Development of the Rodent Gastrointestinal Tract:

The regulation of the development of the GI tract is unique and complex. With respect to the GI tract there are several considerations that need to be borne in mind when designing juvenile toxicity studies.

In man, a proportion of adult digestive functions are in place at birth, and many digestive enzymes are present in concentrations that are relatively close to adult levels. The rat GI tract by contrast, is relatively undeveloped at birth and practically all of the functional development is in the post-natal period. The rodent GI tract is functionally immature for the first 2 weeks of life, this is followed by extensive changes in Week 3. If we measure maturity by the availability of digestive enzymes, then it can be considered that the GI tract has adult status by end of Week 4. This maturation process occurs by the replacement of cells rather than modification of existing cells and the mucosal mass of the rat becomes fairly constant after about 40 days.

Clearly weaning constitutes a significant change in dietary composition so, unsurprisingly, a major change in function is also required. Although weaning is an obvious developmental milestone, does the change in diet force the changes in the GI tract or vice-versa?

Research has indicated that the alteration in dietary composition at weaning does not in itself have a causal role in metabolic and digestive development in the intestine, but probably modulates levels of enzyme activity.

Day 7 of age rat stomach.
For further images click here
So, if the composition of the diet is not the main driver, then where should we be looking? There appear to be two main drivers for GI tract development: above all it seems that the basis of the developmental process is "hardwired" into DNA, so the development is set to explode at around Day 14 of age, irrespective of most other factors. However, the process is also dependent on a corticosterone surge. After an initial birth peak, low levels of circulating corticosterone prevail in the first weeks of life in the rat. This is comparable to intrauterine conditions in other species. Glucocorticoid levels surge from Day 14 and it is this surge that controls the rate of expression of the intestinal programme. Interestingly, the small intestine loses its sensitivity to Glucocorticoids at Day 17, thus leaving only a very small window for well ordered development. In the stomach this surge stimulates antral gastrin secretion and the development of gastrin receptors.

There are other factors that may have an important but lesser role. It seems certain that some factors in milk such as epidermal growth factor act as developmental cues. Although the ingestion of food is almost certainly relatively unimportant for intestinal development, nibbling of food may play an important role in the development of pancreatic enzymes. Metabolic hormones, including insulin and somatotrophin, also have a part to play. With regard to the development of the stomach, it seems that gastrin has a major influence, but other major GI tract hormones have comparatively little impact on GI tract development.

Intraluminal nutrients look to be important for normal mucosal growth but do not prevent maturation, although, their absence may slow the process as malnutrition results in a profound reduction in villous height and mucosal mass.

The rodent GI tract prior to Day 17 bears very little resemblance to man. The pH and enzyme environment is bland and targeted towards absorption of large molecules, consequently protein denaturation is minimal. The human small intestine is also considerably less permeable to macromolecules than its rodent counterpart. Although gastric secretion is lower at birth in humans when compared to the adult, the contrast is far greater in pre-weaned rodents. HCl and pepsinogen secretion in human neonates are around 50% of adult levels, with a pH of 4.5, while normal adult pH and pepsinogen secretion are established by 1 year of age.

Hence, unless a NCE is specifically being developed for preterm and infant children, oral dosing in rats dosed before Day 21 of age is likely to result in bioavailability data unrepresentative of that to be expected in older children. This serves to emphasise the importance of toxicokinetic data taken at early time-points in juvenile rat studies. Indeed there is considerable merit in gathering such data as part of preliminary range-finding studies to assess whether relevant exposures are being attained.

There is, of course, one class of drugs which may find this benign environment extremely favourable. One of the difficulties of many biological drugs and, in particular, monoclonal antibodies, is the hostile environment the GI tract represents for protein based technology. In addition, drugs that have similar structures to rodent antibodies may be actively absorbed during this early dosing period. Absorption of maternal immunoglobulins and antibody transfer in rats is high for the first 14 days of life, but continues to take place at a lower rate up to Day 24 of age or thereabouts.

Overall, in comparison to humans, there are some significant differences in the development of the rodent GI tract. Thus to avoid a scientifically flawed approach, it is imperative that these differences are fully taken into account when designing a juvenile study. Oral dosing regimens in pre-weaned rodents need to be carefully considered and we would encourage a tailored approach based on sound scientific justification.
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Appendix 1 – Rodent Stomach Images

**Day 4 of age x200**

Compared to the Day 28 stomach, the neonatal stomach is less than half of the height; the mucosal height at this stage is only around 250 microns compared to around 600 microns at Day 28.

The surface mucous cells appear well established but there are virtually no secretory cells.

There is little in the way of cell types that respond to gastrin.

**Day 7 of age x200**

Anatomically there is very little development in the first week of life. By Day 7, early signs of proliferation can be seen in the deeper layers. Mucosal height has increased to about 275 microns.

In the days prior to weaning, the stomach appears to be sensitive to trophic effects of secretin.

**Day 14 of age x200**

If one compares this Day 14 stomach to the Day 7, the striking aspect is the appearance of some cellular differentiation in the gastric glands. The pale, eosinophilic (pink) Parietal cells with their classic “fried egg” appearance can be clearly seen in the superficial areas and, deeper in the mucosa, the basophilic (deep blue) chief cells are also becoming apparent. In this period the height of the mucosa increases to around 350 microns.

Functionally, however, the stomach is still largely immature, with minimal pepsinogen and acid secretion. The result being that the rodent stomach, which is broadly neutral at birth, remains so for the first 2 weeks of life, although there is a small increase in secretions in this second week of life and the pH drops from 6 to 4. This corresponds with a rise in plasma corticosterone levels, thought to be involved in the maturation and regulation of parietal cells.
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Appendix 1 - Rodent Stomach Images

Day 21 of age x200

The mucosal cell population continues to increase between Days 15 and 21 of age. Mucosal height is now around 450 microns. Anatomically, the cell population and differentiation is comparable to the adult stomach. Antral gastrin secretion increases dramatically from Day 18 until weaning and at around Day 20, the mucosa becomes responsive to histamine and pentagastrin.

Day 21 (the start of the weaning period) is a significant landmark for the development of the rat stomach. As a result of these changes, a significant increase in mucosal pepsin activity is seen from around Day 19. Given the close relationship between acid secretion and pepsinogen activation, significant increases in acid secretion also occur around weaning, corresponding with a peak in corticosterone levels.

Day 28 of age x100

By Day 28 the adult anatomy of the stomach is in place. From the surface the mucous neck cells stretch deeper into the gastric glands where the hydrochloric acid secreting, eosinophilic parietal cells can be found. Deeper still at the base of the gastric glands the more basophilic pepsin secreting chief cells can be clearly seen.

From Day 28 through to final maturation at about 6 weeks, the height of the mucosa increases from around 600 microns to 800 microns. The levels of pepsin and acid have been steadily increasing from Day 19 and are close to adult levels by this stage, although final levels of secretion in the stomach are not seen until around Week 6.
References


