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Anatomical and physiological differences between various species used in studies on the pharmacokinetics and toxicology of xenobiotics. A review of literature.

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Glossary

ASA	acetylsalicylic acid
CDNB	1-chloro-2,4-dinitrobenzene
CCK	Cholecystokinine
C. perfringens	Clostridium perfringens
Coprophagy	ingestion of faeces
CYP450	cytochrome P450
DCNB	dichloronitrobenzene
E.coli	Escherichidae coli
GSH	glutathione
GST	glutathione *S*-transferase
IMMC	interdigestive migrating motility complex
Log $K_{o/w}$	log K octanol/water
MALT	mucosa associated lymphoid tissue
Microsomes	membrane phragments of endoplasmatic reticulum
PEG	polyethylene glycol
Paracellular transport	transport via the cavity between cells
PNPA	*p*-nitrophenyl acetate
Postprandial	fed state after a meal
Preprandial	fed state before a meal
RIVM	Rijksinstituut voor Volksgezondheid en Milieu
Transcellular transport	transport through a cell
UDPGT	uridine diphosphate glucuronosyltransferase

Abstract

This report is the first report of the project named: Selection of species and interspecies differences in relation to kinetics and dynamics of compounds. An inventory is made of relevant physiological and anatomical characteristics of various species most commonly used in studies on pharmacokinetics and toxicology of oral exposure to xenobiotics. The following species have been studied : man, mouse, rat, rabbit, dog, (mini)pig, and monkey. For each species, anatomical and physiological characteristics of the different compartments of the gastrointestinal tract: mouth, stomach and small intestine, of the bile and of the metabolism in liver and small intestine were reviewed. This report is primarily meant to become a reference work for researcher using animal models to study pharmacokinetics and toxicokinetics of xenobiotics and for risk assessors on pharmacokinetics and toxicokinetics. The aim is that it should be possible to make a better choice of an animal model to study a certain compound using the available information on interspecies differences in anatomical and physiological characteristics.

Contents

Samenvatting

In het geneesmiddelenonderzoek wordt traditioneel gebruik gemaakt van gegevens uit dierstudies om de eerste dosis in de mens te schatten en om de veiligheid en de werkzaamheid te testen van nieuw ontwikkelde geneesmiddelen. In het algemeen worden dierstudies voor risk assessment gebruikt voor: farmacologisch en toxicologisch onderzoek, om voorspellingen te doen over de farmacokinetiek in de mens en om nieuwe doseringen en formuleringen te screenen. Er zijn vanzelfsprekend vele verschillen tussen de verschillende diersoorten onderling én tussen dieren en de mens die van invloed zijn op de farmacokinetiek/farmacodynamiek van een stof. Aangenomen wordt dat met name de anatomische en fysiologische verschillen verantwoordelijk zijn voor de discrepantie in absorptie van stoffen in dieren en mensen. De mate waarin de farmacokinetiek van een stof wordt beïnvloed door interspeciesverschillen is afhankelijk van de fysisch/chemische eigenschappen van de stof. Daarom kan inzicht in de consequenties van anatomische en fysiologische interspeciesverschillen voor de farmacokinetiek van verschillende klassen van verbindingen misschien leiden tot een verbeterde proefdierkeuze en als gevolg daarvan tot een meer optimale extrapolatie van dier naar mens. In 1998 is er een project gestart bij het Laboratorium voor Blootstellingsonderzoek en Milieu-epidemiologie waarin speciaal aandacht wordt besteed aan een beter onderbouwde proefdierkeuze met betrekking tot farmacokinetisch en toxicokinetisch onderzoek. In dit eerste rapport wordt een overzicht gegeven van de meest relevante fysiologische en anatomische eigenschappen van verschillende species die over het algemeen gebruikt worden in farmacokinetisch en toxicologisch onderzoek van orale blootstelling aan xenobiotica. Dit rapport is met name bedoeld als naslagwerk. De volgende species worden in dit rapport behandeld: mens, muis, rat, konijn, hond, (mini) varken en aap. Er wordt een overzicht gegeven van de anatomische en fysiologische eigenschappen van de verschillende delen van het maag-darmkanaal: nl. van de mond, de maag en de dunne darm en van de gal en van het metabolisme in de lever en de dunne darm. De bijdrage van absorptie in de mond in vergelijking met de absorptie in de rest van het maag-darmkanaal zal relatief klein zijn omdat de verblijftijd van verbindingen in de mond relatief kort is en omdat speeksel-enzymen geïnactiveerd worden zodra ze de maag bereiken. Een van de meest opvallende interspecies verschillen in de mond is de gekeratiniseerde mucosa in de rat. Dit maakt dit dier ongeschikt om te gebruiken als model voor het bestuderen van absorptie in de mond (buccale absorptie). De maag daarentegen heeft waarschijnlijk meer invloed op het absorptie proces. De anatomie van de maag is vergelijkbaar in de mens, de hond, het varken en de aap. De maag van knaagdieren echter is complexer en is verdeeld in een glandulair en een niet-glandulair gedeelte. Fysiologische gegevens die belangrijk zijn voor de absorptie van verbindingen zijn: maag motiliteit, maaglediging, pH and microbiële flora in de maag. Op basis van de beschikbare literatuur kon worden geconcludeerd dat motiliteitspatronen en lediging van de maag onder nuchtere omstandigheden in mens en honden in grote lijnen overeenkomen. Aan de ander kant zijn er ook belangrijke verschillen gevonden: de maagledigingssnelheid en de achtereenvolgende terugkeer naar het motiliteitspatroon in de gevastte toestand is langzamer in honden; de basale maagzuur-uitscheiding is lager in honden en de groei van bacteriën in de maag is

hoger in honden. Tenslotte is geconcludeerd dat er weinig literatuur beschikbaar is wat betreft de anatomie en fysiologie van de maag in de overige species (muis, rat, konijn, varken en aap). Het laatste deel van het maag-darmkanaal dat bestudeerd is in dit rapport is de dunne darm. Er zijn veel overeenkomsten gevonden in de anatomie van de dunne darm in de verschillende species, maar ook veel verschillen. Het blijkt bijv. dat knaagdieren en lagomorphen een goed ontwikkeld cecum bezitten, wat hen in staat stelt om cellulose te verteren. Fysiologische eigenschappen van de dunne darm die een rol spelen in de absorptie van verbindingen zijn; verschillen in absorptiemechanismen, motiliteit en verblijftijden en de pH. Er ontbreken nog gegevens over deze eigenschappen in de verschillende species, hetgeen het maken van een compleet overzicht belemmert. Als verbindingen en/of voedsel de dunne darm inkomen, wordt ook gal uitgescheiden in de dunne darm. Gal is belangrijk voor de vertering en absorptie van vetten en vetoplosbare vitaminen in de dunne darm. Wat betreft de eigenschappen van de gal: secretie-snelheid, galzoutconcentratie, galzoutsamenstelling en vetzuursamenstelling van de gal, lijkt geen van de species equivalent te zijn aan de mens. Als laatste zijn de interspeciesverschillen wat betreft metabolisme van stoffen in de lever en in de dunne darm bestudeerd. De conclusie is dat er veel data beschikbaar zijn, maar dat het ook moeilijk blijft om de resultaten van verschillende studies met elkaar te vergelijken omdat de experimentele omstandigheden meestal niet gelijk zijn. Tot nu toe zijn er wel een aantal belangrijke verschillen in enzymactiviteiten tussen species gevonden en bij het kiezen van een diermodel voor een bepaalde verbinding moet rekening gehouden worden met deze verschillen. De uiteindelijke conclusie van dit rapport is dat nog relatief veel informatie over m.n. relevante fysiologische parameters ontbreekt. Daarnaast blijken ook steeds meer specialistische onderwerpen, zoals interspecies verschillen ten aanzien van inductie van metabolisme, verschillen tussen stammen binnen één species, effecten van leeftijd, etc. van belang te zijn. Als vervolg op dit rapport zal worden geprobeerd om deze ontbrekende informatie en informatie over de meer specialistische onderwerpen alsnog te verkrijgen.

Summary

In drug development animal data traditionally are used to estimate first-dose to man and to assess safety and efficacy of newly developed drugs. More generally, the objectives of animal studies in risk assessment are to support research on pharmacology and toxicology, to predict human pharmacokinetics and to support screening of new dosage forms and formulations. There are of course many differences between animal species among themselves and between animals and man, which may influence the pharmacokinetics/pharmacodynamics of a compound. It is assumed that anatomical and physiological differences are the main factors that cause discrepancy between human and animals with respect to the absorption of xenobiotics. The extent to which the pharmacokinetics of a xenobiotic is affected by interspecies differences is depending on the physical/chemical characteristics of the compound. Therefore, insight into the consequences of interspecies differences in anatomy and physiology on pharmacokinetics of various classes of compounds may lead to improved species selection and subsequently to improved animal-human extrapolation. In 1998 a project has been started at the Laboratory of Exposure Assessment & Environmental Epidemiology in which attention is focussed on improved species selection with regard to pharmacokinetic/toxicokinetic studies. This is the first report in which inventory was made of relevant physiological and anatomical characteristics of various species most commonly used in studies on pharmacokinetics and toxicology of oral exposure to xenobiotics. This report is primarily meant as a review. The following species were taken into account: man, mouse, rat, rabbit, dog, (mini)pig, and monkey. The anatomical and physiological characteristics of the different compartments of the gastrointestinal tract: mouth, stomach and small intestine, of the bile and of the metabolism in liver and small intestine were reviewed. It appeared that the transit time of compounds in the mouth is relatively short and that salivary enzymes become inactive reaching the stomach. For these reasons it is expected that the attribution of buccal absorption to the total absorption process will be small. The most pronounced interspecies difference found for the mouth is the keratinised oral mucosa of the rat, which makes this animal not suitable for studying absorption in the mouth (buccal absorption) . The stomach on the on other hand may have more influence on the absorption process. The stomach is fairly similar in humans, dogs, pigs and monkeys regarding anatomy. The stomach of rodents, however, is more complex and is divided into a glandular and a non-glandular portion. Physiological characteristics, which may be important in absorption of compounds, are gastric motility, gastric emptying, pH and microbial flora in the stomach. Based on the available literature, it can be concluded that the gross physiology of the stomach in humans and dogs is similar in the fasted state, with similar motility patterns and gastric emptying of indigestible solids and liquids. However, there are also some important differences, such as the meal emptying rate and subsequent return of the fasting motility pattern being much slower in dogs, basal rate of gastric acid secretion being lower in dogs, and bacterial growth in the stomach being higher in dogs. Finally, it was also concluded that there is very little scientific literature concerning the anatomy and physiology of the stomach of other species

(mouse, rat, rabbit, pig and monkey). The last part of the gastrointestinal tract, which was taken into account in this report, is the small intestine. There are many similarities regarding the anatomy of the small intestine in the various species, but also many differences. It was for instance found that rodents and lagomorphs possess a very well developed cecum, which enables them to efficiently utilise cellulose. Physiological characteristics of the small intestine playing a role in the absorption of compounds are differences in absorption mechanisms, motility and transit times and pH. It was concluded that data regarding the small intestine in the different species studied are far from complete as well and this makes it difficult to give a complete overview. As compounds and/or food enter the small intestine, bile is excreted into the small intestine as well. Bile is important for digestion and absorption of fats and fat-soluble vitamins in the small intestine. Considering bile characteristics (flow-rate, bile salt content, bile salt composition and bile lipid content) no animal species was found to be equivalent to humans. Finally, interspecies differences in hepatic and intestinal metabolism were studied. It was concluded that there is quite some data available, but it always remains difficult to compare the results of different studies, because of differences in experimental methods. Several important differences in enzyme activities have been found so far between species and these differences should be taken into account when choosing an animal model to study a certain compound, which could be metabolised by such enzymes. It can be concluded that important information on physiological parameters is lacking. Apart from that it appeared that more specialised topics, like interspecies differences in relation to induction of metabolism, differences between strains of a species, effects of age, need also to be reviewed. As an ongoing action of this project it will be tried to obtain this information.

1. Introduction

1.1 Outline of project

During the last decade, the use of experimental animals has reduced dramatically in countries like the Netherlands and the U.S.. Nevertheless, animals will still be required for predicting pharmacokinetics and pharmacodynamics of a compound in humans. In drug development animal data traditionally are used to estimate first-dose to man and to assess safety and efficacy of newly developed drugs. More generally, the objectives of animal studies in risk assessment are to support research on pharmacology and toxicology, to predict human pharmacokinetics and to support screening of new dosage forms and formulations.
Numerous studies in which a variety of compounds were tested have demonstrated that interspecies differences can be substantial, hampering reliable extrapolation of the results to the human situation. There are of course many differences between animal species as well as among themselves as between animals and man. These differences may influence the pharmacokinetics/ pharmacodynamics of a compound, e.g. long nose (inhalatory exposure) and tails, furry (topical application), diet, coprophagy, intestinal anatomy and physiology, differences in metabolic pathways, extent of biliary excretion, body surface relative to body weight, metabolic rates, etc. There are, on the other hand also various similarities between animals and man, e.g. warm blood, liver/lung is important for metabolism, liver/kidney important for elimination, comparable circulatory system, specialised mechanisms for elimination of xenobiotics.
It is assumed that anatomical and physiological differences are the main factors that cause discrepancy between human and animals with respect to the absorption of xenobiotics. These differences, in contrast to differences in the process of elimination, are rarely related to size, body weight, etc. [http://www.shef.ac.uk, ...]. This implicates that extrapolation by means of scaling factors will fail. The extent to which the pharmacokinetics of a xenobiotic is affected by interspecies differences is depending on the physical/chemical characteristics (e.g. molecular weight, pKa) of the compound. Therefore, insight into the consequences of interspecies differences in anatomy and physiology on the pharmacokinetics of various classes of compounds may lead to improved species selection and subsequently to improved animal-human extrapolation. [Jezyk *et al., 1992*].

In 1998 a project has been started at the Laboratory of Exposure Assessment & Environmental Epidemiology in which attention is focussed on improved species selection with regard to pharmacokinetic/toxicokinetic studies. The aim of the project is gaining insight into the impact of anatomical and physiological differences between species on the

pharmacokinetics (especially on oral bioavailability) of various classes of compounds. The information gathered should be implemented into a decision tree as a tool for optimised species selection.

1.2 Rationale of literature study

The planning of the project is divided into four steps. Step 1 deals with making up an inventory of species which have to be taken into account. Step 2 concerns making up the inventory of relevant physiological and anatomical characteristics for the selected species. In the third step, the pharmacokinetics of various classes of compounds, varying in physical/chemical properties, is compared for the selected species. Finally, the feasibility of making a decision tree for optimisation of species selection will be determined and if possible the decision tree will be developed. The project will first be focussed on *oral bioavailability* since information on this topic is most relevant for risk assessment on xenobiotics. In a later phase the project might be extended for topical bioavailability, since this route of exposure is especially relevant for risk assessment on contaminants, additives, etc.. Moreover, it is to be expected that this route of exposure demonstrates substantial interspecies differences.

Step 1 of the project revealed that the following species had to be taken into account: 1) Man, 2) Mouse, 3) Rat, 4) Rabbit, 5) Dog, 6) (Mini)pig, and 7) Monkey. These species were selected because they are commonly used in studies on pharmacokinetics and toxicology of xenobiotics. Otherwise, the pig was chosen since general statements can be found in literature, that the gastrointestinal tract of pigs is quite comparable to that of man. In step 2; mainly reviewing literature information on anatomy and physiology of these species was derived. Literature was divided into 5 topics, i.e. 1) mouth, 2) stomach, 3) small intestine, 4) bile, and 5) metabolism (liver and intestine). For each relevant species information was gathered per item. This literature was read and summarised by a group of 5 reviewers. Per review meeting one topic was discussed; 2 articles per reviewer. Each meeting was summarised by one reviewer, taking the reviews, the discussion and additional information into account.
Moreover, pharmacokineticists of pharmaceutical industries and research institutes were asked to contribute to this project. Their knowledge on interspecies differences often is based on daily research practice but is rarely to be found in scientific publications.

At April 22, 1999 a workshop was organised at the National Institute of Public Health & the Environment. The workshop was attended by pharmacokineticists of pharmaceutical industries and research institutes and by pharmacokineticists and risk assessors of the National Institute of Public Health & the Environment. The information gathered by reviewing literature was presented at that workshop and discussed by the attendees (appendix I, VERSLAG of the workshop + list of attendees).

As an ongoing action, the participants of the workshop will be asked to fill in a questionnaire. This questionnaire should help to fill in the gaps of information and should gain insight into the every day practice of dealing with interspecies differences.

1.3 Outline of report

The present report covers the first two steps of the project and therefore only contains a review of literature on anatomy and physiology of man and various species of experimental animals. This review is meant to become a reference work for those who are interested in physiological and anatomical differences of the gastrointestinal tract between man and those animals. During the realisation of the report, it became clear that such a reference work needs some time for development. It appeared that, reviewing biomedical and veterinary databases, various parameters were not available. The authors of this report intent to give an overview of figures and facts mainly obtained from biomedical literature databases. Interpretion of consequences for pharmacokinetics/toxicokinetics of compounds will be discussed in future reports.
Chapters 2-6 give a review of various compartments of the gastrointestinal tract. Each chapter is started with an overview of the anatomy of the concerning compartment in *humans*. Subsequently, interspecies differences concerning anatomy are discussed. In the second part of the chapters, physiological features, like pH and transit time are discussed for the relevant species. Chapter 7 discusses the information gathered so far. The report concludes with suggestions for ongoing research.

2. Mouth

2.1 Anatomy

2.1.1 Anatomy in man

2.1.1.1 The buccal cavity

The buccal cavity is formed by the cheeks, the hard and soft palates (the roof of the mouth) and tongue. The cheeks form the lateral walls of the buccal cavity, which are covered by non-keratinised stratified squamous epithelium. The hard palate, which is formed by the roof bones, is covered by mucous membrane and forms a bony partition between the buccal cavity and the nasal cavity. The soft palate forms the back (muscular) portion of the roof and is also lined by mucous membrane [Christie *et al.*, 1995].

The tongue, together with its associated muscles, forms the floor of the buccal cavity. It is composed of skeletal muscle covered with mucous membrane [Tortora and Grabowski, 1996]. The so-called extrinsic muscles move the tongue from side to side and in and out, and the intrinsic muscles alter the shape and size of the tongue for speech and swallowing. To roughen the surface of the tongue the upper and lateral faces are covered with papillae, which are projections of the lamina propria (see oral mucosa) covered with epithelium. Humans have three different papillae: filliform, circumvallate and fungiform. The filliform are distributed in parallel rows over the front of the tongue. The fungiform are distributed among the filliform papilla, appear as red dots on the surface and are more numerous near the tip of the tongue. The circumvallate papillae are arranged in the form of an inverted V on the back part surface of the tongue. The circumvallate and fungiform papilla contain taste buds [Guyton, 1991; Tortora and Grabowski, 1996].

2.1.1.2 The oral mucosa

The buccal cavity is covered by a moist stratified squamous epithelium. It functions as a barrier for ions and molecules between the internal and external environment [Barsuhn *et al.*, 1988; Orlando *et al.*, 1988]. The oral or buccal mucosa consists of 3 layers: the epithelium, a lining layer in direct contact with the contents of the buccal cavity, an underneath it a layer called the lamina propria and a thin layer of smooth muscle, the muscularis mucosae (see Figure 2.1). The epithelial layer in humans is mainly non-keratinised stratified squamous epithelium, which has a protective function in the mouth and oesophagus. The lamina propria contains many blood and lymphatic vessels and scattered lymphatic nodules. This layer supports the epithelium, binds it to the muscularis mucosae and provides it with blood and lymph. The blood and lymphatic vessels are the avenues by which absorbed molecules reach other tissue of the body. The muscularis mucosae in turn consist of mucosa, submucosa and muscularis. Next to connective tissue, it contains bloodvessels, glands and lymphatic tissue membrane [Guyton, 1991; Tortora and Grabowski, 1996]. Between the basal layer of the

epithelium and the connective tissue of the lamina propria and submucosa lays a basement membrane, which has two functions:

1. Provide adherence between the epithelium and the connective tissue beneath and to provide mechanical support for the epithelium.
2. Form a barrier to the passage of cells and some large molecules across the mucosa.

The thickness of epithelia in the buccal cavity is not similar throughout the cavity. The buccal tissue is 500 – 600 μm thick, as sublingual tissue (100 – 200 μm) and the roof of the cavity (250 μm) are much thinner [Chen and Squier, 1984; Veillard *et al.*, 1987].

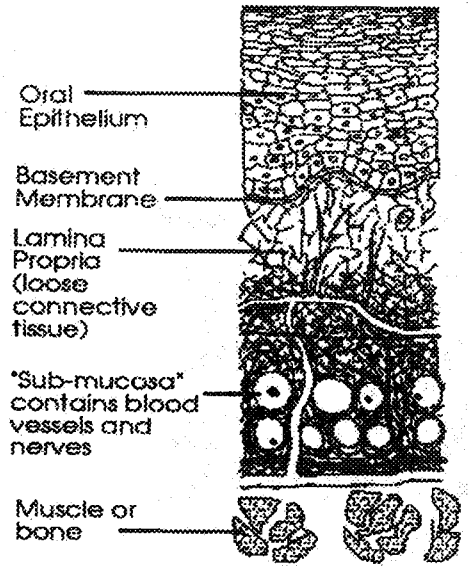

Figure 2.1 Cross section trough the human oral mucosa [Wilson and Washington, 1989, p22]

2.1.1.3 The salivary glands

Humans have three pairs of salivary glands (see Figure 2.2): parotid, submandibular (or submaxillary) and sublingual glands. The parotid glands are located inferior and in front to the ears and secrete saliva via the parotid duct into the buccal cavity. The submandibular glands are located beneath the base of the tongue in the back part of the floor mouth. Next to these greater salivary glands also minor salivary glands are found (labial, buccal and palatal glands). The minor salivary glands are situated in or immediately below the oral mucosa [Wilson and Washington, 1989; Guyton, 1991; Manning *et al.*, 1994; Christie *et al.*, 1995].

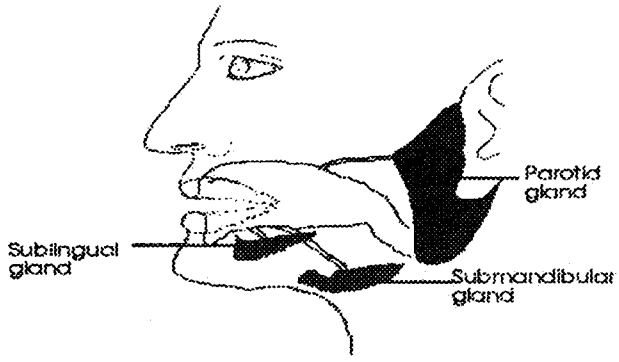

Figure 2.2 The position of the human salivary glands [Wilson and Washington, 1989,p24]

2.1.1.4 The oesophagus

The oesophagus is 25 cm long and has a diameter of ca. 2 cm. It connects the buccal cavity with the stomach. The stratified squamous non-keratinised epithelium lining the buccal cavity is continued through the pharynx down into the oesophagus. The lowest part of the oesophagus (ca. 2 cm) is lined with gastric mucosa and covered by peritoneum. The epithelium is supported by the lamina propria and by the muscularis mucosae. The muscular band consists of two types of layers: an inner circular and an outer longitudinal muscle layer. These layers are responsible for the peristaltic wave to transport food trough the oesophagus. The main body of the oesophagus is lined with small, simple mucous glands. Each gland opens into the lumen by a long duct which pierces the muscularis mucosae [Wilson and Washington, 1989]. A sphincter is situated at the point where the oesophagus enters the stomach to prevent gastro-oesophageal reflux, i.e. to prevent acidic gastric contents from reaching stratified epithelia of the oesophagus, where they can cause inflammation and irritation [Wilson and Washington, 1989; Brown *et al.*, 1993].

2.1.2 Interspecies comparison

2.1.2.1 The buccal cavity

Tongue papillae are also found in other species, as is shown in Table 2.1. Humans, rabbits (New Zealand White) and rats have similar papillae. As observed for humans each vallate and fungiform papilla of the rat and rabbit contains a taste bud. Rabbits have also taste buds on the foliateform papilla. Like humans, rat fungiform papilla are more numerous at the tip of the tongue [Millar and Preslar, 1975; Tortora and Grabowski, 1996]. For the rat 3 types of taste buds are observed, each with their own distinct expression (dark and light) and contents (containing granula, vesicles and smooth endoplasmic reticulum) [Takeda and Hoshino, 1975]. No differences in size or number of taste buds were observed in young (5 – 7 months old) and old rats (23 – 24 months old) [Mistretta and Baum, 1984]. Next to the papillae on the rat tongue bottom also excretory glands and mucous cells emerge that are considered to play a part in cleansing, circulation of tastants and assistance for continuous bathing of the taste

pores by oral secretion. Lymphatic tissue is found in the rat tongue, but lymphatic organs such as lymph follicles are absent.
No information was found with regard to the mouse, dog, pig and monkey.

Table 2.1:Tongue papillae in human, rabbit and rat [Hebel and Stromberg, 1988; Manning *et al.*, 1994; Tortora and Grabowski, 1996]

papillae	human	rabbit (New Zealand White)	rat
(circum) vallate	*	*	*
filliform	*	*	*
fungiform	*	*	*
foliate		*	
linguales			*

*: Present

2.1.2.2 The oral mucosa

The thickness of the buccal mucosa does not vary to a great extent in different species (see Table 2.2). The mucosa of rat and hamster is keratinised which makes these species unsuitable for research on buccal drug delivery. More useful models are the rabbit and dog buccal mucosa both of which have been claimed to be broadly similar in structure and composition to human buccal mucosa species [Harris and Robinson, 1992]. No information was found with regard to the mouse and monkey.

Table 2.2: Characteristics of buccal mucosa from various species [Harris and Robinson, 1992].

species	thickness (µm)	keratinisation
Human [1)]	500 – 600	-
Rabbit [2)]	600	-
Dog [3,4)]	770	-
Pig [3)]	770	-
Rat	--	+

-: absent, +:present, --: no data; ; [1)] [Chen and Squier, 1984]; [2)] [Chen, 1970]; [3)] [Squier and Hall, 1984]; [4)] [Squier and Hall, 1985].

2.1.2.3 The salivary glands

The three major salivary glands, parotid, submandibular and (major) sublingual glands, which are found in man, are also found in rabbit and rat. The parotid and submandibular gland are also found in dog, but it is not known whether dogs also have a sublingual gland [Chauncey *et al.*, 1963; Hebel and Stromberg, 1988; Manning *et al.*, 1994; Höld *et al.* 1998].
Minor salivary glands are observed in the rat (buccal, palatal and lingual glands) and rabbit (zygomatic gland). The rabbit sublingual gland should be named actually the minor sublingual gland, as the major sublingual gland seen in many mammals is not present. On the other hand a zygomatic salivary gland is present in rabbit, which is not present in man, rat and dog [Manning *et al.*, 1994. For monkey, pig and mouse information is lacking.

2.1.2.4 The oesophagus

Only little information was found on the oesophagus in rat, rabbit and pig. The oesophagus of rat (75 x 2 mm) and rabbit has no mucous glands and the cardia of the stomach has a well-developed sphincter, which prevents them from vomiting [Hebel and Stromberg, 1988; Manning *et al.*, 1994]. Morphologically the oesophagus is similar in man and pig; both are omnivores and have a non-keratinised epithelium, submucous glands and similar membrane enzymes. Like in humans, pigs can suffer from reflux oesophagitis and stress ulceration of the oesophagus. The pig oesophagus may therefore be a good model for investigation compared to the human oesophagus [Christie *et al.*, 1995].

2.2 Physiology

2.2.1 Physiology in man

2.2.1.1 The buccal cavity and oral mucosa

In the mouth, food, nutrients etc. are chewed, mixed with saliva, partially digested and absorbed and than transported through the oesophagus to the stomach. The teeth and tongue are involved in the chewing process. The tongue is also involved in taste and swallowing. Saliva plays an important role in the process of digestion, absorption and swallowing.

1. Before swallowing nutrients may already be absorbed in the mouth. Therefore they have to cross the buccal or oral mucosa [Barsuhn *et al.*, 1988]. The outer layer of epithelium is the rate-limiting step of mucosa penetration. Passage across the buccal mucosa is believed to occur by facilitated or passive diffusion through the lipid membrane [Orlando *et al.*, 1988]. But also active transport occurs, which was indicated by measurement of the potential difference across the mucosa (mechanism explored in Ussing chambers). This active transport is primarily mediated by active Na^+ absorption, in which Na^+-K^+-ATPase activity is involved [Utoguchi *et al.*, 1997].

Although normally the contact time between a compound and the mucosa in the buccal cavity is short, some compounds are delivered in this way. Mucosal drug delivery offers several advantages over traditional methods of delivery, including a non-invasive means of administration, avoidance of pre-systemic and hepatic first-pass metabolism and ease of use. An example to deliver drug by this way is the peptide drugs. This delivery route avoids the contact with gastric acid, enzyme-mediated degradation in the gastrointestinal tract, and hepatic metabolism, factors that limit a good bioavailability of the peptide drugs. Drug delivery via the oral cavity can be subdivided in three routes of delivery of which the sublingual and buccal route are commonly used [Harris and Robinson, 1992]:

- Sublingual delivery, which is the administration of drug via the sublingual mucosa (membrane of the ventral surface of the tongue and the floor of the mouth) to the systemic circulation.

- Buccal delivery, which is the administration via the lining of the cheek to the systemic circulation.
- Local delivery, for the treatment of local and periodontal disease.

The characteristics of the sublingual and buccal delivery routes are summarised in Table 2.3.

Table 2.3: Differences between the sublingual route and buccal route in man [Harris and Robinson, 1992]

	Sublingual	buccal
permeability	relatively permeable	much less permeable
absorption rate	Rapid	slower
	easily accessible	suitable for high-molecular weight
	suitable for low-molecular weight molecules	molecules
mucus	No	yes
epithelium	< 40 cell layers	40 – 50 cell layers
epithelium thickness	100 – 200 μm	500 – 800 μm

2.2.1.2 Saliva

In man saliva is mainly produced by the greater salivary glands (parotid gland 23%, submandibular gland 65%, sublingual gland 4%). The minor salivary glands produce only 8% of the total volume of saliva [Höld *et al.*, 1998]. The parotid glands produce only serous, the mandibular glands serous and mucin and the sublingual predominantly mucin. Overall, saliva consists mainly of water (98%). The further 2% consist of proteins, amino acids, mucin, electrolytes such as potassium, sodium, calcium, phosphate and chloride, bicarbonate and cholesterol. In addition, drugs, which are present in the systemic circulation, can be secreted by saliva into the mouth. Saliva contains several enzymes for digestion, such as ptyalin, an α-amylase, which hydrolyses polysaccharides such as glycogen and starch to smaller saccharides, and (lingual) lipase, which hydrolyses triglycerids. Ptyalin has an optimum pH of 6.9, but is stable within the pH range of 4 to 11 and hence it will continue to act until the food is acidified by gastric acid [Wilson and Washington, 1989; Höld *et al.*, 1998].

Salivary secretion is a reflex response controlled by both parasympathetic and sympathetic secretomotor nerves by presentation and ingestion of food [Höld *et al.*, 1998]. The quantity and quality of saliva secretion vary with the nature of nutrition. For example, liquid food decreases the quantity of saliva and a high polysaccharide content increases the ptyalin concentration in saliva. Saliva production is ca. 1 l/day, with a rest flow of approximately 0.5 ml/min. After stimulation the production increases to 7 ml/min whereby the concentration of sodium doubles and the concentration of chloride and bicarbonate also increases strongly. As a result of the increase in bicarbonate saliva concentration, the salivary pH rises with increasing rates of secretion. Saliva pH can range from 6.2 (rest flow) to 7.4 (increased, stimulated flow) [Wilson and Washington, 1989; Höld *et al.*, 1998].

General examples of the functions of saliva regarding humans and animals are [Höld *et al.*, 1998]:

1. Moisten the mucous membranes of the upper aerodigestive tract, to facilitate speech and to control the bacterial flora of the mouth.
2. Supply of enzymes, designated to play an important role in preparing food for digestion.
3. Secrete hormones and other pharmacologically active compounds.
4. Wet the fur of animals with saliva in response to heat stress, thereby obtaining the same cooling possibility available to man by sweating.
5. Defence and killing, although this is observed only by the American screw, a mammalian known to produce toxic saliva.

2.2.1.3 The oesophagus

The pH of the normal oesophagus lumen is usually between 5 and 6. Mucous with a pH of 5 – 6 is secreted into the oesophagus for easy gliding of food. With mucus, also bicarbonate and epidermal growth factor are secreted, which together with bicarbonate present in saliva, protect the lower part of the oesophagus for the acid gastric juice [Wilson and Washington, 1989; Brown *et al.*, 1993]. Liquid passes directly, but for instance capsules taken without water can stick to the oesophagus wall for 2 hours.

2.2.2 Interspecies comparison

2.2.2.1 The oral mucosa

The pH membrane permeability relationship in the mucosa observed for humans was also observed in dogs [Barsuhn *et al.*, 1988]. The active transport way, indicated by measurement of the potential difference across the mucosa, was also found in dogs (Mongrel) and rabbits (New Zealand White). The observed potential difference across the buccal mucosa of humans, dogs and rabbits differed not much [Utoguchi *et al.*, 1997]. It is not clear whether a similar potential difference over the mucosa indicates a similar extent of active transport in these species. Carrier-mediated transport of monocarboxylic acids was observed in epithelial cells from rabbit (Japan White) oral mucosa, indicating the involvement of this mechanism in the absorption of compounds [Utoguchi *et al.*, 1997]. For rats (Sprague-Dawley) it was found that the permeability of compounds across the tongue mucosa is higher when the $\log K_{o/w}$ is higher [Siegel, 1984]. Important is that the pKa of the compound has to be between 5.5 – 7.5 for a good absorption.

2.2.2.2 Saliva

The enzyme activity of different enzymes in saliva from the parotid and submandibular glands obtained from man, dog, rabbit and rat was determined by Chauncy *et al.* (1963) . Saliva was collected from anaesthetised animals of which the glands were stimulated by

pilocarpine. From the rat only the mixed saliva from the parotid and the submandibular gland could be obtained (see Table 2.4 and 2.5).

Table 2.4: The parotid saliva enzyme activity in saliva obtained from man, dog, rabbit and rat [Chauncey *et al.*, 1963]

enzyme	human	dog	rabbit	rat*
acid phosphatase	+++	++	+++	+++
alkaline phosphatase	-	-	-	++++
non-specific esterase	+	++	++	+++
pseudo-cholinesterase	+	++	-	+++
β-D-galactosidase	-	++	+++	+++
amylase	++++	-	++++	++++

-: None; +: weak; ++: moderate; +++: marked; ++++: intense (scaling is used to indicate a degree of reactivity: intense is 1000-fold greater than weak, marked 100-fold and moderate 10-fold; *: mixed saliva from parotid and submandibular glands

The enzyme activities of the enzymes excreted by the parotid or by the submandibular gland are very similar. Only for rabbit, the amylase activity is pronounced different in the two glands: in the saliva from the parotid gland the enzyme activity is intense, whereas no amylase enzyme activity is observed in the saliva from the submandibular gland. However, enzyme activities exhibited moderate differences between different species. For instance alkaline phosphatase is only secreted in rat saliva, β-D-galactosidase is not secreted in human saliva and pseudo-cholinesterase not in rabbit saliva. It is unclear whether the activity can be extrapolated to concentrations in saliva. It is difficult to predict to what extent these differences in enzyme activities may have an impact on the outcome of the digestion step, as normally food stays only a short time in the buccal cavity.

Table 2.5: The submandibular saliva enzyme activity in saliva obtained from man, dog and rabbit [Chauncey *et al.*, 1963].

enzyme	human	dog	rabbit
acid phosphatase	+++	++	++
alkaline phosphatase	-	-	-
non-specific esterase	++	+++	++
pseudo-cholinesterase	+	++	-
β-D-galactosidase	-	+++	+++
amylase	++++	-	-

-: None; +: weak; ++: moderate; +++: marked; ++++: intense (scaling is used to indicate a degree of reactivity: intense is 1000-fold greater than weak, marked 100-fold and moderate 10-fold.

A last point to consider is that saliva can also influence micro-flora in the buccal cavity. For instance parotid proteins (high molecular weight glycoproteins), submandibular and sublingual mucin induce aggregations of oral bacteria strains in humans [Koop *et al.*, 1990], which are also observed in other animals such as streptococcus and actinomyces viscous in rats [de Jong *et al.*, 1984]. Although almost no information could be found about this topic (and even no information regarding the pig, monkey and mouse), it is not inconceivable that

microflora in the buccal cavity of the different animal species can differ to a great extent. This should be kept in mind.

2.2.2.3 The oesophagus

Information towards interspecies comparison regarding the physiology of the oesophagus was not obtained.

2.3 Conclusions

The first step in digestion and absorption of orally taken food or drugs can already take place in the mouth. Enzymes present in saliva excreted by the salivary glands are involved in the digestion. The three major salivary glands were present in man, rabbit, rat and dog. The excreted saliva exhibited moderate differences, both in activity as well as in type of enzymes present. It is not clear whether differences in the activity of the enzymes can be extrapolated to differences in salivary concentrations of these enzymes. In the absorption process, a compound has to cross the oral mucosa. The thickness of the oral mucosa was comparable in rabbit, dog and pig. However, the oral mucosa in rat is keratinised which limits the buccal drug absorption. Information with regard to the mouse and monkey are lacking in these fields.

After passing the buccal cavity, food will enter the oesophagus, which main function is the transportation of the food to the stomach. The morphology of pig oesophagus is similar to that of man. Furthermore, pigs can suffer, like humans, from reflux oesophagitis and stress ulceration of the oesophagus. Therefore, pig oesophagus may be a good model to investigate the human oesophagus.

Considering the short transit time in the buccal cavity and the inactivity of the saliva enzymes, when they have reached the stomach, it can be expected that the contribution of the buccal absorption process to the total absorption of a compound will be small. Only when delivery is focussed on the oral mucosa, the impact of the buccal absorption process will be larger with regard to the total absorption process and interspecies differences can be more pronounced.

3. Stomach

3.1 General

The major function of the stomach is to temporarily store food and release it slowly into the duodenum. It processes the food to a semi-solid chyme, which enables better contact with the mucous membrane of the intestine, thereby facilitating absorption of nutrients. In addition, the stomach is an important site of enzyme production.

3.2 Anatomy and histology

3.2.1 Man

In humans, the stomach connects the oesophagus to the duodenum, the first part of the small intestine. The stomach has four main areas: cardia, fundus, body, and pylorus (Figure 3.1).

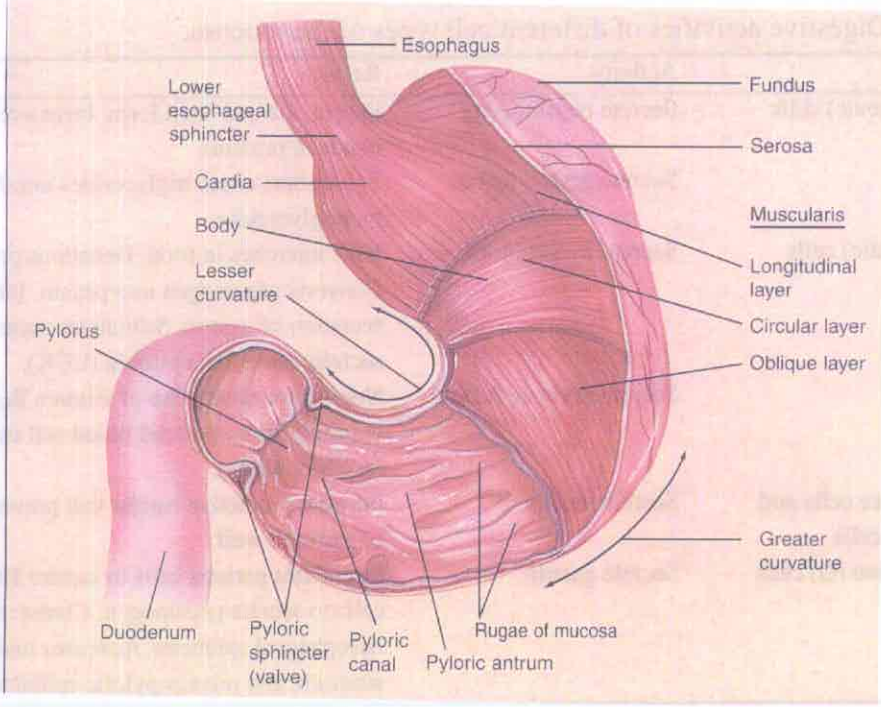

Figure 3.1 External and internal anatomy of the stomach of man[Tortora and Grabowski, 1996].

The cardia surrounds the superior opening of the stomach. The rounded portion superior to the body and to the left of the cardia is the fundus. Inferior to the fundus is the large central portion of the stomach, called the body. The region of the stomach that connects to the

duodenum is the pylorus. It has two parts, the pyloric antrum, which connects to the body of the stomach, and the pyloric canal, which leads into the duodenum. The pylorus communicates with the duodenum of the small intestine via the pyloric sphincter (valve). This valve regulates the passage of chyme from stomach to duodenum and it prevents backflow of chyme from duodenum to stomach [Tortora and Grabowski, 1996].

The stomach wall is composed of four layers: mucosa, submucosa, muscularis and serosa (Figure 3.2).

Mucosa

The mucosa contains a lamina propria (areolar connective tissue) and a muscularis mucosa (smooth muscle). The surface of the mucosa is a layer of simple columnar epithelial cells called mucous surface cells. Epithelial cells also extend down into the lamina propria, forming narrow channels called gastric pits and columns of secretory cells called gastric glands. The glands contain three types of exocrine gland cells that secrete their products into the stomach lumen: mucous neck cells, chief cells, and parietal cells. In addition, gastric glands include one type of hormone-producing enteroendocrine cell. These cells are located mainly in the pyloric antrum.

Table 3.1: Digestive activities of different cell types of the mucosa.

Cell type	Activity	Result
Chief (zymogenic) cells	Secrete pepsinogen	Pepsin, the activated form, breaks certain peptide bonds in proteins.
	Secrete gastric lipase	Splits short-chain triglycerides into fatty acids and monoglycerides.
Parietal (oxyntic) cells	Secrete hydrochloric acid	Kills microbes in food. Denatures proteins. Converts pepsinogen into pepsin. Inhibits secretion of gastrin. Stimulates secretion of secretin and cholecystokin (CCK).
	Secrete intrinsic factor	Needed for absorption of vitamin B_{12}, which is required for normal red blood cell formation (erythropoiesis).
Mucous surface cells and mucous neck cells	Secrete mucus	Forms a protective barrier that prevents digestion of stomach wall.
Enteroendocrine (G) cells	Secrete gastrin	Stimulates parietal cells to secrete HCl and chief cells to secrete pepsinogen. Contracts lower oesophageal sphincter, increases motility of the stomach, and relaxes pyloric sphincter.

The secretions of the mucous, chief, and parietal cells are collectively called gastric juice, which totals about 2000-3000 ml per day.

Submucosa

The submucosa of the stomach is composed of areolar connective tissue, which connects the mucosa to the muscularis.

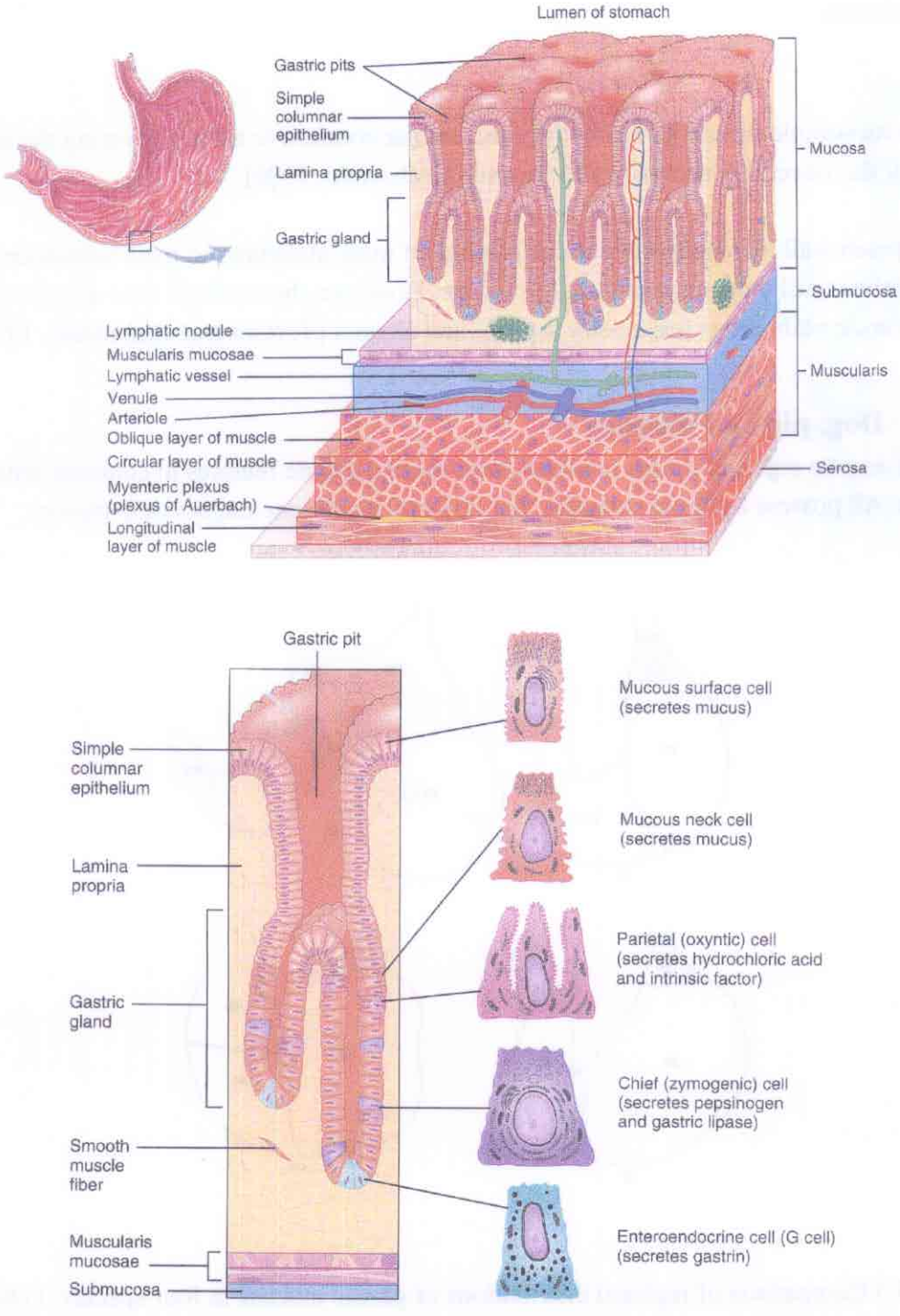

Figure 3.2 Histology of the stomach. Above: Three-dimensional view of layers of the stomach. Under: Sectional view of the stomach mucosa showing gastric glands. [Tortora and Grabowski, 1996].

Muscularis

The muscularis has three layers of smooth muscle: an outer longitudinal layer, a middle circular layer, and an inner oblique layer. This arrangement of smooth muscle fibers allows the stomach to churn food, break it into small particles, mix it with gastric juice, and pass it to the duodenum.

Serosa

The serosa (simple squamous epithelium and areolar connective tissue) covering the stomach is part of the visceral peritoneum [Tortora and Grabowski, 1996].

The stomach wall is impermeable to the passage of most materials; so most substances are not absorbed until they reach the small intestine. However, the stomach does absorb some water, ions, certain drugs (especially aspirin) and alcohol [Tortora and Grabowski, 1996].

3.2.2 Dog, pig and monkey

Dogs, domestic pigs and rhesus monkeys have several gastric features in common with humans. All possess a simple stomach; that is, there is only one major compartment.

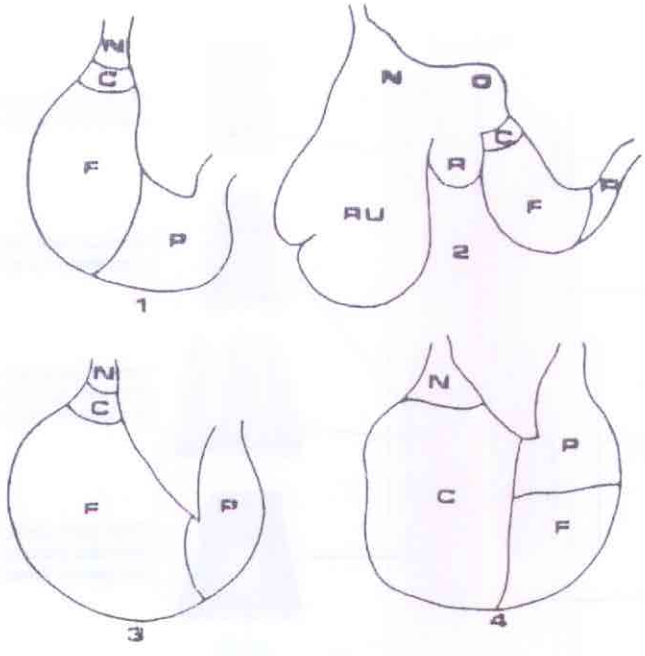

Figure 3.3 Comparison of regional distributions of gastric mucosa in four species: 1) human, 2) ruminant, 3) dog, and 4) pig. N corresponds to nonglandular mucosa, C to the cardiac region, F to the fundic region, P to the pyloric region, R to the reticulum and RU to the rumen [Banks, 1974].

Furthermore, the stomach is lined with a glandular mucosa that secretes substantial quantities of gastric acid. The amount of glandular and aglandular mucosa in the stomach of four species is depicted in Figure 3.3 [Banks, 1974]. The fundus and pyloric regions, which

contain the parietal cells responsible for secretion of gastric acid, are much more extended in humans than in the pig, while being fairly similar between human and dog.

The stomach of domestic pigs is much larger than that of humans, in the order of several liters. This is probably partly due to the larger body size, but also may be partly attributed to dietary preferences. Although officially an omnivorous species, domestic pigs are predominantly herbivorous and, as a result, the gastric chamber is somewhat modified. For example, there is a diverticulum or pouch at the top of the stomach which is probably a site for microbial metabolism of ingesta and the percentage of parietal cell-containing mucosa is smaller than in humans [Dressman and Yamada, 1991]. Another relevant aspect in pigs lies in their susceptibility to gastric ulcers. Many studies have been conducted in domestic pigs, with the incidence of gastric ulcers reported between 5 and 50% of the general pig population [Pond and Houpt, 1978], with usual incidence of about 20% [Tumbleson, 1986]. Pigs restricted to a laboratory setting are often administered cimetidine on a routine basis because of this propensity to ulcers.

In the primate family, the anatomy of the stomach varies between the different type of monkeys. This is due to a huge variation in the dietary habits, from insectivores, through omnivores, to types that survive on vegetarian or fruit and nut diets [Jones, 1972]. This naturally leads to a range of structural arrangements and relative dimensions within the gastrointestinal tract. For example, some types of monkeys that live on a totally herbivorous diet, such as the leaf eater monkey, have multicompartment stomachs. For the commonly used primates, like the rhesus monkey, the stomach is simple [Dressman and Yamada, 1991].

3.2.3 Mouse, rat and rabbit

The stomachs of rodents are divided into a glandular and a non-glandular portion. The non-glandular stomach is generally thin walled and transparent (see Figure 3.4). This part is used in the storage and digestion of food. The glandular stomach is thick walled. Gastric glands containing mucus-secreting neck cells, pepsinogen-secreting chief cells, and HCl-secreting parietal cells occupy the lamina propria of this part. The glandular stomach is a relatively small part of the total stomach compared with the glandular stomach of man [Kararli, 1995].

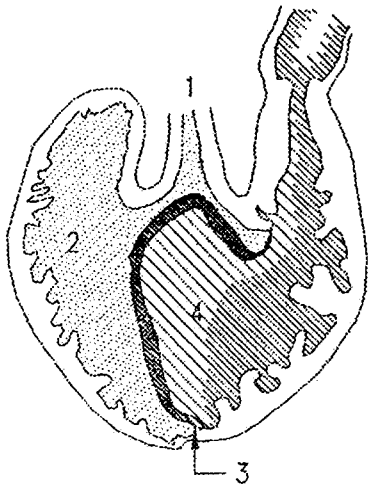

Figure 3.4 Regional distributions of gastric mucosa in the rat; 1) cardiac region, 2) cutaneous (nonglandular) area; 3) line of transition from cutaneous to glandular mucous membrane, 4) cardiac glandular region. [Hebel and Stromberg, 1976].

The stomach of the rabbit is simple and lacks specialised regions. The stomach is lined entirely with glandular epithelium, and, largely, with proper gastric glands [Stevens and Hume, 1995]. Occurrence and distribution of the cells in the gastric glands differ considerably among the mouse, rat, and rabbit. In mice and rats, a varying proportion of parietal and chief cells occupies the lower one-third of the glandular lamina propria. In rabbits, the predominant chief cells are distributed in the lower three-quarters of the glands intermingling with parietal cells. In all these species, the parietal cells are the dominant cell type in the upper one-third of the gastric glands, often extending up to the neck of the gland interspersing between mucus neck cells and occasionally chief cells [Kararli, 1995].

3.3 Gastric motility

3.3.1 Man

In humans, gastric motility has two distinct modes: the fasted and fed (postprandial) state.

Fasted state

In the fasted state, there is a cyclic pattern of motility in the upper gastrointestinal tract consisting of four phases. The sequence consists first of a quiescent phase with rare contractions, phase 1. During phase 2 contractions gradually increase in intensity and frequency. When these progress into a maximal amplitude and frequency of contraction, this is designated phase 3 activity. Phase 3 activity in the stomach is usually associated with the initiation of an interdigestive migrating motility complex (IMMC) in the duodenum, which then proceeds to migrate through the small intestine toward the ileum. It is the phase, which gives the cycle the term the "housekeeper" sequence, since it serves to remove the large undigested fragments of food from the stomach. The last phase, phase 4, is a short transitional

period between the intensive activity of phase 3 and the quiescence of phase 1[Dressman, 1986; Wilson and Washington, 1989]. Thus, in the fasting state, contractile activity in the stomach ranges from resting to maximal amplitude and frequency. Of all contractions during fasted and fed state, phase 3 contractions are the strongest.

Fed state (or postprandial state)
Feeding results in a profound alteration in the gastrointestinal motility pattern. In the stomach the cyclic contractile pattern is replaced by regular tonic contractions which propel food toward the antrum while mixing it with gastric secretions. Antropyloric contractions occur in a manner that permits fine particles and liquids to pass into the duodenum, while resulting in retropulsion of larger particles into the body of the stomach. When the meal has finished the motility pattern of the fasted state is resumed [Dressman, 1986].

3.3.2 Dog, pig and monkey

Dogs and pigs, like humans, tend to ingest periodic 'meals' which are followed by gastric emptying [Weis and LaVelle, 1991]. The basic motility patters, in terms of an IMMC which can be interrupted by feeding, appear to be present in dog, pig and monkey [Dressman and Yamada, 1991]. In dogs, cyclic gastric motility patterns, that are qualitatively similar to the patterns in humans, have been observed [Dressman, 1986]. Sarna *et al.* (1985) and Gelysteen *et al.* (1985) found that phase 3 activity lasts for about 19 min in both humans and dogs. The interval between phase 3 activity cycles was observed to be 106 $\pm$ 8 min in dogs. In humans phase 3 activity fronts are observed every 112.5 $\pm$ 11.4 min (mean $\pm$ SE). The usual cycle period therefore appears to be about 2 h in both species (times ranging between 1 and 3 h are quite common) [Sarna *et al.*, 1985; Gelysteen *et al.*, 1985].

There are some important differences in gastrointestinal transit between pigs and humans. Pigs exhibit the IMMC when fasted, but the fed-state behaviour for gastric emptying is different from that in humans. In pigs, the emptying of food from the stomach is bimodal, with about 30-40% of contents emptying in the first 15 min, followed by a more sustained emptying about 1 h later [Pond and Houpt, 1978]. Emptying also appears to be incomplete, so there may be food present in the stomach 24 h a day. The ability to retain food in the stomach for such a long period may lead to the false assumption that one is studying the pig in the fasted state, when, in fact, there is still food present.

3.3.3 Mouse, rat and rabbit

In contrast to humans, rodents and lagomorphs are 'continuous feeders'. Due to such continuous feeding habits the stomach of the healthy rabbit is never empty. Continuous feeding behaviour allows for maintenance of gastric floral growth required by the rodents and lagomorphs for digestion of cellulose and the release of essential nutrients and vitamins from plant material. Furthermore, rats and rabbits re-ingest faecal matter as an adaptive mechanism allowing for the digestion of cellulose and the absorption of essential nutrients and vitamins from plant material (Figure 3.5) [Weis and LaVelle, 1991].

No information on the gastric motility pattern in these species has been found.

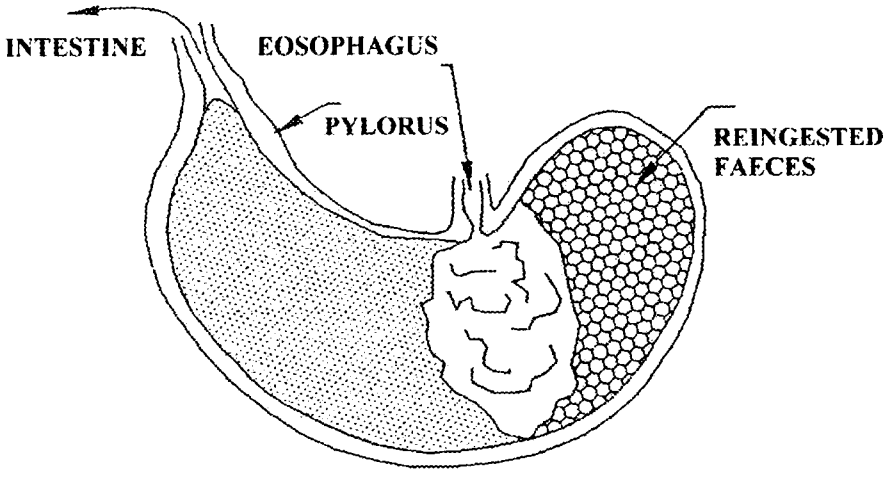

Figure 3.5 In the rabbit stomach ingested food is located in the pyloric region. Re-ingested faecal pellets are located in the large fundus where they remain separated while fermentation proceeds [Weis and LaVelle, 1991].

3.4 Gastric emptying: effect of type of liquid and meal

3.4.1 Man

In considering gastric emptying, four cases are relevant: emptying of non-nutrient liquids in the fasted state, emptying of nutrient liquids, and emptying of liquids consumed with meals, and emptying of solid meals [Dressman, 1986]. Differences in gastric emptying between humans and dogs have been studied for each of these four cases:

Non-nutrient liquids in the fasted state

When water or normal saline is ingested in the fasted state, there is usually no interruption of the fasting motility pattern and emptying follows an approximately exponential pattern. The half-emptying time in humans has typically been reported in the 8- to 15-min range [Hunt, 1956; Brener *et al.*, 1983].

Nutrient liquids

When nutrient fluids are ingested, the fasting motility pattern is interrupted. Under these circumstances, feedback mechanisms in the duodenum result in a slower, approximately linear emptying rate.

Liquids consumed with meals

When fluids are ingested with a solid meal, the stomach tends to empty more slowly than when given liquids alone. For smaller meal sizes, liquid is emptied faster than the solid

fraction of the meal, and emptying follows an approximately exponential relationship. In humans, consumption of very large meals has been shown to result in convergence of the solid and liquid emptying. Half-emptying times in the order of 30 min after a small meal have been reported [Meyer *et al.*, 1976; Moore *et al.*, 1984], whereas after a large meal this was prolonged to 3 h.

Rate of emptying of solid meals
Typical rates of solid-meal emptying are shown in Table 3.2 [Dressman, 1986].
Emptying rates are quite dependent on meal size and composition. The time for return to the phase 3 activity was found to be dependent on the meal size.

Table 3.2: Gastric emptying of solid meal fraction. [Dressman, 1986].

Species	Meal	$t_{1/2}$ (min)
Human	213 g stew, 50 g liver	117
	225 g stew, 30 g liver	70
	150 g stew, orange juice	77
	208 kcal pate, lettuce, oil	130
	60 g steak, 30 g liver	115
Dog	60 g steak, 30 g liver	180
	100 g liver	180

3.4.2 Dog, pig and monkey

Non-nutrient liquids in the fasted state
Data reported for dogs are consistent with those in humans. Increasing viscosity or administering hyperosmotic solutions results in slower gastric emptying rate [Hunt *et al.*, 1951; Hunt, 1963].

Liquid consumed with meals
In dogs, the liquid empties also faster than the solid fraction, like in human, but the half-time of emptying is about 90 min even with a fairly small meal. Unfortunately, no direct comparison, using identical meal and fluid, of liquid emptying in the two species is available. Overall, it appears that gastric handling of liquids is qualitatively very similar in dogs and humans, but while liquid emptying in the fasted state is quantitatively similar, emptying in the postprandial state may take considerably longer in dogs.

Rate of emptying of solid meals
For meaningful comparison a standard meal must be given to each species. Meyer *et al.* (1979, 1981) studied emptying of a liver and steak meal in both dogs and humans (see Table 3.2). In both species there is substantial interindividual variation in the meal emptying rate, but the results clearly indicate that emptying is considerably slower in dogs than in humans. A dependency of the time for return of phase 3 activity on the meal size is also apparent for

the dog. The delay in return of phase 3 activity after feeding is observed to be much longer in dogs than in humans [Dressman, 1986].

No information has been found concerning the pig and the monkey.

3.4.3 Mouse, rat and rabbit

No information has been found.

3.5 Gastric emptying: effect of particle size

3.5.1 Man

The rate of gastric emptying of a compound depends on its particle size. In humans, particles of less than 0.5 mm appear to empty with fluid, particles of 0.5- 3 mm empty sometimes during the course of the meal and objects larger than 4.5 mm are usually delayed until the meal has emptied [Dressman, 1986].

3.5.2 Dog

In both humans and dogs qualitatively similar gastric emptying rates with increasing particle size have been observed. In dogs, the gastric residence time increased with increasing particle size and with particles $\geq$ 5 mm in diameter approached a plateau value both in the fasted state and after feeding (Itoh *et al.*, 1986) (see Figure 3.6). However, the gastric emptying time of particles and tablets after feeding was significantly greater than in the fasting state, except with 1 mm particles. These results strongly suggest that in both the fed and fasted state, large tablets (5-10 mm diameter) will empty from the stomach only during the strong contractions from the IMMC. On the other hand, the small particles will behave more like digestible food and will empty from the stomach with weaker contractions before the IMMC wave occurs.

In a study conducted by Aoyagi *et al.* (1992), rates of gastric emptying of nondigestible tablets (diameter 11 mm) and granules (diameter 1 mm) in humans were compared with those in three animal species: dogs, minipigs and stomach-emptying controlled rabbits. In this study, the rates of gastric emptying of both dosage forms in dogs tended to be faster than or similar to those in humans (see Figure 3.7). Food delayed gastric emptying of non-digestible tablets and granules in both humans and dogs. However, this effect was larger in dogs. In a study of enteric-coated tablets of pyrodoxal phosphate very slow gastric emptying (>10 h) was observed in fed dogs, while the emptying in fed humans was 3.3 h on average.

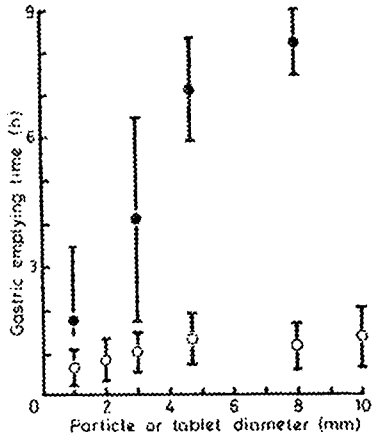

Figure 3.6 Mean emptying times of radio-opaque particles and tablets as a function of particle size in the beagle dog. (•) after feeding and (o) fasting. Error bars are standard deviations. [Itoh *et al.*, 1986].

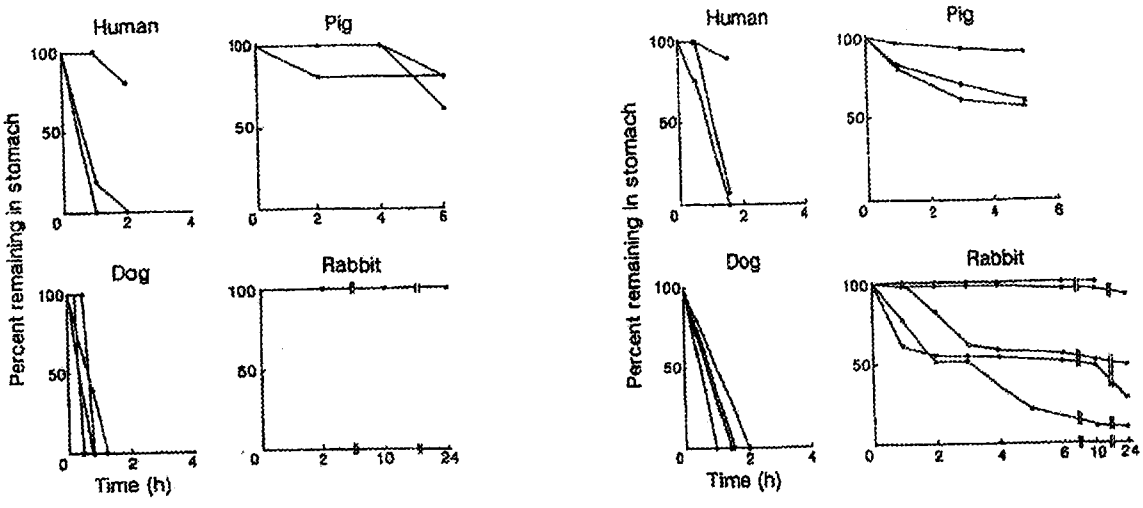

Figure 3.7 **A)** Fractions of enteric-coated tablets of barium sulphate (diameter 11 mm) remaining in the stomachs of individual humans (n=3), dogs (n=4), minipigs (n=3), and stomach-emptying-controlled rabbits (n=5) under fasting conditions, except for rabbits, which where fed after dosing. Five tablets were given to humans, dogs and pigs, and two tablets were given to rabbits.
B) Fractions of ethyl cellulose-coated granules (diameter 1 mm) remaining in the stomachs of individual humans (n=3), dogs (n=4), minipigs (n=3), and stomach-emptying-controlled rabbits (n=5) under fasting conditions, except for rabbits, which where fed after dosing. Fifty granules were given to humans and animals [Aoyagi *et al.*, 1992].

The gastric emptying time of > 10 h in fed dogs corresponds to the long disruption time of the IMMC after feeding. This effect of food on gastric emptying was also observed with granules, although to a much lesser extent. This suggests that, under nonfasting conditions, the expulsion of granules from the stomach should depend less on interdigestive gastric contraction than should that of tablets [Aoyagi *et al.*, 1992].

3.5.3 Pig and monkey

In the study conducted by Aoyagi *et al.* (1992) (see above), rates of gastric emptying of nondigestible tablets (diameter 11 mm) and granules in minipigs tended to be slower than to those in humans and dogs (see Figure 3.7). Most of the tablets and granules were emptied within 2 h in dog and human, but less than 50% of the granules and even fewer tablets were emptied within 2 h by the minipig stomach. Similar effects were later observed by following salicylate serum levels after administration of enteric-coated tablets [Aoyagi, 1986]. Onset of levels was observed within 1 h in dogs and humans, but not for 8 h after administration to minipigs. The retention of enteric-coated dosage forms by minipigs that had been restricted from food overnight may be explained by the lack of complete emptying (see paragraph 3.3.2). Therefore, to ensure that one is studying drug absorption in the fasted state, it may be advisable to evacuate the pig's stomach before administering the dosage form [Dressman and Yamada, 1991].

No information about gastric emptying and particle size in monkeys has been found.

3.5.4 Mouse, rat and rabbit

In the study conducted by Aoyagi *et al.* (1992) the rate of gastric emptying of granules in rabbits was slow and variable compared to humans and dogs (see Figure 3.7). In this study stomach-emptying-controlled rabbits have been used. These rabbits were modified by the method of Maeda *et al.* (1979) to compensate for the tendency of rabbits to empty slowly from the stomach. The rate of gastric emptying of granules was faster when the granules were given before feeding, in comparison with that after feeding or under fasting conditions This may give rise to the speculation that granules given before feeding might be expelled from the stomach by food. The non-digestible tablets were not emptied from the stomach because of the large size of the tablets (diameter 11 mm). The same phenomenon was observed by Takahashi *et al.* (1985). They revealed that 7.7 mm-diameter tablets were not expelled from the stomach of rabbits.

No information about gastric emptying and particle size in mouse and rats has been found.

3.6 pH

3.6.1 Man

pH plays an important role in the digestion of food in the stomach. When food enters the stomach, chemoreceptors monitor the pH of the stomach chyme (= a semifluid mixture of food with gastric secretions). When pH is increased, because proteins have entered the stomach and buffered some of the stomach acid, stretch receptors and chemoreceptors are activated. From the receptors, nerve impulses travel to the submucosal plexus, where they activate parasympathetic fibers. The resulting nerve impulses in the parasympathetic fibers cause waves of peristalsis and stimulate the flow of gastric juice from parietal cells, chief cells, and mucous cells. The peristaltic waves mix the food with gastric juice, and when they become strong enough, a small quantity of chyme, about 10-15 ml, squirts past the pyloric sphincter into the duodenum. As the pH of the stomach chyme returns to a low level and the stomach walls are stretched less because chyme has passed into the small intestine, this negative feedback cycle turns down secretion of gastric juice [Tortora and Grabowski, 1996]. The pH, gastric acid and fluid secretion rates and gastric volume for humans are given in Table 3.3. However, for pH, no exact values can be given: pH values vary between and within people and thus between different studies. The resting pH of healthy people is around 1.8 but can be as low as 1.0. A meal can increase the pH to between 3 and 5, but foods such as milk can raise gastric pH to over 6 [Wilson and Washington, 1989]. As gastric acid is released in response to eating, the pH gradually returns to premeal values over a period of 60 to 90 min. Mean data for postprandial gastric pH in humans are shown in Figure 3.8 [Dressman, 1986]. Similar profiles have also been observed by Malagelada *et al.* (1967).

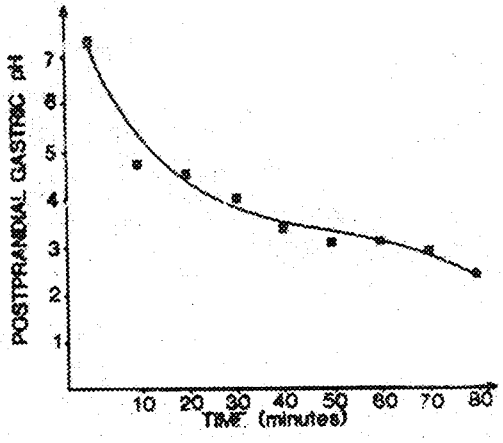

Figure 3.8 Postprandial gastric pH in human subjects [Dressman, 1986].

The median daytime pH for eight subjects over an 24 hour period was 2.7 (range 1.8 to 4.5) in the body of the stomach and 1.9 (range 1.6 to 2.6) in the antrum (see Figure 3.9) [McLauglan *et al.*, 1989]. The preprandial pH for the two regions was similar, but the difference in the median pH observed was due to the different pH curves produced after meals. On commencing to eat, the pH in the body rose after approximately 30 seconds (range

8-60 seconds) which was earlier than the antral pH that began to rise after 6 minutes (range 2-30 minutes). The time for pH to return to preprandial levels was approximately 2 hours for both regions of the stomach. The difference in pH observed demonstrates that different patterns exist between the body and antrum. While sleeping, the pH increases and decreases several times (see Figure 3.9). The increases in pH may probably indicate phase 3 of the IMMC. During phase 3 of the IMMC the pylorus remains open and duodenal content may enter the stomach, which causes an increase in pH. This increase is followed by a decrease in gastric pH due to the release of gastric juice.

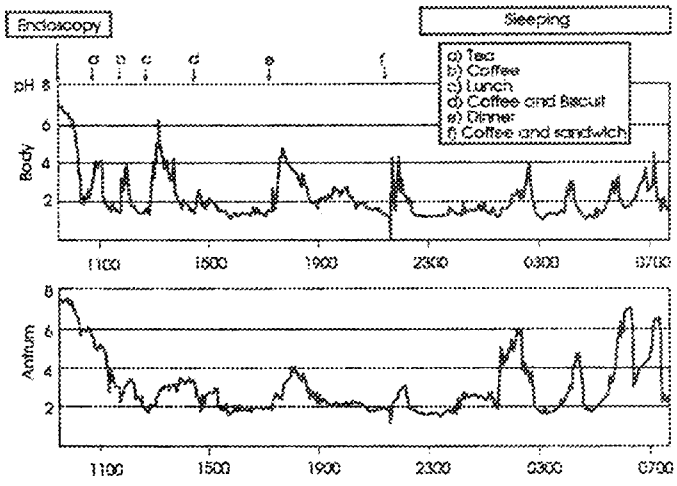

Figure 3.9 pH in the body and antrum of the stomach in a healthy volunteer over a 24 hour period [McLauglan *et al.*, 1989].

3.6.2 Dog, pig and monkey

The gastric acid and fluid secretion rates, gastric volume and pH values for beagle dogs, domestic pigs and Rhesus monkeys are given in Table 3.3 [Dressman and Yamada, 1991]. From Table 3.3 it can be concluded that 1) in dogs, the gastric acid secretion rate at the basal state is low compared to humans, domestic pigs and rhesus monkeys; 2) the gastric pH in the fasted state, however, is quite acid in dogs as well as in humans and in pigs; 3) following stimulation (i.e. food), gastric acid secretion rates in dogs exceed those of the human and pig; 4) gastric pH in the fed state, however, is higher in humans than in dog and pig.

When monkeys are allowed to range freely, their gastric acid production is similar to humans (see Table 3.3). Under restraint conditions, however, the gastric response changes. It has been observed that in Old World monkeys, including the macaque family, the secretion of gastric acid is almost completely shut down when the animal is restrained, and the pH of the gastric contents may be neutral and sometimes even slightly alkaline. In addition, the quantities of gastric juice secreted are also reduced, and gastrointestinal motility appears to be inhibited. New World monkeys, by contrast, appear to secrete greater amounts of acid when restrained than when allowed to range freely. These contrasting responses illustrate the need to choose the type of monkey carefully, depending on the aims of the experimental protocol. Of course,

changes in the physiological status also occur for other species when they are placed in a stressful environment, but perhaps because of the high intelligence level, stress effects on the physiology appear to be quite pronounced in monkeys [Dressman and Yamada, 1991].

Table 3.3: Comparison of gastric acid secretion data of humans, dogs, domestic pigs, and rhesus monkeys [Dressman and Yamada, 1991].

Parameter	Human	Dog	Pig	Monkey
Stomach capacity (l)	1-1.6	1	6-8	0.1
Basal volume (l)	0.024[a]			0.008[b]
BAO				
Volume (ml/min)	1	0.3-1.5	1.05	Similar to humans unless stressed
Rate (mEq/h)	2-5	0.1	5.6 µg/h	
PAO				
Rate (mEq/h)	18-23	39	1.25 $\mu g \cdot kg^{-1} \cdot h^{-1}$	-
PH			2.7 (3.75-4; n=20)	-
Fasted	1.7 (1.4-2.1)[c]	1.5	1.6-1.8 (0.8-3.0)[d]	-
Fed (during meal)	5.0 (4.3-5.4)[d]	2.1 ± 0.1 (SD)	<2 (n=1)	-

- = no data available; [a] From Lentner and Wink, 1981; [b] From Dubois *et al.*, 1977;
BAO = basal acid output; PAO = peak acid output
[c] Interquartile range; [d] Range

The data in Table 3.3 do not reflect the existence of fluctuations in gastric pH during the fasted and fed state in dogs. Fluctuations in fasted gastric pH in dogs have been observed by Itoh *et al.* (1986) They monitored changes in intragastric pH and measured gastric residence times of various particles and tablets both in beagle dogs in the fasted state and after a single meal. The pH in the stomach was variable in the fasted state (see Figure 3.10) but an abrupt pH increase (up to pH 6-7) in the stomach was observed during the emptying of larger tablets. In some instances this high pH in the stomach was maintained until the next IMMC wave occurred. The abrupt pH increase may be due to either reflux from the duodenum or some other means of local neutralisation of stomach acid. The fact that in some cases the high pH (5-7) was maintained until the next IMMC, implies that, at least in those cases, there was minimal acid secretion in the stomach after the IMMC activity. This would be consistent with the fact that the dog is known to have a low basal rate of gastric acid secretion. Fluctuations in gastric pH in the fed state in dogs have also been observed by Itoh *et al.* (1986). They observed a mildly acid pH region after feeding which may reflect the buffering capacity of the food, followed by a steady state low pH for some hours (see Figure 3.11).

No data on fluctuations in gastric pH in fasted and fed state in the stomach of pigs and monkeys have been found.

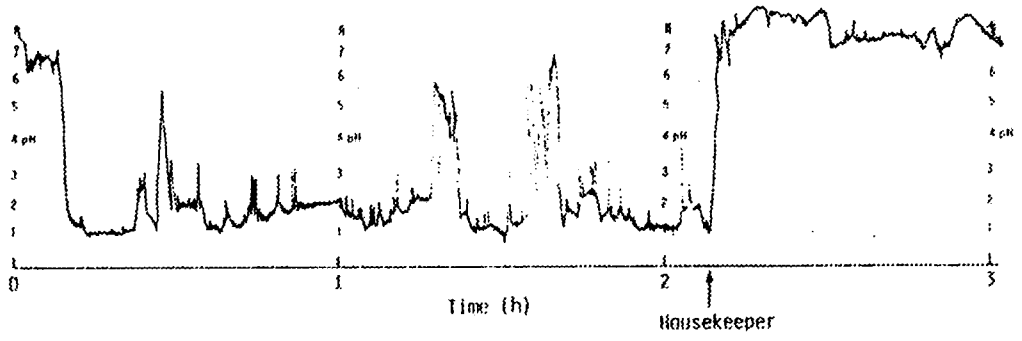

Figure 3.10 Typical intragastric pH-time profile in the fasted beagle dog as measured by the Heidelberg capsule, a radiotelemetric pH sensor. At the time indicated by the arrow large particles were observed by X-ray to exit the stomach 'en masse' indicating the occurrence of an IMMC wave. In the case shown here the elevated pH associated with the IMMC was maintained for more than 1 h [Itoh *et al.*, 1986].

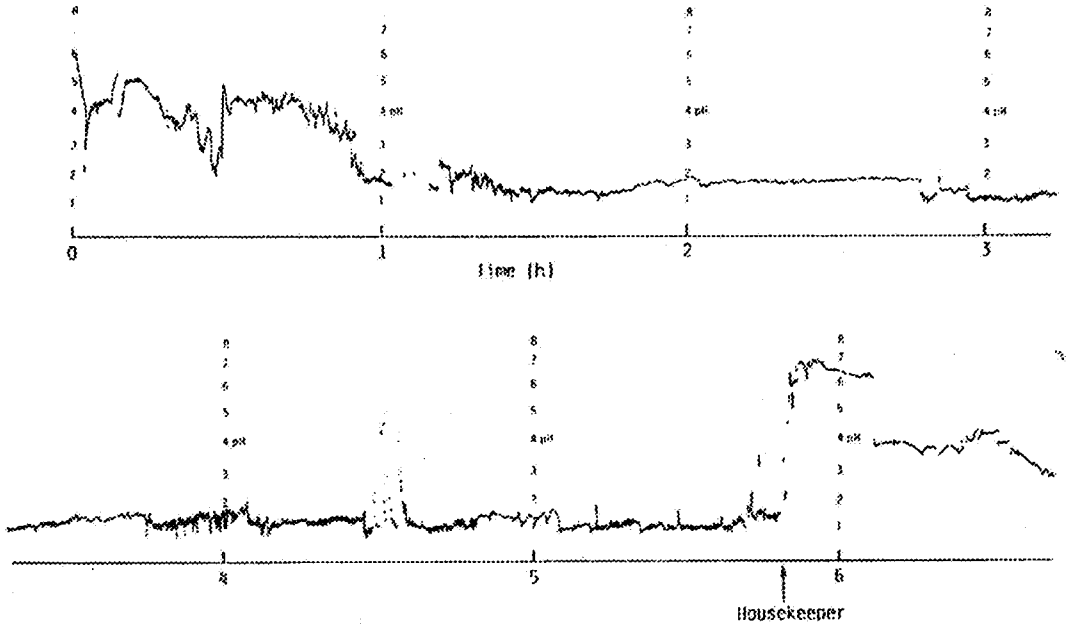

Figure 3.11 Typical intragastric pH-time profile in the beagle dog after a single meal (50 g standard dog chow), as measured by the Heidelberg capsule [Itoh *et al.*, 1986].

3.6.3 Mouse, rat and rabbit

Very few (and old) data on the pH in mouse, rat and rabbit have been found. Smith (1965) has compared the contents of the stomach in dog, pig, monkey, rabbit, rat and mouse (Table 3.4).

Table 3.4: pH value (median for different animals) of the contents of the stomach in different species [Smith, 1965].

Animal	No. examined	anterior portion of stomach	posterior portion of stomach
Monkey	3	4.8	2.8
Dog	3	5.5	3.4
Pig	20	4.3	2.2
Rabbit	11	1.9	1.9
Rat	7	5.0	3.3
Mouse	3	4.5	3.1

In the stomach of the animals examined, except the rabbit, the anterior (cardiac) region was found to have a higher pH value than the pyloric region since the parietal cells tend to be localised in the lower part of the stomach (see Table 3.4). In the rabbit both portions had low pH values. The high acid value throughout the rabbit stomach is probably due to this organ not being separated into compartments, thereby permitting its contents to be sufficiently mixed. In addition, the rabbit is a good producer of gastric acid [Smith, 1965; Calabrese, 1991].

3.7 Microbial flora

3.7.1 Man

The stomach of humans is sterile in the fasting state because of the low gastric pH. The presence of the microflora is governed to a great extent by the acidity of the region. Few bacteria grow at pH 3 or less. However, both food and saliva entering this organ neutralise gastric acid and allow colonisation by transient flora of predominantly acid-resistant bacteria such as lactobacilli, bacteroides and enterococci. Proliferation of bacterial growth occurs in diseases such as gastric achlorhydria. Patients with this disease tend to have large numbers of enterobacteria, streptococci and bacteroides [Ilett *et al.*, 1990].

3.7.2 Dog, pig, monkey, mouse, rat, and rabbit

Like in humans, the stomach of the rabbit contains very few organisms. This is due to the low gastric pH. The higher gastric pH in other species (see Table 3.4) enables large numbers of bacteria to colonise the stomach. Table 3.5 represents a selection of the findings of Smith (1965), who compared the numbers of the six major kinds of microflora in the contents of the stomach in 20 different species. In rodents, the bacterial counts are highest in the anterior part of the stomach (Table 3.5). This can be explained by the anatomy of the stomach: rodents display a two-compartment stomach (non-glandular and glandular compartment), which permits the bacteria to proliferate faster in the non-acid producing anterior part of the stomach (non-glandular compartment).

Table 3.5: Numbers of different kinds of organisms in the contents of the stomach of different species [Smith, 1965].

Species	Microflora	Log_{10} viable count (median) per gram contents of the stomach anterior portion	posterior portion
Monkey	E. coli	3.0	2.6
	C. perfringens	1.7	1.7
	Streptococci	6.5	5.0
	Lactobacilli	8.7	8.0
	Yeasts	5.2	5.3
	Bacteroides	N	N
Dog	E. coli	4.5	4.4
	C. perfringens	5.2	5.2
	Streptococci	5.5	5.5
	Lactobacilli	4.5	4.2
	Yeasts	N	N
	Bacteroides	N	N
Pig	E. coli	5.3	3.0
	C. perfringens	2.4	N
	Streptococci	6.0	4.4
	Lactobacilli	8.6	7.0
	Yeasts	4.3	4.3
	Bacteroides	N	N
Rabbit	E. coli	N	N
	C. perfringens	N	N
	Streptococci	N	N
	Lactobacilli	N	N
	Yeasts	N	N
	Bacteroides	4.2	4.2
White rat	E. coli	2.0	N
	C. perfringens	N	N
	Streptococci	5.0	4.2
	Lactobacilli	8.4	7.5
	Yeasts	6.2	6.0
	Bacteroides	N	N
White mouse	E. coli	2.0	N
	C. perfringens	1.7	N
	Streptococci	6.0	5.3
	Lactobacilli	7.7	7.0
	Yeasts	6.7	6.3
	Bacteroides	N	N

N=no viable organisms found in 0.02 g of chyme, i.e., log_{10} viable count per gram < 1.7.

According to Scheline (1973), the microflora of the rabbit most closely simulates the human, based on the general lack of microbes in the highly acidic environment of the stomach and the proximal portion of the small intestine of both species (see Chapter 4). However, at this point, the data based upon which interspecies assessments can be made is quite limited and old.

3.8 Conclusions

Differences in the stomach between humans and dogs have been studied extensively. Literature about differences between humans and the other species (pig, monkey, mouse, rat and rabbit) is brief or even lacking.

Based on the available literature, it can be concluded that there is a similarity between the anatomy and histology of the stomach in dogs and those in humans. In addition, the gross physiology of the stomach in humans and dogs is similar in the fasted state, with similar motility patterns and gastric emptying of indigestible solids and liquids. However, there are also some important differences in gastric physiology between dogs and humans in stomach physiology. The meal-emptying rate and subsequent return of the fasting motility pattern (phase 3) are much slower in dogs, basal rate of gastric acid secretion is lower in dogs and bacterial growth in the stomach is higher in dogs.

4. Small intestine

4.1 Anatomy and histology

4.1.1 Anatomy in man

The small intestine is divided into three segments. The duodenum, which means '12'; the structure is 12 fingers breadth in length, extends about 20-30 cm. The jejunum is approximately 1 m in a living person and approximately 2.5 m in a cadaver due to loss of smooth muscle tone after death. Jejunum means 'empty', since at death it is found empty. The final portion of the small intestine, the ileum, measures about 2 m (ca. 3.5 m in cadavers) [Tortora and Grabowski, 1996].

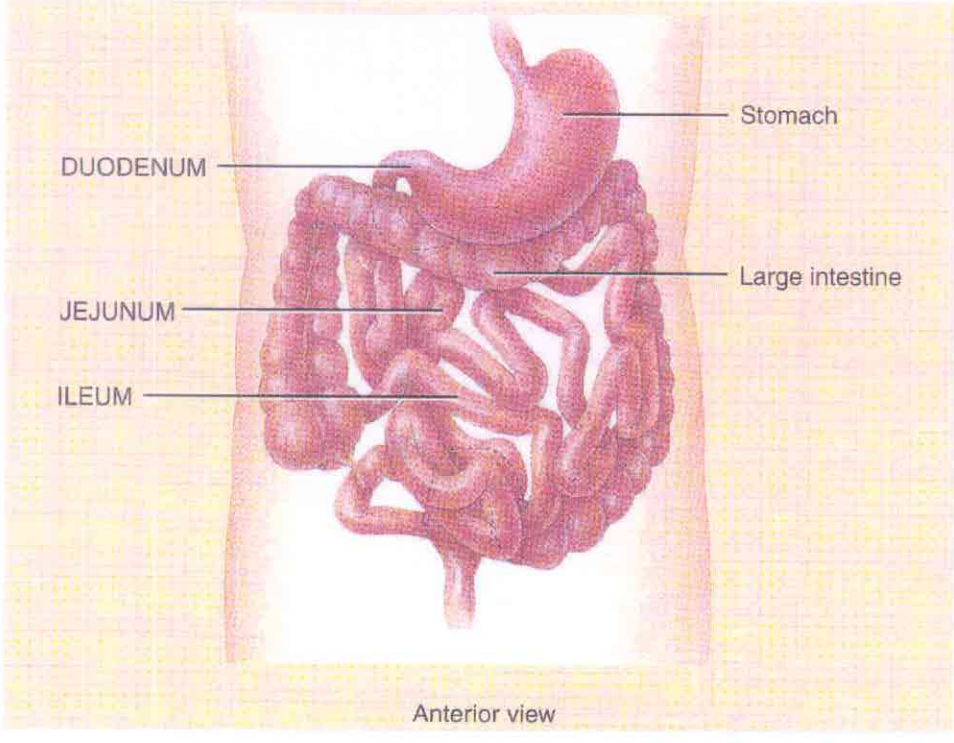

Figure 4.1 Divisions of the small intestine [Tortora and Grabowski, 1996].

4.1.2 Histology in man

If examined closely, the appearance of the lumen surface of the small looks like velvet due to the covering by millions of small projections called villi, which extend approximately 1 mm into the lumen. This large number of villi (20-40 per square millimetres) vastly increases the surface area of the epithelium available for absorption and digestion. Villi are only the most striking feature of the mucosa. The mucosa houses a dynamic, self-renewing population of epithelial cells including goblet cells, which secret mucus, endocrine cells, which produce hormones and peptides, Panted cells, which secrete large amount of protein rich materials, undifferentiated cells for renewal of the intestinal mucosa, and absorptive cells, which take up nutrients from the lumen and transport them into blood, fulfilling the basic function of the digestive system (Figure 4.2). The lamina propria of the small intestine has an abundance of mucosa-associated lymphoid tissue (MALT). The muscularis of the small intestine consists of two layers of smooth muscle. The outer, thinner layer contains longitudinally arranged fibers. The inner, thicker layer contains circularly arranged fibers [Tortora and Grabowski, 1996; http://arbl.cvmbs.colostate.edu].

The structure of the intestinal wall is not consistent throughout the entire small intestine. The duodenum contains a thick wall, with deeply folded mucous membrane and duodenal digestive glands. The foldings are called the 'folds of Kerckring'. They are well developed both in the duodenum and in the jejunum. The jejunum has a thicker wall and is more vasculated than the duodenum. Furthermore, it has larger and more villi than the ileum. The ileum has more lymphatic follicles than elsewhere in the intestine. The size and number of villi throughout the intestine varies upon diet and between populations.

The apical membrane of the absorptive cells features *microvilli*. Each microvillus is a 1 µm-long cylindrical membrane-covered projection. The microvilli form the so-called brush border. Larger amounts of digested nutrients can diffuse into the absorptive cells of the intestinal wall because the microvilli greatly increase the surface area of the plasma membrane (Figure 4.2). Moreover, the membrane of the microvilli is rich in protein, cholesterol and glycolipids and contains digestive enzymes such as dissaccharidases and peptidases. Also specific receptor proteins are located here, which bind substances before absorption, like vit. B12 and conjugated bile salts. There are an estimated 200 million microvilli per square millimetre of small intestine The mucosa contains many cavities lined with glandular epithelium. Cells lining the cavities form the intestinal glands (crypts of Lieberkühn) and these cells secrete intestinal juice (Figure 4.2). The submucosa of the duodenum contains duodenal (Brunner's) glands. They secrete alkaline mucus that helps to neutralise gastric acid in the chyme. Total volume secreted by crypts: 1800 ml/day (most extracellular fluid), pH 7.5-8.0. The fluid is absorbed rapidly by the villi and serves as watery vehicle for absorption. The lamina propria of the small intestine has an abundance of mucosa-associated lymphoid tissue (MALT). The muscularis of the small intestine consists of two layers of smooth muscle. The outer, thinner layer contains longitudinally arranged fibers. The inner, thicker layer contains circularly arranged fibers [Tortora and Grabowski, 1996; http://arbl.cvmbs.colostate.edu].

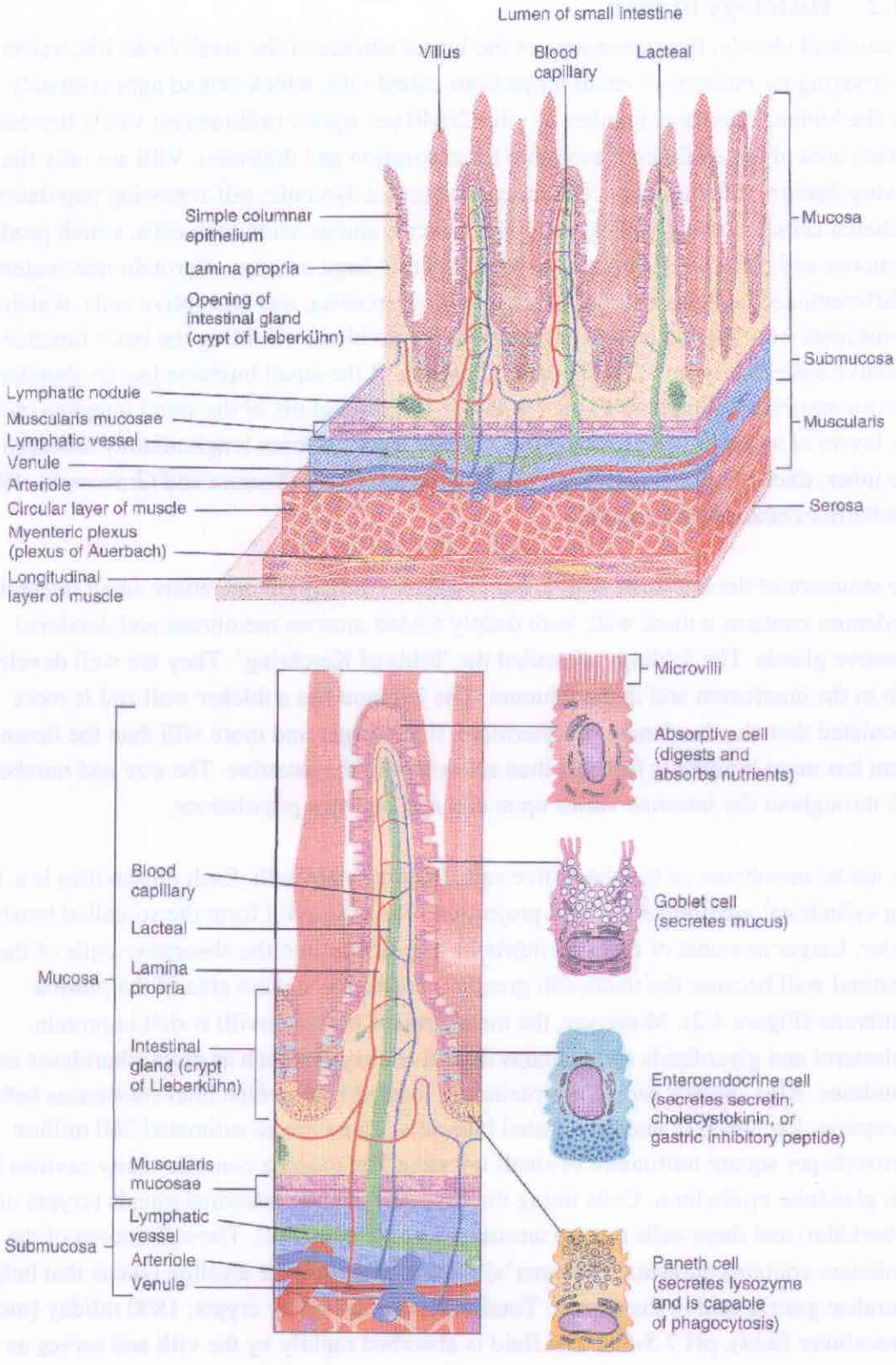

Figure 4.2 Histology of the small intestine. Above: Three-dimensional view of layers of the small intestine showing villi. Under: Enlarged villus showing lacteal, capillaries, and intestinal gland [Tortora and Grabowski, 1996].

4.1.3 Interspecies comparison

The digestive systems of humans, rats, pigs and dogs are, to a first approximation, virtually identical. It becomes apparent in a more detailed examination that each of these species has evolved certain digestive specialisations to adapt to a particular diet.
These differences become particularly apparent when carnivores such as dogs are compared with herbivores like most rodents and rabbits. Parts of their digestive tract have been adapted to massive fermentation vats in order to utilise cellulose efficiently, the major carbohydrate of plants. They belong to the group of caudal fermentors, also known as cecal digesters. These animals are similar to dogs and humans in respect to the stomach and small intestine, but their large intestine, where the fermentation occurs, is complex and exceptionally large. Their main adaptation is the formation of a large cecum. In contrast, dogs evolved from animals that lived on the carcasses of other animals, and their digestive systems reflect this history - extremely small fermentation vats and essentially no ability to utilise cellulose. Bridging the gap between carnivores and herbivores are omnivores like humans, monkeys, pigs and mice whose digestive tracts attest to a historical diet that included both plants and animals [Kararli, 1995]. Although pigs are officially an omnivorous species, domestic pigs are predominantly herbivorous. Their cecum is several times larger than that of humans, and there is significant production of volatile fatty acids from carbohydrates in this region along with catabolism of amino acids and synthesis of B vitamins. In other words, the pig lean toward herbivore anatomy, whereas the human leans toward carnivore, although both are intermediate [Dressman and Yamada, 1991].

The jejunum is considered to be roughly 40% of the small gut in man, but closer to 90% in animals. The ileum which empties into the large intestine is considered to be about 60% of the intestine in man, but veterinary anatomists usually refer to it as being only the short terminal section of the small intestine [Kararli, 1995]. The rhesus monkey has a duodenal length of about 5 cm, much shorter than the 25-30 cm length in humans, so by extrapolation, the overall length of the small intestine is most likely somewhat shorter. The diameter in rhesus monkeys is a little larger than would be expected from the overall size of the animal: the duodenum has a diameter of 1.5-2 cm, and the jejunum and ileum around 2 cm (Table 4.1). This may compensate, to some extent, for the shorter length in terms of absorptive capacity. Also, villi in the rhesus monkey tend to be broad and leaf shaped, as opposed to the filamentous appearance of human villi. Taken together, the dimensional comparisons and villus morphology suggests that the absorptive capacity of the small intestine in the rhesus monkey may be lower than in man. The small intestine is lined with villi and, although these may differ in terms of size and morphology among species, they always serve the purpose of dramatically increasing the surface area available for absorption in each of these species as well as in humans. In pig, the small intestine is about twice as long as in humans. The diameter of the small intestine in pig is bout 2.5-3.5 cm, which is very similar to that of humans. The villi are expected to be fairly similar to those in humans, since both are omnivores. Therefore it is expected that the surface area of the principal absorptive mucosa in the pig is greater than that in humans [Dressman and Yamada, 1991].
Table 4.1: Anatomical characteristics of the (small) intestine for various species [Kararli, 1995].

	Man	Mouse	Rat	Rabbit	Dog	Pig	Monkey
Small intestine							
In m	6.25 [1)]	?	0.13 [1)]- 0.82 [3)]	1.51 [3)]	2.48 [3)] - 4.14 [1)]	14.16 [3)]- 18.29 [1)]	?
In % of total	79 [1)]	?	64 [1)]	60 [1)]	85 [1)]	78 [1)]	?
Diameter small intestine	5 [1)]	?	0.3-0.5 [1)]	?	1 [1)]	2.5-3.5 [1)]	1.2-2 [1)]
duodenum (in cm)	3-4 [4)]				2-2.5 [4)]	2.5-3.5 [4)]	1.5-2 [4)]
Villi	Finger shaped [1)]	Finger shaped [1)]	Tongue shaped [1)]	?	?	Finger shaped [1)]	Tongue shaped in duodenum/ finger shaped in jejunum + ileum [1)]
Relative surface area (region/body)							
Duodenum	10.3 [2)]	?	1.8 [2)]	?	?	?	?
Jejunum	74.9 [2)]	?	19.8 [2)]	?	?	?	?
Ileum	22.9 [2)]	?	0.4 [2)]	?	?	?	?
Absorbing surface area (m^2)							
Duodenum	0.09 [1)]						
Jejunum	60 [1)]						
Ileum	60 [1)]						
Cecum							
In m	0.15 [1)]	?	0.04 [3)] - 0.06 [1)]	0.44 [3)] - 0.61 [1)]	0.08 [1.3)]	0.17 [3)]- 0.23 [1)]	0.05-0.06 [4)]
In % of total	2 [1)]		31 [1)]	11 [1)]	2 [1)]	1 [1)]	
Colon							
In m	1.50 [1)]	?	0.01 [1)]- 0.26 [3)]	1.23 [3)] - 1.65 [1)]	0.34 [3)] - 0.60 [1)]	4.27 [3)] - 4.99 [1)]	0.4-0.5 [4)]
In % of total	19 [1)]		5 [1)]	28 [1)]	13 [1)]	21 [1)]	
Total (in m)	7.9 [1)]	?	0.2 [1)]-0.9 [3)]	5.8 [1)]	4.8 [1)]	23.5 [1)]	?
Ratio*	1: 4.5 [1)]		?	1:10 [1)]	1:6 [1)]	1:14 [1)]	
Location of Peyer's Patches	Ileum [4)]				duodenum, jejunum, ileum [4)]	jejunum, ileum, colon [4)]	ileum [4)]

*) Ratio of body length to intestine length; [1)] [Kararli, 1995]; [2)] [Weis and LaVelle, 1991]; [3)] [Clemens and Stevens, 1980]; [4)] [Dressman and Yamada, 1991]

It should be stressed that data mentioned in table 4.1 were obtained from post mortem examination of the tissues. The dimensions of the tissues *in vivo* appear to be smaller, especially the length of the small intestine. For example, the post mortem length of the small intestine in humans is approximately 7 m, while the estimated length *in vivo* is close to 3 m.

The number of microvilli per unit area of villi surface varied between 65 microvilli per mm^2 of villi surface in rat and 34 microvilli per mm^2 of villi surface in dog. However, after taking into account the sizes of the villi, the effective surface area per unit villus surface appeared to

be a constant value of approximately 25 μm^2 μm^{-2}. As in man, the jejunum in dog, rat and rabbit has larger and more villi and microvilli than the ileum [see references in Bijlsma et al, 1995, Kararli, 1995].

4.2 Absorption mechanisms

4.2.1 Overview

The extent of absorption of a compound in a segment of the gastrointestinal tract depends generally on the rate of absorption, exposed surface area and time available for drug absorption (transit time). Important factors determining the absorption rate are the physicochemical properties of a compound but also the potential metabolic conversion by enzymes and/or microorganisms in the intestinal lumen or in the intestinal cells, local pH differences, mechanism by which a compound is absorbed by the intestinal cells.
The intestinal epithelium is the one cell layer thick contact between the intestinal lumen and blood (Figure 4.2). At the lumen side of the absorptive cells, a thin water layer "bound" to the microvilli (the so called unstirred water layer) forms the direct contact with the absorptive cells. Thus, passage across the absorptive cell layer (and unstirred water layer) is often the determining step in intestinal drug absorption. Various absorption mechanisms can be distinguished (Figure 4.3). Basically, a compound can be absorbed either by crossing the cell membranes through the cell (transcellular pathway) or by transport along the cells (paracellular pathway). Since the transcellular pathway occupies more than 99.9% of the total surface area, most drugs are absorbed by the transcellular route.

1. Paracellular transport: The tight junctions form an aqueous pore between the absorptive cells. Since the surface area in between the tight junction is only 0.1% of the total surface area (absorptive cells with microvilli), drug transport across the intestinal epithelium by this route is minimal (pathway A). The epithelial junctions become progressively tighter from small intestine to the colon, which mainly decreases the permeability of polar compounds along the intestinal tract [Rouge *et al.*, 1996]. Small hydrophilic molecules (ions) are allowed to pass between cells through the tight junctional opening but the tight junctions form a transport barrier for the absorption of large molecules (>400 D) and are impermeable for macromolecules. This transport is not inversely proportional to the molecular weight of the drug, but depends more on the molecular size. Furthermore, the tight junctional permeability is charge (cations) selective, but the influence of the size is dominant over the charge of a molecule. The tight junctional barrier function is physiologically regulated, for example, by serosal peptide hormones [Hochman and Artursson, 1994], but it can also be hampered in patho-physiological conditions [Hollander 1988, Schulzke et al., 1998]. Furthermore, the tight junctions can be opened by a variety of nutritional and xenobiotic compounds [Hochman and Artursson, 1994]. By opening of the tight junctions (pathway B), the oral absorption of hydrophilic drugs can be increased, which may be of interest of the pharmaceutical companies.

2. Transcellular passive transport: For a molecule to cross the apical and basolateral membrane by passive diffusion (pathway C), it must have the appropriate physicochemical properties (e.g. size, charge, lipophilicity, hydrogen bonding potential) . Lipophilic compounds ($0<\log K_{o/w}<4$) are rapidly absorbed due to their solubility in the lipid bilayers. For very lipophilic compounds ($\log K_{o/w} >4$), low solubility in the unstirred water layer may decrease the permeability. The passive transcellular diffusion of a drug is depended on the net effect of these factors.
3. Transcellular active transport: Hydrophilic compounds ($\log K_{o/w}<0$) can not readily pass the lipid bilayers but their transcellular absorption can be facilitated by substrate specific carriers or by active transport proteins (pathway D). This transport route is important for the absorption of nutrients such as monosaccharides, amino acids and di/tripeptides, but is also important for the absorption of electrolytes and bile salts and for specific classes of drugs. For example, L-dopa and D-cycloserine are absorbed by intestinal amino acid transport systems, while orally available cephalosporins and renin antagonists are substrates for the intestinal oligopeptide transporter [Tsuji and Tamai, 1996].
4. Endocytosis: The tight junctions are normally impermeable for macromolecules, and macromolecules such as peptides and proteins can be transported via specific receptor-mediated endocytosis or via non-specific endocytosis (pathway F). Specialised, so-called M cells in the Peyer's patches have less microvilli and show high endocytic activity but the absorptive cells show also endocytic activity [Neutra, 1998]. Endocytosis of compounds is minimal in adult small intestine and is not a quantitatively significant mechanism for drug absorption in the intestine. However, the intimate contact with lymphocytes suggests that endocytosis is probably an important absorption route for potentially antigenic macromolecules [Mayer, 1998, Rouge *et al.*, 1996].[

Thus, compounds that are well absorbed in the intestine must meet various conditions. The compound must either be small ($M_r < 350$) and hydrophilic to access the diffusive paracellular pathway, or lipophilic for passive transcellular absorption, or be transported across the intestinal enterocyte by specific carrier-mediated mechanisms. For passive transcellular absorption, the molecule must have the appropriate physicochemical properties to cross both the apical and basolateral membrane, lipophilic barriers. However, compounds that are adapted for absorption by this route may be substrates for enterocytic intracellular metabolism (pathway C*) and for apically polarised efflux mechanisms (pathway E) [Hunter and Hirst, 1997].

In this way, by metabolism and secretion, the intestinal epithelium plays an important role in protecting the body against the absorption of ingested xenobiotics. No single efflux system explains the diversity of drugs secreted by the intestine but the role of P-glycoprotein in intestinal secretion has received great attention [Hunter and Hirst, 1997]. The role of P-glycoprotein, product of the MDR1 gene, as a drug transport protein is first discovered in cancer cells. P-glycoprotein is a membrane glycoprotein, member of the ABC-transport family, and transports a variety of hydrophobic anti-cancer agents out of tumour cells. In humans two *mdr* genes have been detected, but only the *MDR1* gene encodes for the multidrug-related P-glycoprotein efflux pump. The second MDR gene product in humans,

MDR3, has a discrete substrate specificity and transports cholesterol and phosphatidylcholine into the bile [Smit et al., 1993].
In normal tissue MDR1 P-glycoprotein is expressed in many secretory organs including liver, kidney and intestinal tract epithelia. In the intestinal tract P-glycoprotein is localised in the brush border of the mature enterocyte [van der Valk et al., 1990, Meyers et al., 1991]. The localisation of P-glycoprotein in the intestine is in agreement with a secretory function of P-glycoprotein, and P-glycoprotein has been shown to limit the oral bioavailability for various pharmaceuticals [see references in Hunter and Hirst, 1997]. However, more insight in the function of P-glycoprotein in the intestine and in the P-glycoprotein mediated pharmacokinetics of compounds has been gained from knockout mice.
Rodents have 3 *mdr* genes from which the mdr1a and mdr1b P-glycoproteins show similar characteristics as the human MDR1 P-glycoprotein. Absence of mdr1-type P-glycoprotein in mice has a profound effect on the tissue distribution and on the hepatic and intestinal clearance of hydrophobic cationic drugs [Smit et al., 1998]. It was shown in these knockout mice that P-glycoprotein in the blood-brain barrier is very important in preventing the accumulation (up to a factor 20) of a variety of drugs in the brain [Schinkel et al., 1994, 1998, Jonker et al., 1999]. Moreover, intestinal P-glycoprotein has a prominent role in the extrusion of several drugs from the blood into the intestinal lumen, and in preventing drugs in the intestinal lumen from (re-entering) the bloodstream.
It has recently become apparent that the role of P-glycoprotein in intestinal absorption of compounds can not be studied without considering the role of the metabolising P450-3A family [Soldner et al., 1999, Watkins 1997, see also chapter 6.3]. P450-3A4 is present, like P-glycoprotein, in the villus tip enterocyte. Furthermore, the substrate specificity of P-glycoprotein and P450-3A4 show a significant overlap [Wacher et al., 1995]. Thus, P-glycoprotein together with P450-3A4 may form a co-ordinated intestinal barrier resulting in a limited oral availability [Hall et al., 1999, Watkins et al., 1997]. Bioavailability of a compound could be maximised by inhibition of increasing drug absorption (inhibition P-glycoprotein) and reducing drug biotransformation (inhibition P450-3A) as was shown for example by co-administration of verapamil and digoxin [Pedersen et al., 1983]. As other efflux systems and cellular metabolism pathways are present in the intestine, other interactions between efflux and metabolism are likely to exist, for example the interaction between the glutathione S-conjugate transporter proteins (MRPs) and glutathione S-transferases. A better understanding of the interactions between intestinal secretory efflux systems and cellular metabolism pathways will allow optimisation of oral drug absorption.

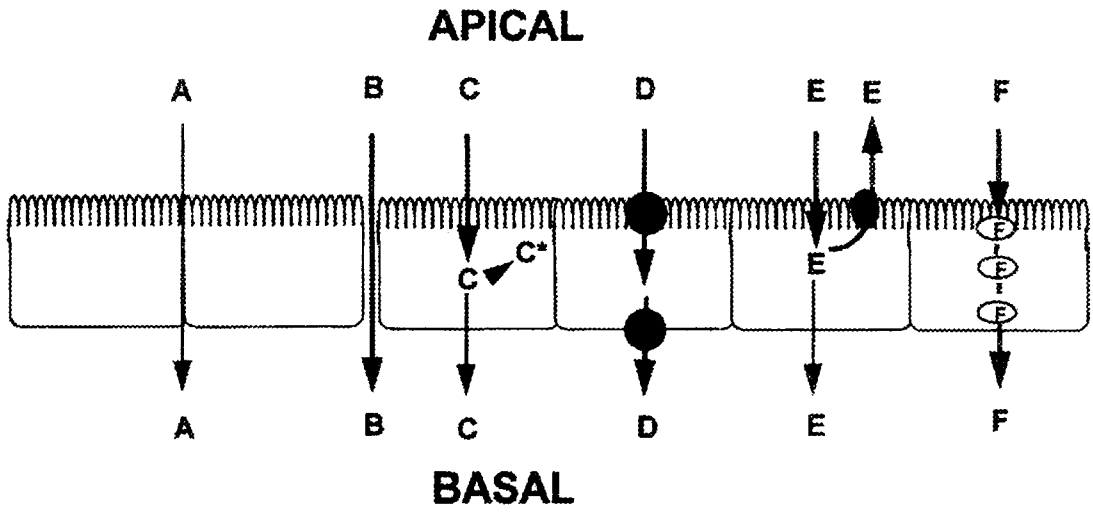

Figure 4.3: Pathways of intestinal absorption. (A) paracellular diffusion; (B) paracellular diffusion enhanced by a modulator of tight junctions; (C) transcellular passive diffusion, (C*, intracellular metabolism); (D) carrier mediated transcellular transport; (E) transcellular diffusion modified by an apically polarised efflux mechanism; (F) transcellular vesicular transcytosis [Hunter and Hirst, 1997].

4.2.2 Permeability in man

Several clinical and preclinical models are being used to predict the human *in vivo* absorption of a compound. The permeability (P) of a drug can be determined in man by a so called 'multi lumen tube' [Lennernas, 1998]. With the use of 2 balloons a 10 cm long intestinal segment can be occluded. In this segment the drug can be introduced and disappearance from the segment can be measured. Permeability can then be determined and was found to be correlated with the absorption of drugs. Other, preclinical permeability models to predict the human *in vivo* absorption are for example in situ perfusion , the Caco-2 cell culture model and excised intestinal segments in the Ussing chamber.

4.2.3 Interspecies comparison

In a study by Jezyk *et al.* (1992) permeability of various compounds was tested *in vitro* (side-by-side diffusion, Ussing chamber type cell) in four segments of the small intestine of rabbit, cynomolgus monkey, and dog (Table 4.2). The dog demonstrated the lowest permeability, independent of the site in the intestine. The authors concluded that complementary studies on tissue resistance suggested that the interspecies differences were caused by differences in intestinal absorptive surface area and/or cell density between species. In this way it may be possible to correlate the permeability in these tissue segments to human tissue [Jezyk *et al.*, 1992].

Table 4.2: Rank order permeability of several compounds tested *in vitro* in rabbit, monkey and dog [Jezyk *et al.*, 1992].

test compound	MW/LOG K_{OW}	PERMEABILITY IN DECREASING ORDER		
methanol	32 / -0.74	rabbit	monkey	dog*
mannitol	182/ -3.10	rabbit	monkey	dog*
ganciclovir	255/ -1.65	monkey	rabbit	dog*
naproxen	250/ 0.42	monkey	rabbit	dog*
PEG 900	900	rabbit =	monkey=	dog**
hydrocortisone	362/ 1.20	monkey	rabbit*	dog*
progesterone	315/ 3.87	rabbit	monkey*	dog*

*Significantly lower; ** only in ileum is permeability in dog significantly lower than in rabbit and monkey

The oral absorption of a wide range of polyethylene glycol (PEG 400 and PEG 900) and a series of D-peptides (236-406 D) was evaluated *in vivo* in rats and dogs [He *et al.* (1998)]. The absorption was correlated with morphometric information on the density distribution of tight junctions in these species. Absorption data in rat and dog have also been compared with literature values for PEG oligomers and some hydrophilic drugs in human. Qualitatively both the rat and dog showed a similar biphasic dependency of bioavailability on molecular mass, with cut-offs around 600 and 800 D, respectively. This was interpreted as due to the existence of two populations of tight junctions with smaller (restrictive) and larger pore dimensions, while the restrictive pore in dog has a larger radius than in rat. Quantitatively, dogs showed a much higher bioavailability of especially the larger oligomers, which implies that the larger tight junctions in dogs compared to rats have either a larger pore radius or are more prevalent, or a combination of both. In both dog and rat, the bioavailability versus molecular mass relationship for PEGs and peptides did not overlap. This illustrates that mass alone fail to predict the absorption of these two different types of molecules. Size and charge are also important. Generally there was a good correlation between the bioavailability of PEG compounds and several hydrophilic drugs (acyclovir, atenolol, cimetidine, nadolol and hydrochlorothiazide) in human and rat, with the rat tending to slightly underpredict bioavailability in human. In contrast, there was no apparent correlation between dog and human, with dog grossly overpredicting bioavailability in human. For example, the oral bioavailability of both acyclovir and nadolol are virtually 100 % in dog, and only 25 and 28 % in human and 16 and 15 % in rat, respectively. Furthermore, transport systems may have different efficiencies or specificities in the dog, since several compounds, that are poorly absorbed in humans (such as acyclovir, methyldopa, and nadolol), were almost completely absorbed in the dog [Dressman and Yamada, 1991]. This suggests that the rat is a better animal model than dog for predicting human bioavailability of paracellular and carrier mediated absorbed compounds [He *et al.*, 1998].

No information was found concerning the mouse and pig.

4.3 Motility and transit times

4.3.1 Man

Co-ordinated contractions of smooth muscle participate in several ways to facilitate digestion and absorption in the small intestine. They enable food to be mixed with digestive enzymes from the pancreas and bile salts from the biliary system. On the other hand, chyme is moved down the digestive tube by these contractions. In most species, the small intestine cycles through two states, each of which is associated with distinctive patterns of motility. Following a meal, when the lumen of the small intestine contains chyme, two types of motility dominate (see Figure 4.4):

1. *segmentation contractions:* If ingested materials were simply propelled through the digestive tube, very poor digestion and absorption could be expected, because the digestive enzymes would not be adequately mixed with the ingesta and the bulk of the ingesta would not come in contact with the epithelial cells that absorb nutrients. Segmentation contractions are a common type of mixing motility seen especially in the small intestine - segmental rings of contraction chop and mix the ingesta. Alternating contraction and relaxation of the longitudinal muscle in the wall of the gut also provides effective mixing of its contents.
2. *peristalsis:* Food must be propelled along the length of the digestive tube in order to be subjected to the sequential series of processing involved in disassembly and absorption. The principal type of propulsive motility, seen particularly in the oesophagus and small intestine, is peristalsis - a ring of muscle contraction appears on the oral side of a bolus of ingesta and moves toward the anus, propelling the contents of the lumen in that direction; as the ring moves, the muscle on the other side of the distended area relaxes, facilitating smooth passage of the bolus.

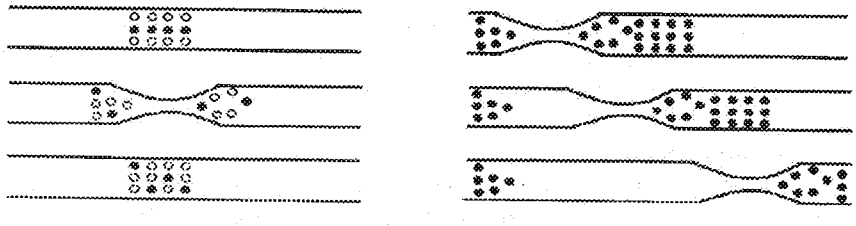

Figure 4.4 Segmental contractions (left) and peristaltic contraction (right) of the small intestine [Wilson and Washington, 1989].

The interdigestive state is seen between meals, when the lumen is largely devoid of contents. During such times, so-called housekeeping contractions propagate from the stomach through the entire small intestine, sweeping it clear of debris (see also chapter 3). Motility in the small intestine, as in all parts of the digestive tube, is controlled predominantly by excitatory and inhibitory signals from the enteric nervous system. These local nervous signals are however

modulated by inputs from the central nervous system, and a number of gastrointestinal hormones appear to affect intestinal motility to some degree.

In a study by Degen *et al.* (1996) individual differences in transit during the menstrual cycle in healthy women were quantified and compared to the intrinsic variability in healthy men. Women were tested during the follicular and luteal phase and men were also tested twice. It appeared that no sex differences and no effect of the menstrual cycle could be detected for the small bowel transit. However, irrespective of the cycle phase in women, colonic transit was significantly faster in men. Median small intestinal transit time was 7.5 h in healthy children. Judged by the number of observations in each segment of the small intestine, a marker capsule was located in the duodenum for 8%, in the proximal part for 5%, in the mid part for 12%, and in the distal part for 75% of the time. Median colonic transit time was 17.5 h. For 43% of this time, the capsule was located in the cecum [Degen *et al.*, 1996].

4.3.2 Interspecies comparison

In dogs it was discovered that a band of large-amplitude action potentials begins in the duodenum and traverses the small bowel during the interdigestive state. This motor activity is similar to what was found in humans [Itoh and Takahashi, 1981]. Also in rhesus monkeys mixing and propulsive motility, similar to those found in humans, are found in the intestine [Dressman and Yamada, 1991].
The efficiency of digestion and absorption is highly dependent on the rate at which digesta move through the gastrointestinal tract. The rate of passage can be estimated by the use of digesta 'markers', substances which are not normally secreted, digested or absorbed by the gut. Data on intestinal transit times in experimental animals appeared to be scarce. Especially this information can be of importance for predicting intestinal absorption. One of the rare studies on transit times in various species was reported by Clemens *et al.*(1980). They state that in many mammals with a simple stomach, gastric emptying and small intestinal transit of fluid and 2 mm particles occurred within 4 to 8 hours after feeding. Based upon this assumption and simply using the combined length of cecum, and colon-rectum, calculations were made with regard to the transit of the markers through the hindgut for each species. It is interesting to note that the rate of marker movement was reasonably constant between the species (2-5 cm/h). Three exceptions were noted. The pig demonstrated a transit of both fluid and particle markers 5-15 times more rapid than that of other species. Transit of markers through the cecum and colon of the rabbit was slightly faster than many species, but considerably slower than that observed in the pig [Clemens *et al.*, 1980].

Table 4.3: Interspecies comparison on transit times in the intestine.

Transit time (hours)	Human	Mouse	Rats (Wistar)	Rabbit	Dog	Pig	Monkey
small intestine	3-4 [1), 3)] 2.7- 8.5 [2)] 5.1-9.2 [4)] 4 [5)]		1.5 [5)]		1.8 [5)] 0.5-2 [6)]		
colon	6.2-54.7 [4)]		6.0-7.2 [7)]	3.8 [7)]			

[1)] Kararli, 1995; [2)] transit time depending on particle size and caloric density of the meal. Overall it appears that transit consistently takes 3-5 hr, independent of particle size, when subjects are fasted or have ingested a light meal.; [3)] Degen and Philips., 1996; [4)] in children (8-14 years of age) [Fallingborg *et al.*, 1990]; [5)] Davies and Morris, 1993; [6)] Miyabayashi *et al.*, 1986; [7)] Sakaguchi *et al.*, 1987.

Data on transit time through the small intestine are even more rare. The residence time in the small intestine in the pig is expected to be longer than in humans, owing to the greater length of this segment of the gastrointestinal tract. In dogs it was found that the difference in length correlates well with the observed transit times in man and dogs. The small intestine of the dog is only about half as long as in humans and, in the fasted state, the transit time in the dog is only about half that for humans [Dressman and Yamada, 1991]. In studies in beagle dogs and humans, the mean transit time of a Heidelberg capsule in the small intestine was observed to be approximately 2 h in dogs, compared with almost 4 h in humans [Youngberg, 1984]. However, the range in dogs was found to be much broader than in humans, suggesting that drug absorption is likely to be more variable and less complete in dogs [Dressman, 1986].

4.4 pH

4.4.1 Man

The lumen of the proximal jejunum has a pH of 5.0-6.5, which rises slowly along the length of the small intestine to pH 6-7 (sometimes up to pH 9) [Wilson and Washington, 1989]. Several fluids are secreted in the small intestine to neutralise the acidic food particles from the stomach. Secretion from glands in small intestine:

1. Brunner glands (secrete protective alkaline secretion (bicarbonate) and mucus).
2. Intestinal cells (secrete mucus and a few enzymes).

pH of the intestinal juice from these glands is ca. 7.5 - 8.0.

4.4.2 Interspecies comparison

Humans, dogs, pigs and monkeys secrete a pancreatic juice rich in enzymes and bicarbonate. As a result of the balance between gastric acid and pancreatic secretions, intestinal pH is close to neutral for these species, with a slight tendency to more basic values in the monkey, and a slight tendency toward more acidic values in humans (see Table 4.4) [Dressman and Yamada, 1991]. Generally, in the small intestine, the measured pH becomes progressively more alkaline in the distal portions within the same animal. Intestinal pH is consistently 1

unit higher in dogs than in humans when comparison is made at times normalised to gastric emptying of the pH measuring device [Dressman, J.B. (1986)].

Table 4.4: Interspecies comparison on pH in the intestine

	Human	Rat (Lister hooded)	Rats (Wistar)	Rabbit	Dog	Pig	Monkey
pH during fasted state:			6.5-7.1 [1]	6.0-8.0 [1]	6.2-7.5 [1]	6.0-7.5 [1]	5.6-6.0 [1] 7-9 [4]
- duodenum	5-7 [1] 5.6-6.4 [4]	7.1 (6.9) [1]	(6.9) [1]		4.5-7.5 [5]	7.2 [4]	
- jejunum	6-7 [1]						
- ileum	7.0 [1] 7.4 [2] 7-8 [3]						
-jejunum/ ileum		8.0 (7.4) [1]	(7.8) [1]				
- cecum	5.9 [2]	7.2 (6.4) [1]	6.8 (6.7) [1]	6.6 [1]	6.4 [1]	6.3 [1]	5.0 [1]
- colon	5.5-7 [1, 2]	7.6 (6.8) [1]	6.6 (7.1) [1]	7.2 [1]	6.5 [1]	6.8 [1]	5.1 [1]
- rectum	7 [1]						

Between brackets: in fed state, during meal; [1] [Kararli, 1995]; [2] [Fallingborg *et al.*, 1990]; [3] [Rouge *et al.*, 1996]; [4] [Dressman and Yamada, 1991]; [5] [Youngberg *et al.*, 1985]

Base-line duodenal pH values in dogs in the fasted state varied during the first 5 min. after gastric emptying between 4.5 and 7.5 (mean 6.1 ± 0.1). A significant linear upward trend in pH over the 60-minute post-gastric emptying period was found (mean pH = 6.2 + 0.017 t) [Youngberg *et al.*, 1985]. The duodenal pH in the fasted state in pigs is a 0.5-1 pH unit higher than in humans. There is no information about the intestinal pH in the fed state, however [Dressman and Yamada, 1991]. Marked host species differences in the pH of the gut lumen are associated with varying population densities, species and distribution of microflora in the gut lumen (see for more details Chapter 6.3.2).

McEwan *et al.* (1990) measured the mucosal surface pH of pig jejunum *in vivo*. They found that when the pH in the bulk phase of the perfusing buffer solution was 7.10, the pH at the mucosa was 6.19 ± 0.04 (n=19). Mucosal surface pH did not change over 60 minutes of perfusion with Krebs phosphate buffer. This relatively acidic mucosal surface pH is not an anoxic artefact since anoxia resulted in a significant alkalisation of the mucosa. This finding, that the pig, like man and the rat, has a jejunal "acid microclimate" has important implications for weak electrolyte absorption from the upper small intestine of this species as well as for any other pH dependent brush border process. According to the original microclimate hypothesis the acid microclimate is the predominant controlling factor influencing the rate at which weakly dissociable compounds with appropriate dissociation constants are absorbed from the upper small intestine. Alterations in the microclimate pH could, therefore, have profound effects on the rates at which these compounds are transported [McEwan *et al.*, 1990]. No information was found according the existence of a similar jejunal "acid microclimate" in mouse, rabbit, dog and monkey.

4.5 Conclusions

Some data concerning transit times and pH values are still lacking and more information is needed about the microflora and the effect of food on the absorption of compounds in different species. Transport systems (i.e. active transporter proteins, carriers, efflux transporters, tight junctions) may be expressed to a different extent among species, therefore, it is important to know by which mechanism a compound is absorbed in the intestine. However, a species comparison on specific transport systems has not been made in this report.

It could be concluded that the relative absorptive area among species are not very different. Although based on relative lengths and diameter of the small intestine it could be expected that the absorptive capacity of the small intestine in rhesus monkeys is somewhat lower than in human, whereas the surface area of the principal absorptive mucosa in the pig is greater than that in humans. Furthermore, the rat seems to be a better model than the dog for paracellular transport in humans since dogs overpredict the bioavailability in humans. This could be due to larger tight junctions having larger pore radius, being more prevalent, or both in dogs than in rats or humans. The motor activity found in the small intestine of most species seems to be very similar to those found in humans. It is, however, very difficult to obtain data on the transit time through the small intestine of different species. The residence time in pigs is expected to be longer than in humans, owing to the greater length of this segment of the gastrointestinal tract. However, it is also mentioned that pigs demonstrate a higher transit of both fluid and particle markers (5-15 times) than other species. It was found that the differences in length in humans and dogs correlates well with the observed differences in transit times in both species. The intestinal pH in most species is close to neutral, with a slight tendency to more basic values in the monkey. The pH becomes progressively more alkaline in the distal portions within the same animal.

5. Bile

5.1 Anatomy of the gallbladder

5.1.1 Man

The gall bladder is a small sac, pear-shaped, about 7 – 10 cm long and is the storage depot for bile. It is located (in a depression) at the back part surface of the liver. It is connected with the liver via the cystic duct and the hepatic bile duct (see Figure 5.1). Together they form the common bile duct, which leads into the small intestine. Just before the common bile duct enters the small intestine it joins with the pancreatic duct at the ampulla of Vater. A valve around this ampulla is called the Sphincter of Oddi and controls the release of bile into the small intestine. The mucosa of the gall bladder consists of simple columnar epithelium arranged in rugae resembling those of the stomach. The gall bladder lacks a submucosa. The middle, muscular coat of the wall consists of smooth muscle fibers, which play a role in the contraction of the gall bladder [Tortora and Grabowski, 1996; Benneth and Plum, 1996].

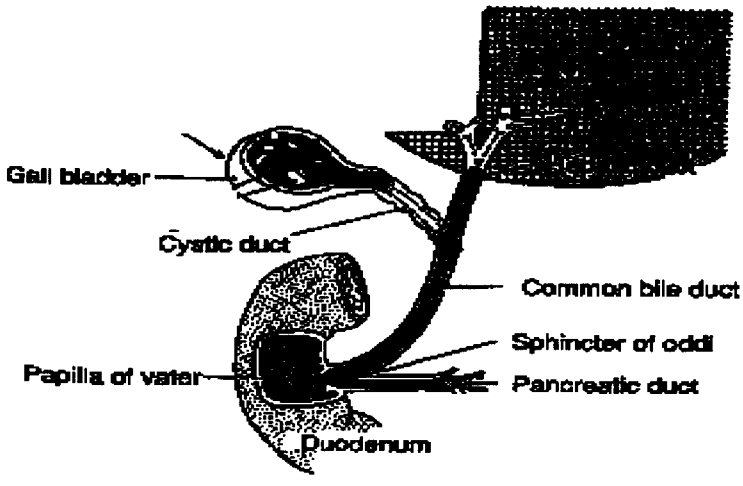

Figure 5.1 The human liver and gall bladder with their ducts [Guyton, 1991]

5.1.2 Interspecies comparison

Like humans the gall bladder of the rabbit (New Zealand White) is also situated in a deep depression of the caudal surface of the liver. The hepatic ducts unite to form the common bile duct which receives the cystic duct from the gall bladder and enters the dorsal surface of the duodenum [Tortora and Grabowski, 1996]. The rat has no gall bladder. Bile from the rat liver flows directly through the (hepatic) bile duct into the small intestine [Hebel and Stromberg, 1988]. It is known that dogs have no Spincter of Oddi. No information was found with regard to the anatomy and histology of the gall bladder in other animal species.

5.2 Physiology

5.2.1 The human bile

Bile is continuously formed in bile canaliculi in the liver hepatocytes, were it is collected and secreted into the hepatic bile duct. Bile is a complex fluid containing water, electrolytes and a battery of organic molecules including bile acids (water-soluble derivatives of cholesterol), cholesterol, phospholipids and bilirubin. Adult humans produce up to 800 ml bile daily. When the small intestine is empty the Sphincter of Oddi is closed and the backed-up bile flows into the cystic duct to the gall bladder for storage. Here it is concentrated about 5-fold, but sometimes it can be even concentrated up to a maximum of 12- to 20 fold. Concentration occurs by absorption of water and ions by the mucosa from the gall bladder. Human gall bladder bile consists of approximately 84% water, 11.5% bile salts/acids, 3% lecithin, 0.5% cholesterol and 1% other components, such as bile pigments, inorganic ions and proteins [Kararli, 1995; Tortora and Grabowski, 1996; Benneth and Plum, 1996]. In response to the intestinal presence of digestion products (especially lipid digestion products) the gall bladder contracts and the Sphincter of Oddi relaxates, leading to expulsion of bile into the small intestine, with peak flows occurring ca. 30 min after meal ingestion [Charman *et al.*, 1997]. In the small intestine the two fundamentally important functions of bile in all species are:

1. Bile contains bile acids, which are critical for digestion and absorption of fats and fat-soluble vitamins in the small intestine.
2. Many waste products are eliminated from the body by secretion into bile and subsequent elimination in faeces.

The involvement of bile in the digestion and absorption process is a complex process. Presence of lipids in the small intestine stimulates secretion of bile salts, and biliary lipids. Lipids enters the duodenum as a crude emulsion through the hydrolysis of lipids by lipase. The biliary lipids adsorb to the surface of this crude emulsion, stabilising it and further reducing it to droplet size and micelles are formed. The reduction to droplet size (emulsification) occurs through the detergent action of the bile acids on particles of dietary fat. Emulsification is not digestion per se, but is of importance because it greatly increases the surface area of fat, making it available for digestion by lipases, which can not access the inside of lipid droplets. Formation of micelles occurs by the solubilisation of lipids by bile acids. The micelles are thus aggregates of fatty acids, cholesterol and monoglycerids that remain suspended in water. Bile may also improves the bioavailability of poorly water soluble substances, such as drugs, by enhancing the rate of dissolution and/or solubility. An increase in the rate of dissolution can occur via:

- A decrease in the interfacial energy barrier between for instance the solid drug and the dissolution medium (via enhanced wetting) leading to an effective increase in the surface area.
- An increase in the solubility via micellar solubilisation.

The wetting process predominates at a bile salt concentration below the critical micelles concentration (concentration at which micelles starts to form), whereas enhanced solubility is dominant at concentrations above the critical micelles concentration. The wetting and solubilisation process is compound dependent due to the specificity of the interactions associated with these processes [Tortora and Grabowski, 1996; Charman *et al.*, 1997]. Some models (relationships) are developed to predict the extent to which bile salts can enhance the solubility of a drug, based on the physicochemical properties of the compound (partition coefficient oct/water and aqueous solubility) [Mithani *et al.*, 1997]. Poorly water-soluble drugs partition between the emulsion droplets and the micellar phase. The character of these colloidal phases is controlled by the relative concentrations of bile salt, biliary lipids and lipid digestion products and is therefore continually changing. As time progresses after ingestion of a meal the concentration of the lipid emulsions droplets decreases relative to the micelles and the nature of the available 'dissolution media' changes correspondingly. Therefore, dissolution is subject to change not only as a function of the fed/fasted cycle but also to change the kinetics of lipid digestion [Charman *et al.*, 1997]. The constituents of the micellar phase affect on the intestinal permeability of poorly water-soluble drugs via 3 major processes:

1. Lipid digestion products and bile salts characteristic for the fed state may alter the intrinsic permeability of the intestinal membrane leading to increased penetration via paracellular or transcellular routes.
2. Solubilisation of poorly water-soluble drugs within bile salt micelles may facilitate diffusion through the unstirred water layer leading to increased absorption.
3. Conversely, solubilisation may decrease the intermicellar 'free' fraction of drug that could lead to a decrease in absorption (total fraction of drug outside the micelles decreases and thus also the free fraction).

These divergent effects are probably the basis for often contradictory reports concerning drug absorption when drugs are administered as solubilised systems [Charman *et al.*, 1997].

After secretion into the small intestine most bile salts are re-absorbed. Although large amounts of bile acids are secreted into the intestine every day, only a relative small part is lost from the body by excretion with faeces. This is because approximately 95% of the bile acids delivered to the duodenum are re-absorbed into blood within the ileum. The bile acids are then extracted from the venous blood (which goes from the ileum straight into the portal vein) by the liver. The net effect of this so-called enterohepatic recirculation is that each bile salt molecule is reused about 20 times, often two or three times during a single digestive phase [http://arbl.cvmbs.colostate.edu]. Presence of anaerobic bacteria in the duodenum especially bifidobacteria and bacteroids play an important role in this process. These bacteria are capable of deconjugating bile acids and thereby increasingh the re-absorption. Therefore, antibiotics can have a negative impact on the recirculation process, as by killing the bacteria in the duodenum deconjugation will decrease [Northfield and McColl, 1973]. The enterohepatic recirculation can also play a role in the recirculation of drugs. After absorption drugs enter the systemic circulation. When passing the liver, extraction from the blood may

occur, followed by excretion into the duodenum with the bile. In the duodenum re-absorption can take place.

5.2.2 Composition and excretion rate of the bile

The hepatic bile flow in humans is ca. 2 – 22 ml/d/kg. As mentioned before it consists mainly of water. Bile salts are the most important constituents (3 – 45 mmol/l), with minor proportions of bilirubin, cholesterol, fatty acids (phospholipids) and lecithin [Dressman and Yamada, 1991; Smeets-Peeters *et al.*, 1998]. Bile salts consists mainly of glycocholic acid (trihydroxy bile salt, ca. 37% of the total bile salts) and glycochenodeoxycholic acid (dihydroxy bile acid, ca. 33%), next to taurocholic acid (7%), taurochenodeoxycholic acid (ca. 7%) and taurodeoxycholic acid (ca. 2%) (see also Table 5.4). The bile salts are thus mainly conjugated by glycine [Wildgrube *et al.*, 1986; Washizu *et al.*, 1991; Smeets-Peeters *et al.*, 1998]. Duodenal bile acids, which were measured after given a test meal to healthy volunteers, showed an almost similar profile compared to hepatic bile. Bile acids were conjugated in the same proportions with glycine and taurine, which is different in hepatic bile, as glycine conjugates prevail here. Relative amounts of cholic acid (ca. 40% of the total amount), chenodeoxycholic acid (ca. 40%) and deoxycholic acid (ca. 20%) were comparable with the relative amounts in the hepatic bile [Fausa, 1974]. This indicates that hepatic bile (but also bile from the gall bladder; see paragraph 5.4.1) can be used for the prediction of the duodenal bile acid profile.

Table 5.1: Concentrations of electrolytes in human hepatic bile [Erhlinger, 1987]

	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-
conc.* (mEq/l)	132 - 165	4.2 – 5.6	1.2 – 4.8	1.4 – 3.0	96 - 126	17 - 55

*numbers indicate range of published values

Total bile salt concentrations obtained from the gall bladder were ca. 14 mmol/l [Bloch *et al.*, 1980]. This is within the range observed for the hepatic bile, which is not expected because as bile is concentrated in the gall bladder higher concentrations are expected. On the other hand comparison of these values are difficult as gall composition may fluctuate in relation to food intake and composition. The total phospholipid concentration in bile of the gall bladder was ca. 2.8 mmol/l [Bloch *et al.*, 1980].

5.2.3 Interspecies comparison

5.2.3.1 Composition and excretion rate of the bile

As can be seen in Table 5.2 large differences are observed in bile flow within one species. Probably one of the causes is that the rate of bile fluid secretion is dependent on the circadian rhythm of the animal [Vonk *et al.*, 1979]. The bile flow of the rodents and rabbits is rather high compared with humans, monkeys and dogs. In rats this can be explained by the lack of a gall bladder. Bile is than secreted continuously in dilute form and in large volumes [Kararli, 1995]. In mice and rabbits the cause of the high bile flow is unclear. Differences in food

intake and kind of food compared to humans, monkeys and dogs can be one of the reasons. Like humans, dogs show also a bile peak flow at ca. 30 min after meal ingestion [Charman *et al.*, 1997]. The pig has substantially less capacity to concentrate bile in the gall bladder [Dressman and Yamada, 1991]. Bile obtained from the dog and pig gall bladder contains similar total amounts of bile acids, which is 3-fold higher compared to human gall bladder bile. The amounts of phospholipids were similar in man and pig, but were 2-fold higher in dogs. The total lipid content differed markedly between these species. The lipid content in pig gall bladder bile was ca. 2-fold higher compared to man and even 3-fold higher in dogs [Nakayama, 1969]. The values were expressed as mg/ml which makes a comparison with the values in Table 5.2 difficult.

Table 5.2: Flow rates and composition of hepatic bile from different animal species [Dressman and Yamada, 1991; Davies and Morris, 1993; Kararli, 1995; Kararli, 1989; Smeets-Peeters *et al*, 1998, Smit et al., 1993].

	mouse	rat	rabbit	dog	monkey
bile flow (ml/d/kg)	80-120*	48 – 92	130	19 - 36	19 - 32
total bile salt rate (mmol/d/kg)	5.2	0.85 – 1.0	--	1.6 – 2.9	0.36
phospholipid rate (mmol/d/kg)	0.39	0.15 – 0.30	--	--	0.08
cholesterol rate (mmol/d/kg)	0.06	0.030 – 0.035	--	--	0.014
total bile salts (mmol/l)	--	8 – 25	6 - 24	16 - 187	22
phospholipid conc. (mmol/l)	--	2.6 – 3.5	--	--	4.3
cholesterol (mmol/l)	--	0.4 – 0.6	--	--	0.75

*numbers indicate range of published values; --: no data

Table 5.3: Concentrations of electrolytes in hepatic bile obtained from the rat, rabbit, dog, monkey and human.

	Rat [1)]	Rabbit [1)]	Dog [1)] (beagle)	Pig [2)] (Poland-China)	Monkey [2)] (baboon)	Human [2)]
Na^+	157 - 166	148 - 156	141 - 230	161 ± 3	157 ± 5	170 ± 8
K^+	5.8 - 6.4	3.6 - 6.7	4.5 - 11.9	4.6 ± 0.1	4.3 ± 0.6	5.2 ± 0.4
Ca^{2+}		2.7 - 6.7	3.1 - 13.8	11.7 ± 0.4*	5.4 ± 1.6*	9.5 ± 1.0*
Mg^{2+}		0.3 - 0.7	2.2 - 5.5	2.4 ± 0.1*	2.2 ± 0.7*	4.2 ± 0.7*
Cl^-	94 - 98	77 - 99	31 - 107	96.0 ± 5.0	93.0 ± 4.0	99.0 ± 7.0
HCO_3^-	22 - 26	40 - 63	14 - 61	27.5 ± 0.7	45.4 ± 5.5	21.6 ± 3.7

concentrations in mEq/l; numbers indicate range of published values; * mg/dl;
[1)] Ehrlinger, 1987; [2)] Kobayashi *et al.*, 1998

The concentrations of electrolytes in the hepatic bile of different species are listed in Table 5.3. Compared with man, dogs (beagle), rabbits and rats show similar electrolyte profiles in hepatic bile.

As mentioned before bile salts are important in the wetting and solubilisation process (and thereby in the absorption process) of lipids and especially poorly water soluble drugs. The total bile salt output is rather similar between the species, only the dog has a higher output. On the other hand the composition of bile salts showed marked differences between the species, as is shown in Table 5.4.

Table 5.4: Biliary bile salts composition (% of total) in man and various animal species [Alvaro *et al.*, 1986; Wildgrube *et al.*, 1986; Washizu *et al.*, 1991; Chan *et al.*, 1995; Smeets-Peeters *et al.*, 1998].

	Human (hepatic)	Rabbit (hepatic)	Dog (gall bladder) (mongrel)	Rat (S-D)	Pig (gall bladder)
Tauro-β-muricholic acid	--	--	--	13 ± 1	--
Tauro-HDCA	--	--	--	--	4 ± 0.5
Tauro-CA	7 ± 3	--	81 ± 10	48 ± 5	--
Tauro-CDCA	7 ± 3	--	6 ± 4	32 ± 4	3 ± 0.3
Tauro-DCA	2 ± 2	--	13 ± 1	6 ± 1	--
Glyco-HCA	--	--	--	--	13 ± 2
Glyco-HDCA	--	--	--	--	48 ± 4
Glyco-CA	37 ± 8	5 ± 4	--	--	1 ± 0.2
Glyco-CDCA	33 ± 9	--	--	0.2 ± 0.02	31 ± 3
Glyco-DCA	10 ± 6	87 ± 6	--	--	--

Abbreviations: CA: cholic acid, CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; HCA: hyocholic acid; HDCA: hyodeoxycholic acid; S-D: Sprague-Dawley

Bile salts obtained from human, dog and rabbit gall bladder showed similar bile acids profile [Coleman *et al.*, 1979; Alvaro *et al.*, 1986; Washizu *et al.*, 1990], which could be expected as bile is only concentrated in the gall bladder. Relative profiles (% of total amount) will then remain similar. Therefore bile obtained from the liver or gall bladder can be normally compared.

Bile salts showed marked differences in their hydrophilic properties, owing to their hydroxyl structure and type of conjugation. Considerable differences in the type of bile salts (i.e. number and position of hydroxyl groups, presence of oxo groups) and in conjugation pattern with glycine or taurine are observed among the various animal species and human. Trihydroxy bile salts (i.e. cholic acid) where predominantly found in dog and rat bile, whereas trihydroxy and dihydroxy bile salts (i.e. chenodeoxycholic acid, deoxycholic acid and hyodeoxycholic acid) were predominantly found in pig and in man and dihydroxy bile salts were predominantly found in the rabbit. From the type of conjugation it can be concluded that the rat and dog conjugate their bile salt almost exclusively with taurine and pig, rabbit and man prevailing with glycine [Alvaro *et al.*, 1986; Verkade *et al.*, 1993]. Bile salts in rat and dog are more hydrophilic compared to pig and man, since tauroconjugates and trihydroxy bile salts are more hydrophilic than glycoconjugates and dihydroxy bile salts, respectively. The phospholipid composition in dog, rat, pig, and man was similar, in which

phosphatidylcholine represents more than 95% of the total phospholipids [Alvaro *et al.*, 1986]. Information with regard to monkey and mouse is lacking.

5.2.3.2 Factors influencing composition and rate of bile excretion

In literature several studies are described in which food composition, fasting/feeding and radiation are factors affecting biliary excretion and bile salt composition (in human and several animal species). The fasting state influences the lipid flow and composition of the bile. In the rhesus monkey biliary lipid secretion decreased after a 7-day fast, the molar percentage of cholesterol and phospholipids increased in hepatic bile. This returned to a normal range 72 hours after reconsumption of standard oral feed [Redinger *et al.*, 1973]. In humans the molar percentage of cholesterol increased 15 hours after fasting, but decreased after fasting for 4 to 6 days [Duane *et al.*, 1976; Bloch *et al.*, 1980]. Parental nutrition could not overcome these problems when fasting is needed, as rabbits (New Zealand White) receiving parental nutrition showed a decreased bile flow of almost 50%, compared with normal feeding rabbits. Bile acid concentration was also decreased to 50%, cholesterol stayed constant and phospholipids concentration decreased ca. 60%. From the bile acids, glyco-chenodeoxycholic acid (-40%), glycolithocholic acid (+500%) and glycodeoxycholic acid (-50%) changed strongly [Das *et al.*, 1996]. The change in glycolithocholic acid is probably due to bacterial biotransformation to lithocholic acid and its increased absorption from the cecum.

Addition of dietary phospholipids such as ethanolamine influences the bile composition of the rat (Wistar). Cholesterol concentration decreased and bile acid concentration and biliary phospholipids increased. The increase in biliary phospholipids was also observed by adding lecithin to the food [Peled and Gilat, 1994]. Biliary lipid composition can thus be modulated by dietary phospholipids. The protein source in food seems to have no effect on bile composition, as observed in vervet monkeys fed with different protein source. No difference was observed in bile composition when casein or soybean was added to a high-cholesterol diet [Jaskiewicz *et al.*, 1987]. It is questionable to what extent this study was sensitive to detect a difference as the high-cholesterol diet can mask the outcome. Not only food affects the composition and excretion rate of bile, also "external" factors do. Pigs (Large WhitexLandrace) started vomiting after irradiation and the food consumption decreased. This correlated strongly with a decrease in bile flow, in which the dihydroxylated bile acids were increased. Dihydroxylated bile salts are known to interfere with gut functions [Scanff *et al.*, 1997], which probably explains the observed vomiting and decrease in food intake.

5.3 Conclusions

Considering bile flow rate and total hepatic bile salt concentration monkey and dog hepatic bile are comparable with the hepatic bile of man (bile flow rate and hepatic bile salt

concentration of bile from the pig are not known). Pig bile is rather similar to man considering the hepatic bile salt composition: both conjugate their bile mainly with glycine and the bile salts are predominantly trihydroxy and dihydroxy bile salts. On the other hand bile obtained from the pig gall bladder showed marked differences compared with gall bladder bile of man: total bile acid content is in man 3-fold higher and lipid content ca. 2-fold higher. As it was found that hepatic bile and gall bladder bile are comparable when taken into account the relative amount of constituents similarities between human and pig bile are less. Overall there is no animal species which shows similar flow-rate, bile salt content, bile salt composition and bile lipid content compared to humans.

The obtained values from literature showed marked differences also within one species, which makes comparison difficult. The general functions of the bile is wetting and solubilisation of lipids (and eventually low water soluble compounds). These two processes are dependent on not only the bile salts (composition, concentration) but also on the physicochemical properties of the lipids or compounds to be absorbed. It can be expected that differences in these aspects between the species and within the species will result in marked differences in the wetting and solubilisation processes. This may lead to differences in drug absorption for especially poorly water soluble compounds.

6. Metabolism

6.1 Introduction

The processes of metabolism and disposition have a major bearing upon the biological properties of xenobiotics, determining both the chemical natures and target concentrations of the compound-derived materials in the body. Interspecies differences in metabolism represent a major complication in toxicity testing, being responsible for important differences both in the nature and magnitude of toxic responses. In particular, these differences represent probably the single greatest complicating factor in the use of animal toxicity data as an indication of potential human hazard. Although it is considered desirable to identify a species which metabolises the test compound like man, this ideal is generally not attainable. While metabolic and toxicokinetic data should be used in the selection of animal species, in reality the choices are constrained by other major factors such as availability, background pathological knowledge and regulatory acceptability. On the other hand, species differences in metabolism may present exploitable opportunities for insights into mechanisms of toxicity and with appropriate supporting data may thereby increase confidence in the animal-to-human extrapolation [Caldwell, 1992].

6.2 Liver metabolism

The species differences which are encountered in the pathways of xenobiotic metabolism may be qualitative or quantitative in nature. Qualitative differences may arise from either reactions being restricted in their occurrence to particular species or groups of species (Table 6.1) or from a species being (relatively) defective in a metabolic reaction of otherwise widespread occurrence (Table 6.2) [Caldwell, 1992].

Table 6.1: Metabolic reactions whose occurrence is restricted to primate species [Caldwell, 1986]

Aromatisation of quinic acid
Glutamine conjugation of arylacetic and aryloxyacetic acids
O-Methylation of 4-hydroxy-3,5-diiodobenzoic acid
N-Glucuronidation of sulfadimethoxine
C-Glucuronidation of pyrazolones
Quaternisation by glucuronidation of tertiary amines
Carbamate acyl glucuronidation

Table 6.2: Some examples of species defects for common metabolic reactions [Smith, 1988]

Metabolic pathway	Substrates affected	Species/strain affected	Reference
C-oxidation	Debrisoquine and sparteine	Rat (DA, female) Guinea pig	Al-Dabbagh *et al.*, 1981
	Amphetamine	Guinea pig	Dring *et al.*, 1970
	Phenmetraxine		Franklin *et al.*, 1974
N-hydroxylation	2-acetamidofluorene	Guinea pig	Weisburger, 1964
	clorphentermine	Rat (Wistar albino)	Caldwell *et al.*, 1975
N-oxygenation	Trimethylamine	Chicken (Rhode Island Red)	Bolton *et al.*, 1976
Epoxide hydration	Styrene 7,8-oxide	Rat (F344)	Oesch *et al.*, 1983
Acetylation	Arylamines	Dog and related species	Bridges and Williams, 1963; Williams, 1974
Sulphation	*p*-nitrophenol and phenol	Mouse (brachymorphic)	Capel *et al.*, 1972
Glucuronidation	Bilirubin	Pig	
	Androsterone and various xenobiotics	Rat (Gunn and other Wistar strains) Bolivian squirrel monkey	Popper, 1985; Portman *et al.*, 1984

6.2.1 Phase I metabolic activities: Cytochrome P450 monooxygenase

Cytochrome P450 comprises a superfamily of enzymes that catalyse oxidation of a variety of xenobiotic chemicals such as drugs, toxic chemicals and carcinogens as well as endobiotic chemicals including steroids, fatty acids, prostaglandins and vitamins. The liver microsomal P450 enzymes contribute significantly to the biotransformation of a number of xenobiotic chemicals. Therefore it is important to determine if there are marked species-related differences in the catalytic roles of individual P450 enzymes towards these chemicals between experimental animal models and humans [Shimada *et al.*, 1997]. Apart from the catalytic role of individual P450 enzymes, also general physiological characteristics of the liver will influence the amount of biotransformation of a compound. Table 6.3 gives information concerning the body and liver weights of four species (rat, monkey, dog and human), mg of protein per gram of liver and per. The mg protein per gram of liver is in these species is very similar [Knaak *et al.*, 1993].

Table 6.3: Liver characteristics of different species (mean ± SE, n=4) [Knaak *et al.*, 1993].

Measurements	Human	Rat (Sprague-Dawley)	Dog (beagle)	Monkey (macaca)
body weight (kg)	70[1]	0.29 ± 0.0	8.6 ± 0.4	2.8[1]
liver weight (g)	1700[1]	10.6 ± 0.3	360 ± 20	62.5[1]
mg protein per:				
g liver	131 ± 7	164 ± 14	120 ± 5	126 ± 3
whole liver	222700	1738 ± 123	43200 ± 4199	7875

[1] Estimated weight

In a number of *in vitro* studies the metabolic activities of various CYP450 isoenzymes were studied in different species. To compare the results of different studies, the enzyme activities

in different animal species are also calculated as ratio of the enzyme activity measured in humans (see bold numbers in Tables).

Table 6.4: P450 contents and drug oxidation activities in liver microsomes of various animal species (data are of mean ± SD) [Shimada *et al.*, 1997]

P450 content[1] and drug oxidation[2]	Major human P450	Human (n=18)	Monkey (cynomolgus) (n=5)	Dog (beagle) (n=6)	Rat (S-D) (n=3)
P450 content[1]		307 ± 160	1030 ± 106	385 ± 36	673 ± 50
		1[3]	**3.4**	**1.3**	**2.2**
Phenacetin *O*-deethylation	1A2	32 ± 30	110 ± 20	28 ± 10	36 ± 20
		1	**3.4**	**0.82**	**1.1**
Coumarin 7-hydroxylation	2A6	21 ± 22	209 ± 126	12 ± 5	< 1
		1	**10.0**	**0.57**	**< 0.05**
Pentoxyresorufin *O*-dealkylation	2B6	5 ± 5	35 ± 5	60 ± 10	70 ± 10
		1	**7**	**12**	**14**
Phenytoin *p*-hydroxylation	2C9	29 ± 15	45 ± 12	84 ± 6	44 ± 8
		1	**1.6**	**2.9**	**1.5**
Mephenytoin 4-hydroxylation	2C19	39 ± 23	144 ± 26	106 ± 13	74 ± 15
		1	**3.7**	**2.7**	**1.9**
Bufuralol 1-hydroxylation	2D6	20 ± 14	471 ± 58	12 ± 2	743 ± 74
		1	**23.6**	**0.6**	**37.2**
Aniline *p*-hydroxylation	2E1	696 ± 986	227 ± 165	280 ± 91	403 ± 57
		1	**0.33**	**0.40**	**0.58**
Benzphetamine *N*-demethylation	3A/2B	544 ± 427	940 ± 200	348 ± 78	2387 ± 639
		1	**1.7**	**0.64**	**4.4**
Ethylmorphine *N*-demethylation	3A/2B	423 ± 543	1080 ± 249	273 ± 149	3370 ± 621
		1	**2.6**	**0.65**	**8.0**
Erythromycin *N*-demethylation	3A4/5	244 ± 212	586 ± 101	144 ± 70	930 ± 164
		1	**2.4**	**0.59**	**3.8**
Nifedipine oxidation	3A4/5	605 ± 691	3826 ± 131	456 ± 126	820 ± 187
		1	**63.8**	**0.75**	**1.4**

[1] pmol P450/mg protein, [2] pmol/min/mg protein, [3] in bold: ratio to human, S-D = Sprague-Dawley

Shimida *et al.* (1997) showed P450-dependent oxidation activities in liver microsomes of four different species including Sprague-Dawley rats, beagle dogs and cynomolgus monkeys (Table 6.4). Antibodies raised against human CYP1A2, 2A6, 2C9, 2E1, 3A4 and monkey CYP2B17, and rat CYP2D1 were used to examine if these antibodies recognise homologous P450 proteins in the liver microsomes of different animal species (Table 6.5). Total P450 levels in liver microsomes of various animals were determined spectrally. These levels were found to be roughly correlated with the activities of CYP1A2, 2B6, 2C19 and 3A4 (correlation not shown). The levels of individual forms of P450 were quantitated for liver microsomes of humans and monkeys using densitometric tracings of the immunostaining bands. These relative intensities were correlated with the activities of P450-dependent drug oxidations of individual forms of P450. Most isoenzyme levels were found to be correlated to their corresponding drug oxidation activity (correlations not shown). However, there were no

significant correlations between CYP2D levels and bufuralol 1'-hydroxylation activities in humans and monkeys.

Table 6.5: Immunoreactive responses of antibodies raised against different P450 isoenzymes with P450 proteins in liver microsomes of various animal species [Shimada *et al.*, 1997].

Antibody against:	Human	Monkey (cynomolgus)	Dog (beagle)	Rat (Sprague-Dawley)
Human 1A2	++ (1A2)	++	+	-
Human 2A6	++ (2A6)	++	+	+
Monkey 2B17	++ (2B6)	++	+	-
Human 2C9	++ (2C9)	++	-	-
Rat 2D1	++ (2D6)	++	-	++
Human 2E1	++ (2E1)	++	++	++
Human 3A4	++ (3A4)	++	+	+

++: high, +: weak, and -: no immunoreactive response; in brackets the corresponding isoenzyme for human

Monkey liver P450 proteins were found to have relatively similar immunochemical properties by immunoblotting analysis to human enzymes, which belong to the same P450 gene families (Table 6.5). Mean catalytic activities (on basis of mg microsomal protein) of P450-dependent drug oxidations with most substrates were higher in liver microsomes of monkeys than of humans (Table 6.4). Humans showed much higher activities for aniline *p*-hydroxylation than those catalysed by monkeys, however. When the catalytic activities of liver microsomes of monkeys and humans were compared on the basis of nmol of P450, both species gave relatively similar rates towards the oxidations of most substrates used, except that aniline *p*-hydroxylation was higher and bufuralol 1'-hydroxylation was lower in humans than in monkeys (not shown). On the other hand, the immunochemical properties of P450 proteins and the activities of P450-dependent drug oxidation reactions in dogs and rats were somewhat different from those of monkeys and humans; the differences in these animals species varied with the P450 enzymes examined and the substrate used (not shown) [Shimada *et al.*, 1997].

In another study by Stevens *et al.* (1993) very similar results were found for CYP450-dependent reactions in human and monkey (rhesus) liver microsomes (Table 6.6). The total amount of P450 that was measured was approximately the same in both studies. In monkey liver microsomes the total amount of P450 was 3 times higher than in human liver microsomes. This partly explains the higher drug oxidations rates of most P450-isoenzymes found in monkeys compared to humans (Table 6.4 and 6.6). However, coumarine 7-hydroxylation activity was found to be the only activity which was higher in humans than in monkeys in this study, but not in the study mentioned above. This might be due to the different strains of monkeys that were used in these studies. Stevens *et al.* also analysed the measurements for sex differences, but no differences were found in both species.

Table 6.6: Cytochrome P450-mediated enzymatic reactions in human and monkey liver microsomes (data are of mean ± SD) [Stevens *et al.*, 1993]

P450 content[1] and drug oxidation[2]	Major human P450	Human (n=12)	Monkey (n=6) (rhesus)
Total P450[1]		290 ± 60	950 ± 80
		1[3]	**3.3**
Ethoxyresorufin *O*-Deethylation	1A1/1A2	25.6 ± 13.7	273 ± 37
		1	**10.7**
Coumarin 7-Hydroxylation	2A6	423 ± 225	97.7 ± 33.1
		1	**0.2**
Pentoxyresorufin *O*-Dealkylation	2B6	0.79 ± 0.22	3.92 ± 0.88
		1	**5.0**
N-Nitrosodimethyl-amine	2E1	620 ± 250	520 ± 60
N-Demethylation		**1**	**0.8**
Ethoxycoumarin *O*-Deethylation	2E1	0.22 ± 0.80	1.23 ± 0.22
		1	**5.6**
Erythromycin *N*-Demethylation	3A4	170 ± 60	1800 ± 160
		1	**10.6**
Benzphetamine *N*-Demethylation	3A4	1070 ± 250	6520 ± 620
		1	**6.1**
Chlorpromazine *S*-Oxygenation	3A	85.3 ± 45.4	348 ± 84
		1	**4.1**

[1] pmol P450/mg protein, [2] pmol/min/mg protein, [3] in bold: ratio to human

Table 6.7: Cytochrome P450-mediated enzymatic reactions in human, dog, cynomolgus monkey and rhesus monkey liver microsomes (data are of mean ± SD) [Sharer *et al.*, 1995]

P450 mediated activities[1]	Major human P450	Human	Dog (beagle)	Monkey (cynomolgus)	Monkey (rhesus)
Ethoxyresorufin *O*-Deethylation	1A1/1A2	21 ± 14	46 ± 25	240 ± 85	295 ± 71
		1[2]	**2.2**	**11.4**	**14.0**
Coumarin 7-Hydroxylation	2A6	448 ± 330	75 ± 17	678 ± 209	289 ± 125
		1	**0.17**	**1.5**	**0.65**
Tolbutamide 4-Hydroxylation	2C9/C10	88 ± 37	-[3]	50 ± 12	47 ± 9
		1	**-**	**0.57**	**0.53**
S-Mephenytoin 4'-Hydroxylation	2C19	44 ± 30	12.7 ± 0.4	106 ± 50	30 ± 7
		1	**0.29**	**2.4**	**0.68**
Bufuralol 1'-Hydroxylation	2D6	34 ± 20	49 ± 11	530 ± 154	558 ± 189
		1	**1.4**	**15.6**	**16.4**
N-Nitrosodimethyl-amine	2E1	761 ± 319	694 ± 220	758 ± 286	610 ± 76
N-Demethylation		**1**	**0.91**	**1.0**	**0.8**
Erythromycin *N*-Demethylation	3A	153 ± 74	876 ± 316	2949 ± 298	1997 ± 437
		1	**5.7**	**19.3**	**13.1**
Midazolam 1'-Hydroxylase	3A	320 ± 182	1053 ± 257	1330 ± 174	1107 ± 326
		1	**3.3**	**4.2**	**3.5**

[1] pmol/min/mg protein, [2] in bold: ratio to human, [3] not detectable

N = 6, except tolbutamide 4-hydroxylase: cynomolgus monkey (n=5), rhesus monkey (n=4); *S*-mephenytoin 4'-hydroxylase: human (n=4), dog (n=2); bufuralol 1'-hydroxylase: human (n=5)

In a follow up study by Sharer *et al.* (1995) apart from humans and rhesus monkeys also dogs and cynomolgus monkeys were studied. In Table 6.7 the results of the CYP450-mediated enzymatic reactions in liver microsomes of these species are given. Liver microsomes from cynomolgus and rhesus monkeys showed significantly higher activities than those from humans for ethoxyresorufin *O*-deethylase, bufuralol 1'-hydroxylase, midazolam 1'-hydroxylase, erythromycin *N*-demethylase and tolbutamide 4-hydroxylase. Cynomolgus monkey had higher activity than humans and rhesus monkeys for *S*-mephenytoin 4'-hydroxylase and erythromycin *N*-demethylase. All other monkey enzyme activities were not significantly different from those in humans. Dog subcellular fractions showed higher activities than humans for midazolam 1'-hydroxylase, erythromycin *N*-demethylase. Furthermore, dog samples had significantly lower activity for coumarin 7-hydroxylase. All other activities were nor significantly different from those in humans. These results reveal minor differences between the cynomolgus and rhesus monkey in drug metabolism capacities *in vitro*, but both species are generally more metabolically active than humans, whereas dogs had more diverse deviations from humans [Sharer *et al.*, 1995).

Table 6.8: Cytochrome P450-mediated enzymatic reactions in human and dog liver microsomes (data are of mean ± SD) [Chauret *et al.*, 1997]

P450 mediated drug oxidation[1]	Major human P450	Human (n=13)	Dog (n≥6) (beagle)
Phenacetin *O*-Deethylation	1A1/1A2	510 ± 641	737 ± 122
		1[2]	**1.4**
Coumarin 7-Hydroxylation	2A6	422 ± 352	73 ± 40
		1	**0.17**
Tolbutamide Hydroxylation	2C8/2C9	40 ± 30	7 ± 5
		1	**0.18**
S-Mephenytoin 4'-Hydroxylation	2C19	35 ± 42	35 ± 13
		1	**1.0**
Dextromethorphan *O*-Demethylation	2D6	125 ± 64	52 ± 14
		1	**0.42**
Chlorzoxazone 6-Hydroxylation	2E1	206 ± 109	253 ± 63
		1	**1.2**
Testosteron 6ß-Hydroxylation	3A4	1690 ± 660	254 ± 108
		1	**0.15**

[1] pmol/min/mg protein, [2] in bold: ratio to human

Chauret *et al.* (1997) compared cytochrome P450-mediated metabolic activities *in vitro* in human, dog, cat and horse liver microsomes. The results of this study in humans and dogs (beagles) are given in Table 6.8. It is not yet known which P450 isoenzymes are important for the metabolism of drugs in the liver of dogs, although the presence of some P450s has been reported. Catalytic activities using specific probes for CYP1A1/1A2, 2A6, 2C19, 2D6, 2E1 and 3A4 have been reported in the dog. Characterisation of some cytochrome P450s by amino acid sequence has shown that P450s belonging to the CYP1A [Ohta *et al.*, 1990; Uchida *et al.*, 1990], CYP2C [Uchida *et al.*, 1990; Shiraga *et al.*, 1994], CYP3A [Ciaccio *et*

al., 1991] and CYP2D [Sakamoto *et al.*, 1995; Nakamura *et al.*, 1995] subfamilies are present in the dog.

Dogs showed significant statistical differences in the coumarin 7-hydroxylase, tolbutamide hydroxylase and testosteron 6ß-hydroxylase activity compared to humans. These activities were lower in dogs than in humans. More variability is seen in the human metabolic activity than in the dog (Table 6.8). This was expected as the humans in this study were taken from the general population, while the dogs were from an inbred laboratory strain. The laboratory animals have therefore many fewer genetic differences. Also their environments and diet are the same, eliminating possible factors that could contribute to differential induction of some of the P450s. There were no marked sex-related differences in the metabolism of the different catalytic activity markers tested in human and dog [Chauret *et al.*, 1997].

In Table 6.9A and B the metabolic activities of liver microsomes of different species are given as they were listed in the product characterisation form of In Vitro Technologies, SanverTech, where these microsomes can be purchased [SanverTech, 1999]. Samples of several animals were pooled and the enzyme activities were determined in these pooled fractions, therefore no interindividual variation was given. Three different strains of rats were characterised and they seem to be very similar in their metabolic activities, but the differences with humans are large. For example, no coumarin 7-hydroxylation could be detected in rat liver microsomes. This is consistent with the results of Shimida *et al.* (1997). Some immunoreactive staining was observed, however, with the antibody raised against human CYP2A6. Therefore it can not be concluded that no CYP2A6 is present in the rat. Large interspecies differences were also found in the metabolic activities in mouse, rabbit and minipig liver microsomes, these species were not studied in any of the other studies mentioned above. Interestingly, no *S*-mephenytoin-4'hydroxylation could be detected in rabbit liver microsomes.

The P450-mediated enzyme activities measured in the different studies show similar results, but also a lot of differences. The varying results obtained between laboratories could be caused by differences in expression of the isoenzymes measured between different groups of animals used. Another reason could be the different substrates used as a marker for a specific isoenzyme or different experimental conditions used to determine the enzyme activities, such as different substrate concentrations. In general these metabolic studies show that monkey P450s are very similar to human P450s in enzymatic structure, but apparent differences in substrate affinities and/or enzyme levels yield generally higher activities. The rhesus and cynomolgus species of monkey of which both are within the same genus, displayed very similar metabolic profiles. Dogs exhibited activities less congruous to humans, with various activities being significantly lower or absent. Another factor which might influence the enzyme activities is the induction of specific isoenzymes by certain compounds. This might also be subject to interspecies differences and should be kept in mind.

Table 6.9: Cytochrome P450-mediated enzymatic reactions in mouse, rat and rabbit (A) and in dog, minipig, monkey and human liver microsomes (B) [SanverTech, 1999].

A

P450 mediated drug oxidation[2]	human P450	Mouse (CD)		Rat (S-D)		Rat (Fischer)		Rat (Wistar)		Rabbit (NZW)	
Gender		M	F	M	F	M	F	M	F	M	F
Total P450[1]		400	540	440	580	330	380	600	620	1800	1960
		1.2[3]	**1.6**	**1.3**	**1.7**	**1.0**	**1.1**	**1.8**	**1.8**	**5.3**	**5.8**
Ethoxyresorufin *O*-Deethylation	1A1/ 1A2	182	188	31	89	71	98	402	281	1320	1701
Phenacetin *O*-deethylation	1A2	489	419	172	196	198	190	436	421	1550	1676
		2.0	**1.7**	**0.7**	**0.8**	**0.8**	**0.8**	**1.8**	**1.8**	**6.5**	**7.0**
Coumarin 7-Hydroxylation	2A6	20	20	0[4]	0	0	0	0	0	410	350
		0.01	**0.01**	**0**	**0**	**0**	**0**	**0**	**0**	**0.2**	**0.2**
S-Mephenytoin 4'-Hydroxylation	2C19	66	47	6	8	13	18	20	18	0	0
		3.3	**2.4**	**0.3**	**0.4**	**0.7**	**0.9**	**1**	**0.9**	**0**	**0**
Chlorzoxazone 6-Hydroxylation	2E1	2585	3239	1825	1102	806	793	1433	1145	3832	4164
		1.6	**2.0**	**1.1**	**0.7**	**0.5**	**0.5**	**0.9**	**0.7**	**2.3**	**2.5**
Ethoxycoumarin *O*-Deethylation	2E1	519	601	209	112	231	173	287	257	2380	2189
		1.5	**1.7**	**0.6**	**0.3**	**0.6**	**0.5**	**0.8**	**0.7**	**6.7**	**6.1**
Dextromethorphan *O*-Demethylation	2D6	87	179	343	154	244	166	127	168	231	243
		0.7	**1.4**	**2.7**	**1.2**	**1.9**	**1.3**	**1.0**	**1.3**	**1.8**	**1.9**
Testosteron 6ß-Hydroxylation	3A4	100	300	1100	100	200	100	1600	100	1800	1900
		0.02	**0.06**	**0.2**	**0.02**	**0.04**	**0.02**	**0.3**	**0.02**	**0.3**	**0.4**

B

P450 mediated drug oxidation[2]	human P450	Human	Dog (beagle)		Minipig (Yucatan)		Monkey (Cynomolgus)		Monkey (Rhesus)	
Gender			M	F	M	F	M	F	M	F
Total P450[3]		340	570	740	850	1350	730	990	1260	810
		1	**1.7**	**2.2**	**2.5**	**4.0**	**2.1**	**2.9**	**3.7**	**2.4**
Ethoxyresorufin *O*-Deethylation	1A1/ 1A2	n.d.	151	561	n.d.	n.d.	n.d.	334	503	631
Phenacetin *O*-deethylation	1A2	240	506	971	164	441	450	1027	360	1227
		1	**2.1**	**4.0**	**0.7**	**1.8**	**1.9**	**4.3**	**1.5**	**5.1**
Coumarin 7-Hydroxylation	2A6	1700	130	310	90	1750	8200	2570	1640	3760
		1	**0.07**	**0.2**	**0.05**	**1.0**	**4.8**	**1.5**	**1.0**	**2.2**
S-Mephenytoin 4'-Hydroxylation	2C19	20	17	0	3	3	n.d.	209	80	42
		1	**0.9**	**0**	**0.2**	**0.2**		**10.5**	**4**	**2.1**
Chlorzoxazone 6-Hydroxylation	2E1	1658	2274	2968	2115	3027	1491	714	605	507
		1	**1.4**	**1.8**	**1.3**	**1.8**	**0.9**	**0.4**	**0.4**	**0.3**
Ethoxycoumarin *O*-Deethylation	2E1	357	2519	2270	781	8956	1723	2958	2423	2757
		1	**7.1**	**6.4**	**2.2**	**25.1**	**4.8**	**8.3**	**6.8**	**7.7**
Dextromethorphan *O*-Demethylase	2D6	127	96	52	1084	802	n.d.	905	422	821
		1	**0.8**	**0.4**	**8.5**	**6.3**		**7.1**	**3.3**	**6.5**
Testosteron 6ß-Hydroxylation	3A4	5300	1100	500	5800	5000	2800	2500	2200	3400
		1	**0.2**	**0.09**	**1.1**	**0.9**	**0.5**	**0.5**	**0.4**	**0.6**

[1] pmol P450/mg protein, [2] pmol/min/mg protein, [3] in bold: ratio to human, [4] 0 = not detectable, n.d.: not determined

6.2.2 Phase II metabolic activities

Phase II conjugation reactions involve a large number of enzymes acting on a diverse group of compounds, usually resulting in the formation of a water-soluble product that can be excreted in the bile or urine. In the study of Stevens *et al.* (1993) several phase II metabolic reactions were studied in humans and rhesus monkeys (Table 6.10). No sex differences were found for the phase II metabolic activities in both species measured in this study [Stevens *et al.*, 1993]. In the follow up study by Sharer *et al.* (1995) apart from humans and rhesus monkeys also dogs and cynomolgus monkeys were studied. Table 6.11 list the results of the six phase II enzyme activities measured in hepatic subcellular fractions from each of these species.

6.2.2.1 Glucuronosyltransferase

Glucuronide formation is generally regarded as a major pathway for the conjugation on xenobiotics and the enzyme responsible for this reaction is uridine diphosphate glucuronosyltransferase (UDPGT). In humans, nine hepatic UDPGT cDNAs have been cloned, and these are dividable into two major families: UGT1 and UGT2. Traditionally, the forms of UGT1 were believed to glucuronidate bilirubin and phenols preferentially, such as acetaminophen, whereas the forms of UGT2 glucuronidate bile acids and steroids, such as 17α-EE. In Table 6.10 the results of this study are shown for human and monkey liver samples. Acetaminophen glucuronosyltransferase activity was 6.7 times higher in rhesus monkey liver microsomes compared with human liver microsomes. However, glucuronosyl-transferase activity toward 17α-EE was significantly lower (0.6 times) in monkey liver microsomes compared to human liver microsomes [Stevens *et al.*, 1993]. The results in Table 6.11 that the incubates from both monkey species (rhesus and cynomolgus) exhibited higher activities than those of humans for acetaminophen glucuronosyltransferase (cynomolgus 6.3-fold, rhesus 8-fold), whereas no significant differences were observed for 17α-EE UDP-glucuronosyltransferase. Between the two monkey species, no significant differences were. Dog liver subcellular fractions had higher activities than human fractions for acetaminophen glucuronosyltransferase (5.2-fold) and no significant difference from human in activity of 17α-EE glucuronosyltransferase [Sharer *et al.*, 1995].

6.2.2.2 Sulfotransferase

Sulfation is also an important conjugation pathway for the elimination of phenols, alcohols, and amines. Sulfotransferases have also been divided into two groups, based on substrate selectivity: one catalysing the sulfation of steroid/bile acid substrates and the other being more specific for phenols. Comparison of human and rhesus monkey hepatic 17α-EE sulfotransferase activity showed no species differences (Table 6.10). In contrast, cytosolic acetaminophen sulfotransferase activity was 3.9-fold higher with the rhesus monkey samples [Stevens *et al.*, 1993]. In Table 6.11 it is shown that incubates from both rhesus and cynomolgus monkeys exhibited higher activities than those of humans acetaminophen sulfotransferase (cynomolgus 3.9-fold, rhesus 4.1-fold), whereas no significant differences were observed for 17α-EE sulfotransferase. Between the two monkey species, no significant differences were observed. Dog liver subcellular fractions had higher activities than human

fractions for acetaminophen sulfotransferase (2-fold), 17α-EE sulfotransferase (2.3-fold). [Sharer *et al.*, 1995].

Table 6.10: Phase II enzyme activities in human and monkey liver samples (data are of mean ± SD) [Stevens *et al.*, 1993].

Phase II enzyme activity[1]	Human (n=12)	Monkey (n=6) (rhesus)
Acetaminophen Glucuronyltransferase	101 ± 67	675 ± 123
	1[2]	**6.7**
17α-EE Glucuronyltransferase	111 ± 44	65 ± 14
	1	**0.6**
Acetaminophen Sulfotransferase	19.1 ± 17.1	74.9 ± 22.5
	1	**3.9**
17α-EE Sulfotransferase	14.3 ± 7.2	11.6 ± 3.5
	1	**0.8**
Isoniazid Acetylase	90 ± 78	317 ± 114
	1	**3.5**
6-Mercaptopurine Methylase	31.7 ± 5.1	23.5 ± 8.9
	1	**0.7**
DCNB Glutathione Transferase	1230 ± 290	3500 ± 660
	1	**2.8**

[1] pmol/min/mg protein, except DCNB activity: nmol/min/mg protein,[2] in bold: ratio to human; DCNB = dichloronitrobenzene, 17α-EE = 17α-ethynylestradiol. All activities were measured in liver cytosol with the exception of glucuronidation.

Table 6.11: Phase II enzyme activities in human, dog and monkey liver samples (data are of mean ± SD) [Sharer *et al.*, 1995].

Phase II enzyme activity[1]	Human (n=6)	Dog (n=6) (beagle)	Monkey (n=6) (cynomolgus)	Monkey (n=6) (rhesus)
Acetaminophen Glucuronyltransferase	78 ± 33	407 ± 111	489 ± 93	625 ± 267
	1[2]	**5.2**	**6.3**	**8.0**
17α-EE Glucuronyltransferase	85 ± 42	142 ± 55	62 ± 32	95 ± 19
	1	**1.7**	**0.7**	**1.1**
Acetaminophen Sulfotransferase	88 ± 28	176 ± 36	347 ± 22	360 ± 50
	1	**2.0**	**3.9**	**4.1**
17α-EE Sulfotransferase	39 ± 12	91 ± 19	44 ± 14	43 ± 16
	1	**2.3**	**1.1**	**1.1**
6-Mercaptopurine Methylase	3.5 ± 0.8	1.9 ± 0.5	4.1 ± 1.2	3.3 ± 0.6
	1	**0.5**	**1.2**	**0.9**
DCNB Glutathione *S*-transferase	1070 ± 130	7280 ± 565	2820 ± 560	2720 ± 430
	1	**6.8**	**2.6**	**2.5**

[1] pmol/min/mg protein, except DCNB activity: nmol/min/mg protein,[2] in bold: ratio to human; DCNB = 3,4-dichloronitrobenzene, 17α-EE = 17α-ethynylestradiol. All activities were measured in liver cytosol with the exception of glucuronidation.

6.2.2.3 Methylase

Hepatic S-methyltransferase activity was also compared between species. This reaction is important in the metabolism of thiopurines that are often used in chemotherapy. Hepatic 6-

mercaptopurine S-methyltransferase activity was found to be significantly higher in human liver cytosol when compared with rhesus monkey liver cytosol (Table 6.10), but this difference was very small [Stevens *et al.*, 1993]. No significant differences were observed for 6-mercaptopurine activity for the two monkey species and humans studied by Sharer *et al.* (1995) (Table 6.11). Between the two monkey species, also no significant differences were observed. Dog incubates had lower activity than those of human for 6-mercaptopurine methylase (0.5-fold).

6.2.2.4 N-*Acetyltransferase*

Analysis of the human liver samples for isoniazid *N*-acetyltransferase activity showed high variation between individuals. Three samples had no measurable *N*-acetyltransferase activity probably caused by the *N*-acetylation genetic polymorphism that is know for this enzyme. The mean cytosolic isoniazid N-acetyltransferase activity was 3-fold higher for rhesus monkey liver samples compared with the human liver samples (Table 6.10) [Stevens *et al.*, 1993]. Sharer *et al.* (1995) also found that the isoniazid N-acetylation activities measured in human cytosol exhibited the expected polymorphism of poor and extensive metabolism, whereas dog cytosol had no activity. Cynomolgus and rhesus monkeys displayed an interesting disparity, with all individuals of the cynomolgus species exhibiting rapid metabolism at rate 2-fold higher than the human extensive metabolisers. The activity profile for the rhesus monkey, however, was highly variable, with rates of three individuals similar to those of cynomolgus monkeys, rates of two individuals similar to those of human poor metabolisers, and one individual completely lacking in activity (not shown). Dog cytosol had no measurable activity as has been reported previously (see also Table 6.2) [Sharer *et al.*, 1995].

6.2.2.5 Glutathione S-*transferases*

Glutathione (GSH) conjugation occupies an important role in the detoxification of potential alkylating agents both *in vitro* and *in vivo*. Glutathione *S*-transferases (GSTs) are a family of enzymes found at high levels in the liver and play an important role in the detoxification of electrophilic alkylating agents. Mammalian cytosolic GST isoenzymes have been categorised into four classes designated α, μ, π [Mannervik *et al.*, 1985] and θ [Meyer *et al.*, 1991]. Puri and Kohli (1995) determined glutathione S-transferase activity in liver cytosol of rat, rabbit and monkey using three different substrates. 1-Chloro-2,4-dinitrobenzene (CDNB) is the most widely used model substrate for determination of GST activities α, μ and π. Thus, this substrate has been used as a general measure of overall glutathione S-transferase metabolic capacity. DCNB is known to be metabolised mainly by GST-π in the rat and ethacrynic acid is in the rat mainly metabolised by GST-α and GST-π [Mannervik and Danielson, 1988]. The results of the study of Puri and Kohli are shown in Table 6.12. Large interspecies differences were found in the activity towards CDNB and ethacrynic acid, but GSH conjugation towards DCNB occurs in all three species at almost the same rate [Puri and Kohli, 1995].

Table 6.12: Glutathione *S*-transferase activity in rat and rabbit and monkey liver cytosol with different substrates (data are of mean ± SD) [Puri and Kohli, 1995].

Glutathione *S*-transferase substrate[1]	Enzyme class involved in rat	Rat (n=7) (Wistar)	Rabbit (n=5) (NZW)	Monkey (n=4) (Rhesus)
CDNB	α, μ, π	760 ± 170	6440 ± 1400	1930 ± 330
DCNB	μ	12 ± 1.0	10 ± 2.0	12 ± 2.0
Ethacrynic acid	α, π	33 ± 8.0	7 ± 2.0	12 ± 2.0

[1]nmol/min/mg protein, CDNB = chlorodinitrobenzene; DCNB = dichloronitrobenzene; NZW = New Zealand White

It is difficult to compare the data for GST activity towards DCNB of Puri and Kohli (1995) with the data of Stevens *et al.* (1993) and Sharer *et al.* (1995) since no human data were determined. Stevens *et al.* (1993) found that hepatic cytosolic GST activity towards DCNB was nearly 3-fold higher in rhesus monkeys compared with human samples (Table 6.10). The same results were found by Sharer *et al.* (1995) for both the two monkey species studied, but this difference was not statistically significant (Table 6.11). Between the two monkey species, also no significant differences were observed. Dog liver subcellular fractions had higher activities than human fractions for DCNB glutathione *S*-transferase (6.8-fold).

In Table 6.13 the results are shown of an older study in which the glutathione S-transferase activity in liver cytosol of different species was determined. There were marked species differences in the enzyme activities. The activities found in rabbit cytosol were almost 6-fold higher than those found in rat. Since the enzyme activity was determined with a different substrate, it is difficult to compare these data with the data found in the two studies mentioned above (see Table 6.10 and 6.11), however the results in rat and rabbit were quite similar to the results found by Puri and Kohli (1995) (see Table 6.12) [Igarashi *et al.*, 1986].

Table 6.13: Glutathione *S*-transferase activity in mouse, rat and rabbit liver cytosol (data are of mean ± SE) [Igarashi *et al.*, 1986]

	Mouse (ICR)	Rat (Sprague-Dawley)	Rabbit (NZW)
CDNB Glutathione Transferase[1]	3670 ± 490	1200 ± 40	5960 ± 430

[1]nmol/min/mg protein, n = 5, CDNB = chlorodinitrobenzene, NZW = New Zealand white

6.2.2.6 Carboxylesterases

Hepatic microsomal carboxylesterases function in the hydrolysis of a wide variety of endogenous and xenobiotic compounds and play an important role in drug an lipid metabolism in many mammalian species. Several research groups have shown that there are multiple isoenzymes of hepatic microsomal carboxylesterases in various animal species. Table 6.14 shows a comparison of hepatic microsomal carboxylesterase activities in various mammals and humans. Pig was found to have the highest specific activity of liver microsomes toward *p*-nitrophenylacetate and butanilicaine. The highest activity of malathion hydrolase was found in rat liver microsomes. And the highest activity of isocarboxazid hydrolase was found in rabbit liver microsomes. On the other hand, human liver microsomes

were found to have the lowest activity towards all substrates used. These marked species variations of carboxylesterase activities may be explained, at least in part, by the properties of the various carboxylesterase isoenzymes existing in the various species [Hosokawa *et al.*, 1990].

Table 6.14: Hepatic microsomal Carboxylesterases activities in various mammals and humans (data are of mean ± SD) [Hosokawa *et al.*, 1990]

	p-Nitrophenyl-acetaat	Malathion	Butanilicaine	Isocarboxazid
Rat (n=5)	1.93 ± 0.1	74.6 ± 9.2	0.12 ± 0.03	60.1 ± 12
Mouse (n=5)	8.52 ± 0.5	14.0 ± 2.4	0.01 ± 0.01	78.8 ± 6.4
Rabbit (n=2)	10.3	59.9	1.08	197
Pig (n=1)	10.9	28.8	1.62	125
Dog (n =1)	2.42	26.7	1.06	68.8
Monkey (n=3)	4.37 ± 0.32	42.5 ± 2.5	< 0.005	169 ± 23
Human (n=1)	1.06	<0.002	0.07	19.0

p-Nitrophenylacetate hydrolase (µmol/mg protein/min), malathion hydrolase (nmol/mg protein/min), butanilicaine hydrolase (nmol/mg protein/min), isocarboxazid hydrolase (nmol/mg/ protein/30 min).

6.3 Intestinal metabolism

Metabolism in the gut lumen and wall can make a variable contribution to the overall bioavailability and hence to the pharmacological activity of a wide range of drugs. Bacterial flora in the gut, the environmental pH and oxidative or conjugative enzymes present in the intestinal epithelial cells can all contribute to the process. Bacterial biotransformation is greatest in the colon, while gut wall metabolism is generally highest in the jejunum and decreases distally. When such degradation occurs within the gut lumen, it simply reduces the amount of drug available for absorption. Metabolism in the gut wall can occur during the absorptive process and such presystemic elimination further decreases the amount of active drug available within the body. Occasionally, pro-drugs are administered orally with the intention that their metabolism in the gut lumen and/or wall will lead to the local production of an active drug species. Lastly, it should be realised that drugs are not the only foreign compounds to be metabolically altered in the gut and a variety of dietary and environmental xenobiotics may be substrates for chemical and/or enzymatic activation or deactivation in this area. The occurrence of presystemic intestinal elimination for drugs may be indicated by large differences in the pattern of metabolites after oral and intravenous administration to conventional and germ-free animals. Such findings are suggestive of presystemic elimination. Proof of such metabolism in man is difficult to obtain since direct measurement of metabolites in the gut lumen and/or portal venous blood is required [Ilet *et al.*, 1990].

6.3.1 Metabolising enzymes in the gut wall

Enzyme activity resides primarily in the mucosal epithelial cells. Since most orally administered drugs must pass through these cells during absorption, biotransformation may

occur if suitable enzymes are present. The extent of such first-pass metabolism depends partly on enzyme concentration and also on the rate of drug transfer through the mucosal cells or the route of transfer (transcellular or paracellular). Drugs may also undergo postabsorptive transfer from the systemic circulation to the intestinal epithelial cells and be metabolised. In such cases, both the drug pKa and its oil/water partition coefficient would be expected to control access to the metabolic sites and hence the extent of postabsorptive gut clearance. The enzyme activity is greatest in the villous tips and decreases progressively towards the crypts. Metabolic activity is generally higher in the duodenum and jejunum than in the ileum and colon [Ilet *et al.*, 1990].

6.3.1.1 Phase I metabolic activities: Cytochrome P450 monooxygenase

Small intestinal cytochromes P450 provide the principal, initial source of biotransformation of ingested xenobiotics. P450s occur at highest concentrations in the duodenum, near the pylorus, and at decreasing concentrations distally, being the lowest in the ileum. Highest concentrations occur from midvillus to villous tips, with little or none occurring in the crypts of Lieberkuehn. There is now overwhelming evidence that drugs that are substrates for P450-3A4 can undergo profound first-pass metabolism within the small intestine [Hall et al., 1998]. Selective inhibition of the intestinal P450-3A4 activity by grapefruit juice increased the oral bioavailability of many P450-3A4 substrates (e.g. felodipine, nifedipine, saquinavir). Grapefruit juice, however, appears to enhance the P-glycoprotein-mediated drug transport [Soldner et al., 1999], and this may, therefore, potentially counteract the P450-3A4 inhibitory effect. Microsomal P450-2C8-10, and 2D6 forms have also been identified in human small intestine. P450s 2B1, possibly 2B2, 2A1 and 3A1/2 were located in the endoplasmic reticulum of rodent small intestine, while P450-2B4 has been purified to electrophoretic homogeneity from rabbit intestine [Kaminsky and Fasco, 1992].

Little is known regarding species differences in the intestinal drug-metabolising enzymes. Studies of intestinal enzymes have been conducted primarily in rat and human intestines, but rarely in other species. P450-3A is known to be a multigene family both in rats and in humans [de Waziers and referneces therein]. However, several compounds that are supported by P450-3A4 in humans are supported by P450-2B1/B2 in rats. In humans, this P450 isoenzyme is not usually expressed. In the pharmaceutical industry, dogs and monkeys are common non-rodent species used in early toxicology and metabolism studies of compounds intended for human use. Therefore, information about species differences/similarities with respect to the drug-metabolising enzymes in these animals is important as well. The results of P450 enzyme activity determined in the small intestinal mucosa in humans, beagle dogs and rhesus monkeys are shown in Table 6.15. Ethoxyresorufin O-deethylation activity (P450-1A1/2) was below the limit of detection in all samples. Generally, humans and monkeys exhibited higher intestinal enzyme activities than dogs. Intestines from humans and monkeys were equally active in bufuralol 1'-hydroxylase and testosterone 6β-hydroxylase activity. In addition, low but significant tolbutamide methylhydroxylase activity was observed in human and monkey intestines. Only testosterone 6β-hydroxylase activity was detected in dog intestine [Prueksaritanont *et al.*, 1996].

Table 6.15: P450 enzyme activities in intestinal microsomal fractions of human, monkey and dog (data are of mean ± SD) [Prueksaritanont *et al.*, 1996].

P450 mediated activities[1]	Major human P450	Human (n=5)	Dog (n=4) beagle	Monkey (n=5) rhesus
Testosterone 6β-Hydroxylase	3A4	1580 ± 1560	150 ± 40	1130 ± 970
		1[2]	**0.09**	**0.7**
Bufuralol 1'-Hydroxylase	2D6	15.3 ± 6.95	< 0.5	16.7 ± 8.00
		1		**1.1**
Tolbutamide Methyl-hydroxylase	2C8/9	5.1 ± 3.82	< 0.5	2.3 ± 2.05
		1		**0.5**

[1] pmol/min/mg protein, [2] in bold: ratio to human

6.3.1.2 Phase II metabolic activities

The potential of the epithelial cells of the villus-to-crypt surface of the small intestine of the rat to conjugate xenobiotics was studied by Dubey and Singh (1988). The cells were isolated sequentially in the villus-to-crypt gradient and were found to exhibit heterogeneous distribution patterns and inducer-sensitivities of the conjugating enzymes and their cofactors. The mature upper villus cells were rich in UDP-glucuronosyltransferase activity, which declined toward the highly replicating undifferentiated crypt cells. The specific enzyme activities were four times lower in crypt cells than in the upper villus cells. The UDP-glucuronosyltransferases of crypt cells were highly sensitive to inducers, in comparison to the villus cells. The cofactor content (UDPGA) ranged from about 0.07 to 0.2 mM in cells from crypts to villus-tip respectively. The highest GST activity was also found in de villus cells, which was approximately 4 times higher than in the crypt cells. The crypt cells were again more sensitive to induction (3-5-fold) in comparison with the villus cells (2-fold). The endogenous GSH content, however, was lower in the villus cells compared to the crypt cells. The differential and higher sensitivity of the intestinal cells to inducers appears to provide protection of the intestine against xenobiotics during intestinal 'first pass' [Dubey and Singh, 1988].

Table 6.16: Conjugating enzyme activities in subcellular fractions obtained from human, monkey and dog intestines (data are of mean ± SD) [Prueksaritanont *et al.*, 1996]

	Human (n=5)	Dog (n=4) beagle	Monkey (n=5) rhesus
p-Nitrophenol Glucuronidase[1]	0.80 ± 0.68	0.09 ± 0.06	2.11 ± 0.98
	1[3]	**0.1**	**2.6**
p-Aminobenzoic acid Acetyltransferase[1]	< 0.05	< 0.02	< 0.05
Sulfamethazine Acetyltransferase[2]	71.0 ± 123.3	< 5	31.3 ± 16.1
	1		**0.4**
Acetaminophen Sulfotransferase[2]	130 ± 54.6	9.63 ± 3.84	20.6 ± 10.0
	1	**0.07**	**0.2**
CDNB Glutathione Transferase[1]	0.28 ± 0.13	0.04 ± 0.02	0.28 ± 0.11
	1	**0.1**	**1.0**

[1] nmol/min/mg; [2] pmol/min/mg; [3] in bold: ratio to human; CDNB: chlorodinitrobenzene

Prueksaritanont *et al.* (1996) determined several conjugating enzyme activities in intestines from humans, monkeys and dogs (Table 6.16). The substrates used in this study can be metabolised by various isoforms of the different conjugating enzymes, therefore they serve as general indicators of these enzymatic activities. All of the conjugating enzymes investigated were found to be present in monkey and human intestine. *N*-Acetyltransferase activity was not observed in dog intestine. The absence of *N*-acetyltransferase activity has also been reported previously in dog liver. In general, the activities of the conjugating enzymes were much lower in dog intestine than in human and monkey intestine [Prueksaritanont *et al.*, 1996].

Table 6.17: Carboxylesterase activities in subcellular fractions obtained from human, monkey and dog intestines (data are of mean ± SD) [Prueksaritanont *et al.*, 1996]

	Human (n=5)	Dog (n=4) beagle	Monkey (n=5) rhesus
Microsomal PNPA Hydrolase	2760 ± 1420	40 ± 20	1000 ± 360
	1[1]	**0.01**	**0.4**
Cytosolic PNPA Hydrolase	130 ± 140	40 ± 10	250 ± 200
	1	**0.3**	**1.9**
Microsomal ASA Hydrolase	< 0.1	< 0.1	3.95 ± 1.72
Cytosolic ASA Hydrolase	0.96 ± 0.52	0.36 ± 0.18	2.22 ± 0.86
	1	**0.4**	**2.3**

nmol/min/mg; PNPA: *p*-nitrophenyl acetate; ASA: acetylsalicylic acid; [1] in bold: ratio to human

Carboxylesterase activities in microsomal and cytosolic fractions are shown in Table 6.17. In human, microsomes were much more active in hydrolysing PNPA, whereas cytosolic fractions were more active in catalysing the hydrolysis of ASA. The microsomal PNPA hydrolase activity in human intestine was as high as that reported in human liver. In monkeys, PNPA hydrolase activities were higher in the microsomal than in the cytosolic fractions, whereas ASA hydrolase activities in both fractions were comparable. Dogs contained relatively low PNPA hydrolase activity and measurable ASA hydrolase activity only in the cytosolic fraction [Prueksaritanont *et al.*, 1996].

6.3.2 Drug metabolism in the lumen of the gastrointestinal tract

Potential substrates for biotransformation by the gut microflora include xenobiotics which are administered orally as well as those which are transferred to the gut lumen from other sites within the body. Both biliary excretion, and secretion by the cells of the gastrointestinal mucosa/submucosa will contribute to such transfer of drugs from the systemic circulation to the gut lumen. The microflora of the gastrointestinal tract in man are a remarkably well-balanced and highly complex ecosystem comprising both anaerobes and aerobes and the gut contents contain some 10^{11} bacteria per gram with representation from 400 species. The dominant gastrointestinal bacteria in man and in most laboratory animals are the nonsporing anaerobic lactobacilli and bacteroides. Because few bacteria grow at pH 3 or less, the distribution of the microflora throughout the length of the gut is governed to a great extent by the acidity of the region. In man, the stomach, duodenum, jejunum and upper ileum are sparsely populated with micro-organisms. Whereas the stomach is sterile in the fasting state, both food and saliva entering this organ neutralise gastric acid and allow colonisation by transient flora of predominantly acid-resistant bacteria such as lactobacilli, bacteroides and enterococci. Such microflora are more prolific in the anterior part of the stomach. Marked host species differences in the pH of the gut lumen (Table 6.18) are associated with varying population densities, species and distribution of microflora in the gut lumen (Table 6.19). Mice have abundant lactobacilli and clostridia throughout the gut. In man and rabbit, the gastric pH is low enough to be bactericidal while the higher pH of species such as mouse and rat enables large numbers of bacteria to colonise in the stomach and upper intestine [Ilett *et al.*, 1990].

Table 6.18: The pH of the content of the gastrointestinal tract [Ilett *et al.*, 1990]

Species	Stomach		Intestine		Cecum	Faeces
	Anterior	Posterior	Upper	Lower		
Man	1-3		6.8-8.6		7.5-8.0	
Rabbit	1.9	1.9	6.0	8.0	6.6	7.2
Rat	3.0	3.8	6.5	7.1	6.8	6.9
Mouse	4.5	3.1				

The similarity between the human and the rabbit does not stop with the number and location of micro-organisms but continues with the type of microflora (Table 6.19). For example, the upper intestine of both species contains mainly bacteroides and bifidobacteria, whereas other common domestic and laboratory animals display considerably more varied flora in the proximal intestine. With respect to the lower portion of the small intestine humans and common laboratory animals display comparable flora. Below the ileocecal valve in humans there is a notable change in the intestinal microflora, as illustrated by an increase in anaerobic micro-organism (i.e. bacteroides and bifidobacteria) that exceed the aerobic and facultative flora by a ratio of 1000-10.000 to 1. The large intestine in humans and most common laboratory animals displays the most abundant microflora of the entire alimentary tract, with the types of organisms of the common animal models being similar to those in humans. However, numerous differences between species do exist. Among the more striking

differences are the following: (1) clostridia are found in the intestine of carnivores only; (2) yeasts are lacking in rabbits, whereas they are numerous in other warm-blooded animal species [Smith, 1965].

Table 6.19: Numbers of different kinds of organisms in the contents of the intestine of different species [Smith, 1965].

		Log_{10} viable count (median) per gram contents of contents of					
		Small intestine portion					
Species	Microflora	1	3	5	7	Cecum	Faeces
Monkey	E. coli	2.4	3.4	3.4	4.7	5.7	6.4
	C. perfringens	2.0	N	N	2.4	2.6	N
	Streptococci	5.0	5.7	6.4	6.7	8.0	7.8
	Lactobacilli	7.8	8.6	8.6	9.0	9.7	9.5
	Yeasts	5.1	5.8	5.7	5.6	6.4	5.2
	Bacteroides	N	N	N	N	9.0	9.0
Dog	E. coli	2.7	3.4	4.4	5.2	7.2	7.2
	C. perfringens	4.5	4.8	5.2	6.2	7.7	7.8
	Streptococci	5.9	5.6	5.8	7.0	8.2	9.3
	Lactobacilli	3.3	4.7	4.7	5.8	8.7	9.0
	Yeasts	N	N	N	N	N	N
	Bacteroides	N	N	N	N	8.5	9.3
Pig	E. coli	3.4	3.7	4.5	5.3	6.5	6.8
	C. perfringens	N	N	1.7	3.0	N	N
	Streptococci	4.2	4.5	6.0	6.5	7.0	7.2
	Lactobacilli	6.5	7.0	7.6	8.0	8.6	8.8
	Yeasts	3.9	3.9	4.4	4.0	4.0	4.2
	Bacteroides	N	N	N	N	7.4	7.6
Rabbit	E. coli	N	N	N	N	N	N
	C. perfringens	N	N	N	N	N	N
	Streptococci	N	N	N	N	N	N
	Lactobacilli	N	N	N	N	N	N
	Yeasts	N	N	N	N	N	N
	Bacteroides	3.8	4.1	5.0	6.7	8.5	8.7
White rat	E. coli	N	3.4	3.6	4.5	5.2	5.6
	C. perfringens	N	N	N	N	2.0	1.7
	Streptococci	3.0	4.7	4.7	5.0	6.0	6.2
	Lactobacilli	7.0	7.4	7.8	8.0	8.4	8.6
	Yeasts	6.0	6.7	6.8	6.7	7.3	7.0
	Bacteroides	N	N	N	N	8.2	8.4
White mouse	E. coli	1.7	2.0	3.4	4.0	5.4	6.0
	C. perfringens	N	N	N	N	N	1.7
	Streptococci	5.0	5.0	6.0	6.7	7.0	6.7
	Lactobacilli	6.3	6.8	7.5	8.0	8.8	9.0
	Yeasts	6.2	6.0	6.0	6.7	7.0	6.6
	Bacteroides	N	N	N	N	9.4	9.4

N=no viable organisms found in 0.02 g of chyme, i.e., log_{10} viable count per gram < 1.7.

Factors that influence the microflora are:

- Individual differences
- Ethnic differences

- Presence of foreign compounds such as antimicrobials. Antibiotics have profound effects on the bacterial enzymes responsible for the hydrolysis of drug conjugates undergoing enterohepatic recycling (e.g. in humans: failure of oral contraceptives when administered concurrently with antibiotics has been suggested to be due to a decreased enterohepatic recycling of steroid conjugates excreted in the bile).
- Changes in intestinal transit time and defecation frequency
- Coprophagy in rats (not in rabbits, probably due to low pH throughout the stomach)
- Diet (in humans conflicting effects, in animal species the host's diet has a significant influence on the numbers and types of bacteria found in the GI-tract)
- Disease
- Age (at birth in humans: enterobacteria and enterococci are the predominant flora. Bifidobacteria are absent but appear after 7 days and become predominant flora after 13 days. Elderly people have less lactobacilli but more E. coliforms. In some instances, the lack of xenobiotic metabolising ability observed in infants is not due to absence of certain microflora but rather to immaturity of the bacterial enzyme systems in the gut lumen (e.g. the extent of metabolism of several compounds increases with age and this reflects the developmental processing in bacterial enzymes such as β-glucosidase and reductases))

Metabolism of foreign compounds and nutrients by the microflora in the gut lumen is small in comparison with metabolism by the gut mucosa and liver. However, the intestinal microflora may play an important role in the first-pass metabolism of compounds which are poorly or incompletely absorbed by the gut mucosa (Azo dyes are completely reduced by gut bacteria). Bacterial metabolism is largely degradative, hydrolytic and reductive with a great potential for both metabolic activation and detoxification of xenobiotics. The intestinal microflora also play an important role in the enterohepatic recirculation of xenobiotics (e.g. digoxin, ouabain, oral contraceptives, penicillin) and endogenous compounds (e.g. steroid hormones, bile acids, folic acid and cholesterol) which are well absorbed and subsequently reenter the gut via the bile. Compounds which undergo enterohepatic recirculation are generally conjugated with polar groups in the liver prior to their secretion in to the bile. Once secreted into the small intestine, bacterial enzymes including β-glucuronidase, sulfatase and various glycosidases catalyse the hydrolysis of the conjugates [Ilett *et al.*, 1990].

Table 6.20: Species differences in ß-glucuronidase activity in the microflora [Ilett *et al.*, 1990]

Species	Proximal region	Distal region
Human	0.02	0.9
Rabbit	2.4	45.4
Rat	304.0	139.0
Mouse	1200.0	5015.0

µmol *p*-nitrophenyl glucuronide degraded/hr/g content

Activity of bacterial enzymes varies markedly among different host species and this is a reflection of species differences in the population density, distribution and species of gut lumen microflora (Table 6.20). More specifically, differential patterns of intestinal β-glucuronidase activity were found in the rat and mouse when compared with rabbits and

humans. The mouse and rat displayed very high activity in the small intestine, presumably because of the relatively large numbers of micro-organisms located in the upper portion of the gastrointestinal tract [Smith, 1965]. Generally, gut microflora contain a wide variety of metabolising enzymes with differing levels of activity (Table 6.21).

Table 6.21: Activity of β-galactosidase, β-glucosidase, β-glucuronidase, α-galactosidase, α -glucosidase, azoreductase of rat intestinal microflora [Ilett *et al.*, 1990]

	β-Galactosidase[1]	β-Glucosidase[1]	β-Glucuronidase[1]	α –Galactosidase[1]	α–Glucosidase[1]	Azoreductase[2]
Enterobacteria	42.4	5.8	24.7	12.7	5.9	0.4
Enterococci	58.8	192.7	2.9	20.8	14.0	0.9
Lacatobacilli	91.6	26.0	1.6	97.7	26.6	13.0
Clostridia	13.7	22.1	11.3	53.1	30.1	26.7
Bacteroides	50.7	35.1	6.0	24.0	9.8	0.2
Bifidobacteria	39.1	29.3	1.9	28.2	20.7	0.8

[1] Activity expressed as μmol of the appropriate nitrophenyl substrate metabolised per 10^8 cells per hour

[2] Activity expressed as pmol of sulfasalazine metabolised per 10^8 cells per hour

6.4 Stomach metabolism

Metabolism in the stomach is of minor importance compared to metabolism in liver and intestine, but it can be important for certain compounds. It has, for example, been shown that the stomach mucosa is the primary site for first pass metabolism of ethanol by alcohol dehydrogenase (ADH). Over 80 % of an orally administered dose is metabolised by the stomach (i.e., the bioavailability is only 17 %). Human gastric ADH and rat gastric ADH appear similar and the rat appears to be a good model [Barr, 1991].

Metabolism by the microflora present in the stomach may be important in some species. However, in humans this will not play a major role, because due to the low pH in the human stomach very few microfloral organisms are present (see also Chapter 3). No information concerning metabolism by stomach microflora of other species was found.

6.5 Conclusions

It is difficult to compare the results of the different studies, because different experimental procedures and different substrates for specific isoenzymes are used in the different studies. In general the metabolic studies show that monkey P450s are very similar to human P450s in enzymatic structure, but apparent differences in substrate affinities and/or enzyme levels yield generally higher activities. Dog P450s show larger differences compared to humans. For example, dogs have very low CYP2A6 (coumarine hydroxylation) and CYP2C8/2C9 (tolbutamide hydroxylation) levels compared to humans. It is also interesting that in rats, both Sprague-Dawley, Fischer and Wistar strains, no CYP2A6 activity (coumarine hydroxylation) could be detected. These differences should be taken into account when choosing an animal model for studying the toxicity or kinetics of a compound which is metabolised by one of

these P450. Furthermore, it should be kept in mind that comparable isoenzymes found in the various animal species might not have exactly the same properties as those found in humans. No information was found concerning most phase II enzymes (except glutathione *S*-tranferase and carboxylesterase) in mouse, rat, pig and rabbit. Differences in enzyme activities were very variable between species, depending on the enzyme studied or substrate used. The most pronounced difference was the lack of *N*-acetylase activity in dogs.

Phase I enzyme activity (P450) in the gut wall is highest in the duodenum and decreasing distally. The highest concentrations occur from midvillus to villous tips. Little is known regarding species differences in the intestinal drug-metabolising enzymes. In general, intestinal enzyme activities are higher in humans and monkeys than in dogs. No information about other species was found sofar. The same was found for the phase II metabolic activities in the gut wall. Metabolism in the gut lumen is mainly determined by the microflora present in the gut. In man and rabbit, the gastric pH is low enough to be bactericidal while the higher pH of species such as mouse and rat enables large numbers of bacteria to colonize in the stomach and upper intestine. The mouse and rat displayed very high β-glucuronidase activity in the small intestine, presumably because of the relatively large numbers of microorganisms located in the upper portion of the gastrointestinal tract. The large intestine in humans and most common laboratory animals displays the most abundant microflora of the entire alimentary tract, with the types of organisms of the common animal models being similar to those in humans. However, numerous differences between species do exist. Among the more striking differences are the following: (1) clostridia are found in the intestine of carnivores only; (2) yeasts are lacking in rabbits

7. Conclusions and ongoing research

This report is primarily meant as a review for those who are interested in physiological and anatomical differences of the gastrointestinal tract between man and experimental animals. This information is of interest both for researchers using animal models as well as for risk assessors on pharmacokinetics and toxicokinetics. During the realization of this report it became clear that such a reference work needs some time for development. In first instance it was planned to describe differences on anatomy and physiology of species commonly used in toxicological and (pharmaco) kinetic research. However, it appeared that, reviewing biomedical and veterinary databases, various parameters were not available. More detailed information on omissions and interspecies differences for the various parts of the gastrointestinal tract can be found in the conclusions of each chapter of these report. As an ongoing action of this project it will be tried to obtain lacking information. In a workshop, organised by the Laboratory of Exposure Assessment and Environmental Epidemiology/R.I.V.M. at April 22, 1999, attended by pharmacokineticists from pharmaceutical compagnies and biomedical research institutes, it was demonstrated that more information is available than is published in international literature. Much of this information is based on many years of experience in the application of animal models. For that reason, questionnaires were send to the attendees of the workshop in order to draw up an inventory of this 'hidden' knowledge. As a second action, other types of databases, e.g. agricultural databases will be consulted.
Beside this attempt to fill the gaps of information, more specialised topics like induction of metabolism, differences between various strains of a species, effects of age, practical considerations, etc. will be considered. Both these actions will result in a supplement to the present report.

The information obtained by the questionnaires will serve as a basis for comparing compounds with varying physical/chemical properties with pharmacokinetics of various classes of compounds in the species selected. The results of these comparison will be discussed in a workshop which will be organised in 2000. Finally, the feasibility of making a decision tree for optimisation of species selection will be determined and if possible the decision tree will be developed.
During the last two steps of the project, the necessity for extending the project to topical and inhalatory exposure will be investigated.

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APPENDIX 1 Verslag Discussiebijeenkomst: "Interspecies extrapolatie" van 22 april 1999

Deze bijeenkomst werd georganiseerd als onderdeel van project " Specieskeuze en interspeciesverschillen in relatie tot kinetiek en dynamie van stoffen". In het kader van dit project is bij het laboratorium voor Blootstellingsonderzoek en Milieu-epidemiologie literatuuronderzoek gedaan naar verschillen in fysiologie van het maagdarmkanaal en verschillen in metabolisme tussen veel gebruikte proefdieren (rat, muis, konijn, hond, (mini)varken en aap) en de mens. Bij het uitvoeren van de literatuurstudie bleek dat het niet mogelijk was een volledig overzicht van deze interspeciesverschillen te verkrijgen d.m.v. de wetenschappelijke literatuur. Door middel van deze discussiebijeenkomst, waarvoor mensen uit de farmaceutische industrie, TNO Voeding, RITOX etc. uitgenodigd zijn, wordt getracht de hiaten in het overzicht op te vullen met ervaringen uit de praktijk.
De bijeenkomst bestond uit een viertal voordrachten gevolgd door een discussie.

Voordrachten:
- Inleiding: doel van de bijeenkomst (Adrienne Sips)
- Interspeciesverschillen m.b.t. maag (Cathy Rompelberg)
- Interspeciesverschillen m.b.t. de dunne darm (Jan Welink)
- Interspeciesverschillen m.b.t. metabolisme (Loeckie de Zwart)

Aan de hand van deze voordrachten werd gediscussieerd met de deelnemers aan deze bijeenkomst over de ervaringen in de praktijk. Hierna volgt een verslag van deze discussies.

Na de voordracht over de interspeciesverschillen m.b.t. de maag blijkt dat fysiologische gegevens van de maag niet in de eerste plaats belangrijk zijn voor toxiciteits-studies. De matrix waarin een stof zich bevindt is in geneesmiddelenonderzoek over het algemeen niet erg belangrijk. In het preklinisch onderzoek (bij proefdieren) wordt een stof eigenlijk nooit toegediend in tabletvorm, maar direct als oplossing of als suspensie. Pas als er zich problemen blijken voor te doen met de formulering in de mens wordt hier naar gekeken (A. Slikkerveer). Bij toxiciteits-studies wil men nl. hoge blootstellingen bestuderen in de proefdieren en dus hoog doseren (H. Koster). Bij het geneesmiddelenonderzoek worden voedingseffecten niet in proefdieren onderzocht maar alleen in de mens. Dit geldt niet voor andere soorten onderzoek, zoals bijv. bij biobeschikbaarheidsstudies van contaminanten in verontreinigde grondmonsters (zoals uitgevoerd bij LBM), of bij bio-equivalentie studies, waar de matrix wel van belang kan zijn.
Uit de literatuurstudie kwam naar voren dat de grootte van een tablet die nog door de maagportier kan verschilt per species, wat kan leiden tot het blijven steken in de maag van te grote tabletten bij kleinere proefdieren. K. Groen deelt mee dat een tablet bij de hond een maximale grootte kan hebben van 1 cm diameter. Verder werd gemeld dat dit voor de overige

species niet relevant is voor het geneesmiddelenonderzoek. Er wordt alleen onderzoek gedaan naar enteric-coated tabletten in de hond, niet in andere species.
D. van den Dobbelsteen meldt dat een substantieel deel van de dosis al geabsorbeerd kan worden in de maag. Er worden hiervoor al grote verschillen in absorptie gevonden tussen de muis en de rat.
Coprofagie, een eigenschap van knaagdieren, kan een rol spelen bij de absorptie omdat bijv. de teststof hierdoor gerecycled kan worden. In verschillende onderzoeken wordt beschreven dat dit voorkomen kan worden door de dieren op roosters te plaatsen. N. Bode meldt echter dat dat weinig effect zal hebben omdat de knaagdieren een deel van de keutels direct vanuit de anus opeten.
J. Bessems vraagt of het tijdstip van blootstelling van belang is. Het blootstellen van knaagdieren gebeurt nl. vaak in de ochtend, dus voor ze gaan rusten. Dit komt niet overeen met de praktijksituatie: zo wordt in de arbeidstoxicologie de mens voornamelijk overdag blootgesteld, nadat ze geslapen hebben. A. van Iersel vertelt hierover dat op het RIVM bij inhalatietoxicologie er wel degelijk verschillen werden gevonden tussen het 's avonds doseren of het 's ochtends doseren. Ook D. van den Dobbelsteen vertelt dat uit onderzoek bij Organon blijkt dat CNS-effecten groter zijn als 's avonds wordt gedoseerd i.p.v. 's ochtends, terwijl dit niet weerspiegeld wordt door de AUC. Er wordt verondersteld dat dit een dynamie-effect is.
A. Slikkerveer geeft aan dat in de mens veel onderzoek gedaan is naar de maag pH i.v.m. de Helicobacter bacterie. Wellicht dat via deze route nog extra informatie gezocht kan worden.

E. van der Aar vraagt waarom er niet gekeken is naar P-glycoproteinen (PGP's) omdat uit de literatuur van de laatste tijd blijkt dat deze een belangrijke rol spelen bij opname en excretie van stoffen in de vnl. darm. Hierover was al wel informatie verzameld, maar dit was nog niet aan de orde gekomen in de voordrachten zoals die deze middag waren behandeld. Het belang van PGP's wordt zeker ingezien en dit zal ook verder uitgezocht worden. De tot nu toe gevonden informatie over PGP's was vooral gericht op de mens. E. van der Aar heeft onlangs zelf literatuuronderzoek hiernaar gedaan en kan informatie hierover aan het LBM verstrekken.
J. Bessems geeft aan dat R. Havenaar bij TNO veel weet over de microflora in de darm in verschillende species. Daar zou dus informatie ingewonnen kunnen worden.

Een groot gedeelte van de discussie ging over interspeciesverschillen in metabolisme. H. Koster liet enkele voorbeelden zien van in vivo metabolisme studies in proefdieren die nauwelijks voorspellende waarde bleken te hebben voor de mens. Ook kwam naar voren dat er een duidelijk verschil in aanpak is in het bestuderen van het metabolisme. Bij Duphar wordt het metabolisme-patroon vnl. onderzocht in vivo in proefdieren, maar bij bijv. Yamanouchi wordt ook veel in vitro metabolisme-onderzoek gedaan.
Er wordt opgemerkt dat metabolismepatronen niet alleen afhangen van de gekozen species, maar dat ook de verschillende stammen en soorten binnen een species tot verschillen kunnen leiden. Gevraagd wordt waarop de keuze voor een bepaalde soort van een species is gebaseerd. Bijv. bij apen blijkt dit gebaseerd te zijn op vele factoren, die niet altijd primair

gericht zijn op wat het beste overeenkomt met de mens. Hier speelt met name de beschikbaarheid van de dieren een grote rol. Bavianen worden in het wild gevangen en dit veroorzaakt een grote intraindividuele spreiding. Daarom wordt liever voor gekweekte soorten gekozen. Een andere overweging is geld. De marmoset-apen zijn kleiner, dus is er minder teststof nodig. H. Koster zegt dat deze apen in chronische studies echter niet de voorkeur hebben omdat ze moeilijk handelbaar zijn. Bij Organon is wat betreft de steroiden weer overgegaan op de cynomolgus aap omdat het metabolismepatroon in deze soort toch beter overeen kwam met de mens.
E. van de Aar merkt op dat haar ervaring is dat de metabolisme-verschillen tussen rattenstammen vooral verschillen zijn in snelheid van metabolisme, maar niet zozeer in metaboliet-patronen. Dit is in vivo gevonden.
J. Bessems vraagt of wij ons voornamelijk richten op P450-enzymen en of er ook nog gekeken wordt naar minder voor de hand liggende enzymen zoals β-lyase of S-methyltransferases. Deze enzymen worden zeker niet uitgesloten, maar de aandacht die deze enzymen krijgen is afhankelijk van de informatie die hierover te vinden is. Er wordt wel in eerste instantie vooral gekeken naar de belangrijkste fase 1 en fase 2 enzymen.

N. Bode vertelde dat bij Janssen een project loopt waarbij gekeken wordt naar de kinetiek van een stof in zowel mens als proefdier in relatie tot de fysisch-chemische eigenschappen van deze stof. Hierbij wordt de problematiek van achter naar voren bekeken, op basis van de humane kinetiekdata en de proefdierdata wordt gekeken of er een relatie te ontdekken is met de fysisch-chemische eigenschappen van de stof.

Het was een zeer waardevolle bijeenkomst gezien de grote opkomst van de door ons uitgenodigde deelnemers en hun bijdrage aan de discussie. Hieruit blijkt wel dat er veel belangstelling bestaat voor dit onderwerp. Tevens bleek ook dat er toch nog veel informatie mist en dat er dus nog wel veel werk ligt om een compleet overzicht te kunnen maken.

Appendix 2 Mailing list

1 Directeur Generaal van de Volksgezondheid
2 dr. F. Schuring, Hoofdinspecteur Gezondheidsbescherming
3 drs. P. de Greeve, VWS, Inspectie W&V
4 mr. J. de Haan, VWS, GZB
5 dr. A.A.J. van Iersel, ZON, Programmacommissie Alternatieven voor Dierproeven
6 drs. H. Verburg VWS, Inspectie V&W
7 prof.dr. M. Balls, ECVAM
8 prof. dr. J.J. Sixma, Voorzitter Gezondheidsraad
9 Directie RIVM
10 dr. ir. G. de Mik, directeur sector stoffen en risico's, RIVM
11 drs. J. van Eijkeren, LBM-RIVM
12 dr. J. W. van der Laan
13 dr. H.E.M.G. Haenen, LGM-RIVM
14 dr. ir. Sj. De Boer, LGM-RIVM
15 dr. F. Hajdarevic, LGM-RIVM
16 dr. L. van Aerts, LGM-RIVM
17 dr. E. Putman, LGM-RIVM
18 dr. H. Stevenson, LGM-RIVM
19 drs. S. van de Plas, LGM-RIVM
20 dr. C.F.M. Hendriksen, CDL-RIVM/Coördinatiepunt Alternatieven voor Dierproeven
21 dr. A.A.J. van Iersel, LGM-RIVM/Coördinatiepunt Alternatieven voor Dierproeven
22 dr. A.H. Piersma, LEO-RIVM
23 dr. ir. M.J Zeilmaker, LBM-RIVM
24 dr. W.H. Könemann, CSR-RIVM
25 drs. T.G. Vermeire, CSR-RIVM
26 dr. G.J.A. Speijers, CSR-RIVM
27 drs. M. Olling, College Beoordeling Geneesmiddelen
28 drs. P. Baede-van Dijk, College Beoordeling Geneesmiddelen
29 dr. D. van de Dobbelsteen, Organon
30 dr. J.J.Tukker, Rijksuniversiteit Utrecht
31 dr. A. Slikkerveer, Yamanouchi Europe BV
32 dr. E. van der Aar, Yamanouchi Europe BV
33 dr. R. Witkamp, TNO-Pharma
34 dr. J. Bessems, TNO-Voeding
35 dr.P. Bos, TNO-Voeding
36 dr. F. Schurz, TNO-Voeding
37 dr. H. Koster, Solvay
38 dr. S. Ouwerkerk, Solvay
39 dr. C. Groen, Kinesis
40 dr. N. Bode, Janssen Research Foundation
41 dr. J. Meulenbelt, VIC-RIVM
42 dr. A. Rutten, Intervet Internationaal
43 drs. P. Bollen, Ellegaard, Denemarken
44 Depot van Nederlandse Publikaties en Nederlandse Bibliografie
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