemical demonstration of alkaline phosphatase provides a useful tool to examine the effects of xenobiotics on intrinsic membrane glycoproteins in the small intestine (Hasegawa et al., 1987).

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 man and animals. Immunocytochemical studies have demonstrated that in man this enzyme is located in the duodenum and proximal jejunum but not ileum, colon and stomach (Hermon-Taylor et al., 1977). These enzymes constitute integral structural proteins of the cell membrane with active sites protruding from the cell surface. They are synthesised by the absorptive cells (Blok et al., 1984).

The lateral surfaces of absorptive cells are in direct contact with neighbouring cells and firmly attached to each other by terminal bars or junctional complexes. A terminal bar comprises an apically situated tight junction or zonula accludens, a central zone, the zonula adherens, below which is situated a desmosome or macula adherens. The junctional complexes are relatively impermeable to macromolecules. Studies with labelled tracers in the rat jejunum have shown that horseradish peroxidase (molecular weight 40,000, diameter 5 nm) and ferri-

tin (molecular weight 100,000, diameter 10 nm) do not penetrate junctional complexes (Yamamoto, 1982).

Below the junctional complexes, the cell membranes interdigitate and the intercellular space widens towards the cell base, a feature that may be important in the movement of electrolytes and water across the intestinal epithelium (Rhodin, 1974).

The cytoplasm of absorptive cells contains smooth and granular endoplasmic reticulum, free ribosomes and mitochondria. The Golgi apparatus is located above the nucleus. The apical part of the cytoplasm is devoid of organelles except for a tight meshwork of filaments called the terminal web. Filaments of actin within the microvilli are linked to the terminal web and this is believed to be important in the movement of microvilli (Moosker and Tilney, 1975; Moosker et al., 1978).

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 defence against intestinal pathogens. Goblet cells are characterised by the presence of abundant mucous droplets formed by the Golgi complex and which accumulate in the apical part of the cell cytoplasm. Histochemical study shows that neutral mucosubstances are present in the goblet cells found in crypts and on the villi in the entire small bowel mucosa of most species including man but there is an interspecies variation in the population of sialo- and sulphomucins (Sheahan and Jarvis, 1976). In the mouse, sulphomucins predominate but among rats considerable individual variation in the proportion of sialo- and sulphomucins is reported. In the hamster, sulphomucins are more prominent in the proximal and sialomucins in the distal small bowel. In the dog, both sulphomucins 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 man compared with non-human primates but sialomucins are predominant in both species. A few goblet cells in the distal ileum in man also contain sulphated mucins (Sheahan and Jarvis, 1976).

Paneth cells remain located near the crypt base throughout the small intestine (Sandow and Whitehead, 1979). They are found in rodents and man but typically not in carnivores such as dog and cat (Rhodin, 1974; Satoh et al., 1986). They are characterised by the presence of numerous eosinophilic cytoplasmic secretory granules between about 1.0 and 2 $\mu$m 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 (Lewin, 1969). 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 (Speece, 1964; Riecken and Pearse, 1966;

Ghoos and Vantrappen, 1971; Satoh et al., 1986). Staining for lysozyme appears to be the most practical immunocytochemical stain for the detection of Paneth cells in formalin fixed paraffin wax embedded tissue sections. However, other immunocytochemical reagents have been employed in human and rodent tissues. These include antibodies to the CD15 antigen (Ariza et al., 1996) and to a rat Paneth cell zinc binding protein (Sawada et al., 1994) as well as the use of pokeweed lectin for murine Paneth cells (Evans et al., 1994).

The Paneth cell granules not only contain lysozyme but also a range of antimicrobial peptides such as secretory phospholipid $A_2$, $\alpha$-defensins, also called cryptins. These are believed not only to possess antimicrobial activity but also important in the regulation of cell volume, chemotaxis, mitogenesis and inhibition of natural killer cell activity (Ouellette, 1997). It has been shown that these substances are released from Paneth cells when germ free rats are dosed with the intestinal flora from specific pathogen free rats (Satoh and Vollrath, 1986; Satoh et al., 1986). However, it has been shown that Paneth cells develop under germ free conditions and do not require luminal bacteria or dietary material for their development (Ouellette, 1999).

Endocrine cells are also scattered throughout the small intestinal mucosa. They are of both argentaffin and argyrophil types and are situated predominately 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 (Inokuchi et al., 1983).

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 (Pabst, 1987). These lymphocytes are termed intraepithelial lymphocytes and are predominantly of T-suppresser/cytotoxic type in man and laboratory animals (Selby, 1981; Martin et al., 1986; Pabst, 1987).

Most lymphocytes in the lamina propria are also T cells but T-helper (CD4 positive) cells outnumber the T-suppresser/cytotoxic or CD8 positive phenotype (Hirata et al., 1986; Pabst, 1987; Bruder et al., 1999). Intraepithelial lymphocytes are also mainly T cells (Bruder et al., 1999). Many plasma cells present in the lamina propria produce IgA, the major immunoglobulin of mucosal secretions (Michalek et al., 1975). IgA represents another important component of the mucosal barrier between the gastrointestinal mucosa and intraluminal antigens. The main function of IgA is to effect immune exclusion by intimate co-operation with non-specific defence mechanisms (Brandtzaeg et al., 1985). Plasma cells in the lamina propria produce dimeric IgA with two dimeric molecules joined by a joint (J) piece. A secretory component (SC), a glycoprotein expressed on the basolateral surface of epithelial cells acts as a receptor for dimeric IgA and as a transport system for IgA to the gut lumen where monomeric IgA is secreted (Brandtzaeg et al., 1985). 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 (Rodning et al., 1983). A significant reduction in IgA-containing lymphocytes and plasma cells was observed following microbial reduction associated with gnotobiosis, probably reflecting decreased microbial antigenic stimulation. Experimental studies using labelled mesenteric lymphocytes also suggests that local microenvironments are important in the distribution of there cells in the intestinal wall (McDermott et al., 1985).

Peyer's patches are the most 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. Peyer's patches are located on the ante-mesenteric wall of the small bowel and consist principally of collections of lymphoid follicles. In man, Peyer's patches are more common in the ileum (Cornes, 1965) but in mice they are more uniformly distributed (Owen and Neumanic, 1978). In rats they are also more numerous in the distal than in the proximal small intestine and the number of follicles in patches usually varies from 2 to 6 but sometimes many more may be seen in any particular section (Martin et al., 1986). Peyer's patches vary between rat strains. A comparative study showed that in Fischer 344 rats they are smaller than those in Wistar rats (Bruder et al., 1999). Particular care in selection and orientation of tissue blocks is therefore essential for any form of quantitative or semiquantitative assessment of Peyer's patches.

Peyer's patches are more than simple aggregates of lymphoid follicles. They consist of lymphoid follicles surrounded by a corona of small lymphocytes principally of B-cell type. The interfollicular area contains post-capillary venules with specialised cobblestone type epithelium (Yamaguchi and Schoefl, 1983) and many T lymphocytes. Beneath the epithelium, over the bulging follicles (dome area), mixtures of T and B lymphocytes, plasma cells and macrophages can be seen (Pabst, 1987). Immunohistochemical study in rats demonstrates the presence of W3/13-positive T (CD43) cells in the interfollicular area. In the rat, many cells with macrophage morphology also stain with the W3/25 (CD4) monoclonal antibody in the interfollicular zone (Bland and Warren, 1985; Martin et al., 1986). Immunocytochemical study of the Peyer's patches in the mouse has also shown considerable heterogeneity of staining patterns, particularly under the dome epithelium (Ermak and Owen, 1986). Similar patterns have been observed following immunohistochemical study of Peyer's patches in man (Spencer et al., 1986).

The epithelium overlying the Peyer's patch follicles (dome area) contains specialised epithelial cells, called microfold, membranous or simply M cells. These cells have been identified in many species including rats, mice, hamsters, dogs, monkeys and man (Owen and Bhalla, 1983; Wolf and Bye, 1984). 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 (Owen, 1977; Jeurissen et al., 1985). 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 (Wolf and Bye, 1974; Smith et al., 1987). M cells therefore form weak 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 (Jeurissen et al., 1985). Understanding of these cellular and molecular characteristics is critical for the design of mucosal vaccines for pathogens that exploit this pathway (Neutra, 1998).

No simple, specific histological markers for M cells have been identified for use in routine histological sections although many specialist techniques can be applied (reviewed by Gebert et al., 1996). This has hampered their study because they are still best identified on the basis of their ultrastructural characteristics or ability to capture and transport macromolecules (Hamzaoui and Pringault, 1998). The M cell shares tight junctions and desmosomes with adjacent epithelial cells but it has fewer and shorter microvilli than absorptive cells. There is lack of a well organised terminal web. Vesicles are abundant in the apical cytoplasm but lysosomes are reduced in number. Attenuated cytoplasmic processes may be seen embracing lymphocytes (Owen and Nemanic, 1978; Wolf and Bye, 1984). A cardinal feature is the presence of a intraepithelial pocket on the basal membrane which acts as an internal docking site for lymphocytes, such that they are filled by B and CD4 positive lymphocytes, macrophages and dendritic cells that move between underlying follicles and the epithelium (Ermak et al., 1990; Farstad et al., 1994).

With care, these attenuated microvilli can be seen at light microscopy in semithin plastic sections (Wolf and Bye, 1984). Cytochemical analysis has also demonstrated that they can be distinguished at light microscopic level by markedly reduced alkaline phosphatase activity on their terminal surface, in contrast to the dense reaction product produced by other enterocytes (Owen and Bhalla, 1983). As the glycoproteins on the surface of M cells are different to those on surrounding cells, they can be selectively stained by labelled lectins, although these patterns are different in different species. For instance, mouse M cells are labelled by the fucose-specific probe *Ulex europaeus* and to some extent *Anguilla anguilla* (Giannasca et al., 1994).

Mucosal mast cells also appear to be involved in the immunological defence of the gastrointestinal tract. They respond by proliferation, migration and discharge of granules during nematode infestations (Miller, 1980). 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 employed for the demonstration of mast cells in tissue sections (Wingren and Enerbäck, 1983). Histochemical study suggests that mucosal mast cells differ from connective tissue mast cells by a lower degree of sulphation of glycosaminoglycans and different spatial relationships of protein and glycosaminoglycans in their granules. These cross link following formalin fixation in a way, which is sufficient to prohibit cationic dye binding. Wingren and Enerbäck (1983) showed that these staining

difficulties can be surmounted in tissues fixed in formaldehyde by staining in toluidine blue for prolonged periods of time (5–7 days), a procedure which allows adequate penetration of the toluidine blue molecule.

## 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 artefact caused by washing may confound interpretation of changes induced by xenobiotics (Roe, 1984). Combination of artefact 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. Rolling the unfixed, opened rodent intestine around a wooden stick prior to freezing or fixation is one proposed method (Moolenbeck and Ruitenberg, 1981), although this method risks undue manipulation of the unfixed tissue. 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 (Filipe and Branfoot, 1974).

## 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 or as a direct result of the effects of xenobiotics or ionising radiation. Antimitotic or radiomimetic agents as well as ionising radiation are liable to adversely affect the rapidly dividing cells of the small intestine with resulting breakdown of the mucosal barrier. The ulcerogenic activity of non-steroidal anti-inflammatory drugs may also be expressed in the small bowel mucosa.

Different agents also act in synergy to enhance damage to the small bowel mucosa. An important example is the effect of drugs that depress the immune system and permit the development of pathological infections by microorganisms of the opportunistic type in the small intestine.

The histological features of the inflammatory process in the small intestine are not usually specific for a particular agent. It is important to search for evi-

dence 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 or foreign substances 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 favour the proliferation of potentially pathogenic organisms in sufficient quantities to cause overt damage to the mucosa. Certain bacterial flora may also act synergistically with intestinal protozoans to produce pathological changes (Boorman et al., 1973).

In non-human primate colonies, gastrointestinal disease remains one of the most important causes of death (Holmberg et al., 1982). In contrast to other laboratory species, histological evidence of intestinal infectious disease is relatively common and may confound the interpretation of gastrointestinal alterations occurring in toxicity studies. Although the majority of potentially pathogenic organisms affect the primate colon, a number of bacteria, protozoa and metazoa occur in the small intestine. A detailed review of the protozoa and metazoa occurring in the primate gastrointestinal tract has been published (Toft, 1982). Owen (1993) has reviewed the parasites of laboratory animals including those of the gastrointestinal tract and the principle reference for identification of metazoa in tissue sections remains that of Chitwood and Lichtenfels (1973).

Organisms which can cause inflammatory disease in the small bowel but which are primarily agents that produce inflammatory disease in large bowel are reviewed under 'Colon' (see below).

*Bacterial infections*

*Bacillus piliformis* is 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 F344 rats appear more resistant to infection than outbred Syrian hamsters (Waggie et al., 1987). Lesions of variable severity usually occur in the ileum but may also extend into the caecum and colon. Severe infections are characterised histologically by ulceration of the mucosa, oedema 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 (Ganaway, 1985). 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 visualised in haematoxylin and eosin stained sections (Weisbroth, 1979). 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 (Ganaway, 1985). *Salmonella typhimurium* and *Salmonella enteritidis* are regarded as the organisms typical of murine salmonellosis (Weisbroth, 1979). Lesions occur in the ileum and may extend into the jejunum and caecum. They are characterised 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 a pseudomembranous colitis in man 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 organism has not been cultivated, but has been suggested that it is a campylobacter (Jacoby, 1985) although other organisms have been identified in this condition (Fox et al., 1993; Peace et al., 1994).

Although it is characterised by hyperplasia of the ileal mucosa in its early stages, an inflammatory phase intervenes in which there is focal necrosis and haemorrhage 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 (Jacoby, 1985).

*Helicobacter jejuni* (*Campylobacter jejuni*) is a common cause of diarrhoea in man 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 commonly appreciated. It is important to recognise that dogs colonised with these agents may be susceptible to stress-induced, acute onset gastroenteritis (Fox et al., 1988; Reed and Berridge, 1988). In man this form of bacterial disease is characterised histologically by mucin-depletion, flat-

tening and reactive changes in the small bowel epithelium, crypt abscesses, oedema and infiltration of the mucosa by neutrophils, lymphocytes and plasma cells. Similar histological findings have been reported in dogs infected with this organism (Prescott and Monroe, 1982). The organisms are gram-negative curved, slender rods, which can be visualised in tissue sections with the Warthin–Starry stain, a recognised technique for spiral bacteria.

The carbol fuchsin technique of Gimenez (1964), first used for the identification of Ricketsiae in yolk sac culture and a cresyl fast violet technique are also useful methods for the identification of Campylobacter species in paraffin sections (Burnett et al., 1987; McMullen et al., 1987). Another Campylobacter-like organism, *Helicobacter pylori* (*Campylobacter pylori*) has been identified in human patients with gastritis, gastric and duodenal ulcers. It is an aetiological or predisposing factor in these forms of gastrointestinal disease (Marshall and Warren, 1984; Rouvroy et al., 1987; Richter-Dahlfors et al., 1998).

*Protozoan parasites*

*Spironucleus 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, oedema and leucocyte infiltration (Boorman et al., 1973; Wagner et al., 1974). The morphological expression of damage is accompanied by decreased levels of disaccharidases such as maltase, sucrase and lactase, which may represent a direct effect of the trophozoites on the brush border enzymes (Gillon et al., 1982). Trophozoites are characteristically elongated symmetrical flagellates approximately 2–5 $\mu$m wide, 12–20 $\mu$m long.

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 man 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 (Gillon et al., 1982). This has led to the suggestion that the response to infection by these parasite is primarily a cell-mediated immune reaction similar to experimental graft versus host reaction in the small intestine of mice (Mowat and Furguson, 1981; Gillon et al., 1982). Depression of the immune response by treatment with corticosteroids has been shown not only to increase parasite numbers in murine giardiasis but also cause recrudescence of occult infections (Nair et al., 1981). It has also been suggested that decreased gastric acidity can predispose to giardiasis in man (Nalin et al., 1978).

*Giardia muris* (*Lamblia muris*) is sometimes found in the small intestine of rat and mouse but it is common in the hamster (Fig. 50). Trophozoites appear in histological sections as crescent-shaped structures on the brush border of the intestinal mucosa or in the adjacent lumen. Mucosal lesions may be totally ab-

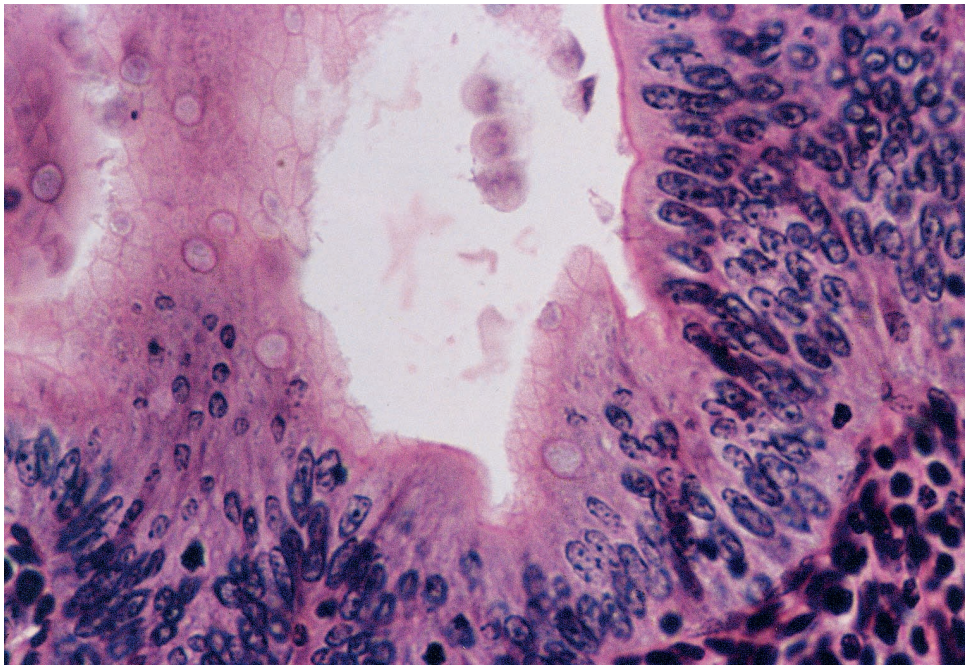

Fig. 50. Section through the duodenal mucosa of an untreated aged hamster showing the presence of *Giardia muris* in the lumen just above the epithelial surface. (HE, ×100.)

sent 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 (Boorman et al., 1973). Another important finding is that lactase, sucrase and maltase levels have been shown to decrease in the small intestine in mice infested with *Giardia muris* (Gillon et al., 1982).

*Giardia lamblia* may colonise the small intestine of non-human primates and of man and produce similar morphological appearances to those found with infestations in rodents by *Giardia muris*. Other flagellates such as *Tritrichomonas muris* are also be 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 recognised in the gastric glands of mice by Tyzzer in 1907 and has since been confirmed as a cause of diarrhoea in animals and as a human pathogen. It causes mild diarrhoea in normal subjects especially children and young adults but it can produce severe intestinal disease in immunocompromised individuals (Casemore et al., 1985).

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 man, with other parasites or infections. They are

rounded, weakly basophilic structures 1–4 $\mu$m diameter in haematoxylin 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 visualised 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 (Casemore et al., 1985). These organisms have been demonstrated in laboratory species including mice, hamsters, rabbits, dogs and non-human primates (Cockrell et al., 1974; Rehg et al., 1979; Toft, 1982; Fukushima and Helman, 1984; Davis and Jenkins, 1986).

*Metazoa*

*Hymenolepis nana* (dwarf tapeworm) and *Hymenolepis diminuta* (rat tapeworn) are described in the intestine of rats, mice, hamsters, non-human primates and man (Hsu, 1979). A variety of other metazoan patients are found in the small intestine of non-human primates, see review by Toft (1982).

*Viruses*

Certain viruses produce inflammatory small bowel changes in mice. 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 (Barthold, 1985).

Murine rotavirus (epidemic diarrhoea 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 $\mu$m diameter, are characteristic findings (Barthold, 1985). Electron microscopic examination shows cytoplasmic vesicles arising from the rough endoplasmic reticulum, which contain virus particles and electron dense granular material (Barthold, 1985). A mouse adenovirus may also produce large basophilic intranuclear inclusions in the epithelial cells of the small intestine and caecum.

K virus infection produces lesions in the jejunal and ileal mucosa of mice. Histological features are characterised by mild polymorphonuclear infiltration, with ballooning of occasional endothelial cells within intestinal villi. Intranuclear inclusions can be demonstrated in these endothelial cells by light microscopy using appropriate fixation (Greenlee, 1985).

A variety of viruses have been isolated from the gastrointestinal tract of non-human primates including viruses of man (Kalter, 1982). However, they appear to be relatively infrequent causes of gastrointestinal disease and when disease is caused by these viruses it usually affects other organs.

*Drug-induced inflammation and ulceration*

Not only can non-steroidal anti-inflammatory agents such as indomethacin and phenylbutazone produce gastric ulceration but also penetrating ulcers of the small bowel of laboratory animals (Shriver et al., 1975). Imaging studies with $^{111}$indium-labelled leukocytes in man have also suggested that subclinical intestinal inflammation is associated with long-term therapy with non-steroidal anti-inflammatory drugs (Bjarnason et al., 1987). It has been postulated that indomethacin-induced intestinal ulcers in rats and dogs are produced by a prostaglandin-independent mechanism, different from the manner in which gastric ulceration is induced (Tabata and Okabe, 1980; Whittle, 1981). Conversely, Satoh et al. (1981) have suggested that similar mechanisms are responsible for both gastric and intestinal ulceration. They showed that indomethacin-induced gastric ulceration developed in the body mucosa in fasted rats but in the antrum and small intestine in rats given indomethacin 30 minutes after a 1-hour period of refeeding following a 24-hour fast. There was good temporal correlation between the development of intestinal ulcers and inhibition of prostaglandin synthesis (Satoh et al., 1981). The ulcers in the small intestine were morphologically similar to those occurring in the stomach and they were distributed mainly in the mucosa on the mesenteric aspect of the bowel wall.

Single-dose studies with indomethacin and ibuprofen in rats conducted by Suwa et al. (1987) have demonstrated differences between pathology of induced gastric and intestinal damage. Gastric damage was superficial, occurred within 6 hours and was fully repaired 2 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 through the muscularis mucosa and were accompanied by inflammation and oedema. Ulcers were still present 2 weeks later.

Rainsford (1978) has shown that potent intestinal ulcerogens such as indomethacin inhibit the incorporation of radioactive $^{35}$S sulphate into glycoproteins of the upper intestinal mucosa as well as the stomach of rats. This may decrease the capacity of the mucus in the intestine to act as a buffer for hydrogen ions.

Indomethacin given orally to dogs in doses of 2.5 mg/kg/day for 1–23 days was also shown to produce intestinal ulceration. These ulcers were deep, punched-out lesions, many of which were lying over Peyer's patches (Stewart et al., 1980). 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 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. These antigens may have been produced by inhibition of suppresser cells in Peyer's patches, following depression of prostaglandin synthetase by indomethacin (Stewart et al., 1980).

Special dye techniques, scanning and transmission electron microscopy have also shown that non-steroidal anti-inflammatory drugs also produce adverse effects on the small intestine mucosa without overt pathological changes being

evident by light microscopy (Brodie et al., 1970; Djaldetti and Fishman, 1981). Following administration of aspirin to mice for 5 weeks, shortening and erosion of microvilli and increased numbers of goblet cells were only demonstrated in the duodenum and jejunum by scanning and transmission electron microscopy (Djaldetti and Fishman, 1981). Morphometric studies of the intestinal mucosa of indomethacin-treated mice have also shown widespread alterations to columnar cells, goblet cells and Paneth cells suggesting generalised effects on mitotic activity and crypt loss (Ettarh and Carr, 1996).

Such submicroscopic findings support the idea that non-steroidal anti-inflammatory agents may induce damage to the small intestine more commonly than supposed.

Although non-steroidal, anti-inflammatory drugs are the best-known drugs with adverse effects on gastrointestinal mucosa, small intestinal inflammation and ulceration can also produced by other agents through different mechanisms.

Anticancer drugs and other therapeutic agents, which affect cell proliferation, depress the bone marrow or the immune system can also produce intestinal mucosal necrosis, haemorrhage, inflammation and opportunistic gastrointestinal overgrowth when administered to dogs or rodents in high doses (Fig. 51) (Martin et al., 1985; Bregman et al., 1987). Lymphoid infiltrates without tissue

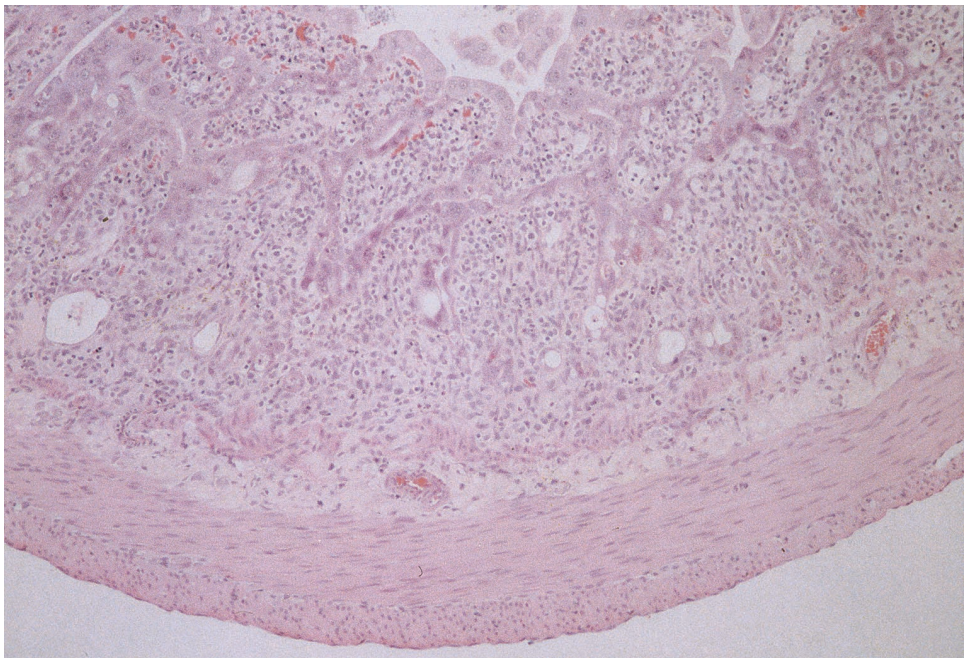

Fig. 51. Section of small intestine from a Wistar rat treated with a 5-day course of an antiproliferative anticancer drug. The mucosa shows mucus-depletion, loss of villous architecture as well as a reparative and inflammatory response. (HE, ×25.)

damage were also reported in the small intestine of rats treated with human recombinant interleukin-2 (Anderson and Hayes, 1989).

Agents of particular toxicological interest are cysteamine, propionitrile and their structural analogues 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 (Szabo and Cho, 1988). 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 neutralisation of gastric acid in the first part of the duodenum and this may contribute to the development of ulceration (Szabo and Cho, 1988). Furthermore, these effects can be attenuated or prevented by dopamine agonists or their precursors whereas dopamine antagonists can potentiate their effects. This suggests that the central or peripheral dopamine-mediated actions of these agents may be involved in the pathogenesis of duodenal ulceration (Szabo, 1979; 1984).

*Fatty change (lipidosis)*

Using appropriate fixation and staining procedures, fine granular lipid droplets can be normally visualised in the apical parts of epithelial cells covering the upper third of 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.

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 (Visscher et al., 1980).

After administration of Sa H51-055 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. Ultrastructural examination revealed uniform electron-lucent droplets within profiles of the smooth endoplasmic reticulum and Golgi apparatus. Lipid droplets increased with time, accumulated and coalesced 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 most active in lipid absorption, resynthesis and transport (Dobbins, 1969). It was suggested fatty change might have taken place because of alterations in the sugar moiety of chylomicra brought about by interference with glucose transport (Visscher et al., 1980).

Lipid droplets which stained with oil-red-O in formalin-fixed frozen sections and showed uniform electron density 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 (Gray et al., 1974). 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 (Friedman and Cardell, 1972) and ethionine (Hyams et al., 1966), agents which have inhibitory effects on protein synthesis. Detailed morphological study 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 (Friedman and Cardell, 1972). 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 which are important for the 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 mucosal 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 may also be observed (see following).

*Phospholipidosis (myelin figures, myeloid bodies, myelinoid bodies)*

The small intestinal mucosa is also one of the many sites at which 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 both man and laboratory animals probably as a result of drug interaction with polar lipids rendering them difficult to digest (Lüllmann-Rauch, 1979). Species differences in susceptibility and tissue distribution of phospholipid are probably not only related to physiochemical characteristics of the inducing drugs which influences their ability to permeate selective biomembranes and react with different lipids, but also to tissue concentrations of drugs achieved and the ability of organs to metabolise parent drug to less amphiphilic products.

In general terms, this form of disorder is characterised by membrane-bound, acid phosphatase-positive cytoplasmic inclusions, which on ultra-structural study are seen as lamellated or crystalloid structures in lysosomes. These appearances are characteristically reversible on cessation of treatment with the inciting agent.

An example of this phenomenon occurring in the small intestine is provided by the iodinated amphiphilic drug, amiodarone, which has been used clinically in Europe for the past 20 years in the treatment of angina and more recently in the control of supraventricular cardiac arrhythmias. Its adverse effects in man are believed to be the result of accumulation of drug in lysosomes particularly in liver, skin and eye (D'Amico et al., 1981; Shepherd et al., 1987).

When high doses of amiodarone were administered orally to rats and beagle dogs, multilamellated lysosomal inclusion bodies accumulated first in the jejunal mucosa and mesenteric lymph nodes before becoming widely distributed in other organs particularly in the lungs (Mazué et al., 1984). In both rats and dogs the small intestinal lesions were characterised by the presence of foamy macrophages with pale finely vacuolated cytoplasm and condensed eccentric nuclei within the lamina propria of the jejunal villi (Mazué et al., 1984). 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 (Vic et al., 1985). Electron microscopy confirmed the presence of lamellated 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 lipidosis was seen in many organs following intravenous administration in dogs, more lipidosis 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 were almost completely resistant to lipidosis induced by amiodarone under similar conditions (Mazué et al., 1984).

Similar cytological changes have been reported in cells of some organs in patients treated with amiodarone (D'Amico et al., 1981; Shepherd et al., 1987).

*Villous atrophy, hypoplasia*

Villous shortening or stunting results when the proliferative activity of the crypt epithelium is reduced or under circumstances in which crypt cell proliferation is insufficient to compensate for increased cell loss as a result of mucosal cell damage. Decreased cell proliferation can be seen segments, which are surgically bypassed, or following decreased food intake, parenteral nutrition, hypophysectomy or thyroidectomy (Williamson, 1978; Bastie et al., 1982).

As adrenergic factors are important in the control of small intestinal epithelial cell division, drugs that alter $\alpha$ or $\beta$ adrenoreceptor activity may influence the proliferative capacity of the epithelium. In mice, increased $\alpha 1$ or $\beta$ receptor

stimulation by appropriate agonists (e.g. phenylephrine) diminishes proliferation of crypt cells but proliferation is increased by stimulation of $\alpha 2$ receptor activity (Kennedy et al., 1983). Yohimbine, a $\alpha 2$-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 (Williamson, 1978).

The detailed morphological study of the small intestinal mucosa in the rat following hypophysectomy by Bastie et al. (1982) has shown a reduction in the height of the small intestinal villi associated with reduction in mitoses in the crypt epithelium. The number of goblet cells was shown to fall particularly in the jejunum and the number of Paneth cells increase in the ileum. Ultrastructural examination showed decreased height of the microvilli of absorptive cells and a lower number of their intracytoplasmic organelles and ribosomes. There were also significant decreases in brush border enzyme activities of alkaline phosphatase, aminopeptidase, maltase and lactase reported about 1 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 characterised by blunting, shortening or 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, haemorrhage and secondary infection of the gut wall ensue if there is overwhelming cell damage.

Comparison of the gastrointestinal toxicity expressed by antimitotic anticancer drugs of different classes in rodents, dogs, non-human primates and man have suggested that there is a higher degree of correspondence between effects in man and dog than between man and other species (Owens, 1962; Schein et al., 1970). In studies with the antiviral agent acyclovir, a radiomimetic effect was noted in the gastrointestinal tract of dogs at high doses but not rodents (Tucker et al., 1983).

Another example is the villous atrophy described in rats following treatment with an antibacterial agent ICI 17,363. This was believed to arise as a result of both interference with cell division and a direct effect on the surface epithelial cells (Murgatroyd, 1980). The effects of ICI 17,363 were characterised 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 enterectomy, increased feeding and stimulation of autonomic nerves. Administration of neurotransmitters, thyroxine, growth hormone, corticosteroids, testosterone, gastrin, glucagon and recombinant epidermal growth factor may also stimulate epithelial cell proliferation (Williamson, 1978; Breider et al., 1996; Reindel et al., 1996; Vinter-Jensen, 1999). In rats hypothalamic damage, hyperthyroidism, tube feeding, diabetes mellitus and insulin injections have been shown to produce intestinal hyperplasia (MacKay et al., 1940; Levin and Smyth, 1963; Jarvis and Levin, 1966; Forrester, 1972). 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 (see previous).

The compensatory response to the surgically resected or bypassed intestine has been the focus of the most detailed studies of increased cell renewal in the small intestine. Partial resection in both rats and man is accompanied by increased villous height and crypt length (Hanson et al., 1977). This is primarily the result of hyperplasia for it has been shown that the numbers of cells per unit length of villus remains unchanged (Hanson et al., 1977) but there is an overall increase in the cell population of villus and crypt (Hanson and Osborne, 1971). DNA/RNA ratios also remain largely unaltered (Williamson, 1978). 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 achieved by a larger number of cells, the individual absorptive capacity of which is not increased (Williamson, 1978).

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 (Olubuyide et al., 1984). Mucin histochemistry using the high-iron diamine and alcian blue techniques have 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 sulphomucins in the distal ileal segment. Sialomucin production may reflect relative cellular immaturity of the more rapidly proliferating cells under these circumstances. However, as sialic acid conveys more viscoelastic properties to mucin, it has been suggested that the goblet cells change following intestinal bypass fulfils a protective function against the increased flow of gastrointestinal contents (Olubuyide et al., 1984).

A number of nutritional factors, particularly dietary fibre, can influence the proliferative characteristics of the small bowel mucosa. Carefully controlled studies in rats given different forms of dietary fibre have shown that the prolif-

erative characteristics of the small intestine can be modified by both the quantity and the quality of the fibre. One study has shown a decrease in the length of villi, crypt cell hyperplasia and shorter transit times in rats fed pectin-supplemented diet but an increase in mucosal growth without alteration in relative differences in crypt and villous length with guar supplementation compared with rats fed fibre-free diet (Jacobs, 1983). Another rat study has shown that pectin feeding leads to increased mucosal area and height associated with an increase muscle mass (Stark et al., 1996). 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 fibres. Interactions between dietary constituents are complex. For instance, in rats the effects of 2% dietary cholestyramine, a non-absorbable ion exchange resin, on small intestinal histomorphology have be shown to depend on interaction of dietary factors (Burkhardt et al., 1998).

Administration of an inhibitor of cholesterol biosynthesis, 5$\alpha$-cholest-8-(14)-en-3$\beta$-ol-15-one, to rats for up to 9 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 (Smith et al., 1989). The enlargement was most marked in the proximal segment of the small intestine and progressively diminished towards the ileocaecal junction, sparing the stomach, caecum 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 Lieberkuhn 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 (Smith et al., 1989). The mechanism for this change in the rat was not clear, 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, particularly 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 (Balas et al., 1985). 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 sulphated mucosubstances (Gona, 1981).

In this context, it is of interest that chronic treatment with the Rauwolfia neuroleptic, reserpine, causes an increase in the sulphation 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 the goblet cell numbers (Park et al., 1987).

Agents which affect activity of the sympathetic nervous system can also alter epithelial cell proliferation in the small (and large) intestine. Treatment of rats with adrenaline, isoprenaline, phenylephrine, phentolamine and yohimbine all result in decreased mitotic activity of jejunal and colonic crypt cells (Tutton and Helme, 1974; Kennedy et al., 1983). By contrast, administration of metaraminol, clonidine, propranolol, prazosin and labetolol as well as simultaneous injection of propranolol and adrenaline all resulted in an increased rate of crypt cell proliferation (Kennedy et al., 1983). These results suggest that agents that stimulate $\alpha 2$ adrenergic receptor activity and those that are $\alpha 1$-antagonists and $\beta$-adrenergic receptor antagonists increase proliferative activity in the rodent intestinal mucosa. Caffeine is also reported to produce an increase in thickness of the intestinal mucosa when administered in high doses to rats (Lachance, 1982). This raises the possibility that intestinal mucosal hyperplasia can be produced by phosphodiesterase inhibition and resultant increases in intracellular cAMP in a similar way to the hypertrophy induced in salivary tissue.

This is supported by recent findings in rats treated for periods of up to 6 months with the inotropic vasodilator, ICI 153,110, a phosphodiesterase inhibitor intended for treatment of congestive cardiac failure. Administration of high doses not only produced salivary gland hypertrophy but also marked thickening of the small and large intestinal mucosa. This was characterised histologically 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 (Westwood et al., 1990).

Although prostaglandin E analogues produce most of their effects in the stomach, increased thickness of the small intestine characterised by longer villi, deeper crypts and increase in cell size have been reported in rats treated with these agents (Levin, 1988).

*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 (Rowlatt and Chesterman, 1979). The glands are lined by hyperchromatic columnar cells, which show 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 man.

The cause of these changes in the mouse small intestine is unknown but their

prevalence can be altered by dietary fibre and panthothenic acid deficiency as well as by administration of drugs and chemicals (Hare and Stewart, 1956; Seronde, 1965; 1970; Ito et al., 1981).

In their study of DBA mice, Hare and Stewart (1956) considered that the lesions were not genuine neoplasms since they were composed of a mixture of cell types, which normally populate the mucosa. 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. Seronde (1965, 1970) reported these lesions in mice fed purified diets, particularly when deficient in panthothenic acid. Panthothenic acid deficiency was also associated with inflammation and deep penetrating chronic ulcers of the duodenum in affected mice, compatible with an inflammatory aetiology of the lesions.

An increase in the prevalence of these duodenal changes was described in CD-1 mice treated with the synthetic prostaglandin E1 analogue, misoprostol for 21 months (Port et al., 1987). These authors 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 the mouse 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 (Port et al., 1987). They were not seen in rats treated with misoprostol for 2 years (Dodd et al., 1987).

Lesions characterised by such intense proliferative activity may be difficult to distinguish from neoplastic lesion. Indeed chronic administration of hydrogen peroxide to C57BL/6J mice in drinking water was not only shown to potentiate the development of a similar type of duodenal hyperplasia but also to produce frankly invasive adenocarcinomas (Ito et al., 1981).

## LARGE INTESTINE

Anatomically the large intestine is broadly similar in man and laboratory animals but there are significant functional differences. The rat colon is probably one of the best studied of the laboratory animal species because the rat is widely used for experimental work on colon carcinogenesis (Shamsuddin and Trump, 1981). As the canine model is popular for oral dosage-form testing, differences in colonic physiology between dog and man may be better understood than between man and many other species (Dressman, 1986).

In man, as well as in the non-human primates, the large intestine can be divided anatomically into caecum, appendix, ascending colon, transverse colon, sigmoid colon rectum and anal canal. Like the small bowel, the colon comprises mucosa, submucosa, muscularis mucosa and serosa. Mucosal plicae are only found in the rectum although plicae semilumaris, 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 man 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 caecum in dog is a small diverticulum, similar to that found in other carnivorous species and it communicates directly with the colon.

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 caecum. Its size is intermediate between the large and anatomically complex caecum of herbivores such as the rabbit and the small caecum of carnivorous species. This probably reflects the omnivorous nature and flexibility of the rat and mouse in their dietary habits, particularly their ability to breakdown cellulose (Rerat, 1978).

The caecum 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 reasonably controlled milieu (Snipes, 1981). In rat and mouse, the caecum is the site of absorption of many substances including calcium, magnesium, water and electrolytes vitamin K and fatty acids (Snipes, 1981). Caecectomy has been shown to decrease digestion of carbohydrates and protein and increase loss of faecal water in these species (Ambuhl et al., 1979).

The activity of intestinal microflora in the metabolism of both endogenous and exogenous substances has been demonstrated in the rodent caecum (Rowland et al., 1986; Rowland, 1988). The usual stock diets for rodents contain abundant plant fibre which provides bulk and fermentable carbohydrate for the microbial population in the caecum. Rats fed stock diets have been shown to possess high levels of reductive and hydrolytic enzyme activity (e.g. azoreductase, nitroreductase, nitrate reductase, $\beta$-glucosidase and $\beta$-glucuronidase) in their caecal contents compared with rats fed purified fibre-free diets (Wise et al., 1986). Intestines of germ free animals have thinner lamina propria, lower cell turnover, enlarged caecum, altered metabolism of cholesterol, bilirubin and bile salts and larger amounts of mucin in faeces compared with animals possessing normal gastrointestinal microflora (Midtvedt, 1987).

Species differences in microflora are also reported. Comparative studies with human and rat intestinal microflora have suggested that each population of organisms possesses a degree of autonomous self-regulation and capable of responding quite differently to dietary changes (Mallett et al., 1987).

Comparative studies have shown large differences in the numbers of facultative anaerobic gram-negative bacteria in the gastro-intestinal tract of mice from three, major specific pathogen free units in Australian laboratories. It was shown that these differences could influence the immune system, susceptibility to infection and experimental results (O'Rourke et al., 1988).

## Histological and histochemical characteristics

The colon and caecum in man 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 of Lieberkuhn 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 man and laboratory animals is not entirely flat but shows a slightly corrugated or uneven pattern, which varies with the particular site within the colon. In histological sections of the colon in man, this corrugated pattern is seen as an anthemion-like structure of crypts reminiscent of a Greek architectural feature (Filipe and Branfoot, 1974). This is also seen in larger laboratory animal species. In rats and mice the crypts of the caecal 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 (Snipes, 1981).

The proliferative zone in the large bowel is found in the lower part of the gland and mitotic figures are normally limited to this zone. As in the small bowel, multipotent, undifferentiated stem cells situated in the gland base give rise to the principle cell types which migrate to the cell surface with subsequent differentiation and alteration of their enzyme activities and morphological features (Chang and Leblond, 1971). In studies with mouse aggregation chimaeras in which mosaic cell populations of the intestinal epithelium were localised immunocytochemically, it was demonstrated that the entire epithelium of each adult gland descended from a single progenitor cell (Ponder et al., 1985). The single progenitor may itself give rise to several stem cells 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. Microvilli of absorptive cells have been shown by electron microscopic study to become longer and denser with increasing distance distally in the gastro-intestinal tract. Thus, they are longer and more dense in the ileum than in the caecum and least dense and shortest in the colon, perhaps reflecting their relative absorptive capacity (Snipes, 1981). This is reflected at light microscopic level by the less conspicuous brush border in the large intestine compared with that in the small intestine.

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 man both sulphomucins and sialomucins may be seen in the brush border (Sheahan and Jervis, 1976). 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 (Sithigorngul et al., 1983).

Although there has been some debate about the precise nature of the mucous cell populations based on structural studies in mice and rats (Chang, 1981; Shamsuddin and Trump, 1981), for practical purposes two principle types of mucous cell can be defined. One of these is the typical goblet cell with cytoplasmic mucous droplets forming a goblet shape, which is found both in glandular and surface epithelium. The other type, the so-called vacuolated cell or mucous cell, is found only in the crypts (Chang and Leblond, 1971; Thomopoulos et al., 1983). These vacuolated or mucous cells show empty-appearing vacuoles in the cytoplasm which rather than being empty contain abundant sialomucins of a type different from those in goblet cells (Spicer, 1965; Wetzel et al., 1966). Detailed structural studies and cytochemistry using lectin probes have suggested that these vacuolated cells are able to differentiate into absorptive cells with the cellular apparatus to produce the cell surface glycoconjugates of the glycocalyx (Thomopoulos et al., 1983).

Sulphomucins, as demonstrated by the high-iron diamine technique (Table 2), generally predominate in the distal colonic segment. In both rat and mouse, goblet cells of the proximal colonic mucosa contain largely sialomucins in the lower parts of the crypts with sulphomucins predominating in the upper parts of the crypt. The distal colon contains largely sulphomucins. The only difference between rat and mouse appears to be the fact that the mouse caecum contains almost exclusively sulphomucins but sulphomucins and sialomucins are found in the rat caecal mucosa. In hamsters, the entire colon contains predominantly sulphomucins. Neutral mucins and sulphomucins predominate throughout the dog colon with occasional goblet cells containing sialomucin. In non-human primates, neutral mucins, sialomucins and sulphomucins are seen throughout the colon with sialomucins generally more prominent in the proximal colon and sulphomucins in the distal segment.

In man, neutral mucins are found mostly in the caecum. In the caecum and ascending colon, sulphomucins are found in the upper crypts and sialomucins in the crypt base. The converse occurs in the distal colon where sulphomucins predominate in the lower two-thirds of the glands and sialomucins in the upper third of the glands and in the surface epithelium. Lectin-labelling (Table 1) shows even greater heterogeneity of mucins in the colonic mucosal cell population, probably reflecting differentiation patterns and changes in glycosyltransferase activity as cells migrate upwards (Freeman et al., 1980; Thomopoulos et al., 1983). It has been suggested by Jass and Roberton (1994) that the two principle changes in pathological processes in the human colonic mucins are loss of O-acylation substituents at sialic acid $C_4$ and $C_{7,8,9}$ and increased sialylation, neither being specific for neoplasia.

The colon, like many other tissues also possess drug metabolising activity, although less than in the liver. It has been shown that the activity of cytochromes P450 involved in hydroxylation of benzo[a]pyrene in microsomes prepared from the colons of Sprague-Dawley rats retain their activity and responsiveness to

inducers better than those in the liver with advancing age (Sun and Strobel, 1986).

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 defence 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 out numbering the T-suppresser phenotype (Hirata et al., 1986; Pabst, 1987). 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.

This distribution of lymphocyte subsets is seen immunocytochemically in the rat colon using the monoclonal antibodies W3/13, W3/25 and MRC OX8 (see Haemopoietic and Lymphatic Systems, Chapter III). The pan T-cell marker W3/13 shows the presence of T lymphocytes in the lamina propria and most of these are labelled by W3/25 demonstrating their CD4 helper phenotype (Bland and Warren, 1985). MRC OX8 demonstrates that few lymphocytes in the lamina propria are of suppresser/cytotoxic (CD8) type, which contrasts, with the high proportion of MRC OX8 positive lymphocytes in the colonic epithelium (Bland and Warren, 1985). The monoclonal antibodies MRC OX6 and MRC OX17, specific for the rat Ia antigen, also label numerous cells with macrophage and dendritic cell morphology in the large bowel of the rat (Bland and Warren, 1985; Martin et al., 1986).

Mature, small B lymphocytes are relatively uncommon in the colonic lamina propria of man and laboratory animals. However, the lamina propria contains large numbers of plasma cells, which are mainly of IgA type, followed by smaller numbers of IgM an IgG subtypes (Pabst, 1987).

A feature of the colonic mucosa is the presence of lymphoid aggregates also called lymphoid nodules, patches, lymphoid-glandular complexes or microbursa. 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 centres with interfollicular and perifollicular zones composed of T cells (Pabst, 1987). 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 diameter except in the distal colon where they attain sizes of up to 10 mm in maximum diameter (Martin et al., 1986).

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 man and laboratory animals (Kealy, 1976; Scott, 1982; Martin et al., 1986). In some strains of rat, cells in these glands express the Ia antigen, unlike the other parts of the colonic epithelium (Martin et al., 1986). 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 (Kealy, 1976). It has been proposed that these glandular structures represent sites of predilection for the spread of inflammatory disease to the submucosa by allowing microorganisms to pass through the muscularis mucosa (Scott, 1982). It has also been suggested that they constitute physical weak points in the bowel wall and may play a part in the pathogenesis of diverticular disease of the colon in man (Kealy, 1976). Colonic carcinomas induced by dimethylhydrazine in the rat also appear to develop more commonly in the lymphoid aggregates than in other zones (Martin et al., 1986). M cells have been described over the lymphoid aggregates in the caecum of the mouse (Owen and Nemanic, 1978), see Small Intestine.

### Inflammation, ulceration, colitis, proctitis

Although microorganisms are important causes of inflammatory disease in the large intestine of man and animals, among laboratory animals they are usually only 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. Nevertheless, 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 organisms may also confound the histological assessment of drug-induced changes in the colon.

Ulceration and inflammation of the colon as a direct result of administration of potential therapeutic agents is reported in humans although less commonly that in the small intestine. It has been suggested from studies of the effects of anticancer compounds on neoplastic colonic cells that intestinal cells may possess inherent protective properties in the form of an accelerated efflux pump which can serve to protect them from potentially damaging agents (Klohs and Steinkampf, 1986). 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 man as rectal suppositories.

Although inflammatory conditions of the large bowel may possess morphological features typical for some inducing agents, inflammation of the large intestinal mucosa is usually characterised by non-specific histological features. In early or mild inflammation, the surface and glandular mucosa remains intact but shows mucin depletion. This is characterised 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 hyperaemic and congested and contains increased numbers of mononuclear cells.

Severe changes are characterised 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 characterised by lengthening, irregularity and cystic dilatation of glands which are often lined by hyperplastic epithelial cells and goblet cells distended with mucin. 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

*Clostridium difficile* may cause inflammatory changes in the colon of laboratory animals, particularly hamsters, and this may extend into the distal ileum. As in man this form of colitis, often referred to as pseudomembranous colitis, is usually associated with antibiotic therapy. In man it was originally associated with lincomycin and clindamycin therapy but other antibiotics have been implicated. It has been shown that both in man and the hamster experimental model that the enteritis is the result of the toxin produced by *Clostridium difficile* (Bartlett et al., 1977; 1978; Milligan and Kelly, 1979).

In man this condition is histologically characterised 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 (Milligan and Kelly, 1979).

Similar features are observed in the antibiotic-treated hamster although the pseudomembrane is less prominent and it may be distributed more proximally with involvement of the terminal ileum (Rehg, 1985). In the hamster, the condition is characterised histologically by erosion of the colonic epithelium and the variable 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 hyperaemic and oedematous lamina propria and submucosa by polymorphonuclear cells (Rehg, 1985). 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 (Rehg and Lu, 1982) and those treated with antineoplastic drugs (Cudmore et al., 1980). Similar changes have been reported in antibiotic-treated guinea pigs and rabbits (Rehg and Lu, 1981; Rehg and Pakes, 1981). Guinea pigs are particularly sensitive to antibiotics especially those active against gram-positive organisms (Young et al., 1987). As in man, these drugs are believed to alter the intestinal flora, permitting overgrowth of *Clostridium difficile* as well as gram-negative organisms, resulting in a severe and frequently fatal enterocolitis. A study of the disposition

of ampicillin administered parenterally to guinea pigs showed that this drug was rapidly eliminated from the systemic circulation and excreted in urine and bile, possibly favouring this effect on flora in the colon (Young et al., 1987).

*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. This agent usually produces a mild or even asymptomatic enteritis in susceptible mouse populations, although it is a cause of rectal prolapse in mice (Ediger et al., 1974). Marked strain differences have been noted in mice infected with this organism. NIH Swiss mice show the most severe histological changes and C57BL/6J mice appear the least affected (Barthold et al., 1977). Rats and hamsters seem to be unaffected by *Citrobacter freundii* (Barthold et al., 1977).

Microscopic changes are found primarily in the descending colon, although proximal segments of the colon and the caecum may also be affected. An important morphological feature is epithelial hyperplasia, which occurs maximally 2–3 weeks after experimental inoculation with *Citrobacter freundii* (Barthold et al., 1977). 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 visualised in routine haematoxylin, and eosin stained sections. Crypt abscesses, inflammatory cells in the lamina propria, mucosal erosions and ulceration are also features (Barthold et al., 1976; 1978). 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 (Barthold et al., 1978).

Most laboratory animals are naturally resistant to shigella infections but this is not the case for most non-human primates (Takeuchi, 1982). In infections with shigella, the colon shows a superficial acute inflammatory reaction comprising oedema, congestion, haemorrhage and infiltration by acute inflammatory cells. The surface epithelium shows mucin loss and formation of microulcers 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 (Takeuchi, 1982). 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 (Holmberg et al., 1982). Large intestinal lesions are characterised 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, characterised by the presence of similar large macrophages in the lamina propria of villus tips. Superficial ulcers may occur in severely affected segments of intestine (Holmberg et al., 1982). 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 multinucleated giant cells.

## Protozoa and metazoal infections of the colon

Numerous protozoa and metazoa have been described as inhabitants of the caecum and colon of the non-human primate (Toft, 1982). 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 characterised histologically by the presence of necrotizing ulcers, which reach the muscularis mucosa to form typical flank-shaped ulcers containing or surrounded by trophozoites. Extensive haemorrhage may be seen as well as an inflammatory infiltrate composed of neutrophils and mononuclear cells (Toft, 1982).

The ciliate, *Balantidium coli*, can also cause an ulcerative process in the colon of primates, characterised by ulcers which extend down to the muscularis mucosa accompanied by lymphocytic infiltrate and *Balantidium coli* trophozoites of up to 150 $\mu$m in greatest diameter (Toft, 1982).

A variety of metazoan parasites can be observed in the primate colon and usually can be reasonably well identified in tissue sections (see review by Chitwood and Lichtenfels, 1973). 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 man, non-human primates and rodents. *Enterobius vermicularis* is found in the large intestine and appendix of man 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 caecum and adjoining mesentery as well as in other sites in the peritoneal cavity. 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 (Lumb et al., 1985).

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 non-human primate.

## Drug-induced inflammation, erosions, ulcers

Although the stomach and to a certain extent the small intestine remain the primary sites of predilection for the ulcerogenic action of non-steroidal anti-inflammatory, the colonic mucosa may become involved under certain conditions. Less common complications of non-steroidal anti-inflammatory drugs and potassium

chloride therapy in humans are colonic strictures. It appears that nonsteroidal anti-inflammatory drugs produce local inflammation followed by focal scarring of the submucosa with constriction and formation of a mucosal diaphragm whereas potassium causes segmental full thickness scarring and constriction (Fellows et al., 1992; Haque et al., 1992; van Velzen et al., 1996; Wolfe et al., 1999). Another form of induced colon damage has been reported in children with cystic fibrosis, the majority of who take high strength pancreatic-enzyme supplements to control malabsorption (Smyth et al., 1994; FitzSimmons et al., 1997). This condition has distinctive pathological features. There is involvement a long segment of ascending colon by a fusiform stenosis primarily as a result of submucosal thickening by deposition of mature collagen. The mucosa appears relatively spared but shows some ulceration and reparative changes (van Velzen et al., 1996). Although it has been suggested that the changes may have been linked to the methylacrylate 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 more recent availability of high dose forms (FitzSimmons et al., 1997). In view of their usage for over 50 years, preclinical data on this material is scarce.

Colonic damage can be induced experimentally by administration of therapeutic agents. Dogs administered 2.5 mg/kg indomethecin orally each day for periods of up to 23 days developed not only gastric and small intestinal ulceration but also scattered haemorrhagic erosions in the colon and rectum. Histologically, these lesions were characterised by loss of superficial epithelial cells, mucus-depletion of glandular epithelium, crypt abscesses, frequently with acute inflammation in adjacent lymphoid aggregates in the submucosa (Stewart et al., 1980).

An example of chemically induced colitis of relevance to safety assessment of therapeutic agents is that induced by degraded carrageenans or synthetic sulphated dextrans. Carrageenans are a heterogeneous group of sulphated polysaccharides composed mainly of long chains of D-galactose subunits (D-galactan) derived from red seaweed species which are widely used as food emulsifiers, stabilisers, thickeners and gelling agents (Ishioka et al., 1987). When carrageenans are degraded by acid hydrolysis into smaller molecular weight fragments of about 20,000–40,000 and administered orally in high doses (e.g. 10% of diet) to rats, mice, guinea pigs, rabbits and rhesus monkeys, colitis results (Sharratt et al., 1970; Marcus and Watt, 1971; Benitz et al., 1973; Fath et al., 1984; Kitano et al., 1986). Similarly, colitis has been induced in rats following administration of a 5% dietary admixture of dextran sulphate sodium, a sulphated polymer of glucose (a D-glucose) of molecular weight of 54,000 (Hirono et al., 1981) and a very high molecular weight D-glucan, amylopectin sulphate (Ishioka et al., 1987).

Although histological features of this form of induced colitis vary between study, species and strain, the colitis is generally characterised mucosal ulceration mainly in the caecum but also in the distal ileum, distal colon and rectum. There is mucus-depletion with variable acute inflammatory infiltrate of the in-

tact epithelium, crypt abscesses, inflammatory infiltrate of the lamina propria with oedema, hyperaemia and even vascular thrombosis in the submucosa (Hirono et al., 1981; Fath et al., 1984). Increased proliferative activity of the mucosa is confirmed by an increase in the tritiated thymidine index compared with controls (Fath et al., 1984).

In the caecum of rats, ulcers are linear but often circulating the entire circumference of the intestinal wall with subsequent scarring and stricture formation (Oohashi et al., 1981). 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 (Oohashi et al., 1981). Foamy macrophages containing metachromatic material, presumably polysaccharide, are also seen in the lamina propria, submucosa, regional lymph nodes, liver and spleen (Hirono et al., 1981; Oohashi et al., 1981).

The cause of this colitis is 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 20,000–60,000 tend not to incite colitis. An exception to this is the agent amylopectin sulphate, 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 (Ishioka et al., 1987).

It has been suggested that colonic disease produced by these agents is in some way linked to induced changes in intestinal microflora (Marcus and Watt, 1971) although the evidence for this is conflicting (Ishioka et al., 1987). A recent study in guinea pigs and rats using permeability markers of different molecular weights has suggested that degraded carrageenans enhance intestinal permeability in the absence of overt ulceration (Delahunty et al., 1987). It was therefore proposed that carrageenan-induced colitis could be the result of increased intestinal permeability to antigenic or inflammatory substances normally resident in the large intestine. Moreover, long-term administration of high doses of these agents to rats leads to the development of colorectal cancer despite their being devoid of any mutagenic potential (see below). The only obvious features, which are common to a number of these non-genotoxic agents, is chronic inflammation and increased proliferative activity.

The rectal administration of therapeutic agents and surfactants may also induce similar ulcerative and inflammatory changes. Chemical colitis resembling pseudomembranous colitis has been reported in man as a result of chemical cleaning agents accidentally induced by endoscopic examination (Jonas et al., 1988). Cellular degeneration, with loss of mitotic activity and mucin depletion can also occur in the colon following treatment with antimitotic drugs.

Lymphoid infiltrates without tissue damage were reported in the large bowel of rats treated with human recombinant interleukin-2 (Anderson & Hayes 1989).

## Pigmentation

Melanosis coli is a well-described phenomenon in man associated with chronic ingestion of anthraquinone purgatives. It is considered to be due to the excessive accumulation of lipofuscin-like pigment in the macrophages of the colonic lamina propria (Schrodt, 1963; Ghadially and Parry, 1966; Steer and Colin-Jones, 1975). This pigment probably originates from organelles of epithelial cells or macrophages, which are damaged by treatment. Similar morphological changes have been induced in laboratory animals (guinea pigs) by treatment with anthraquinones (Walker et al., 1988). As a result of these animal studies, Walker et al. (1988) 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.

Lipofuscin and iron pigment is occasionally observed in the lamina propria of untreated rodents, notably hamsters, presumably a result of ageing, previous inflammatory processes and haemorrhage.

## Hyperplasia, focal hyperplasia, diffuse hyperplasia, atypical hyperplasia, dysplasia and 'transitional mucosa'

As in other glandular epithelial tissues, hyperplasia may be focal or diffuse with or without atypical cellular features. The term used for hyperplasia with atypical features is *atypical hyperplasia* in the IARC classification (Mohr, 1997) although others use the term *dysplasia*.

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 is sufficient to initiate hyperplasia including thickening of the muscle coats (Dowling et al., 1967; Stragand and Hagemann, 1977).

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, Barkla and Tutton (1985) showed that the remaining proximal segment of the right side of the colon showed an increase in the thickness of the mucosa and the muscularis externa as well as enlargement of lymphoid aggregates. Histologically, the mucosa of the right side of the colon was uniformly thickened showing accentuated folds, elongated mucosal glands with increased height of the surface columnar cells. The changes were most marked up to 30 days following surgery but were less pronounced after 72 days. There was also a significant increase in the mitotic index in the proximal segment at 7 days although at 14 days and later the mitotic index had returned to normal. The distal, down-stream segment showed 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 (Barkla and Tutton, 1985).

A similar form of uniform colonic hyperplasia affecting principally the caecal and right-sided colonic mucosa also occurs in rats following oral administration of sulphated dextrans of molecular weight of approximately 400,000 (Figs 52 and 53). Oral administration of a wide range of compounds such as raw and chemically modified starches, various dietary fibres, caramels, sugar alcohols (lactitol, sorbitol, mannitol, xylilol), lactose, a synthetic polydextrose, polyethylene glycol and magnesium sulphate to rats or hamsters has also been linked to an increase caecal size and colonic mucosal hyperplasia (Leegwater et al., 1974; Roe and Bär, 1985; Newberne et al., 1988; Stark et al., 1996). The characteristic histological appearance of the caecum following administration of these agents is lengthening of the mucosal glands which are lined by epithelium composed of increased numbers of enlarged epithelial cells (i.e. hypertrophy and hyperplasia) showing increased proliferative activity and more rapid incorporation of tritiated thymidine (Newberne et al., 1988). In addition, mucosal and submucosal oedema has been reported in association with the administration of lactose and increased mucosal lymphoid aggregates following lactose or xylitol feeding (Newberne et al., 1988). Changes in the colon due to fibre are complex. Morphometric analysis has shown that changes to the mucosa depend on the fibre type (Stark et al., 1996). There may also be an interaction between dietary fibre content and colonic microflora that influences mucosal growth, although the mechanism is unclear (Whiteley et al., 1998). Hypertrophy of the

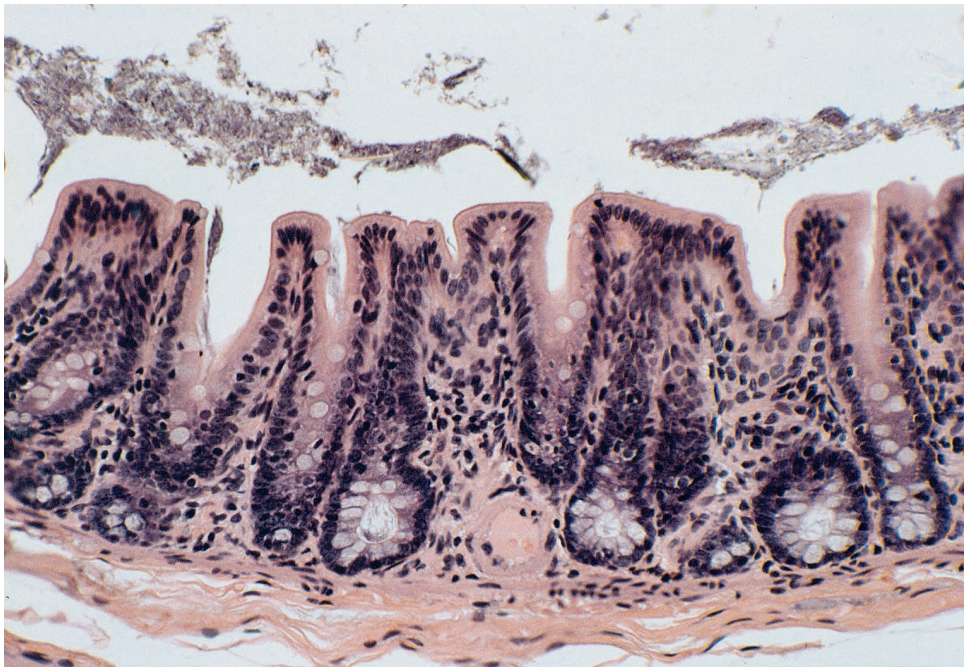

Fig. 52. Normal colonic mucosa from a Sprague-Dawley rat. (HE, ×40.)

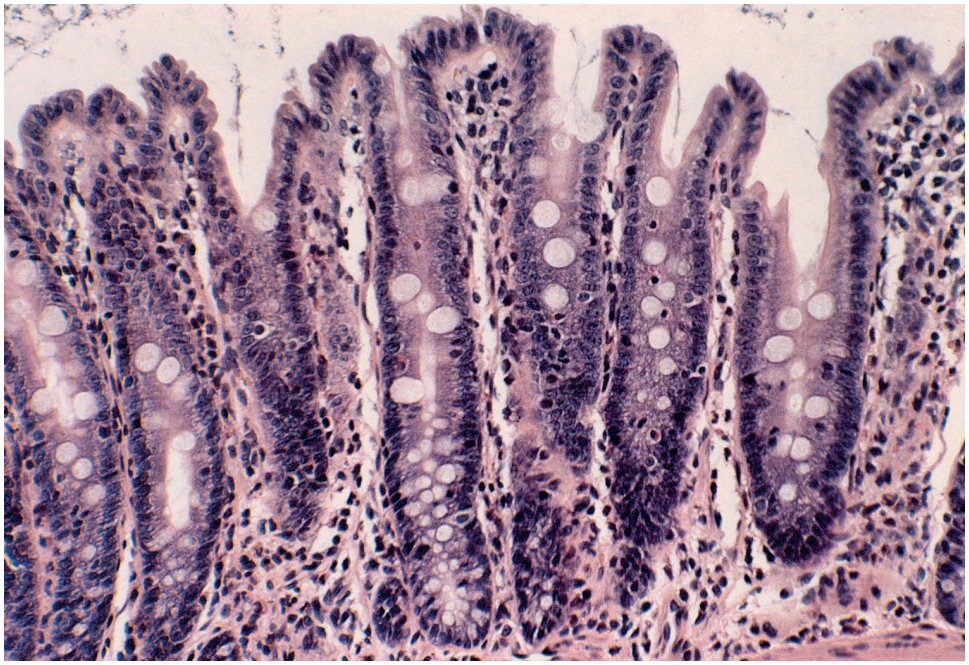

Fig. 53. Similar area of colonic mucosa to that seen in Fig. 52 at the same magnification but from a rat treated with 10% dextran (molecular weight 500,000) in the diet for 2 weeks. This shows diffuse hyperplasia of the mucosa with elongation of colonic glands that are lined by relatively normal epithelial cells with abundant mucin and prominent vesicular nuclei. (HE, ×40.)

muscularis external is also reported in rats fed high fibre diets (Stark et al., 1996).

As the increase in caecal size and mucosal hypertrophy appears generally related to the osmotic activity of the caecal contents in rodents treated with these agents, it has been postulated that the changes represent a physiological adaptation to increased osmotic forces, irrespective of the contributing compounds (Leegwater et al., 1974).

Treatment of rodents with antibiotics also causes caecal enlargement or dilatation without significant histopathological changes, probably as a result of changes in caecal microflora. It has been suggested that the enlargement relates to accumulation of urea as a result of inhibition of bacterial ureases (Juhr and Ladeburg, 1988). 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 posses the potential to directly influence absorption and metabolic functions of mucosal cells (van Leeuwen et al., 1986).

Long-term administration of 16,16-dimethyl prostaglandin E2 to rats also produced thickening of the proximal colonic mucosa, although this was less

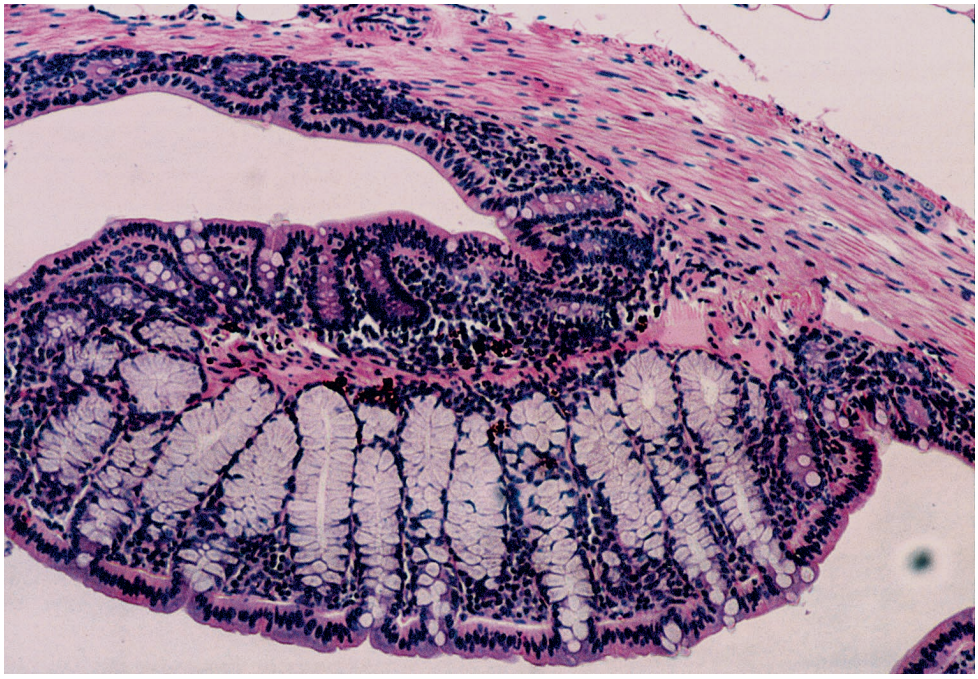

Fig. 54. Section from the colon of an aged hamster from a colony that developed intestinal inflammation and neoplasia spontaneously. This shows focal mucous cell hyperplasia characterised by enlargement and lengthening of the colonic glands with lining cells replete with mucins. (HE, ×25.)

marked than in the stomach and duodenum (Reinhart et al., 1983) and similar changes have been reported in rats treated with other prostaglandin E analogues (Levin, 1988).

As in the small intestine, administration of epidermal growth factor to rats and cynomolgus monkeys induces hyperplasia of the colonic mucosa characterised 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 (Breider et al., 1996; Reindel et al., 1996).

In common with other epithelial surfaces, atypical hyperplasia is associated with the development of colonic cancer in both man 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 characterised by lengthening, dilatation and branching of glands. The epithelium lining these glands shows mucous cell hyperplasia (goblet cell hyperplasia, see Fig. 54), goblet cells containing predominantly sialomucin instead of the normal sulphomucin (Filipe and Branfoot, 1974; Filipe, 1975; Shamsuddin and Trump, 1981; Olubuyide et al., 1985). Despite mucin alterations, activities of glucose-6-phosphatase, glucose-6-phosphate dehydrogenase and gly-

ceraldehyde-3-phosphate dehydrogenase were shown to be normal in this epithelium in rats treated with 1,2-dimethylhydrazine (Mayer et al., 1987). In man, this form of hyperplastic mucosa associated with cancer, has been termed 'transitional mucosa' (Filipe and Branfoot, 1974).

As lesions become more atypical, these dilated, branched glands become more complex and lined by epithelium that shows increasing pseudostratification and vesicular cell nuclei. For example 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 (Shamsuddin and Trump, 1981). In contrast to goblet cell hyperplasia, these atypical zones show increased activity of glucose-6-phosphate, glucose-6-phosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase (Mayer et al., 1987). Whiteley et al. (1996) described similar alterations in rats treated with azoxymethane and the non-genotoxic agent dextran sulphate. These authors also demonstrated that these atypical foci could be identified by low power microscopy as aberrant crypt foci by translumination of the whole mounts of the fixed mucosa stained with methylene blue.

*Note:* 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 7 days. The colonic mucosa showed an increase in sulphomucin (high-iron diamine positive) containing goblet cells in the surface epithelium (Park et al., 1987).

## Neoplasia

Adenomas and adenocarcinomas of the small and large intestine are infrequent spontaneous neoplasms in laboratory animals compared with man 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 man these are located most frequently in the distal colon and rectum (Lingeman and Garner, 1972). 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 ileocaecal valve (DePaoli and McClure, 1982).

In rats and mice, spontaneous intestinal neoplasms are uncommon although adenocarcinomas are occasionally observed in the ileum or colon in mice and rats used in chronic toxicity studies and carcinogenicity bioassays (Burn et al., 1966; Wells, 1971; Maeda et al., 1985; Greaves and Faccini, 1992; Zwicker et al., 1992). Most of these arise in the small intestine and appear to originate in the distal part of the small intestine, caecum and right side of the colon. They may produce metastases, mostly to liver and lungs. In a review of spontaneous adeno-

carcinomas developing over a 17-year period in Wistar rats, Vandenberghe et al. (1985) identified 17 adenocarcinomas, all in ascending colon. In 15 of these cases there appeared to be an intimate relationship with campylobacter-like organisms together with diverticulae and chronic inflammation. These authors suggested that the organisms and the associated inflammation were involved in the pathogenesis of these cancers. 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 (Heyer et al., 1999).

Some hamster colonies, liable to develop inflammatory bowel disease (see above), also have a high incidence of small and large intestinal polyps, adenomas and adenocarcinomas (Fortner, 1957; van Hoosier et al., 1971; personal observations). 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 (van Hoosier et al., 1971).

Adenocarcinomas are 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 (Ward, 1974; Shamsuddin and Trump, 1981; Freeman, 1983). However, there are differences between reported studies. Some have shown that these experimental carcinomas arise from pre-existing adenomas consistent with the 'adenoma-carcinoma sequence' theory (Ward, 1974; Ward et al., 1977). Others suggest that they arise 'de novo' from altered mucosa as microinvasive carcinomas (Sunter et al., 1978; Maskens and Dujardin-Loits, 1981; Rubio et al., 1986). These differences may be partly the result of different dosage schedules. Rubio et al. (1988) have shown that a single dose of 1,2-dimethyhydrazine produces non-polypoid, micro-invasive carcinomas, particularly in the mucosa overlying lymphoid aggregates, whereas in their earlier studies using multiple doses in the same strain of rat, an adenoma-carcinoma sequence was more evident. In addition, there are undoubtedly species and strain differences in the response to these agents. Teague et al. (1981) demonstrated clear differences in the distribution and both macroscopic and histological types of adenomas and adenocarcinomas between three different inbred strains of rat given a similar dosage regimen of 1,2-dimethylhydrazine. In general, many of these carcinomas develop in the distal colonic segments similar to the distribution of human colorectal cancer, although tumours also develop in the proximal colon and in the ileum in rats treated with this agent (Ward, 1974; Shamsuddin and Trump, 1981; Teague et al., 1981).

Neoplasms occurring in the rat colon following administration of high doses of degraded carrageenans and sulphated dextran also commonly occur in the distal colon and rectum and are commonly polypoid adenomas and adenocarcinomas (Hirono et al., 1981; Oohashi et al., 1981; Ishioka et al., 1987). However, in these models adenomas and adenocarcinomas also occur in the caecum and proximal colon and squamous carcinomas are sometimes seen in association with squamous metaplasia at the colorectal junction (Oohashi et al., 1981). The

pathogenesis of neoplasms induced by carrageenans and dextrans remains unexplained. Although they are biologically active compounds, they are non-mutagenic in the usual short-term tests (Ishioka et al., 1987). It has been suggested that carrageenans act as tumour promoters as they potentiate the appearance of carcinomas in rats treated with standard intestinal carcinogens (Watanabe et al., 1978; Hirono et al., 1981). Conversely it has been proposed that these agents are tumour initiators based on the development of carcinomas in rats treated with degraded carrageenans for only 2 months (Oohashi et al., 1981). 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.

The only consistent association of carrageenans with development of carcinoma in rats is that of chronic inflammation and increased cell proliferation. Although dose levels needed to produce inflammation are far higher than any exposure likely to be achieved in man, interpretation of this inflammation-cancer sequence in rats remains a challenge in safety assessment for similar xenobiotics. This situation is interest in view of the unquestionable risk of carcinogenesis in ulcerative colitis in man (Riddell et al., 1983).

A similar range of neoplasms can be defined histologically in both human and experimental pathology. It is appropriate, to use the same classification for all species including man. Lingeman and Garner (1972) who reviewed a range of tumours from domestic and laboratory animals were able to employ the classification for human gastrointestinal neoplasms. A similar approach has been used in the IARC classification of rat intestinal tumours (Mohr, 1997). This classification can be summarised as follows:

*Adenoma (adenomatous polyp)*

These represent localised, sessile or polypoid neoplasms composed of proliferating tubular glands, which show varying degrees of nuclear hyperchromatism, pseudostratification and cellular pleomorphism. A useful scheme for grading the carcinogenic potential of hyperplastic mucosa and adenomatous polyps in man based on the degree of epithelial pseudostratification has been proposed by Kozuka (1975). Although experimental neoplasms may not always show the full spectrum of these changes reported in man, this grading provides a useful baseline concept for the assessment of these non-invasive proliferative lesions.

With increased nuclear pseudostratification and atypical branching of the glandular structures of these polyps becomes more prominent. If neoplastic cells or glands are seen in the stroma of the stalk or base the diagnosis of carcinoma is made.

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 I).

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