CONTROL MECHANISMS OF CELL PROLIFERATION IN INTESTINAL EPITHELIUM

PROEFSCHRIFT

ter verkrijging van de graad van doctor in de geneeskunde aan de Erasmus Universiteit te Rotterdam, op gezag van de Rector Magnificus Prof.Dr. B. Leijnse en volgens besluit van het College van Decanen.
De openbare verdediging zal plaats vinden op woensdag 12 januari 1977, des namiddags te 3.00 uur

door

RUDOLF PIETER CORNELIS RIJKE

geboren te Rotterdam in 1950

1977
Drukkerij Pasmans 's-Gravenhage

Promotor	: Prof.Dr. H. Galjaard
Co-referenten	: Prof.Dr. D.W. van Bekkum Prof.Dr. L.F. Lamerton

Dit proefschrift werd bewerkt binnen de vakgroep Celbiologie en Genetica van de Erasmus Universiteit te Rotterdam.

Het onderzoek werd mede mogelijk gemaakt door financiële steun van de stichting voor Medisch Wetenschappelijk Onderzoek (FUNGO).

CONTENTS

GENERAL INTRODUCTION

1. Cell population kinetics in the adult organism

In the adult organism some organs and tissues still contain proliferating and differentiating cells, whereas other organs only consist of non-dividing specialized cells. On the basis of their proliferative activity cell populations may be classified into three categories (135, 138,208). In some cell populations, e.g. neurons, no cell divisions occur in the adult organism, and these have been referred to as "static" or "stable" cell populations. In other cell populations the number of cells keeps increasing in the adult organism at a slow rate with scattered mitoses, e.g. parenchymal cells of liver and kidney; such cell populations are called "growing" or "expanding" cell populations. In some organ systems cell proliferation occurs at a high rate in the adult organism, and mitotic figures are abundant. These cell populations do not increase in size, or only to a slight degree, and cell production is balanced by continuous cell loss. These cell populations are called "renewing" cell populations (141). Examples of "cell renewing systems" are epidermis and derivatives, gastrointestinal epithelia, testis and blood-forming tissues. The size and the functional capacity of such a cell population is dependent on the precise balance between cell production and cell loss, while some flexibility is needed to meet with possible perturbations of the system.

The regulatory mechanisms responsible for maintaining the balance between cell production and cell loss in renewing cell populations are still largely unknown. A feedback control of cell proliferation by the functional cell compartment has been described for some renewing systems (25, 100,120,184,252). However, little is known about how the size of the proliferative cell compartment is regulated,

and how the proliferative activity in that compartment is controlled. Knowledge of the underlying control mechanisms would yield more insight into the growth and maintainance of the organs concerned. It could also contribute to the understanding of how the balance between cell production and cell loss is disturbed under aplastic and cancerous conditions.

For a study on control mechanisms of cell proliferation in cell renewing systems the epithelium of the small intestine offers certain advantages. The turnover time of the epithelium of the small intestine is the shortest of all organs (17), which enables accurate analyses of the cell population kinetics under various experimental circumstances. The generative cell cycle of the intestinal epithelium is one of the shortest in the organism, being approximately 12 hours (48). The orderly microscopic arrangement of the different developmental stages in the proliferative, maturing and functional cell compartments makes the investigation of ultrastructural and biochemical characteristics of the epithelial cells in relation to cell age possible (20, 40,57,85,173,226). Finally, the small intestine is readily accessible for biopsy in man, and experimental changes of its functional environment may easily be induced in animals.

2. The small intestinal epithelium as a cell renewing system

a. Epithelial cell types

The intestinal epithelium is localized on the villi and in the crypts of the small intestine (see Figure 1). This structure arises in the rat during fetal and early postnatal development from a simple tube lined by a stratified epithelium, which changes into a structure with villi lined by a simple columnar epithelium (106,125,158,159). Dividing epithelial cells have been observed on rat fetal villi, but already before birth cell proliferation is limited to the

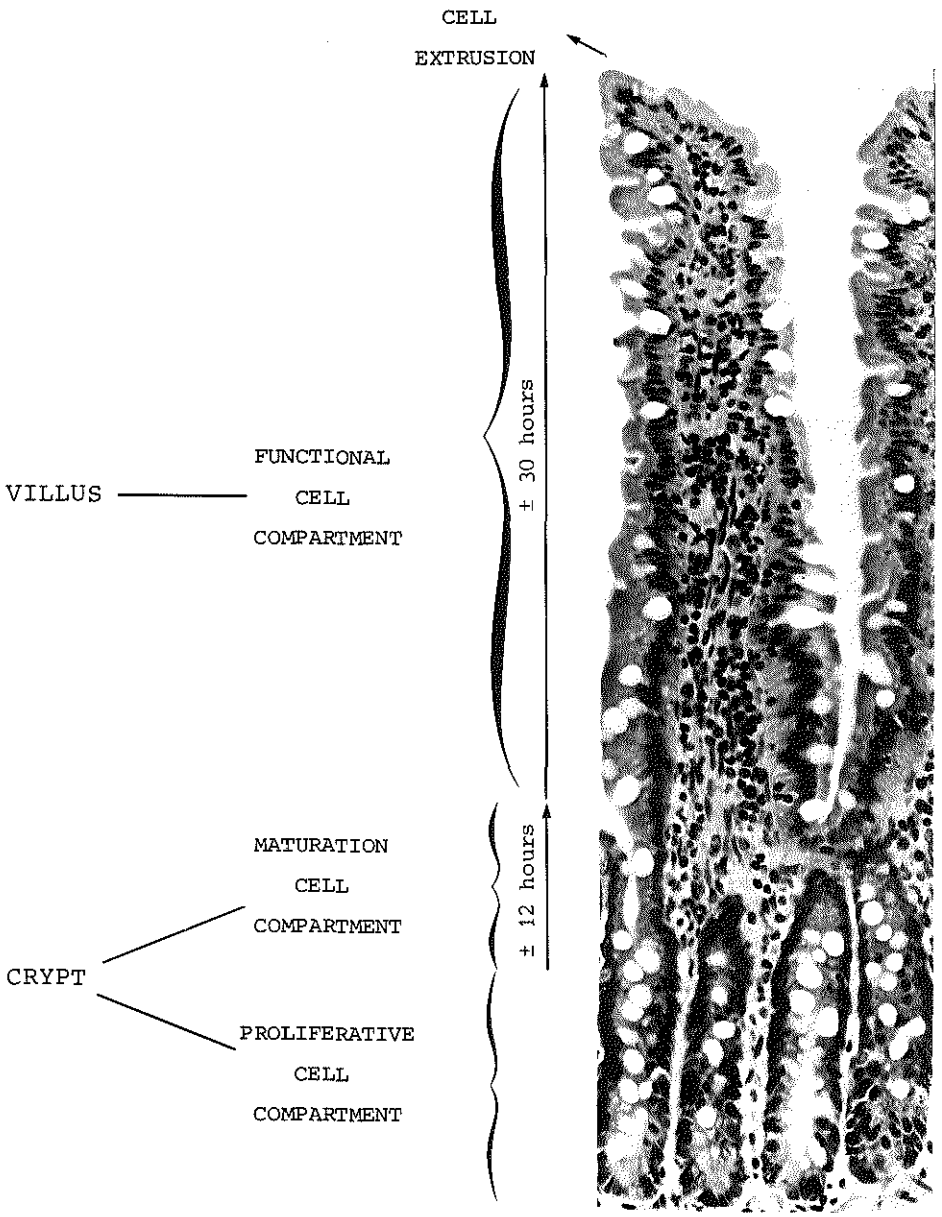

Fig. 1. Localization of proliferative, maturation, and functional cell compartment in jejunal epithelium.

basal parts of the villi (106). In the rat crypts are formed shortly after birth, and cell proliferation is confined to these (106). In man a similar development seems to occur during the first and second trimester of gestation (92).

In the adult animal the epithelium of the small intestine consists of four cell types, which probably arise from a common precursor cell (38-42). The majority of the epithelial cells, more than 85%, consists of columnar absorptive cells (38). The columnar absorptive cell arises by cell division in the lower half of the crypt and subsequently migrates during a twelve hours' period along the upper half of the crypt, which has been designated the maturation cell compârtment (34,193). During its course through the crypt a gradual development of the ultrastructural cell components takes place (38,57,61,173). Also the activity of a number of enzymes shows a gradual increase in the crypt (20,77,84,210). A more sudden development takes place at the crypt-villus junction. Considerable changes in the ultrastructure, e.g. microvilli and endoplasmic reticulum, are then observed (57,61,173), and the activity of several enzymes involved in intestinal function shows a large increase (20,21,84,85,121,170,173,210). On the villus the columnar absorptive cells, which are now actively involved in intestinal function, migrate in the rat in approximately 30 hours to the villus top where they are extruded into the lumen (53,86,149).

A second cell type of the intestinal epithelium is the goblet cell, which produces a mucinous secretion which may serve as a protective or lubricant agent (167). In the lower half of the crypt the precursor cell of the goblet cell, the oligomucous cell, arises from early stages of the columnar absorptive cell. After one or more cell divisions the mature goblet cell develops (31,38,59,164,167). The goblet cell, too, migrates along crypt and villus and is

eventually extruded from the villus top (31,38,164,167).

A third cell type in the intestinal epithelium is the Paneth cell, which is characterized by its specific granulae and its location in the bottom of the crypt. The Paneth cell may function as a source of intestinal lysozyme or as a specific cell type for the phagocytosis of intestinal microorganisms (66-68,172,181). The Paneth cell is not able to divide and probably also arises from early stages of the columnar absorptive cell (39,99). The Paneth cell remains located at the bottom of the crypt, degenerates after a relatively long life-time and is eventually phagocytosed by an adjacent columnar absorptive cell (39).

The least frequent cell type, making up less than 1% of the intestinal epithelial cells, is the entero-endocrine cell, which is a heterogenous cell population of probable endocrine cells. The entero-endocrine cells are responsible for the production of several putative hormones (8,41,72, 179,187). Although it has been suggested that these cells originate from the neural crest (180), this view seems unlikely (9,42,73,142,183). Thus, entero-endocrine cells are now thought to arise from dividing precursor cells, which differentiate from early stages of columnar absorptive cells (41). The entero-endocrine cells also migrate to the villus to be eventually extruded from the villus top (41).

b. Epithelial cell kinetics in the small intestine

Already in 1888 Bizzozero suggested that the epithelial cells arising from the observed mitotic figures in the crypt were to replace damaged villus cells (18). However, it was not until 1945 that Friedman demonstrated by using X-irradiation that goblet cells migrate from the crypt to the villus and subsequently to the villus top (78). Shortly thereafter Leblond and co-workers were able to follow newly-formed epithelial cells from the crypt up to the top of the

villus by means of autoradiography after labelling with radioactive phosphate (140). In the following years it was found that cell division in the crypt is independent of luminal contents, and it was concluded that the epithelial lining of the small intestine constitutes a renewing cell population with an unusually high rate of cell replacement (16,139,141,222). The number of cells produced and lost every 30 days in the rat small intestinal epithelium was found to equal the total number of cells in the body (135).

In 1951 Howard and Pelc described that DNA synthesis occurs during a specific part of the generative cell cycle (S-phase), separated time-wise from the mitotic phase by "gaps", which they later designated as G_1- (pre-DNA synthesis) and G_2-phase (post-DNA synthesis) of the generative cell cycle (114,115). After several attempts to find specific radioactive labelled DNA-precursors (138,141), the introduction of tritiated thymidine (^{3}H-thymidine) was an important step forward (224). Tritiated thymidine is rapidly taken up by dividing tissues, it has a short half-life time in the blood, and it is incorporated only into DNA only during the S-phase of the generative cell cycle, whereas it is released from DNA only after death of the cell (7,48,54, 70,116,202). Furthermore, tritium (^{3}H) gives a high resolution in autoradiography, which facilitates the localization of DNA-synthesizing cells (48,116). Although high doses of thymidine may disturb cell proliferation and high doses of tritium may give local radiation damage, the required dose of ^{3}H-thymidine for cell kinetic studies in vivo is low enough to prevent these side effects (19,48,94,188,206,221). An important prerequisite for the use of ^{3}H-thymidine as a parameter for cell proliferation is that every cell actually divides after DNA synthesis, and this has been amply demonstrated for the intestinal epithelium (26,128,165,207).

A large number of investigations were performed to

study epithelial cell proliferation in the small intestine using ^{3}H-thymidine (116,137,138,165,193). These studies confirmed that epithelial cells divide in the crypt, migrate to the villus and are lost from the villus top. Furthermore, using the model of the generative cell cycle as proposed by Howard and Pelc (115), it became possible to determine the duration of the generative cell cycle phases by following the appearance and disappearance of labelled mitotic figures after pulse labelling with ^{3}H-thymidine (193). More detailed cell kinetic studies were subsequently performed, and models were proposed for the cell renewing system of the intestinal epithelium (33,34,193). Also methods were developed for a more exact quantitative description of the small intestinal epithelium, especially in the mouse (2,35,95,97,129,212,241). As differences do exist in the quantitative description of the intestinal epithelium between mice and rats, quantitative data of rat small intestine will only be referred to in the following, since the investigations described in this thesis dealt with rat epithelium.

In the crypts of the rat small intestine cell proliferation is confined to the lower half of the crypt (33,83, 193,246). In the lower part of this proliferative cell compartment each dividing cell gives two proliferating cells, whereas in the upper part each dividing cell yields two non-proliferating cells (27,34,193). All columnar absorptive cells in the proliferative cell compartment are proliferating with a maximal duration of the generative cell cycle of 24 hours (31), and the proliferating cells are evenly distributed over the phases of the generative cell cycle (207). The mean duration of the generative cell cycle is approximately 12 hours; it is 14-16 hours in the lowest crypt cell positions and 10-11 hours in the upper part of the proliferative cell compartment (1,27,33). This diffe-

rence is due to differences in the duration of G_1- and S-phase, respectively 2-4½ hours and 6-8½ hours. The duration of the G_2- and M-phase of the generative cell cycle is the same throughout the proliferative cell compartment, respectively 1 hour and ½-1 hour (1,33). After having completed 2-3 cell divisions (246), the crypt cell enters the "critical decision zone" (34,193) halfway the crypt where the cell normally stops cell proliferation. After migrating through the upper half of the crypt, which takes 9-12 hours, the cell enters the functional villus compartment (33,86, 246,250). On the villus the epithelial cell performs its function, while migrating from the base to the top of the villus, and 36-42 hours after its last cell division the cell is extruded into the intestinal lumen (53,86,149).

Three-dimensionally, the small intestine of the rat is lined by approximately 2×10^9 villus cells and 2×10^9 crypt cells (5). The total number of villi in the rat small intestine is $1.4\text{-}1.5 \times 10^5$ (46,74). The mean number of crypts per villus, the crypt-villus ratio, throughout the entire small intestine is 16 (44,47,81), which gives $2.2\text{-}2.4 \times 10^6$ crypts for the entire rat small intestine. From these values it may be calculated that there are approximately 15.000 epithelial cells per villus and 800-900 epithelial cells per crypt. Experimentally it was found that the number of cells per crypt is between 530 and 850 (32,110,242,246,250). Assuming 700 cells per crypt, a number of 420 proliferating cells per crypt may be expected, the growth fraction of the crypt cell population being 0.6 (33,248,250). These 420 proliferating cells with a mean generative cell cycle time of 12 hours (1,27,33) produce approximately 35 cells per crypt per hour. This value is in good agreement with experimentally obtained data for the crypt cell production, which was found to be 30-40 cells per crypt per hour (45,246,250). This rate of crypt cell

production, together with a crypt-villus ratio of 16 results in an influx into the villus cell compartment of 560 cells per villus per hour. Since the life span of the epithelial cells is approximately 40 hours, and the transit time along the upper part of the crypt 10 hours (33,86,149,246,250), the villus transit time is approximately 30 hours. This indicates that, with an influx of 560 cells per villus per hour, the number of cells per villus is approximately 17,000. This value agrees well with the 15,000 cells per villus, which was calculated from the number of villus cells and the number of villi in the entire rat small intestine.

c. Regulation of cell proliferation in intestinal epithelium

The mechanisms responsible for the orderly microscopic arrangement and the relative numbers of proliferating, maturing and functional epithelial cells in the small intestine are largely unknown. The same is true for the mechanisms involved in the regulation of epithelial cell proliferation, cell migration and cell death (82).

It has become clear that crypt cell proliferation is influenced by a large number of factors, which is reflected for instance by the existence of a daily rhythm, by changes occurring with aging, and by changes which take place under altered physiological conditions such as lactation (32,52, 79,145,211,213,219). Such changes in the epithelial cell kinetics may be caused by neural, hormonal, or luminal factors (6,87,104,123,136,169,174,218,231,232,247,251). However, nothing is known about the way(s) in which these factors induce changes in epithelial cell kinetics.

A large number of investigators has studied the effects of experimental perturbations of crypt cell proliferation by means of X-irradiation or inhibitors of DNA and protein synthesis (55,56,83,132,143,190,233,240). Relatively low

doses of X-irradiation (300-900R) result in a temporary block of cells in the G_1- and G_2-phase of the generative cell cycle (143,178,191,240). Subsequently many abortive mitoses appear and the crypt cell population is reduced (55,143,146,178,190,191,240). The resulting reduction of crypt cell production leads to a decrease in the number of villus cells (131,178,191,209). A recovery of the crypt cell population takes place from 24 hours after irradiation on, and a temporary overshoot in proliferative activity occurs (55,83,98,143,190,209,240). The latter is the result of some shortening of the generative cell cycle time (143), and especially of a marked expansion of the proliferative cell compartment in the crypt (83). The latter enables cells to go through one extra generative cell cycle before leaving the crypt (83). The overshoot in proliferative activity may be due to the initial damage of the crypt cell compartment, or to the reduction of the villus cell compartment by means of a feedback control mechanism (83,144). The latter possibility was tested by a number of investigators (86,161,205). An inverse relationship was found to exist between the number of villus cells and the proliferative activity in the crypt in normal as well as in germfree rats following 700R X-irradiation (86,161). The small intestinal epithelium was also studied during the recovery period after irradiation by Sato and co-workers, who determined the number of crypt and villus cells (205). They found that the experimental data reasonably fitted curves constructed with a computer model, which was based on a simple two-compartment system with a feedback control of cell proliferation by the functional villus cell compartment (205). However, in the computer model a constancy of the generative cell cycle time and of the growth fraction had been supposed, and this does not agree with experimental findings (83,143).

The first purpose of the investigations presented in this thesis was to test the hypothesis of a feedback control of crypt cell proliferation by the functional villus cell compartment.

Furthermore, it is known that the epithelium of the small intestine may "adapt" to experimentally altered circumstances (64). Especially changes in luminal and/or functional factors seem to result in marked changes in the size of the functional villus compartment (6,63,64). Resection of a large part of the small intestine leads to an enlargement of the villus cell population in the remaining intestine (62,63,71,101,103,160,216). On the other hand, experimental by-pass of a jejunal segment leads to a reduction of the villus cell population in that segment (3,88). Changes in the villus cell compartment were also observed after inducing changes in the luminal contents of the entire small intestine or of parts of the intestine (3,4,6,80,122, 162).

The second purpose of the investigations presented here was to study epithelial cell kinetics following experimental procedures which would lead to a permanent reduction or enlargement of the functional villus cell compartment in an intestinal segment.

d. Cell maturation in the intestinal epithelium

Numerous studies have focussed on the functional capacity of the small intestine under various circumstances, especially by determining the activities of enzymes involved in intestinal function. The activity of a number of intestinal enzymes was determined after changes in luminal or hormonal circumstances, and after disturbing crypt cell proliferation (10,14,37,50,89,107,163,171,238). However, enzyme activities were usually determined in homogenates of

entire small intestinal segments or in homogenates of mucosal scrapings, which does not give much information on the cell maturation of the epithelial cells. For changes in intestinal enzyme activities found in such homogenates may very well be due to changes in the ratio of crypt to villus cells, or to changes in the fraction of proliferating cells.

More promising in this respect are methods which enable (micro)chemical analysis of separated cell compartments of the intestinal epithelium (84,152,170,235,236). These methods have been used by various investigators to study epithelial cell maturation in normal small intestine (75, 105,117,119,168,170,235), extending earlier data obtained by histochemical methods (77,121,173,210,226). Galjaard and co-workers applied such a separation procedure to study epithelial enzyme activities in dissected crypt and villus cell compartments during the recovery period following irradiation (20,21,60,83-85). From these studies it appeared that an expansion of the proliferative cell compartment in the crypt after irradiation is accompanied by a decrease in the activity of a number of enzymes involved in intestinal function (20,21,60,83-85).

The third purpose of the investigations described in this thesis was to study cell maturation in the small intestinal epithelium under various experimental circumstances by determining enzyme activities in separated crypt and villus compartments.

INTRODUCTION AND DISCUSSION OF THE EXPERIMENTAL WORK

To investigate the regulatory mechanisms of epithelial cell proliferation in the small intestine, and to analyse epithelial cell maturation under various circumstances, a number of studies were undertaken, which are dealt with in the following.

After a relatively low dose of X-irradiation the crypt cell population is reduced, and subsequently a decrease in the number of villus cells may be observed (55,178,191,209, 240). During the recovery period an overshoot in proliferative activity occurs (83,98,143,190,209,240), which may be a response to the initial damage of the crypt cell population, or by means of a feedback control mechanism the result of a reduction of the villus cell population (83,144). Data supporting the latter view were obtained by a number of investigators studying the recovery of the intestinal epithelium after X-irradiation (86,161,205). For a correct interpretation of these findings it is necessary to know whether changes in villus length are due to changes in crypt cell kinetics or that (also) variations in the life span of the epithelial cells play a role. Therefore epithelial cell migration along crypt and villus was studied using ^{3}H-thymidine at various time intervals after 700R X-irradiation. These studies, which are described in *appendix publication I*, show that the minimal life span of the epithelial cells is always more than 36 hours, whether the villus cell compartment is markedly reduced or has recovered almost completely. It was found that the cell migration rate is dependent on the rate of crypt cell production. The recovery of the villus cell compartment starts only after crypt cell production has become large enough again to provide a normal cell migration rate along crypt and villus. By determining the radioactivity in dissected villi, after

^{3}H-thymidine labelling at various time intervals after irradiation, it was found that labelled cells were always lost from the villus between 36 and 48 hours after injection of ^{3}H-thymidine. This indicates that the maximal life span of the epithelial cells in the small intestine is less than 48 hours both in normal intestine and during the recovery period after X-irradiation. Thus it appears that the life span of epithelial cells in rat small intestine (36-48 hours) does not change to a considerable extent during recovery from irradiation. Hence the recovery of the villus cell population following irradiation is mainly to be attributed to the increased cell proliferation in the crypt. This finding also implicates that changes in enzyme activities, which were found in the crypt and villus cell compartment during recovery after irradiation (20,21,83-85, 161), are probably only related to the expansion of the proliferative crypt cell compartment.

In the same publication also the recovery of the intestinal epithelium after different doses of X-irradiation was compared. It was found that the number of crypt cells is markedly reduced after 700R X-irradiation, whereas only a small decrease in the number of crypt cells was found after irradiation with 300R. Following both radiation doses the percentage of labelled crypt cells after pulse labelling with ^{3}H-thymidine was reduced after 24 hours, and an overshoot in proliferative activity was observed from 48 hours on. A marked decrease in the number of villus cells was observed from 48 hours after irradiation on, also for both irradiation doses. Thus, the increase in proliferative activity in the crypt seems to be independent of the initial damage of the crypt cell population following X-irradiation with 300R to 700R. Furthermore, an inverse relationship was found between the number of villus cells and the proliferative activity in the crypt.

Although the hypothesis of a feedback control of crypt cell proliferation by the villus cell population was supported by the data from appendix publication I, some contrasting data had been reported from studies on the effects of continuous irradiation. In those studies no increase in proliferative activity was observed during irradiation (29, 132,189,192,242), although a reduction of the villus cell compartment had been found (134,189). The studies, which are described in *appendix publication II*, were meant to follow up these findings by investigating epithelial cell kinetics during the course of continuous irradiation and during the recovery period thereafter. It was found both by autoradiographic studies and by scintillation counting of the separated crypt cell compartment after pulse labelling with ^{3}H-thymidine that the proliferative activity in the crypt was reduced after 1 day of irradiation, but values identical to those in control animals were subsequently found during the remaining period of 5 days of irradiation. Although the percentage of labelled crypt cells was not increased, an increase in the relative size of the proliferative cell compartment in the crypt occurred from 2 days after the start of the continuous irradiation on. At the same time a marked decrease in the number of cells per villus column was observed, whereas the number of cells per crypt column was only slightly affected. After cessation of the irradiation an overshoot in proliferative activity was observed. The number of villus cells as well as the relative size of the proliferative cell compartment in the crypt were normal again at 2 days after cessation of the irradiation.

These findings indicate that also during continuous irradiation an expansion of the proliferative cell compartment in the crypt occurs. The absence of an increase in the percentage of labelled crypt cells may be due to the steri-

lizing action of the continuous irradiation (29,30). This view is also supported by the presence of a decrease in the labelling index of cells in the lower half of the crypt and by the overshoot of proliferative activity immediately after cessation of the irradiation.

In these studies also epithelial cell maturation was investigated by determining the activities of several enzymes involved in intestinal function in separated crypt and villus cell compartments. The activity of non-specific esterases normally increases already during crypt cell maturation and another increase in activity occurs at the crypt-villus junction. During continuous irradiation the esterase activity was reduced in the crypt at all time intervals at which an expansion of the proliferative cell compartment existed in the crypt, and also a decreased activity was observed in the villus cell compartment. This is in agreement with earlier reports, showing an inverse relationship between crypt cell proliferation and esterase activity in the crypt during recovery after a single radiation dose (83,86,161).

The use of X-irradiation as a tool to test the hypothesis of a feedback control of crypt cell proliferation has the disadvantage of reducing the villus cell population by affecting the crypt cell population, the response of which is subsequently studied. A preferable approach would be to affect the villus cell population directly, and subsequently study the effects of this on crypt cell proliferation. Temporary intestinal ischemia is a procedure which seems to affect selectively the villus cell compartment (36,198,199, 201). *Appendix publication III* describes that the number of villus cells was markedly reduced within 2 hours after temporary clamping of the superior mesenteric artery and vein. Subsequently it increased again to approach control values within 24 hours after temporary ischemia. No decrease in

the number of crypt cells was observed, and the specificity of the procedure was further tested by combining cell migration studies with temporary ischemia, which showed that epithelial cells were mainly lost from the upper part of the villi. Although earlier reports mentioned a normal mitotic index following temporary intestinal ischemia (36, 186), an increase in proliferative activity was observed at 8 hours and 16 hours after ischemia. This increase was found to be mainly caused by an increase in the relative size of the proliferative cell compartment in the crypt. These findings strongly support the hypothesis of a feedback control of crypt cell proliferation by the villus cell compartment, and it seems likely that this mechanism acts by changing the relative size of the proliferative cell compartment in the crypt.

In these studies it was also determined whether the response of crypt cell proliferation to a reduction of the villus cell population is of a local or a systemic character. After creating temporary ischemia in half of the small intestine, cell proliferation was studied both in that part of the intestine and in a part which had not been subjected to ischemia. In the part of the intestine which had been ischemic a marked increase in proliferative activity was observed, whereas no changes occurred in the intestinal part which had not been subjected to temporary ischemia. Thus it seems most likely that a feedback control of crypt cell proliferation is a local mechanism.

The experiments so far described were concerned with temporary changes in epithelial cell kinetics of the small intestine. Permanent changes in the epithelium of the small intestine may be observed especially after procedures which lead to changes in luminal and/or functional factors (64). Resection of part of the small intestine, or transposition of an ileal segment to jejunum leads to an increase in the

number of villus cells (3,6,63,101), whereas experimental by-pass of a jejunal segment results in a reduction of the villus cell population (6,88). To study the changes in cell kinetics and cell maturation leading to the "adaptation" of the jejunal epithelium after experimental by-pass, the epithelium in jejunal Thiry-Vella fistulas was studied from 2 to 360 days after surgery. *Appendix publication IV* describes that the number of cells per villus column decreases at 7-14 days after by-pass, and the same is true for the number of cells per crypt column, though to a lesser degree. Subsequently the crypt and villus cell compartments remained constant up to one year after experimental by-pass, which demonstrates the intrinsic nature of the mechanisms maintaining the architecture of the intestinal epithelium. The percentage of labelled crypt cells after pulse labelling with ^{3}H-thymidine does not change in the by-passed segment, except for an increase at 2 days after surgery. Such an increase was also found in the remaining jejunum in continuity, and seems to represent a general response of the epithelium to the extensive manipulation of the small intestine. Also no changes were observed in the relative size of the proliferative cell compartment in the crypt. The three-dimensional crypt size, which was determined by scintillation counting of isolated crypts after ^{3}H-thymidine labelling, was markedly reduced after experimental by-pass. This decrease in the number of cells per crypt, together with an unchanged relative size of the proliferative crypt cell compartment, appears to be responsible for the reduction of the villus cell population. The size of the villus was also reduced three-dimensionally, which was demonstrated by scanning electron microscopy. The number of crypts per unit length of small intestine and the crypt-villus ratio were found to be unchanged in by-passed jejunum. Cell migration studies suggested some shortening of the

life span of the epithelial cells, which would also contribute to the decrease in number of villus cells.

The activities of non-specific esterases and neutral α-glucosidase in the crypt remained unchanged following by-pass, and an increase in the activity of non-specific esterases was found in the villus cell compartment. Thus it seems that the development of these biochemical characteristics of the epithelial cells is not dependent on the presence of normal luminal contents.

From these results it appears that "adaptation" of the intestinal epithelium after by-pass is characterized by a decrease in the number of crypt cells and number of functional villus cells. These changes in the intestinal epithelium after by-pass apparently proceed in a different way than the changes induced by the feedback control of crypt cell proliferation. This view is also supported by the fact that the decrease in the villus cell compartment occurred only at 7-14 days after by-pass, whereas the response of crypt cell proliferation to a reduction of the villus cell compartment is almost immediate.

Appendix publication V describes the recovery of the intestinal epithelium from X-irradiation in by-passed jejunum. Rats were exposed to 700R whole-body X-irradiation at 3-4 months after the experimental by-pass of a jejunal segment. The reduction and subsequent recovery of the crypt cell population occurred in the same way in by-passed jejunum as in jejunum remaining in continuity. Also the recovery of the villus cell compartment proceeded in the same way. These findings show that the response of the crypt cell compartment to an experimental reduction of the villus cell compartment is not dependent on the presence of normal luminal contents.

The results also indicate that the two mechanisms - one responsible for the decrease in number of crypt and villus

cells after by-pass, and the other responsible for the reaction of crypt cell proliferation to a reduction of the villus cell compartment - do not interfere with each other, and indeed may reflect two separate control mechanisms of crypt cell proliferation.

Epithelial cell maturation, as estimated by determining the activities of non-specific esterases, alkaline phosphatase and neutral α-glucosidase in separated crypt and villus compartments, was affected in by-passed jejunum to the same degree as in control jejunum after irradiation. This finding shows once more that the response of the intestinal epithelium to a temporary disturbance of the "steady state growth" is independent of luminal and/or functional factors.

A permanent increase in the number of functional villus cells was reported to occur in an ileal segment after transposition to proximal jejunum (6). Transposition of an ileal segment, in which the number of cells per villus column is considerably smaller than in jejunum, to proximal jejunum offers an excellent model to study the way in which the enlargement of the villus cell population is brought about in transposed ileum, since control ileal and control jejunal epithelium are available in the same rat. *Appendix publication VI* describes the epithelial cell kinetics and cell maturation in an ileal segment from 2 to 30 days after transposition to proximal jejunum. In this study all data obtained from the transposed ileum as well as from the remaining ileum in continuity were expressed as percentages of the values found for jejunum in the same rats. In this way, a correction could be made for the response of the entire small intestine to the extensive manipulation during surgery. The number of cells per villus column increased in the transposed ileum only at 7 days after surgery to reach values of jejunum at 14-30 days after surgery. This increa-

se in the number of villus cells was accompanied by an increase in the number of cells per crypt column up to 130% of values in jejunum and ileum. However, the percentage of labelled crypt cells after ^{3}H-thymidine labelling was the same in jejunum, transposed ileum and remaining ileum at all time intervals after surgery. This was also true for the relative size of the proliferative cell compartment in the crypt. The total proliferative activity per crypt determined by scintillation counting of isolated crypts after ^{3}H-thymidine labelling increased two-fold from 7 days after surgery in transposed ileum. Cell migration studies at 30 days after surgery showed no differences in the life span of the epithelial cells of the three different intestinal segments. Thus, it appears that the enlargement of the villus cell compartment in an ileal segment after transposition to proximal jejunum is caused by an increase in the number of cells per crypt. It is remarkable again that the "adaptation" of the transposed ileal epithelium takes a relatively long time, considering the rapid rate of turnover of the intestinal epithelium. The same was true for changes in the intestinal epithelium after experimental by-pass as described in appendix publication IV.

Epithelial cell maturation was studied by determining the activities of non-specific esterases and neutral α-glucosidase, which are normally lower in ileum than in jejunum (105). After transposition to jejunum the enzyme activities of the ileal epithelium did not increase. Again, it is clear that "adaptation" of the intestinal epithelium takes place by a change in the size of the functional villus cell compartment, and not by alterations in the enzyme activities of the villus cells. These results also suggest that epithelial cell maturation as reflected by enzyme activities is quite independent of the functional environment.

GENERAL DISCUSSION

Although many cell kinetic studies on the epithelium of the small intestine have been performed, the regulatory mechanisms underlying the balance between cell production and cell loss are still largely unknown. The main purpose of the experiments described in this thesis was to study control mechanisms of crypt cell proliferation in the small intestine.

The data described in appendix publication I showed that the recovery of the villus cell population following a single dose of irradiation is not to be attributed to a change in the life span of the epithelial cells. Comparative studies on the recovery from different doses of X-irradiation revealed that the overshoot in proliferative activity in the crypt occurs independent of the degree of initial damage to the crypt cell population following X-irradiation with 300-700R. Furthermore, the hypothesis of a feedback control mechanism of crypt cell proliferation (86, 161,205) was supported by the finding of an inverse relationship between the number of villus cells and the proliferative activity in the crypt. The experiments with continuous irradiation described in appendix publication II demonstrated that an expansion of the proliferative cell compartment may occur in the crypt despite the fact that total proliferative activity in the crypt is not increased. From the inverse relationship between the number of villus cells and the relative size of the proliferative cell compartment in the crypt it appeared that a feedback control mechanism of crypt cell proliferation would act via determining the relative size of the proliferative crypt cell compartment. The following studies described in appendix publication III, clearly showed that a selective reduction of the villus cell population leads almost immediately to an enlargement of the

proliferative cell compartment in the crypt.

Gluten-induced celiac disease in man also presents a situation in which increased cell loss from the villi seems to occur (126,173,228). In this situation an increase in crypt cell proliferation was observed (228,249), as was the case under a number of experimental circumstances in which the villus cell population was reduced (11,51,148,150,182, 196,229). These findings support the hypothesis of a feedback control of crypt cell proliferation by the villus cell population.

From the data presented in appendix publication III it appears that a feedback control mechanism of crypt cell proliferation would be of a local character. This view is also supported by the local response to intestinal damage after transplantation of an ileal segment to the abdominal surface (150). The absence of humoral factors in the feedback control mechanism was also substantiated by the finding that increased crypt cell proliferation during recovery after irradiation was only found in the irradiated partners of parabiotic pairs of rats (58). Luminal and/or functional factors do not influence the action of the feedback control mechanism as is shown by the results described in appendix publication V.

From the studies on experimental by-pass of a jejunal segment and transposition of an ileal segment to proximal jejunum (appendix publications IV and VI), it appears that "adaptation" of the intestinal epithelium to an altered functional environment is primarily achieved by changes in the number of cells per crypt. A change in the size of the crypt, together with an unchanged relative size of the proliferative cell compartment in the crypt, leads to an altered crypt cell production. Consequently this results in a change in the size of the functional villus cell compartment, since important effects on the life span of the epi-

thelial cells were not found. Also the number of crypts per unit length of small intestine and the crypt-villus ratio remained unchanged after experimental by-pass.

Epithelial cell maturation was investigated in the present studies by determining enzyme activities in separated crypt and villus cell compartments during disturbances of epithelial cell renewal caused by X-irradiation or temporary ischemia, and during "adaptation" of the intestinal epithelium to an altered luminal environment (see appendix publications II-VI).

In all instances, an increase in the relative size of the proliferative cell compartment in the crypt was accompanied by a decreased activity in the crypt of non-specific esterases. These enzymes are localized in the endoplasmic reticulum, dense bodies and perinuclear space, and become active during migration along the crypt (20,60,84). A decrease in esterase activity in the crypt was also reflected in a lower activity on the villus. During the enlargement of the proliferative crypt cell compartment after intestinal ischemia also a decreased activity of neutral α-glucosidase was observed. These findings support the view that crypt cell maturation is reduced when the proliferative crypt cell compartment has expanded (85).

However, in the irradiation experiments the activity of those enzymes which are mainly localized on the microvilli and the activity of which is mainly found on the villus, such as alkaline phosphatase, neutral α-glucosidase and leucine aminopeptidase (20,21,60,84,85), was not reduced during expansion of the proliferative cell compartment in the crypt. In earlier reports a decrease in alkaline phosphatase activity was found after irradiation (20,85), but in those studies enzyme activities were determined in proximal duodenum, while in the present investigations jejunum was studied. Also under other circumstances a different effect

was observed on the activity of alkaline phosphatase in various parts of the small intestine (50). The different effects of disturbances of crypt cell proliferation on the crypt cell activities of those enzymes which are mainly developed on the villus are not understood. The existence of inactive precursor proteins of these enzymes in the crypt (234) may play a role, and quantitative data on the presence of these precursor proteins under altered crypt cell kinetics will contribute to a better understanding of crypt cell maturation.

From the enzyme studies in by-passed jejunum and transposed ileum it is clear that the "adaptation" of the intestinal epithelium is solely caused by a change in the size of the functional villus cell compartment and not by changes in crypt cell maturation. This stresses once more the importance of cell kinetic studies of the intestinal epithelium when investigating the response of the epithelium to various circumstances.

Summarizing the data presented in this thesis it seems that at least two different control mechanisms of crypt cell proliferation exist in the epithelium of the small intestine. Firstly, the functional villus cell compartment regulates crypt cell proliferation by determining the relative size of the proliferative cell compartment in the crypt. A second control mechanism determines the number of cells per crypt, thus regulating crypt cell production and hence the size of the functional villus cell compartment. The former control mechanism responds almost immediately to disturbances of the intestinal epithelium. The latter brings about changes in epithelial cell kinetics only after 7-14 days, which is a relatively long period considering the rapid renewal of the epithelium and the reason for this is at presently not understood.

A feedback control mechanism of cell proliferation has

been proposed for a number of cell populations (25,100,120, 184,252). In 1957 Weiss postulated that such a mechanism would act via substances produced by the functional cell compartment inhibiting cell proliferation in the precursor cell compartment (237). Already in 1937 a fraction was extracted from an adult tissue which inhibited cell proliferation in the same tissue in vitro (214). Similar findings were subsequently reported for a large number of organs and tissues (24,112,203,204), and the putative inhibitory substance was called "chalone" (23). The so-called chalones from the various organs and tissues seem to present a biochemically ill-defined and heterogenous group of substances (225). For rat small intestine a putative chalone-fraction was extracted from the crypt cells which holds up cells in the G_2-phase of the generative cell cycle (230). Such a substance, however, is unlikely to be involved in the feedback control of crypt cell proliferation, since it could not be detected in villus cells. Also, an inhibitor of cell proliferation acting on cells in the G_1-phase seems more likely, especially since it has become clear that crypt cells will go through one extra cell cycle when the villus cell population is reduced. More recently, the existence of two chalone-like fractions affecting proliferating embryonic intestinal cells in the G_1- and G_2-phase of the generative cell cycle was described for the newt (22). However, in addition to a more precise biochemical characterization, the biological activity of such "chalones" should be studied in more detail in relation to tissue damage and tissue regeneration before any conclusions can be drawn concerning their physiological role. In the intestinal epithelium such studies will yield numerous problems, since the feedback control of crypt cell proliferation was found to be of a local character, and hence the action of "intestinal chalones" will accordingly be local.

The pathway along which substances produced by the villus cells would reach only crypts nearby could be the epithelial layer itself or the mesenchymal component of the intestinal mucosa. Thus, in addition to the possibility that chalones may be involved in the feedback control of crypt cell proliferation, other factors, such as intercellular communication in the epithelial layer (151) and the interaction between the epithelium and the mesenchyme, may play a role. Especially the possible role of the mucosal mesenchyme with its specific features deserves some attention. The intestinal mesenchyme seems to be very important in the morphogenesis of the intestinal tract epithelia (166,200). In adult animals each crypt in the intestine is surrounded by a continuous layer of subepithelial fibroblasts (155), which form "half-desmosomes" on the epithelial side (185). The epithelial cells make contacts with the fibroblasts via cell processes penetrating the basement membrane, and this phenomenon has putatively been correlated with crypt cell proliferation (158). The subepithelial fibroblasts proliferate along the lower part of the crypt, subsequently migrate to the villus to form a more scattered network, eventually reach the villus top at the same time as the epithelial cells, and degenerate (156). Since the basement membrane seems to be mainly synthesized by the epithelial cells (127), it seems likely that the epithelial cells, subepithelial fibroblasts and basement membrane "migrate" together from the crypt base to the villus top. This is suggestive of some common control mechanism, and it may be worthwhile to investigate the cell kinetics of the subepithelial fibroblasts in relation to the increased crypt cell proliferation after reduction of the villus cell population to determine whether the fibroblasts may play a role in this response.

In the studies on the influence of luminal factors lea-

ding to a permanent local decrease or increase in crypt cell production not only the nature of the signal leading to changes in the crypt, but also the way in which the crypt cell population responds to such a signal is unknown. Also following partial resection of the small intestine and during lactation an increase in crypt cell production is achieved by an enlargement of the crypt without changes in the relative size of the proliferative cell compartment in the crypt (101,104,160). In these studies it was observed that some shortening of the generative cell cycle time occurs in the proliferating crypt cells (102,104,160). Some shortening of the generative cell cycle time in all proliferating crypt cells could lead to an increase in the number of cells per crypt. However, it is also possible that the duration of the generative cell cycle of the cells in the bottom of the crypt, which is longer than that of the remaining crypt cells (1,33,248), is specifically shortened under these circumstances. These cells in the bottom of the crypt could constitute a functional stem cell compartment (133), yielding the different cell types of the intestinal epithelium (42), and may be the origin of the cells repopulating the crypt and forming new crypts after severe damage of the crypt cell population (35,98,243,244). The existence of such a distinct crypt cell compartment with a different generative cell cycle time could also explain the variation found in the duration of the generative cell cycle as determined by the fraction of labelled mitoses method (147, 220).

However, variations in the duration of the generative cell cycle as well as changes in crypt cell production may also be explained by assuming a G_0-state in the generative cell cycle (28). Such a G_0-state (28,178) would constitute a separate "phase" in the G_1-phase of the generative cell cycle, into which all cells enter following mitosis. This

G_0-state may be identical with the proposed "A-state" (217), "B compartment" (197), "dichophase" (24), and "Restriction point" (175) within the G_1-phase. Cells would leave this phase to re-enter the G_1-phase with an equal probability at any time interval after entering the G_0-state (28,197,217). Consequently, the duration of the generative cell cycle and crypt cell production may be regulated by the control of the transition probability from G_0 to G_1, and it has been found that factors influencing cell proliferation may do so by varying the transition probability (197,217). Thus a distinct functional stem cell compartment may not exist in the intestinal crypt, and stem cells, proliferating cells and maturing non-proliferating cells may constitute a continuum of crypt cells in various stages of maturation (133). This continuum may be maintained by local factors influencing the transition probability from G_0 to G_1-phase. This G_0-G_1 transition may also constitute the point in the generative cell cycle where proliferating cells in the "critical decision zone" halfway the crypt (34,193) normally decide to stop cell proliferation, which would be initiated by local factors reducing the transition probability to zero.

One may speculate at this point that crypt cell proliferation is regulated by the interaction of a number of inhibiting and stimulating factors from different origins, which results in a locally determined transition probability from G_0-state to G_1-phase in various parts of the crypt. Such a model for the regulation of crypt cell proliferation resembles the positional information theory for cell differentiation as proposed by Wolpert (245). The number of systemic factors influencing crypt cell proliferation may be very large, but the apparently local character of the control of crypt cell proliferation suggests a specific role for local factors, such as chalones, local hormones and

mesenchymal factors. Also the rate of the intestinal mucosal blood flow, which shows large specific variations (69, 153,154) and which seems to be extremely well regulated (118,153), may play an important role. This is suggested by the finding of a systemic enlargement of the intestinal epithelial cell population after partial resection of the intestine (103), which may be caused by an increased blood flow through the remaining intestine (101,103,227).

In the study on cell proliferation much information has been obtained from experiments in vitro. Besides intracellular factors controlling DNA synthesis and mitosis (12,13, 93,194), a number of extracellular factors has been described to affect cell proliferation. Various factors have been extracted from serum stimulating cell proliferation in vitro (76,109,111,113,195), and these may resemble the "growth factors" which have been described for a number of cell populations (43,49,90,91,108,109,124). Experimental work in vivo has also shown that a number of stimulating factors may be involved in the regulation of blood cell production, of which erythropoietin is well documented by now (130). The cell surface, which has been described as a fluid structure in which movements of specific proteins may occur (215), may also play an important role in the regulation of cell proliferation by the interaction of the cell with its environment (65,157,176,177). Also proteases, calcium ions, cyclic nucleotides, and microtubuli have been mentioned to take part in the interaction of the cell with its environment in the regulation of cell proliferation (15,65,76,177,223,239).

Almost every report on the influence of various factors on cell proliferation in vitro is accompanied by a theory for the regulation of cell proliferation and the origin of uncontrolled cell proliferation. However, the majority of the reports on in vitro cell proliferation deal with un-

physiological stimuli of cell proliferation under unphysiological conditions in cell populations which are normally not involved in continuous cell proliferation in vivo. Therefore a better understanding is primarily needed of the control mechanisms of cell proliferation in vivo in cell populations which undergo a continuous cell renewal. The investigations described in this thesis on the control of cell proliferation in the small intestinal epithelium constitute an attempt to provide more insight into such control mechanisms.

REFERENCES

1. Al-Dewachi, H.S., Wright, N.A., Appleton, D.R. & Watson, A.J. The cell cycle time in the rat jejunal mucosa. Cell Tissue Kinet. 7: 587-594, 1974.

2. Al-Dewachi, H.S., Wright, N.A., Appleton, D.R. & Watson, A.J. Cell population kinetics in the mouse jejunal crypt. Virchows Arch. B Cell Path. 18: 225-242, 1975.

3. Altmann, G.G. Influence of bile and pancreatic secretions on the size of the intestinal villi in the rat. Am. J. Anat. 132: 167-178, 1971.

4. Altmann, G.G. Demonstration of a morphological control mechanism in the small intestine. Role of pancreatic secretions and bile. In: Intestinal adaptation (eds. R.H. Dowling & E.O. Riecken). Schattauer Verlag, New York. pp. 75-86, 1974.

5. Altmann, G.G. & Enesco, M. Cell number as a measure of distribution and renewal of epithelial cells in the small intestine of growing and adult rats. Am. J. Anat. 121: 319-336, 1967.

6. Altmann, G.G. & Leblond, C.P. Factors influencing villus size in the small intestine of adult rats as revealed by transposition of intestinal segments. Am. J. Anat. 127: 15-36, 1970.

7. Amano, M., Messier, B. & Leblond, C.P. Specificity of labelled thymidine as a deoxyribonucleic acid precursor in radioautography. J. Histochem. Cytochem. 7: 153-155, 1959.

8. Andersson, S. Secretion of gastrointestinal hormones. Ann. Rev. Physiol. 35: 431-452, 1973.

9. Andrew, A. A study of the developmental relationship between enterochromaffin cells and the neural crest. J. Embryol. Exp. Morph. 11: 307-324, 1963.

10. Arvanitakis, C. & Olsen, W.A. Intestinal mucosal disaccharidases in chronic pancreatitis. Dig. Dis. 19: 417-421, 1974.

11. Baraona, E., Pirola, R.C. & Lieber, C.S. Small intestinal damage and changes in cell population produced by ethanol ingestion in the rat. Gastroenterology 66: 226-234, 1974.

12. Baserga, R. Biochemistry of the cell cycle: a review. Cell Tissue Kinet. 1: 167-191, 1968.

13. Baserga, R. Non-histone chromosomal proteins in normal and abnormal growth. Life Sci. 15: 1057-1071, 1975.

14. Becciolini, A., Ravina, A., Arganini, L., Castagnoli, P. & DeGuili, G. Effect of ionizing radiation on the enzymes of the intestinal mucosa of rats at different time intervals after abdominal irradiation. Radiat. Res. 49: 213-225, 1972.

15. Berridge, M.J. Control of cell division: a unifying hypothesis. J. Cycl. Nucl. Res. 1: 305-320, 1975.

16. Bertalanffy, F.D. Mitotic rates and renewal times of the digestive tract epithelia in the rat. Acta Anat. 40: 130-148, 1960.

17. Bertalanffy, F.D. & Lau, C. Cell renewal. Int. Rev. Cytol. 13: 357-366, 1962.

18. Bizzozero, G. Über die Regeneration der Elemente der schlauchförmigen Drüsen und des Epithels des Magendarmkanals. Anat. Anz. 3: 781-784, 1888.

19. Blenkinsopp, W.K. Effect of tritiated thymidine on cell proliferation. J. Cell Sci. 2: 305-308, 1967.

20. Both, N.J. de, Dongen, J.M. van, Hofwegen, B. van, Keulemans, J., Visser, W.J. & Galjaard, H. The influence of various cell kinetic conditions on functional differentiation in the small intestine of the rat. A study of enzymes bound to subcellular organelles. Developm. Biol. 38: 119-137, 1974.

21. Both, N.J. de & Plaisier, H. The influence of changing cell kinetics on functional differentiation in the small intestine of the rat. A study of enzymes involved in carbohydrate metabolism. J. Histochem. Cytochem. 22: 352-360, 1974.

22. Brugal, G. & Pelmont, J. Existence of two chalone-like substances in intestinal extract from adult Newt, inhibiting embryonic intestinal cell proliferation. Cell Tissue Kinet. 8: 171-187, 1975.

23. Bullough, W.S. The control of mitotic activity in adult mammalian tissues. Biol. Rev. 37: 307-342, 1962.

24. Bullough, W.S. Mitotic control in adult mammalian tissues. Biol. Rev. 50: 99-127, 1975.

25. Bullough, W.S. & Laurence, E.B. The control of epidermal mitotic activity in the mouse. Proc. Roy. Soc. B 151: 517-536, 1960.

26. Burholt, D.R., Schultze, B. & Maurer, W. Autoradiographic confirmation of the mitotic division of every mouse jejunal crypt cell labelled with ^{3}H-thymidine. Cell Tissue Kinet. 6: 229-237, 1973.

27. Burholt, D.R., Schultze, B. & Maurer, W. Mode of growth of the jejunal crypt cells of the rat: an autoradiographic study using double labelling with ^{3}H- and ^{14}C-thymidine in lower and upper parts of the crypts. Cell Tissue Kinet. 9: 107-117, 1976.

28. Burns, F.J. & Tannock, I.F. On the existence of a G_0-phase in the cell cycle. Cell Tissue Kinet. 3: 321-334, 1970.

29. Cairnie, A.B. Cell proliferation studies in the intestinal epithelium of the rat: response to continuous irradiation. Radiat. Res. 32: 240-264, 1967.

30. Cairnie, A.B. Further studies on the response of the small intestine to continuous irradiation. Radiat. Res. 38: 82-94, 1969.

31. Cairnie, A.B. Renewal of goblet and Paneth cells in the small intestine. Cell Tissue Kinet. 3: 35-45, 1970.

32. Cairnie, A.B. & Bentley, R.E. Cell proliferation studies in the intestinal epithelium of the rat. Hyperplasia during lactation. Exptl. Cell Res. 46: 428-440, 1967.

33. Cairnie, A.B., Lamerton, L.F. & Steel, G.G. Cell proliferation studies in the intestinal epithelium of the rat. I. Determination of the kinetic parameters. Exptl. Cell Res. 39: 528-538, 1965.

34. Cairnie, A.B., Lamerton, L.F. & Steel, G.G. Cell proliferation studies in the intestinal epithelium of the rat. II. Theoretical aspects. Exptl. Cell Res. 39: 539-553, 1965.

35. Cairnie, A.B. & Millen, B.H. Fission of crypts in the small intestine of the irradiated mouse. Cell Tissue Kinet. 8: 189-196, 1975.

36. Cameron, G.R. & Khanna, S.D. Regeneration of the intestinal villi after extensive mucosal infarction. J. Path. Bact. 77: 505-511, 1959.

37. Caspary, W.F., Winckler, K., Lankisch, P.G. & Creutzfeldt, W. Influence of exocrine and endocrine pancreatic function on intestinal brush border enzymatic activities. Gut 16:. 89-92, 1975.

38. Cheng, H. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. II. Mucous cells. Am. J. Anat. 141: 481-502, 1974.

39. Cheng, H. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. IV. Paneth cells. Am. J. Anat. 141: 521-536, 1974.

40. Cheng, H. & Leblond, C.P. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. I. Columnar cells. Am. J. Anat. 141: 461-480, 1974.

41. Cheng, H. & Leblond, C.P. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. III. Enteroendocrine cells. Am. J. Anat. 141: 503-520, 1974.

42. Cheng, H. & Leblond, C.P. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. V. Unitarian theory of the origin of the four epithelial cell types. Am. J. Anat. 141: 537-562, 1974.

43. Cifone, M., Mocarelli, P. & Defendi, V. In vivo production of a macrophage growth factor. Exptl. Cell Res. 96: 96-102, 1975.

44. Clarke, R.M. A new method of measuring the rate of shedding of epithelial cells from the intestinal villus of the rat. Gut 11: 1015-1019, 1970.

45. Clarke, R.M. A comparison of metaphase arresting agents and tritiated thymidine autoradiography in measurement of the rate of entry of cells into mitosis in the crypts of Lieberkühn of the rat. Cell Tissue Kinet. 4: 263-272, 1971.

46. Clarke, R.M. The effect of growth and of fasting on the number of villi and crypts in the small intestine of the albino rat. J. Anat. 112: 27-33, 1972.

47. Clarke, R.M. Control of intestinal epithelial replacement: lack of evidence for a tissue-specific blood-borne factor. Cell Tissue Kinet. 7: 241-250, 1974.

48. Cleaver, J.E. Thymidine metabolism and cell kinetics. North-Holland Publishing Company, Amsterdam, 1967.

49. Cohen, S. Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. J. Biol. Chem. 237: 1555-1562, 1962.

50. Cook, R.M., Powell, P.M. & Moog, F. Influence of biliary stasis on the activity and distribution of maltase, sucrase, alkaline phosphatase, and leucylnaphthylamidase in the small intestine of the mouse. Gastroenterology 64: 411-420, 1973.

51. Creamer, B. Small-intestinal mucosal dynamics and the environment. Brit. Med. J. 2: 1373-1375, 1964.

52. Creamer, B. The turnover of the epithelium of the small intestine. Br. Med. Bull. 23: 226-230, 1967.

53. Creamer, B., Shorter, R.G. & Bamforth, J. The turnover and shedding of epithelial cells. II. The shedding in the small intestine. Gut 2: 117-118, 1961.

54. Cronkite, E.P., Fliedner, T.M., Bond, V.P., Rubini, J.R., Brecher, G. & Quastler, H. Dynamics of hemopoietic proliferation in man and mice studied by H^3-thymidine incorporation into DNA. Ann. N.Y. Acad. Sci. 77: 803-820, 1959.

55. Devik, F. Intestinal cell kinetics in irradiated mice. A quantitative investigation of the acute reaction to whole body roentgen irradiation. Acta Radiol. Ther. Phys. Biol. 10: 129-149, 1971.

56. Devik, F. & Hagen, S. Effects of X-rays and cytotoxic agents on the cell population of the crypts of the small intestine in mice. Virchows Arch. B Zellpath. 12: 223-237, 1973.

57. Dongen, J.M. van. Ultrastructural development and cellular kinetics in intestinal epithelium. Thesis, Erasmus University Rotterdam, 1976.

58. Dongen, J.M. van. Unpublished results, 1976.

59. Dongen, J.M. van, Kooyman, J. & Visser, W.J. The influence of 400R X-irradiation on the number and the localization of mature and immature goblet cells and Paneth cells in intestinal crypt and villus. Cell Tissue Kinet. 9: 65-75, 1976.

60. Dongen, J.M. van, Kooyman, J., Visser, W.J., Holt, S.J. & Galjaard, H. The effect of increased crypt cell proliferation on the activity and subcellular localization of esterases and alkaline phosphatase in rat small intestine. Histochem. J. (in press), 1976.

61. Dongen, J.M. van, Visser, W.J., Daems, W.Th. & Galjaard, H. The relation between cell proliferation, differentiation and the ultrastructural development in rat intestinal epithelium. Cell Tissue Res. (in press), 1976.

62. Dowling, R.H. & Booth, C.C. Structural and functional changes following small intestinal resection in the rat. Clin. Sci. 32: 139-149, 1967.

63. Dowling, R.H. & Gleeson, M.H. Cell turnover following small bowel resection and by-pass. Digestion 8: 176-190, 1973.

64. Dowling, R.H. & Riecken, E.O. (eds.) Intestinal adaptation. Schattauer Verlag, New York, 1974.

65. Edelman, G.M. Surface modulation in cell recognition and cell growth. Science 192: 218-226, 1976.

66. Erlandsen, S.L. & Chase, D.G. Paneth cell function: phagocytosis and intracellular digestion of intestinal microorganisms. I. Hexamita muris. J. Ultrastruct. Res. 41: 296-318, 1972.

67. Erlandsen, S.L. & Chase, D.G. Paneth cell function: phagocytosis and intracellular digestion of intestinal microorganisms. II. Spiral microorganisms. J. Ultrastruct. Res. 41: 319-333, 1972.

68. Erlandsen, S.L., Parsons, J.A. & Taylor, Th.D. Ultrastructural immunocytochemical localization of lysozyme in the Paneth cells of man. J. Histochem. Cytochem. 22: 401-413, 1974.

69. Fara, J.W. & Madden, K.S. Effect of secretin and cholecystokinin on small intestinal blood flow distribution. Amer. J. Physiol. 229: 1365-1370, 1975.

70. Feinendegen, L.E. Tritium-labeled molecules in biology and medicine. Academic Press, New York, 1967.

71. Feldman, E.J., Dowling, R.H., McNaughton, J. & Peters, T.J. Effects of oral versus intravenous nutrition on intestinal adaptation after small bowel resection in the dog. Gastroenterology 70: 712-719, 1976.

72. Ferreira, M.N. Argentaffin and other "endocrine" cells of the small intestine in the adult mouse. I. Ultrastructure and classification. Am. J. Anat. 131: 315-330, 1971.

73. Ferreira, M.N. & Leblond, C.P. Argentaffin and other "endocrine" cells of the small intestine in the adult mouse. II. Renewal. Am. J. Anat. 131: 331-351, 1971.

74. Forrester, J.M. The number of villi in rat's jejunum and ileum: effect of normal growth, partial enterectomy, and tube feeding. J. Anat. 111: 283-291, 1972.

75. Fortin-Magana, R., Hurwitz, R., Herbst, J.J. & Kretchmer, N. Intestinal enzymes: indicators of proliferation and differentiation in the jejunum. Science 167: 1627-1628, 1970.

76. Frank, W., Ristow, H.J., Hoffman, R., Grimm, J. & Veser, J. Growth regulation in cultures of embryonic rat fibroblasts by the serum factors S1 and S2. Adv. Metab. Dis. 8: 337-356, 1975.

77. Friedman, B., Strachan, D.S. & Dewey, M.M. Histochemical and biochemical analysis of the nonspecific esterases of the small intestine of the rat. J. Histochem. Cytochem. 14: 560-566, 1966.

78. Friedman, N.B. Cellular dynamics in the intestinal mucosa: the effect of irradiation on epithelial maturation and migration. J. Exp. Med. 81: 553-561, 1945.

79. Fry, R.J.M., Lesher, S. & Kohn, H.I. Influence of age on the transit time of cells of the mouse intestinal epithelium. Lab. Invest. 11: 289-293, 1962.

80. Fry, R.J.M., Staffeldt, E. Effect of a diet containing sodium deoxycholate on the intestinal mucosa of the mouse. Nature 203: 1396-1398, 1964.

81. Fuji, R. Quantitation of the number of villi and crypts in the intestine of rodent animals. Experientia 28: 1209-1210, 1972.

82. Galjaard, H. Some unresolved questions relevant to intestinal adaptation. In: Intestinal adaptation (eds. R.H. Dowling & E.O. Riecken). Schattauer Verlag, New York. pp. 263-267, 1974.

83. Galjaard, H. & Bootsma, D. The regulation of cell proliferation and differentiation in intestinal epithelium. II. A quantitative histochemical and autoradiographic study after low doses of X-irradiation. Exptl. Cell Res. 58: 79-92, 1969.

84. Galjaard, H., Buys, J., Duuren, M. van & Giesen, J. A quantitative histochemical study of intestinal mucosa after X-irradiation. J. Histochem. Cytochem. 18: 291-301, 1970.

85. Galjaard, H., Meer-Fieggen, W. van der & Both, N.J. de. Cell differentiation in gut epithelium. In: Cell differentiation (eds. D. Viza & H. Harris). Munksgaard, Copenhagen. pp. 322-328, 1972.

86. Galjaard, H., Meer-Fieggen, W. van der & Giesen, J. Feedback control by functional villus cells on cell proliferation and maturation in intestinal epithelium. Exptl. Cell Res. 73: 197-207, 1972.

87. Gleeson, M.H., Bloom, S.R., Polak, J.M., Henry, K. & Dowling, R.H. Endocrine tumor in kidney affecting small bowel structure, motility, and absorptive function. Gut 12: 773-782, 1971.

88. Gleeson, M.H., Cullen, J. & Dowling, R.H. Intestinal structure and function after small bowel by-pass in the rat. Clin. Sci. 43: 731-742, 1972.

89. Gleeson, M.H., Dowling, R.H. & Peters, T.J. Biochemical changes in intestinal mucosa after experimental small bowel by-pass in the rat. Clin. Sci. 43: 743-757, 1972.

90. Gospodarowicz, D. Localisation of a fibroblast growth factor and its effect alone and with hydrocortisone on 3T3 cell growth. Nature 249: 123-127, 1974.

91. Gospodarowicz, D., Greene, G. & Moran, J. Fibroblast growth factor can substitute for platelet factor to sustain the growth of BALB/3T3 cells in the presence of plasma. Biochem. Biophys. Res. Commun. 65: 779-787, 1975.

92. Grand, R.J., Watkins, J.B. & Torti, F.M. Development of the human gastrointestinal tract. Gastroenterology 70: 790-810, 1976.

93. Graves, J.A.M. DNA synthesis in heterokaryons formed by fusion of mammalian cells from different species. Exptl. Cell Res. 72: 393-403, 1972.

94. Greulich, R.C., Cameron, I.L. & Thrasher, J.D. Stimulation of mitosis in adult mice by administration of thymidine. Proc. N.A.S. 47: 743-748, 1961.

95. Hagemann, R.F., Cooper, J. & Poulakos, L. A non-destructive method for measuring intestinal cell transit time. Cell Tissue Kinet. 8: 391-396, 1975.

96. Hagemann, R.F., Sigdestad, C.P. & Lesher, S. A quantitative description of the intestinal epithelium of the mouse. Am. J. Anat. 129: 41-52, 1970.

97. Hagemann, R.F., Sigdestad, C.P. & Lesher, S. A method for quantification of proliferative intestinal mucosal cells on a weight base: some values for C57B1/6. Cell Tissue Kinet. 3: 21-26, 1970.

98. Hagemann, R.F., Sigdestad, C.P. & Lesher, S. Intestinal crypt survival and total and per crypt levels of proliferative cellularity following irradiation: single X-ray exposures. Radiat. Res. 46: 533-546, 1971.

99. Hampton, J.C. Further evidence for the presence of a Paneth cell progenitor in mouse intestine. Cell Tissue Kinet. 1: 309-317, 1968.

100. Hanna, I.R.A. Response of early erythroid precursors to bleeding. Nature 214: 355-357, 1967.

101. Hanson, W.R. Intestinal compensation following intestinal resection in the rat. Ph.D. thesis. University of Iowa, 1975.

102. Hanson, W.R. & Osborne, J.W. Epithelial cell kinetics in the small intestine of the rat 60 days after resection of 70 per cent of the ileum and jejunum. Gastroenterology 60: 1087-1097, 1971.

103. Hanson, W.R., Rijke, R.P.C., Plaisier, H.M., Ewijk, W. van & Osborne, J.W. The effect of intestinal resection on Thiry-Vella fistulae of jejunal and ileal origin: evidence for a systemic control mechanism of cell renewal. Cell Tissue Kinet. (submitted for publication).

104. Harding, J.D. & Cairnie, A.B. Changes in intestinal cell kinetics in the small intestine of lactating mice. Cell Tissue Kinet. 8: 135-144, 1975.

105. Harrison, D.D. & Webster, H.L. Proximal to distal variations in enzymes of the rat intestine. Biochim. Biophys. Acta 244: 432-436, 1971.

106. Hermos, J.A., Mathan, M. & Trier, J.S. DNA synthesis and proliferation by villous epithelial cells in fetal rats. J. Cell Biol. 50: 255-258, 1971.

107. Hietanen, E. & Hänninen, O. Effect of chyme on mucosal enzyme levels in small intestine of the rat. Metabolism 21: 991-1000, 1972.

108. Hogue-Angeletti, R., Hermodson, M.A. & Bradshaw, R.A. Amino acid sequences of mouse 2.5S nerve growth factor. Biochemistry 12: 100-115, 1973.

109. Holley, R.W. & Kiernan, J.A. Control of the initiation of DNA synthesis in 3T3 cells: serum factors. Proc. N.A.S. 71: 2908-2911, 1974.

110. Hopper, A.F., Wannemacher, R.W. & McGovern, P.A. Cell population changes in the intestinal epithelium of the rat following starvation and protein-depletion. Proc. Soc. Exp. Biol. Med. 128: 695-698, 1968.

111. Houck, J.C. & Cheng, R.F. Isolation, purification, and chemical characterization of the serum mitogen for diploid human fibroblasts. J. Cell. Physiol. 81: 257-270, 1973.

112. Houck, J.C. & Hennings, H. Chalones. Specific endogenous mitotic inhibitors. FEBS Letters 32: 1-8, 1973.

113. Houck, J.C., Sharma, V.K. & Cheng, R.F. Fibroblast chalone and serum mitogen (anti-chalone). Nature New Biol. 246: 111-113, 1973.

114. Howard, A. & Pelc, S.R. Nuclear incorporation of P^{32} as demonstrated by autoradiographs. Exptl. Cell Res. 1: 178-187, 1951.

115. Howard, A. & Pelc, S.R. Synthesis of desoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. Heredity suppl. 6: 261-273, 1953.

116. Hughes, W.L., Bond, V.P., Brecher, G., Cronkite, E.P., Painter, R.B., Quastler, H. & Sherman, F.G. Cellular proliferation in the mouse as revealed by autoradiography with tritiated thymidine. Proc. N.A.S. 44: 476-483, 1958.

117. Hülsmann, W.C., Iemhoff, W.G.J., Berg, J.W.O. van den & Pijper, A.M. de. Unequal rates of development of mitochondrial enzymes in rat small intestinal epithelium. Biochim. Biophys. Acta 215: 553-555, 1970.

118. Hulten, L., Jodal, M., Lindhagen, J. & Lundgren, O. Blood flow in the small intestine of cat and man as analyzed by an inert gas washout technique. Gastroenterology 70: 45-51, 1976.

119. Iemhoff, W.G.J., Berg, J.W.O. van den, Pijper, A.M. de & Hülsmann, W.C. Metabolic aspects of isolated cells from rat small intestinal epithelium. Biochim. Biophys. Acta 215: 229-241, 1970.

120. Iversen, O.H., Bhangoo, K.S. & Hansen, K. Control of epidermal cell renewal in the bat web. Virchows Arch. B Cell Path. 16: 157-179, 1974.

121. Jervis, H.L. Enzymes in the mucosa of the small intestine of the rat, the guinea pig, and the rabbit. J. Histochem. Cytochem. 11: 692-699, 1963.

122. Johnson, L.R., Copeland, E.M., Dudrick, S.J., Lichtenberger, L.M. & Castro, G.A. Structural and hormonal alterations in the gastrointestinal tract of parenterally fed rats. Gastroenterology 68: 1177-1183, 1975.

123. Johnson, L.R., Copeland, E.M., Dudrick, S.J., Lichtenberger, L.M. & Castro, G.A. Action of gastrin on gastrointestinal structure and function. Gastroenterology 68: 1184-1192, 1975.

124. Kamely, D. & Rudland, P.S. Induction of DNA synthesis and cell division in human diploid skin fibroblasts by fibroblast growth factor. Exptl. Cell Res. 97: 120-126, 1976.

125. Kammeraad, A. The development of the gastro-intestinal tract of the rat. I. Histogenesis of the epithelium of the stomach, small intestine and pancreas. J. Morphol. 70: 323-351, 1942.

126. Katz, A.J. & Falchuk, Z.M. Current concepts in gluten sensitive enteropathy (celiac sprue). Ped. Clin. N. Amer. 22: 767-785, 1975.

127. Kefalides, N.A. Basement membranes: structural and biosynthetic considerations. J. Invest. Derm. 65: 85-92, 1975.

128. Kember, N.F., Quastler, H. & Wimber, D.R. Adaptation of the rat intestine to continuous irradiation. Br. J. Radiol. 35: 290, 1962.

129. Kovacs, L. & Potten, C.S. An estimation of proliferative population size in stomach, jejunum and colon of DBA-2 mice. Cell Tissue Kinet. 6: 125-134, 1973.

130. Krantz, S.B. & Jacobson, L.O. Erythropoietin and the regulation of erythropoiesis. The University of Chicago Press, Chicago, 1970.

131. Lajtha, L.G. & Oliver, R. Cell population kinetics following different regimes of irradiation. Br. J. Radiol. 35: 131-140, 1962.

132. Lamerton, L.F. Cell proliferation under continuous irradiation. Radiat. Res. 27: 119-138, 1966.

133. Lamerton, L.F. Concluding address at the symposium on stem cells. In: Stem cells of renewing cell populations (eds. A.B. Cairnie, P.K. Lala & D.G. Osmond). Academic Press, New York, pp. 375-382, 1976.

134. Lamerton, L.F., Steel, G.G. & Wimber, D.R. Sensitivity of mammalian tissues to continuous exposure. Brookhaven Symp. Biol. 14: 158-175, 1961.

135. Leblond, C.P. Classification of cell populations on the basis of their proliferative behavior. Nat. Cancer Inst. Monogr. 14: 119-150, 1964.

136. Leblond, C.P. & Carriere, R. The effect of growth hormone and thyroxine on the mitotic rate of the intestinal mucosa of the rat. Endocrinol. 56: 261-266, 1955.

137. Leblond, C.P. & Messier, B. Renewal of chief cells and goblet cells in the small intestine as shown by radioautography after injection of thymidine-H^3 into mice. Anat. Rec. 132: 247-259, 1958.

138. Leblond, C.P., Messier, B. & Kopriwa, B. Thymidine-H^3 as a tool for the investigation of the renewal of cell populations. Lab. Invest. 8: 296-306, 1959.

139. Leblond, C.P. & Stevens, C.E. The constant renewal of the intestinal epithelium in the albino rat. Anat. Rec. 100: 357-371, 1948.

140. Leblond, C.P., Stevens, C.E. & Bogoroch, R. Histological localization of newly-formed desoxyribonucleic acid. Science 108: 531-533, 1948.

141. Leblond, C.P. & Walker, B.E. Renewal of cell populations. Physiol. Rev. 36: 257-276, 1956.

142. LeDouarin, N.M. & Teillet, M.A. The migration of neural crest cells to the wall of the digestive tract in avian embryo. J. Embryol. Exp. Morph. 30: 31-48, 1973.

143. Lesher, S. Compensatory reactions in intestinal crypt cells after 300 Roentgens of Cobalt-60 gamma irradiation. Radiat. Res. 32: 510-519, 1967.

144. Lesher, S. & Bauman, J. Cell proliferation in the intestinal epithelium. In: Normal and malignant cell growth (eds. R.J.M. Fry, M.L. Griem & W.H. Kirsten). Springer-Verlag, Berlin. pp. 49-56, 1969.

145. Lesher, S., Fry, R.J.M. & Kohn, H.I. Age and the generation time of the mouse duodenal-epithelial cell. Exptl. Cell Res. 24: 334-343, 1961.

146. Lesher, S., Sallese, A. & Jones, M. Effects of 1000R whole-body X-irradiation on DNA synthesis and mitosis in the duodenal crypts of the BCF_1 mouse. Z. Zellforsch. 77: 144-146, 1967.

147. Lipkin, M. & Deschner, E. Comparative analysis of cell renewal in the gastrointestinal tract of newborn hamster. Exptl. Cell Res. 49: 1-12, 1968.

148. Loehry, C.A. & Creamer, B. Three-dimensional structure of rat small intestinal mucosa related to mucosal dynamics. Gut 10: 112-120, 1969.

149. Loehry, C.A., Croft, D.N., Singh, A.K. & Creamer, B. Cell turnover in the rat small intestinal mucosa: an appraisal of cell loss. Gut 10: 13-18, 1969.

150. Loehry, C.A. & Grace, R. The dynamic structure of a flat small intestinal mucosa studied on the explanted rat jejunum. Gut 15: 289-293, 1974.

151. Loewenstein, W.R. Membrane junctions in growth and differentiation. Fed. Proc. 32: 60-64, 1973.

152. Lowry, O.H. The quantitative histochemistry of the brain. Histological sampling. J. Histochem. Cytochem. 1: 420-428, 1953.

153. Lundgren, O. The circulation of the small bowel mucosa. Gut 15: 1005-1013, 1974.

154. Madden, J.J., Ludewig, R.M. & Wangensteen, S.L. Effects of glucagon on the splanchnic and the systemic circulation. Amer. J. Surg. 122: 85-90, 1971.

155. Marsh, M.N. & Trier, J.S. Morphology and cell proliferation of subepithelial fibroblasts in adult mouse jejunum. I. Structural features. Gastroenterology 67: 622-635, 1974.

156. Marsh, M.N. & Trier, J.S. Morphology and cell proliferation of subepithelial fibroblasts in adult mouse jejunum. II. Radioautographic studies. Gastroenterology 67: 636-645, 1974.

157. Marx, J.L. Cell surfaces and the regulation of mitosis. Science 192: 455-457, 1976.

158. Mathan, M., Hermos, J.A. & Trier, J.S. Structural features of the epithelio-mesenchymal interface of rat duodenal mucosa during development. J. Cell Biol. 52: 577-588, 1972.

159. Mathan, M., Moxey, P.C. & Trier, J.S. Morphogenesis of fetal rat duodenal villi. Am. J. Anat. 146: 73-92, 1976.

160. McDermott, F.T. & Roudnew, B. Ileal crypt cell population kinetics after 40% small bowel resection. Gastroenterology 70: 707-711, 1976.

161. Meer-Fieggen, W. van der. Regulation of cell proliferation and differentiation in intestinal epithelium. Thesis, Erasmus University Rotterdam, 1973.

162. Menge, H., Bloch, R., Schaumlöffel, E. & Riecken, E.O. Transportstudien, morphologische, morphometrische und histochemische Untersuchungen zum Verhalten der Dünndarmschleimhaut im operativ ausgeschalteten Jejunalschlinge der Ratte. Z. Ges. Exp. Med. 153: 74-90, 1970.

163. Menge, H., Gräfe, M., Lorenz-Meyer, H. & Riecken, E.O. The influence of food intake on the development of structural and functional adaptation following ileal resection in the rat. Gut 16: 468-472, 1975.

164. Merzel, J. & Leblond, C.P. Origin and renewal of goblet cells in the epithelium of the mouse small intestine. Am. J. Anat. 124: 281-305, 1969.

165. Messier, B. & Leblond, C.P. Cell proliferation and migration as revealed by radioautography after injection of thymidine-H^3 into male rats and mice. Am. J. Anat. 106: 247-285, 1960.

166. Mizuno, T. Une hypothèse sur l'organogenèse du tractus digestif. C.R. Soc. Biol. 169: 1096-1098, 1975.

167. Moe, H. The goblet cells, Paneth cells, and basal granular cells of the epithelium of the intestine. Int. Rev. Gen. Exp. Zool. 3: 241-287, 1968.

168. Moog, F. & Grey, R.D. Spatial and temporal differentiation of alkaline phosphatase on the intestinal villi of the mouse. J. Cell Biol. 32: C1-C5, 1967.

169. Musso, F., Lachat, J.J., Cruz, A.R. & Goncalves, R.P. Effect of denervation on the mitotix index of the intestinal epithelium of the rat. Cell Tissue Res. 163: 395-402, 1975.

170. Nordström, C., Dahlqvist, A. & Josefsson, L. Quantitative determination of enzymes in different parts of the villi and crypts of rat small intestine. J. Histochem. Cytochem. 15: 713-721, 1968.

171. Olsen, W.A. & Korsmo, H. Enhancement of intestinal sucrase activity in experimental diabetes: the role of intraluminal factors. J. Lab. Clin. Med. 85: 832-837, 1975.

172. Otto, H.F. Zur funktionellen Bedeutung der intestinalen Paneth-Zellen. Dtsch. Med. Wschr. 98: 220-226, 1973.

173. Padykula, H.A. Recent functional interpretations of intestinal morphology. Fed. Proc. 21: 873-879, 1962.

174. Pansu, D., Berard, A., Dechelette, M.A. & Lambert, R. Influence of secretin and pentagastrin on the circadian rhythm of cell proliferation in the intestinal mucosa in rats. Digestion 11: 266-274, 1974.

175. Pardee, A.B. A restriction point for control of normal animal cell proliferation. Proc. N.A.S. 71: 1286-1290, 1974.

176. Pardee, A.B. The cell surface and fibroblast proliferation. Biochim. Biophys. Acta 417: 153-172, 1975.

177. Pasternak, C.A. The cell surface in relation to the growth cycle. J. Theor. Biol. 58: 365-382, 1976.

178. Patt, H.M. & Quastler, H. Radiation effects on cell renewal and related systems. Physiol. Rev. 43: 357-396, 1963.

179. Pearse, A.G.E. Peptides in brain and intestine. Nature 262: 92-94, 1976.

180. Pearse, A.G.E. & Polak, J.M. Neural crest origin of the endocrine polypeptide (APUD) cells of the gastrointestinal tract and pancreas. Gut 12: 783-788, 1971.

181. Peeters, T. & Vantrappen, G. The Paneth cell: a source of intestinal lysozym. Gut 16: 553-558, 1975.

182. Philipson, B. Morphology in the cat ileal mucosa following construction of an ileal reservoir or transposition of patches to different locations. Scand. J. Gastroent. 10: 369-377, 1975.

183. Pictet, R.L., Rall, L.B., Phelps, P. & Rutter, W.J. The neural crest and the origin of the insulin-producing and other gastrointestinal hormone-producing cells. Science 191: 191-192, 1976.

184. Pinkus, H. Examinations of the epidermis by the strip method of removing horny layers. I. Observations on thickness of the horny layer, and on mitotic activity after stripping. J. Invest. Derm. 16: 383-386, 1951.

185. Pitha, J. The fine structure of clear fibroblast-like cells in the lamina propria of the small intestine. J. Ultrastruct. Res. 22: 231-239, 1968.

186. Pitha, J. The fine structure of regenerating epithelium in the small intestine. Virchows Arch. B Zellpath. 7: 314-341, 1971.

187. Polak, J.M., Pearse, A.G.E., Garaud, J.-C. & Bloom, J. Cellular localization of a vasoactive intestinal peptide in the mammalian and avian gastrointestinal tract. Gut 15: 720-724, 1974.

188. Post, J. & Hoffman, J. Early and late effects of H^3TdR-labeled DNA on ileal cell replication in vivo. Radiat. Res. 34: 570-582, 1968.

189. Quastel, M.R. Continuous irradiation of the rat at high dose rates: a study of jejunal damage, recovery, and radioresistance using tritiated thymidine. Radiat. Res. 18: 46-57, 1963.

190. Quastler, H. The nature of intestinal radiation death. Radiat. Res. 4: 303-320, 1956.

191. Quastler, H. Effects of irradiation on intestinal mucosal cell population. Fed. Proc. 22: 1330-1333, 1963.

192. Quastler, H., Bensted, J.P.M., Lamerton, L.F. & Simpson, S.M. Adaptation to continuous irradiation: observations on the rat intestine. Br. J. Radiol. 32: 501-512, 1959.

193. Quastler, H. & Sherman, F.G. Cell population kinetics in the intestinal epithelium of the mouse. Exptl. Cell Res. 17: 420-438, 1959.

194. Rao, P.N. & Johnson, R.T. Regulation of cell cycle in hybrid cells. In: control of proliferation in animal cells (eds. B. Clarkson & R. Baserga). Cold Spring Harbor, New York. pp. 785-800, 1974.

195. Rechler, M.M., Podskalny, J.M. & Nissley, S.P. Interaction of multiplication-stimulating activity with chick embryo fibroblasts demonstrates a growth receptor. Nature 259: 134-136, 1976.

196. Riecken, E.O., Bloch, R., Menge, H., Idelberger, K., Kramer, F., Miller, B. & Lorenz-Meyer, H. Morphologische und funktionelle Befunde nach Milchsäuredauerinfusion in das Jejunum der Ratte. Z. Zellforsch. 132: 107-129, 1972.

197. Robinson, J.H., Smith, J.A., Totty, N.F. & Riddle, P.N. Transition probability and the hormonal and density-dependent regulation of cell proliferation. Nature 262: 298-300, 1976.

198. Robinson, J.W.L., Haroud, M., Winistöfer, B. & Mirkovitch, V. Recovery of function and structure of dog ileum and colon following two hours' acute ischaemia. Europ. J. Clin. Invest. 4: 443-452, 1974.

199. Robinson, J.W.L. & Mirkovitch, V. The recovery of function and microcirculation in small intestinal loops following ischaemia. Gut 13: 784-789, 1972.

200. Ronzio, R.A. & Rutter, W.J. Effect of a partially purified factor from chick embryos on macromolecular synthesis of embryonic pancreatic epithelia. Developm. Biol. 30: 307-320, 1973.

201. Röttger, P. & Oran, M. Histochemische und biochemische Untersuchungen zur Frage des Enzym-Gehaltes der Dünndarmschleimhaut nach temporärer ischämie. Verh. Dtsch. Ges. Path. 53: 189-195, 1969.

202. Rubini, J.R., Cronkite, E.P., Bond, V.P. & Fliedner, T.M. The metabolism and fate of tritiated thymidine in man. J. Clin. Invest. 39: 909-918, 1960.

203. Rytõmaa, T. Control of cell division in mammalian cells. In: The cell cycle in development and differentiation (eds. M. Balls & F.S. Billett). Cambridge University Press. pp. 457-472, 1973.

204. Saetren, H. A principle of auto-regulation of growth. Production of organ specific mitose-inhibitors in kidney and liver. Exptl. Cell Res. 11: 229-232, 1956.

205. Sato, F., Muramatsu, S., Tsuchihashi, S., Shiragai, A., Hiraoka, T., Inada, T., Kawashima, K., Matsuzawa, H., Nakamura, W., Trucco, E. & Sacher, G.A. Radiation effects on cell populations in the intestinal epithelium of mice and its theory. Cell Tissue Kinet. 5: 227-235, 1972.

206. Sawicki, W. & Dorn, A. Action of exogenous thymidine in vivo on progenitor cell cycle in intestinal epithelium. Acta Histochem. 46: 181-185, 1973.

207. Schultze, B., Haack, V., Schmeer, A.C. & Maurer, W. Autoradiographic investigation of the cell kinetics of crypt epithelia in the jejunum of the mouse. Confirmation of steady state growth with constant frequency distribution of cells throughout the cycle. Cell Tissue Kinet. 5: 131-145, 1972.

208. Schultze, B. & Oehlert, W. Autoradiographic investigation of incorporation of H^3-thymidine into cells of the rat and mouse. Science 131: 737-738, 1960.

209. Sherman, F.G. & Quastler, H. DNA synthesis in irradiated intestinal epithelium. Exptl. Cell Res. 19: 343-360, 1960.

210. Shnitka, Th.K. Enzymatic histochemistry of gastrointestinal mucous membrane. Fed. Proc. 19: 897-904, 1960.

211. Sigdestad, C.P., Bauman, J. & Lesher, S. Diurnal fluctuations in the number of cells in mitosis and DNA synthesis in the jejunum of the mouse. Exptl. Cell Res. 58: 159-162, 1969.

212. Sigdestad, C.P., Hagemann, R.F. & Lesher, S. A new method for measuring intestinal cell transit time. Gastroenterology 58: 47-48, 1970.

213. Sigdestad, C.P. & Lesher, S. Circadian rhythm in the cell cycle time of the mouse intestinal epithelium. J. Interdisc. Cycle Res. 3: 39-46, 1972.

214. Simms, H.S. & Stillman, N.P. Substances affecting adult tissue in vitro. II. A growth inhibitor in adult tissue. J. Gen. Physiol. 20: 621-629, 1937.

215. Singer, S.J. & Nicolson, G.L. The fluid mosaic model of the structure of cell membranes. Science 175: 720-731, 1972.

216. Skála, I., Hromadkova, V. & Skála, J. Hypertrophy of the small intestine after its partial resection in the rat. Size of the mucosal surface. Digestion 2: 23-34, 1969.

217. Smith, J.A. & Martin, L. Do cells cycle? Proc. N.A.S. 70: 1263-1267, 1973.

218. Sprinz, H. Morphological response of intestinal mucosa to enteric bacteria and its implication for sprue and asiatic cholera. Fed. Proc. 21: 57-64, 1962.

219. Sprinz, H. Factors influencing intestinal cell renewal. A statement of principles. Cancer 28: 71-74, 1971.

220. Steel, G.G. The measurement of the intermitotic period. In: The cell cycle in development and differentiation (eds. M. Balls & F.S. Billett). Cambridge University Press. pp. 13-29, 1973.

221. Steel, G.G. & Lamerton, L.F. The turnover of tritium from thymidine in tissues of the rat. Exptl. Cell Res. 37: 117-131, 1965.

222. Steevens Hooper, C.E. Cell turnover in epithelial populations. J. Histochem. Cytochem. 4: 531-540, 1956.

223. Talmadge, K.W., Noonan, K.D. & Burger, M.M. The transformed cell surface: an analysis of the increased lectin agglutinability and the concept of growth control by membrane proteases. In: Control of proliferation in animal cells (eds. B. Clarkson & R. Baserga). Cold Spring Harbor, New York. pp. 313-325, 1974.

224. Taylor, J.H., Woods, P.S. & Hughes, W.L. The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. Proc. N.A.S. 43: 122-127, 1957.

225. Thornley, A.L. & Laurence, E.B. The present state of biochemical research on chalones. Int. J. Biochem. 6: 313-320, 1975.

226. Toner, P.G. Cytology of intestinal epithelial cells. Int. Rev. Cytol. 24: 233-343, 1968.

227. Touloukian, R.J. & Spencer, R.P. Ileal blood flow preceding compensatory intestinal hypertrophy. Ann. Surg. 175: 320-325, 1972.

228. Trier, J.S. & Browning, Th.D. Epithelial-cell renewal in cultured duodenal biopsies in celiac sprue. New Engl. J. Med. 283: 1245-1250, 1970.

229. Troncale, F.J. & Lipkin, M. The cell cycle and the development of pathology in the gastrointestinal tract. Pathobiol. Ann. 2: 293-304, 1972.

230. Tutton, P.J.M. Control of epithelial cell proliferation in the small intestinal crypt. Cell Tissue Kinet. 6: 211-216, 1973.

231. Tutton, P.J.M. The influence of serotonin on crypt cell proliferation in the jejunum of rat. Virchows Arch. B Cell Path. 16: 79-87, 1974.

232. Tutton, P.J.M. Absence of a circadian rhythm in crypt cell mitotic rate following chemical sympathectomy in rats. Virchows Arch. B Cell Path. 19: 151-156, 1975.

233. Verbin, R.S., Liang, H., Saez, L., Diluiso, G., Goldblatt, P.J. & Farber, E. Effects of inhibitors of protein synthesis on structure and function of the crypts of the small intestine. Exptl. Cell Res. 65: 81-93, 1971.

234. Wachsmuth, E.D. & Torhorst, A. Biogenesis of brush border membranes: alkaline phosphatase and aminopeptidase and their possible precursor proteins after X-ray irradiation of small intestine of C3H mice. Beitr. Path. 151: 234-247, 1974.

235. Webster, H.L. & Harrison, D.D. Enzymic activities during the transformation of crypt to columnar intestinal cells. Exptl. Cell Res. 56: 245-253, 1969.

236. Weiser, M.M. Intestinal epithelial cell surface membrane glycoprotein synthesis. I. An indicator of cellular differentiation. J. Biol. Chem. 248: 2536-2541, 1973.

237. Weiss, P. & Kavanau, J.L. A model of growth and growth control in mathematical terms. J. Gen. Physiol. 41: 1-47, 1957.

238. Weser, E. & Hernandez, M.H. Studies of small bowel adaptation after intestinal resection in the rat. Gastroenterology 60: 69-75, 1971.

239. Whitfield, J.F., McManus, J.P., Rixon, R.H., Boynton, A.L., Youdale, T. & Swierenga, S. The positive control of cell proliferation by the interplay of calcium ions and cyclic nucleotides. A review. In Vitro 12: 1-18, 1976.

240. Williams, R.B., Toal, J.N., White, J. & Carpenter, H.M. Effect of total-body X-irradiation from near-threshold to tissue-lethal doses on small-bowel epithelium of the rat. I. Changes in morphology and rate of cell division in relation to time and dose. J. Natl. Cancer Inst. 21: 17-61, 1958.

241. Wimber, D.E., Quastler, H., Stein, O.L. & Wimber, D.R. Analysis of tritium incorporation into individual cells by autoradiography of squash preparations. J. Biophys. Biochem. Cytol. 8: 327-331, 1960.

242. Wimber, D.R. & Lamerton, L.F. Cell population studies on the intestine of continuously irradiated rats. Radiat. Res. 18: 137-146, 1963.

243. Withers, H.R. & Elkind, M.M. Dose-survival characteristics of epithelial cells of mouse intestinal mucosa. Radiology 91: 998-1000, 1968.

244. Withers, H.R. & Elkind, M.M. Microcolony survival assay for cells of mouse intestinal mucosa exposed to radiation. Int. J. Radiat. Biol. 17: 261-267, 1970.

245. Wolpert, L. Positional information and pattern formation. Curr. Top. Dev. Biol. 6: 183-224, 1971.

246. Wright, N.A., Al-Dewachi, H.S., Appleton, D.R. & Watson, A.J. Cell population kinetics in the rat jejunal crypt. Cell Tissue Kinet. 8: 361-368, 1975.

247. Wright, N.A., Morley, A.R. & Appleton, D. The effect of testosteron on cell proliferation and differentiation in the small bowel. J. Endocr. 52: 161-175, 1972.

248. Wright, N.A., Morley, A.R. & Appleton, D. Variation in the duration of mitosis in the crypts of Lieberkuhn of the rat; a cytokinetic study using vincristine. Cell Tissue Kinet. 5: 351-364, 1972.

249. Wright, N.A., Watson, A., Morley, A., Appleton, D., Marks, J. & Douglas, A. The cell cycle time in the flat (avillous) mucosa of the human small intestine. Gut 14: 603-606, 1973.

250. Wright, N.A., Watson, A., Morley, A., Appleton, D., Marks, J. & Douglas, A. The measurement of cell production rates in the crypts of Lieberkuhn. An experimental and clinical study. Virchows Arch. A Path. Anat. Histol. 364: 311-323, 1974.

251. Yeh, K. & Moog, F. Development of the small intestine in the hypophysectomized rat. Developm. Biol. 47: 156-172, 1975.

252. Zajicek, G., Michaeli, Y. & Weinreb, M.M. Kinetics of the inner enamel epithelium in the adult rat incisor during accelerated eruption. Cell Tissue Kinet. 5: 35-39, 1972.

SUMMARY

In the so-called "cell renewing systems" in the adult organism, such as the epidermis, the epithelia of the digestive tract, the testis and the blood-forming tissues, continuous cell loss is balanced by cell production. Normally there is an exact balance between cell loss and cell production, while enough flexibility exists to cope with small perturbations. Although much information has been obtained on the cellular kinetics of the various cell renewing systems, little is known about the regulatory mechanisms which are involved in cell proliferation and cell maturation. Knowledge of such control mechanisms will also contribute to a better understanding of the conditions which lead to unbalanced cell proliferation.

In the study of such control mechanisms the small intestinal epithelium offers a number of advantages. The various stages of cell proliferation and cell maturation may be exactly localized along crypt and villus. This facilitates both cell kinetic studies and biochemical analyses of maturing and functional cells. Furthermore, the short turnover time of the epithelium, which is approximately 70 hours in the rat, allows a rapid and exact determination of the effect of different experimental conditions on cell proliferation and cell maturation.

In the proliferative cell compartment, which is located in the lower half of the crypt, all columnar absorptive cells are engaged in continuous cell proliferation with a mean generative cell cycle time of 12 hours. Halfway the crypt the epithelial cells stop proliferating and subsequently migrate along the maturation cell compartment in the upper half of the crypt to reach the villus after approximately 12 hours. On the villus the epithelial cells are actively involved in intestinal function for approxima-

tely 30 hours, during which period they migrate to the villus top to be extruded into the lumen. In order to perform its various intestinal functions the epithelial cell has to acquire a number of specific structural and biochemical properties. Some of these gradually appear during cell maturation in the crypt, whereas other cell characteristics appear more suddenly on the crypt-villus junction.

A few years ago our group postulated the existence of a feedback control of crypt cell proliferation by the functional villus cell compartment. Indications for such a control mechanism were obtained from comparative studies on the recovery of the intestinal epithelium following low doses of X-irradiation in normal and germfree rats. A relatively low dose of X-irradiation causes a temporary block in the G_1- and G_2-phase of the generative cell cycle. Subsequently many abortive mitoses occur, and the crypt cell population is reduced. The resulting decrease in crypt cell production leads to a reduction of the villus cell population. The recovery of the crypt cell population is accompanied by an overshoot in proliferative activity, which is mainly due to an expansion of the proliferative cell compartment in the crypt. In comparative studies on normal and germfree rats a relationship was found between the overshoot in proliferative activity and the reduction of the villus cell population.

Appendix publication I shows that the recovery of the villus cell population following X-irradiation is not to be attributed to any changes in the life span of the epithelial cells and hence depends on crypt cell production only. The life span of the epithelial cells was found to be independent of villus size. In the same studies it was also observed that the overshoot in crypt cell proliferation following 300R or 700R X-irradiation occurs after the same interval and independently of the degree of initial damage to

the crypt cell population. The hypothesis of a feedback control of crypt cell proliferation by the villus cell compartment was supported by the finding of an inverse relationship between the number of villus cells and the proliferative activity in the crypt during the recovery period after different doses of X-irradiation.

Also during continuous irradiation a decrease in the number of villus cells occurs, whereas the crypt cell population is reduced to a much lesser degree (see *appendix publication II*). No increase was found in the percentage of proliferating crypt cells during continuous irradiation. Yet, an enlargement of the relative size of the proliferative cell compartment in the crypt did occur. These findings indicate that a reduction of the villus cell population is accompanied by an expansion of the proliferative crypt cell compartment, irrespective of the total proliferative activity.

A more direct experimental approach to test the hypothesis of a feedback control of crypt cell proliferation is described in *appendix publication III*. Temporary intestinal ischemia resulted in a selective reduction of the villus cell population without apparent effects on the crypt cell compartment. Within 8 hours after reduction of the villus cell population the proliferative activity markedly increased, which was mainly due to an expansion of the proliferative cell compartment in the crypt. By investigating the effect of villus cell loss in part of the intestine on the proliferative activity in adjacent normal parts of the small intestine it was demonstrated that the feedback control of crypt cell proliferation is a strictly local mechanism.

In *appendix publication V* the recovery of the intestinal epithelium after X-irradiation was studied in a bypassed jejunal segment. The results show that changes in

luminal and/or functional factors do not influence the feedback control mechanism of crypt cell proliferation.

A different approach in the study on the regulation of crypt cell proliferation is presented in *appendix publications IV and VI*, in which the influence of changes in the functional environment of an intestinal segment on epithelial cell kinetics and cell maturation is described.

After experimental by-pass of a jejunal segment a permanent decrease in the number of villus cells was found (see *appendix publication IV*). This was caused by a decrease in crypt cell production, which was the consequence of a decrease in the number of cells per crypt. The relative size of the proliferative cell compartment remained unchanged, and the same was true for the number of crypts per unit length of small intestine and for the crypt-villus ratio. The "adaptation" of the jejunal epithelium to the altered luminal environment occurred after 7-14 days, and subsequently no further changes were observed up to 1 year after surgery.

In the experiments described in *appendix publication VI* an ileal segment was transposed to proximal jejunum to elucidate further the influence of the luminal environment. The number of villus cells in the transposed ileal segment was found to increase after 7-14 days to reach values identical to those in jejunum after 14-30 days. This enlargement of the villus cell population was caused by an increase in crypt cell production. The latter was the result of an increase in the number of cells per crypt without any changes in the relative size of the proliferative crypt cell compartment. Again, the "adaptive" changes in the intestinal epithelium occurred after 7-14 days.

From the experimental data described in *appendix publications IV and VI* it appears that "adaptation" of the small intestinal epithelium to an altered functional environment

consists of a change in the size of the crypt, which influences the size of the functional villus cell population.

In the experiments described in *appendix publications II-VI* epithelial cell maturation was studied by determining the activity of a number of enzymes involved in intestinal function. Microchemical enzyme assays were performed on separated crypt and villus cell compartments after experimental changes in cellular kinetics or functional environment. Expansion of the proliferative crypt cell compartment was found to be consistently accompanied by a decrease in the activity of non-specific esterases in crypt and villus. Following temporary intestinal ischemia a decrease was also observed in the activity of neutral α-glucosidase. These findings suggest that at least some aspects of crypt cell maturation are impaired during expansion of the proliferative crypt cell compartment. However, the activity of a number of enzymes which are predominantly active in villus cells was not reduced during recovery after irradiation. On the contrary, an increase in activity was found during the first two days after irradiation and during continuous irradiation, which might be related to a prolongation of the generative cell cycle.

After by-pass or transposition of an intestinal segment no "adaptive" changes in enzyme activities occurred. It seems that the development of intestinal enzyme activities is "programmed" in the crypt cell and is not influenced by the functional environment. A similar situation seems to exist for the life span of the epithelial cells.

The experimental data described in this thesis suggest the existence of at least two different control mechanisms regulating crypt cell proliferation. The relative size of the proliferative cell compartment seems to be determined by the villus cell population by means of a feedback mechanism. Changes in luminal and/or functional factors seem to

act on the intestinal epithelium by varying the size of the crypt. The way in which these control mechanisms may act on the crypt cells is considered in more detail in the general discussion.

SAMENVATTING

In het volwassen organisme vindt een voortdurende celproliferatie plaats in z.g. "cell renewing" systemen, d.w.z. in celpopulaties waarin een dynamisch evenwicht bestaat tussen celproduktie en celverlies. Voorbeelden van dergelijke celpopulaties zijn de epidermis, de epithelia van het spijsverteringskanaal, de testis en de bloedvormende weefsels. Normaliter is er in deze systemen een evenwicht tussen celaanmaak en celverlies, terwijl de regulatie toch flexibel genoeg is om niet al te grote verstoringen van dit evenwicht het hoofd te kunnen bieden. Hoewel er veel vooruitgang is geboekt bij het onderzoek naar de celproliferatie in verschillende celpopulaties onder diverse omstandigheden, is er nog weinig bekend van de mechanismen die verantwoordelijk zijn voor de regulatie van celproliferatie. Kennis van deze regulatiemechanismen is ook essentieel voor een beter begrip van de achtergronden van de ongekontroleerde celproliferatie in tumoren van deze weefsels.

Het epitheel dat de dunne darm bekleedt is bijzonder geschikt als model om regulatiemechanismen van celproliferatie in een "cell renewing" systeem te onderzoeken. De verschillende stadia van celproliferatie en celmaturatie zijn duidelijk te lokaliseren langs krypt en villus, hetgeen het onderzoek naar de celkinetiek vergemakkelijkt. Bovendien kan d.m.v. mikrochemische analyse van de verschillende celkompartimenten de celmaturatie onderzocht worden. Voorts vormt het dunne darmepitheel de celpopulatie in het organisme die zich het snelst vernieuwt: in de rat wordt het gehele epitheel in ongeveer 70 uur vervangen. Dit maakt het mogelijk om het effekt van verschillende experimentele procedures op de celkinetiek nauwkeurig te bepalen binnen een kort tijdsbestek.

In de onderste helft van de krypt, het proliferatieve

celkompartiment, nemen alle voorstadia van de absorptieve epitheelcel deel aan de celproliferatie, waarbij de gemiddelde duur van de celcyclus ongeveer 12 uur is. Vanuit dit celkompartiment schuiven de cellen langs het bovendeel van de krypt, waar geen celproliferatie plaatsvindt, in ongeveer 12 uur op naar de villus. Vanaf dit ogenblik worden de epitheelcellen aktief betrokken bij de darmfunktie. In ongeveer 30 uur schuiven de cellen langs de villus op naar de villustop waar de epitheelcellen worden afgestoten in het darmlumen.

Het belangrijkste doel van de in dit proefschrift beschreven experimenten was na te gaan welke mechanismen betrokken zijn bij de regulatie van de celproliferatie in het epitheel van de dunne darm. Bovendien werd aandacht besteed aan de invloed van veranderingen in de celkinetiek op bepaalde aspekten van de celmaturatie, en daarmee indirekt op de darmfunktie.

Door bestudering van het herstel van het dunne darmepitheel na een subletale dosis röntgenbestraling bij gewone en kiemvrije ratten werden al eerder aanwijzingen verkregen voor het bestaan van een feedback regulatie van de kryptcelproliferatie door het funktionele villuscelkompartiment. Een relatief lage stralingsdosis blokkeert tijdelijk de delende kryptcellen in de G_1- en G_2-fase van de celcyclus. Vervolgens wordt een groot aantal z.g. abortieve celdelingen waargenomen, en de kryptcelpopulatie wordt gereduceerd. De verminderde celaanmaak in de krypt leidt tot een sterke reduktie van het villuscelkompartiment 36-48 uur na bestraling. Vervolgens vindt een herstel plaats van de kryptcelpopulatie, waarbij de proliferatieve aktiviteit in de krypt tijdelijk de normale waarden overtreft. Gebleken is dat deze "overshoot" voornamelijk berust op een uitbreiding van het proliferatieve celkompartiment in de krypt.

De gegevens beschreven in *publikatie I van de appendix*

tonen aan dat het snelle herstel van de villuscelpopulatie na een eenmalige bestralingsdosis het gevolg is van de toegenomen proliferatieve aktiviteit en dat er geen verandering plaatsvindt in de levensduur van de epitheelcellen. Een verkorting van de villus bleek niet samen te hangen met een verkorte levensduur van de epitheelcel, maar met een verminderde migratiesnelheid. Voorts bleek dat de "overshoot" van de celproliferatie in de krypt tijdens het herstel na bestraling onafhankelijk is van de grootte van de initiële schade die toegebracht wordt aan de kryptcelpopulatie. Wel was er een omgekeerd evenredige verhouding tussen het aantal villuscellen en de proliferatieve aktiviteit in de krypt. Dit ondersteunt de hypotese dat het funktionele villuscelkompartiment betrokken is bij de regulatie van de celproliferatie in de krypt.

Publikatie II van de appendix beschrijft dat er ook tijdens kontinue bestraling gedurende enige dagen een afname van het aantal villuscellen plaatsvond, terwijl het aantal kryptcellen in veel mindere mate gereduceerd werd. Tijdens de kontinue bestraling werd geen toename van het percentage delende cellen in de krypt waargenomen, maar wel was er een uitbreiding van het proliferatieve celkompartiment in de krypt. Ook deze gegevens wijzen erop dat een reduktie van het aantal villuscellen leidt tot een uitbreiding van het proliferatieve celkompartiment in de krypt, ongeacht de totale proliferatieve aktiviteit in de krypt.

Verdere aanwijzingen voor de hypotese dat de villuscelpopulatie de celproliferatie in de krypt reguleert d.m.v. een terugkoppelingsmechanisme werden verkregen door de in *publikatie III van de appendix* beschreven experimenten. Hierbij gelukte het om d.m.v. tijdelijke ischemie van de dunne darm specifiek de villuscelpopulatie te verkleinen zonder duidelijke schade toe te brengen aan de kryptcelpopulatie. Binnen 8 uur na de tijdelijke ischemie nam de pro-

liferatieve aktiviteit in de krypt sterk toe, hetgeen voornamelijk berustte op een uitbreiding van het proliferatieve celkompartiment in de krypt. Tevens werd aangetoond dat de feedback regulatie van de kryptcelproliferatie van lokale aard is, aangezien reduktie van de villuscelpopulatie in een deel van de dunne darm geen effekt heeft op de proliferatie in een aangrenzend darmdeel.

Door onderzoek van het herstel van het dunne darmepitheel na bestraling in een funktioneel uitgeschakelde jejunumlis werd verder aangetoond dat luminale en/of funktionele faktoren geen rol spelen bij de lokale regulatie van de kryptcelproliferatie door het villuscelkompartiment (zie *publikatie V van de appendix*).

Een permanente vergroting of verkleining van de villuscelpopulatie in een deel van de dunne darm kan worden teweeggebracht door langs chirurgische weg een darmlis funktioneel uit te schakelen of te verplaatsen naar een ander deel van de dunne darm. Om te onderzoeken of een regulatiemechanisme van kryptcelproliferatie ten grondslag ligt aan deze veranderingen in de villuscelpopulatie werd onderzocht op welke wijze de "adaptatie" plaatsvindt.

Zoals beschreven is in *publikatie IV van de appendix* vindt er een afname plaats van het aantal villuscellen na het funktioneel uitschakelen van een jejunumlis. Deze verkleining van het villuscelkompartiment werd veroorzaakt door een verminderde celproduktie in de krypt. Dit bleek het gevolg te zijn van een afname van het aantal cellen per krypt, terwijl de relatieve grootte van het proliferatieve celkompartiment in de krypt niet veranderde. Ook het aantal krypten per lengte-eenheid dunne darm en het aantal krypten per villus bleef onveranderd. De gevonden veranderingen in het funktioneel uitgeschakelde jejunumepitheel traden op na 1-2 weken en vervolgens werden geen veranderingen meer waargenomen tot 1 jaar na de chirurgische ingreep.

De in *publikatie VI van de appendix* beschreven experimenten tonen aan dat het aantal villuscellen toeneemt in een ileumlis na transpositie naar proximaal jejunum. Deze toename van het aantal villuscellen, waarbij het aantal cellen per villuskolom in de ileumlis gelijk wordt aan dat in het jejunum, komt tot stand door een vergroting van de celproduktie in de krypt. De toegenomen kryptcelproduktie bleek het gevolg te zijn van een toename van het aantal cellen per krypt, waarbij de relatieve grootte van het proliferatieve celkompartiment in de krypt niet veranderde. Ook na transpositie van een ileumlis naar jejunum traden de beschreven veranderingen pas op 1-2 weken na de operatie.

Uit de in *publikaties IV en VI van de appendix* beschreven experimenten blijkt dat de "adaptatie" van het dunne darmepitheel aan een verandering in het funktionele milieu primair tot stand komt door een verandering in de grootte van de kryptcelpopulatie, wat sekundair de villusgrootte beïnvloedt.

Bij de experimenten beschreven in de *publikaties II-VI van de appendix* werd tevens de ontwikkeling bestudeerd van een aantal enzymen die betrokken zijn bij de funktie van het darmepitheel. Een verlaging in aktiviteit van de niet-specifieke esterasen in de krypt werd voortdurend gevonden bij uitbreiding van het proliferatieve kryptcelkompartiment tijdens herstel na bestraling of tijdelijke ischemie. Na tijdelijke ischemie trad er eveneens een verlaging op in de aktiviteit van neutrale α-glucosidase. Dit suggereert dat een vergroting van de proliferatieve aktiviteit in de krypt ten koste gaat van de ontwikkeling van bepaalde biochemische kenmerken van het darmepitheel. De aktiviteit van een aantal enzymen die normaliter voornamelijk in de villuscel voorkomen bleek echter niet verlaagd te zijn na bestraling. Integendeel, een toename in aktiviteit werd in de krypt gevonden op de eerste dag na een akute bestralingsdosis en

tijdens kontinue bestraling, welke toename mogelijk samenhangt met een verlenging van de celcyclus.

Uit de bepaling van enzymaktiviteiten na het funktioneel uitschakelen van een jejunumlis en na transpositie van een ileumlis naar jejunum bleek dat de "adaptatie" van het epitheel niet gepaard gaat met een verandering in aktiviteit van een aantal enzymen die betrokken zijn bij de darmfunktie. Het lijkt erop dat de celmaturatie niet of nauwelijks beïnvloed wordt door het funktionele milieu. Evenals de levensduur lijkt ook de ontwikkeling van bepaalde enzymaktiviteiten reeds in de kryptcel "vastgelegd" te zijn.

De in dit proefschrift beschreven experimenten wijzen erop dat de kryptcelproliferatie in het dunne darmepitheel langs tenminste twee verschillende wegen gereguleerd wordt. Enerzijds reguleert de villuscelpopulatie de relatieve grootte van het proliferatieve celkompartiment in de krypt, terwijl anderzijds de grootte van de krypt, op nog onbekende wijze, gereguleerd wordt. In de algemene diskussie wordt nader ingegaan op deze regulatiemechanismen en op de mogelijke faktoren die hierbij betrokken zijn.

CURRICULUM VITAE

Na het behalen van het einddiploma gymnasium-B in 1969 aan de Hugo de Groot Scholengemeenschap te Rotterdam begon ik met de studie geneeskunde aan de toenmalige Medische Faculteit Rotterdam; het doctoraalexamen werd in 1974 afgelegd.

Vanaf februari 1972 ben ik werkzaam binnen de vakgroep Celbiologie en Genetica van de Erasmus Universiteit te Rotterdam: van februari tot juli 1972 als keuzepraktikant, van juli 1972 tot juli 1974 als student-assistent en sinds juli 1974 als wetenschappelijk medewerker. Binnen de vakgroep Celbiologie en Genetica werd het in dit proefschrift beschreven onderzoek verricht, deels in samenwerking met Department of Biophysics, Institute of Cancer Research: Royal Cancer Hospital, Sutton, Surrey, Great Britain (Prof.Dr. L.F. Lamerton) en Radiation Research Laboratory, University of Iowa, College of Medicine, Iowa City, Iowa, U.S.A. (Prof.Dr. J.W. Osborne, Dr. W.R. Hanson).

Dankwoord

Graag wil ik iedereen bedanken die op enigerlei wijze heeft bijgedragen aan het tot stand komen van dit proefschrift. In het bijzonder ben ik mijn promotor Prof.dr. T.H. Galjaard erkentelijk voor de stimulerende begeleiding die hij mij gaf bij mijn wetenschappelijke vorming en de grote mate van vrijheid die hij mij daarbij liet.

Hetty Plaisier ben ik zeer dankbaar voor haar aandeel in het praktische werk dat de basis vormde van dit proefschrift en vooral voor de bijzonder loyale en prettige wijze waarop zij steeds met mij heeft samengewerkt.

I am very grateful to Professor Lamerton (Dept. Biophysics, Institute of Cancer Research, Sutton) for his contribution to the experimental work, and for the critical reading of this thesis. Ook Prof.dr.D.W. van Bekkum dank ik hartelijk voor het kritisch doornemen van dit proefschrift.

I thank Professor Osborne (Radiation Research Lab., University of Iowa, Iowa City) for the hospitality during my stay at the University of Iowa. I am very grateful to him and Dr. Wayne R. Hanson (Biological and Medical Research Div., Argonne National Lab., Argonne) for their contributions to some of the experimental work described in this thesis. I also appreciated very much the stimulating discussions in Pittsburgh with Dr. S. Lesher, Dr. Ronald F. Hagemann, Dr. Larry Poulakos and Dr. John W. Cooper. I am very obliged to Dr. Adele B. Kostellow and

Dr. Gene A. Morrill (Dept. Physiology, Albert Einstein College of Medicine, New York) for the opportunity they gave me to broaden my scope of medical research.

Alle (ex-)medewerkers van de afdeling Celbiologie en Genetica dank ik voor hun directe en indirekte bijdragen aan dit proefschrift, en vooral ook voor de plezierige sfeer binnen de afdeling. In het bijzonder ben ik Arthur van der Kamp, Pim Visser, Hans de Ruiter en Joop Bos dankbaar voor de hulp die zij mij op vele gebieden gaven.

Voor de uiteindelijke vormgeving van dit proefschrift dank ik de Heer Vengler (fotografie), Pim Visser en Piet Hardwijk (tekenwerk) en Marja Decae-van Maanen (typen van het manuskript).

Dit proefschrift kon tot stand komen doordat de stichting FUNGO van ZWO mij een tweetal jaren in de gelegenheid stelde als wetenschappelijk medewerker werkzaam te zijn binnen de afdeling Celbiologie en Genetica.

Tenslotte dank ik alle vrienden en vriendinnen, in het bijzonder Laura en Mark, die er voor zorgden dat mijn leven in de afgelopen jaren een veel breder terrein besloeg dan het in dit proefschrift beschreven werk.

Rudy

Appendix: Publications I-VI

Cell Tissue Kinet. (1974) 7, 577–586. Publication I

EFFECT OF VILLUS LENGTH ON CELL PROLIFERATION AND MIGRATION IN SMALL INTESTINAL EPITHELIUM

R. P. C. RIJKE, W. VAN DER MEER-FIEGGEN AND H. GALJAARD

Department of Cell Biology and Genetics, Medical Faculty, Erasmus University, Rotterdam, The Netherlands

(*Received* 27 *December* 1973; *revision received* 3 *June* 1974)

ABSTRACT

For the interpretation of data supporting the hypothesis of a feedback regulation of proliferative activity in intestinal crypts by the functional villus cell compartment the life span and migration rate of epithelial cells on villi of experimentally reduced length should be known. Autoradiographic studies and scintillation counting of isolated villi at different time intervals after ^{3}H-thymidine labelling were carried out 36, 48 and 60 hr intervals after X-irradiation. The results showed that the life span of epithelial cells in rat small intestine (36–48 hr) is independent of the villus length. In villi of reduced length the migration rate of the epithelial cells was found to be decreased compared with controls. Changes in the migration rate in turn seem to be dependent on the production of epithelial cells in the crypt. Comparative studies on the recovery of crypt and villus epithelium after various doses (300 and 700 R) of X-radiation support the hypothesis that increased proliferative activity in the crypt cell compartment is related to a reduction of the number of functional villus cells below a critical villus length. The importance of these findings in the interpretation of data on (micro) biochemical analyses of certain cell differentiation characteristics during increased proliferative activity is discussed.

INTRODUCTION

For a number of cell renewal systems a feedback control of cell proliferation by the functional cell compartment has been described (Bullough *et al.*, 1967; Elgjo, Laerum & Edgehill, 1972; Hanna, 1967; Iversen, 1969; Kivilaakso & Rytömaa, 1971; Rytömaa & Kiviniemi, 1968).

By comparing the recovery of small intestinal epithelium after low doses of X-radiation in conventional and germfree rats, support was obtained for the hypothesis that also in small intestinal epithelium a feedback control of proliferative activity exists (Galjaard, van der Meer-Fieggen & Giessen, 1972a).

These experiments showed that following irradiation an increase in proliferative activity in the crypt occurs after a time interval corresponding to the life span of the epithelial cells in

non-irradiated control animals. This was the fact both for conventional and germfree rats even if the life span of epithelial cells in conventional rats was 12 hr shorter than in germfree rats.

However, during recovery after irradiation, the number of villus cells is still markedly reduced at the time of increased proliferative activity in the crypt (Quastler, 1956; Galjaard *et al.*, 1972a).

For a correct interpretation of experimental results on the relation between functional villus cells and crypt cell proliferation it is therefore important to know what influence the number of villus cells exerts on the migration rate and life span of these cells. Such data are also relevant for a better understanding of studies on crypt and villus cell differentiation.

Microchemical analyses of isolated cell compartments in crypt and villus during recovery after irradiation showed that an increase in the proliferative activity in the crypt is accompanied by insufficient development of certain differentiation characteristics of the epithelial cells (Galjaard & Bootsma, 1969; Galjaard *et al.*, 1972a; Galjaard, van der Meer-Fieggen & de Both, 1972b; de Both *et al.*, 1974). Changes in migration rate and life span of the epithelial cells after irradiation might influence the maturation of crypt cells and villus cells.

The purpose of the present study was to investigate possible effects of a reduction in the number of villus cells on the migration rate and life span of these cells. Furthermore, a study was made of the recovery of rat intestinal epithelium, following different doses of X-radiation with special emphasis on the relation between reduction in number of villus cells and the occurrence of increased cell proliferation in the crypt.

MATERIALS AND METHODS

Male Glaxo-Wistar rats, weighing approximately 250 g, were used and all animals were sacrificed between 09.00 and 10.00 hours throughout the experiments.

Estimation of life span

Thirty animals were exposed to 700 R whole-body X-radiation from a Philips X-ray machine (18 mA, 200 V, 1·5 mm corrected Cu filter, H.V.L. 1·9 mm Cu, dose rate 46 R/min). Three groups of ten rats were injected intraperitoneally with 100 μCi ^{3}H-thymidine (spec. act. 20·4 Ci/mmole) at 36, 48 and 60 hr respectively after irradiation, together with a control group of ten non-irradiated rats. Segments of small intestine (8 cm from the pylorus) were dissected from two rats from each group at $\frac{1}{2}$, 12, 24, 36 and 48 hr after labelling. After fixation in 4% formalin, paraffin sections (5 μm) were processed for dipping autoradiography using Ilford K-2 emulsion. After haematoxylin and eosin staining the microscopic localization of the leading edge of labelled cells along crypt and villus columns and the total number of cells per crypt and villus column were determined. For each rat analyses were carried out in 100 crypt and villus columns; only crypt–villus columns with a single epithelial cell layer and cut longitudinally from the muscularis mucosae to the intact tip of the villus were selected. From the results of these analyses the cell migration rate and minimal life span of the epithelial cells was derived. For an estimation of the maximum life span twelve animals were also exposed to 700 R whole-body X-irradiation. Three groups of four rats were injected intraperitoneally with 100 μCi ^{3}H-thymidine (spec. act. 20·4 Ci/mmole) at 36, 48 and 60 hr respectively after irradiation. From each group two rats were sacrificed at 36 hr and two rats at 48 hr after labelling. Segments of small intestine (8 cm from the pylorus) were frozen

quickly (−70°C) and cryostat sections (20 μm) were freeze-dried *in vacuo*. From the lyophillized sections villi were dissected under the microscope and the segments were weighed on a quartz-fibre balance according to procedures described previously (Galjaard, Van Duuren & Giesen, 1970). Subsequently 50–100 μg of isolated villus material was introduced into a scintillation vial, dissolved in soluene overnight, then 15 ml of scintillation mixture (5 g PPO plus 0·5 g POPOP per litre of toluene) was added. Two samples per rat were counted in a liquid scintillation spectrometer (Packard), and the results were expressed as number of disintegrations per minute per unit dry weight of villus.

Recovery from different doses of X-radiation

Eighteen animals were exposed to whole-body X-radiation with 700 R and two animals were killed atfer intervals of 24, 36, 48, 60, 72, 84, 96, 120 and 168 hr. Ten animals were exposed to whole-body X-radiation with 300 R and two animals were killed at 24, 36, 48, 72 and 168 hr after irradiation. All animals were injected intraperitoneally, with 100 μCi ^{3}H-thymidine (spec. act. 2 Ci/mmole) 30 min before killing. Ten to twenty non-irradiated animals were also labelled with ^{3}H-thymidine in the sample. From each animal segments of small intestine (8 cm from the pylorus) were fixed in 4% formalin, and paraffin sections (5 μm) were processed for dipping autoradiography. After haematoxylin and eosin staining, the number of cells per crypt and villus column and the percentages of labelled cells per crypt column were determined. For each animal 100 longitudinally cut crypt and villus columns were analysed.

RESULTS

Migration of epithelial cells along crypt and villus

The migration of epithelial cells along the length of crypt and villus was studied at various time intervals after 700 R X-radiation. The localization of the leading edge of labelled cells, at ½, 12, 24 and 36 hr after ^{3}H-thymidine labelling, is illustrated for different time intervals after irradiation and in non-irradiated control animals (Fig. 1).

In the non-irradiated control animals the non-dividing maturing crypt cells migrate during 12 hr along the upper half of the crypt, 24 hr after labelling cells are midway the villus and at 36 hr only the upper twelve cell positions of the villus are not yet labelled. After 48 hr all villus tips are covered with labelled cells.

In the group labelled at 36 and 48 hr after 700 R X-radiation the crypt cells also migrate onto the base of the villus within 12 hr, although the distance to be covered is smaller because of an extension of the proliferative cell compartment. Subsequently the migration rate along the villus is reduced compared with that in non-irradiated controls (Fig. 1 and Table 1). At 36 hr after labelling the leading edge of labelled cells has not reached the villus tip, although the villus length is markedly reduced. In the group labelled at 60 hr after irradiation the migration rate approaches the range of control values (Table 1) and again at 36 hr after labelling the villus tip is not yet covered by labelled cells (Fig. 1). At all time intervals after irradiation labelled cells are found at the tips of the villi at 48 hr after labelling.

Estimation of the life span of intestinal epithelial cells

The *minimum life span* of the epithelial cells can be derived from the analysis of the radio-autographs (Fig. 1). Both in the non-irradiated control and at all time intervals after

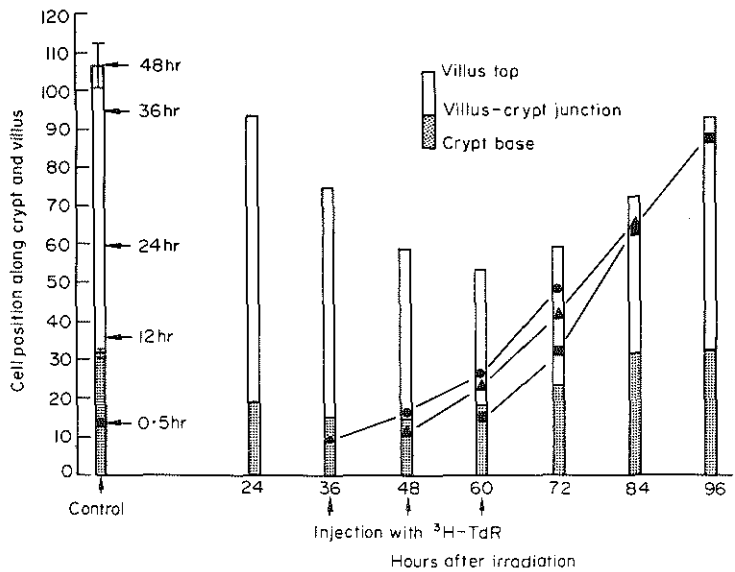

FIG. 1. Cell migration in intestinal epithelium at various time intervals after X-irradiation with 700 R as studied by autoradiography. The histograms show the mean height of the villus and the position of the villus–crypt junctions at various times after irradiation. The leading edge of labelled cells was studied after injection of ^{3}H-thymidine 36 hr (●), 48 hr (▲) and 60 hr (■) after irradiation. The movement of this leading edge is also shown on the control histogram. The mean values of two animals and 100 analyses from different tissue sections per animal are presented for each time interval. The standard deviation for the cell position of the leading edge was four to seven cell positions. The standard deviations for the number of crypt and villus cells are indicated for the controls; detailed data are given in Table 3.

TABLE 1. Migration rate* of epithelial cells on the villus of normal rat small intestine and at different time intervals after X-radiation with 700 R_1

Time interval after labelling	Control	Time interval between X-radiation and labelling		
		36 hr	48 hr	60 hr
12 hr } 24 hr	2·0	0·6	1·2	2·0
24 hr } 36 hr	2·9	1·4	1·3	2·0

* Migration rate is expressed as cell position per hour.

irradiation, *the minimum life span of the epithelial cells was found to be more than 36 hr,* independent of the villus length.

The finding of labelled cells at the tips of the villi at 48 hr after labelling does not necessarily imply that these cells are extruded immediately after reaching the tip of the villus. By quantitative measurements of the radioactivity, at different time intervals after pulse labelling with ^{3}H-thymidine, it was tested whether a reduction in radioactivity, as expressed per unit weight of villus material, occurs. Series of animals were labelled at 36, 48 and 60 hr after X-irradiation. At 36 and 48 hr after ^{3}H-thymidine labelling cryostat sections from small intestine were

freeze-dried, villi were dissected and the dry weight of the pooled fragments was determined. The results of scintillation counting for the three series of irradiated animals are presented in Table 2. Although during the period of investigation labelled crypt cells still migrate onto the villus, the amount of radioactivity per unit weight of villus decreases between 36 and 48 hr after labelling. This is the fact at all three time intervals after 700 R X-radiation, and hence it seems that, independently of the villus length, *the maximum life span of the epithelial cells is less than* 48 *hr*.

TABLE 2. Radioactivity* of isolated villi after ^{3}H-thymidine labelling at various time intervals after X-radiation (700 R)

Time interval between X-radiation and labelling	Time interval after labelling	
	36 hr	48 hr
36 hr	7200 ± 1000	6400 ± 500
48 hr	10200 ± 1800	6500 ± 100
60 hr	22500 ± 1200	13700 ± 1000

Results are the mean of duplicate analyses from pooled villus cell material for two animals per time interval.

* Radioactivity is expressed as d.p.m. per mg dry weight of isolated villi.

Effect of reduction in the number of crypt and villus cells on proliferative activity

Relatively low doses of X-radiation cause a temporary reduction in production of crypt cells which is reflected in a decreased number of crypt cells and functional villus cells. During the recovery period the proliferative activity in the crypt increases again and even exceeds normal values. As the reduction in crypt and villus size will be dependent on the radiation dose, the effect of two different doses of X-radiation on the proliferative activity was studied.

For a number of animals at various time intervals (24–168 hr) after 300 and 700 R of X-radiation the proliferative activity in the crypt was analysed by determining the percentages of labelled cells in radioautographs 30 min after pulse labelling with ^{3}H-thymidine. These results and those of counting of the numbers of crypt and villus cells per longitudinally cut column are given in Table 3. After irradiation with 300 R the length of the crypt hardly decreases and recovery is already completed at 60–72 hr after irradiation.

Irradiation with 700 R, however, results in a considerable decrease in the number of crypt cells during the period of 24–72 hr after irradiation. It takes about 84 hr before the crypt length is normalized. In contrast to the reduction of the crypt length, the decrease in the number of villus cells is approximately the same after irradiation with 300 R and 700 R during the first 48 hr (Table 3). The subsequent recovery of the villus occurs earlier after 300 R than after 700 R irradiation.

After 700 R a minimum villus length (about 50% of normal) is present from 48 to 84 hr after irradiation, and it takes about 5 days before a normal villus length is restored. After 300 R the villus length is minimal at 48–60 hr after irradiation and recovery takes place between 60 and 72 hr.

Both radiation doses result in an initial decrease of the percentages of labelled cells per crypt column at 1 day after irradiation (Table 3), whereas at 48 hr, both after 300 R and 700 R,

TABLE 3. Relationship between proliferative activity in the crypt and reduction in number of crypt and villus cells after X-irradiation*

Hours after irradiation	No. of cells per column				% labelled crypt cells	
	Crypt		Villus			
	300 R	700 R	300 R	700 R	300 R	700 R
Control	32 ± 2	32 ± 1	75 ± 6	75 ± 6	30 ± 6	27 ± 8
24	27 ± 2	19	72 ± 6	80	20 ± 6	12 ± 6
36	27 ± 2	15	64 ± 5	60	28 ± 8	22 ± 11
48	29 ± 2	14 ± 2	50 ± 4	45 ± 5	43 ± 8	47 ± 21
60	—	19 ± 2	—	35 ± 4	—	52 ± 18
72	33 ± 2	22 ± 2	62 ± 4	40 ± 4	37 ± 8	54 ± 14
84	—	32 ± 2	—	40 ± 4	—	50 ± 14
96	—	33 ± 2	—	65 ± 4	—	44 ± 14
120	—	33	—	75	—	32 ± 10
168	32 ± 2	32 ± 2	75 ± 5	75 ± 5	31 ± 6	35 ± 8

* Mean values and standard deviations from two animals and 100 crypt and villus columns from different sections per animal for each interval are presented. Control values are derived from ten to twenty animals.

the percentage of labelled crypt cells shows a considerable overshoot. After 300 R the proliferative activity in the crypt decreases again between 48 and 72 hr. After 700 R high values of the labelling index are observed during the whole period of 48–96 hr after irradiation and a gradual decrease to normal values occurs during the following days.

These results suggest that the initial reduction of crypt cells as well as the subsequent recovery of the crypt are dependent of the radiation dose. The initial decrease in the number of villus cells, however, shows similar values for 300 R and 700 R. The same is true for the

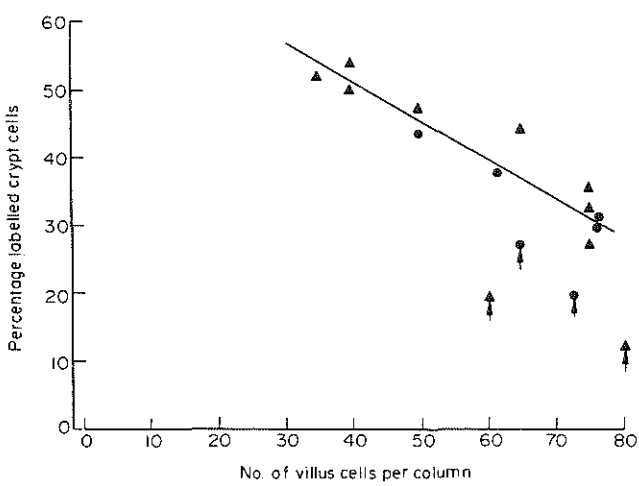

FIG. 2. Relationship between the number of villus cells per column and the percentage of labelled crypt cells per column in controls and at various time intervals after X-irradiation with 300 R (●) and 700 R (▲). The values for thirty-two animals (two animals per point) are presented. The arrows indicate values obtained at 24 and 36 hr after irradiation; all other values are obtained for controls and at later time intervals after irradiation.

overshoot in labelling index which occurs in the crypt at about 48 hr after irradiation. The recovery of the number of villus cells towards control values is much slower after 700 R than after 300 R. As long as the number of villus cells is reduced the percentage of labelled crypt cells was found to exceed control values.

In Fig. 2 the number of villus cells and the percentage of labelled crypt cells are plotted for the different time intervals after 300 R and 700 R. The data show that there is an inverse linear relationship between the number of villus cells and the percentage of labelled crypt cells for all data obtained at 48 hr after irradiation or later. The values observed at 24 and 36 hr do not fit this linear relationship.

DISCUSSION

In previous studies (Galjaard *et al.*, 1972a; van der Meer-Fieggen, 1973) support was obtained for the hypothesis of a feedback control of the proliferative activity in small intestinal crypts by functional villus cells. Using a relatively low dose of X-radiation, the recovery of intestinal epithelium was compared in germfree and conventional rats of the same strain. In these experiments it was shown that an expansion of the proliferative cell compartment in the crypt occurred at 48 hr after X-radiation in conventional rats and at 60 hr in germfree animals. The life span of the epithelial cells in non-irradiated conventional rats was found to be between 36 and 48 hr, whereas in germfree rats this was approximately 12 hr longer (Galjaard *et al.*, 1972a; van der Meer-Fieggen, 1973). In both groups of animals the time interval between irradiation and expansion of the proliferation zone corresponds with the normal life span of the epithelial cells. However, during recovery from X-radiation, the number of villus cells per column is markedly reduced, and this might influence the life span of the epithelial cells and hence the interpretation of experimental data concerning the possibility of a feedback control mechanism.

The results in the present paper show that the life span of the epithelial cells is independent of the villus length, as expressed by the number of cells per villus column.

Combined studies of the localization of labelled cells using autoradiography (Fig. 1) and quantitative analyses using scintillation counting (Table 1) indicate that, as in controls (Leblond & Stevens, 1948), the life span at various time intervals after irradiation remains the same, i.e. between 36 and 48 hr. The extrusion of cells from the tips of the villi was found to continue, even when the villus length is markedly reduced.

In non-irradiated control animals the migration rate seems to increase as the cell migrates from the base towards the tip of the villus (Table 1), and this was also found during the period that the villus length was reduced. During repopulation of the villus this difference in migration rate between base and tip of the villus was not longer observed. In the interpretation of these data on tissue-sections, however, the three-dimensional structure should be kept in mind, as changes do occur during reconstitution of the villi (Loehry & Creamer, 1969).

The constancy of the life span of the epithelial cells, even after marked reduction of the villus length, implies that the number of cells per villus column is dependent on the migration rate of the epithelial cells. The data in Table 1 and Fig. 1 show that following 700 R X-radiation a decreased migration rate of the villus cells coincides with a reduced number of villus cells and also that the recovery of the villus is preceded by an increase in migration rate of the epithelial cells.

The migration rate of the villus cells is restored to normal as soon as the crypt length has

been restored (see Table 1 and Fig. 1), which might indicate that the migration rate of the villus cells is dependent on the crypt length. From the data on crypt length after irradiation (Fig. 1) it can be deduced, from Lajtha's model for cell kinetics following irradiation (Lajtha & Oliver, 1962), that the reduction of crypt size does result in a reduced cell output to the villus and hence in a reduced migration rate of the epithelial cells. During recovery, following irradiation, the crypt is restored before repopulation of the villus occurs.

The results, presented in Table 3, show that the percentage of labelled crypt cells increases above control values at 48 hr after X-radiation with 300 R as well as with 700 R. At this period the number of crypt cells is markedly reduced after 700 R but there is hardly any reduction after 300 R (Table 3). Hence there seems to be no relation between the number of crypt cells and the moment of increase in proliferative activity. However, the number of villus cells is reduced to the same extent at 48 hr after 700 R and after 300 R.

Also in the subsequent period from 48 to 120 hr after irradiation both after 300 R and 700 R an inverse relationship between villus length and the percentage of labelled crypt cells was observed (Fig. 2).

Such a linear relationship, for data from 48 hr after irradiation onwards, is consistent with the hypothesis of a feedback control of proliferation by the functional villus compartment. The data for 24 and 36 hr after irradiation do not fit this linear relationship, representing the fact that during this period the proliferative activity is first blocked and then restored to normal whereas the villus length only shows a slight reduction after 36 hr.

We would like to hypothesize that a reduction of the population of villus cells below a 'critical villus length', probably corresponding with 60–70 % of its normal length, will trigger an expansion of the proliferation cell compartment in the crypt. Such a feedback mechanism might act on cells in 'the critical decision zone' (Cairnie, Lamerton & Steel, 1965) halfway along the crypt in order to initiate one extra cell cycle (Galjaard *et al.*, 1972b).

In view of the digestive function, accompanied by a certain 'wear and tear', it seems reasonable that minor changes in the number of villus cells would not affect crypt cell proliferation.

For a definite proof of the hypothesis of a feedback regulation of crypt cell proliferation the use of X-radiation as a tool for reduction of the villus cell population has the disadvantage of acting via changes in the crypt cell population. It is therefore important to seek other methods which selectively affect the functional villus cell compartment. Such procedures have already contributed in studies of other cell renewal systems (Hanna, 1967; Hennings & Elgjo, 1970; Zajicek, Michaeli & Weinreb, 1972).

Various procedures for the isolation of crypt and villus cell compartments (Nordström, Dahlqvist & Josefsson, 1968; Harrison & Webster, 1969; Galjaard *et al.*, 1970) have enabled quantitative biochemical analysis of differentiation processes in intestinal epithelium (Fortin-Magana *et al.*, 1970; Galjaard *et al.*, 1970; Iemhoff & Hülsmann, 1971; Nordström *et al.*, 1968; Webster & Harrison, 1969). In some instances such studies have also been related to changes in crypt cell kinetics following low doses of X-radiation (de Both *et al.*, 1974; Galjaard & Bootsma, 1969; Galjaard *et al.*, 1972a, b). During the period of increased proliferative activity in the crypt an insufficient development was found of certain differentiation characteristics, i.e. the activity of some enzymes involved in small intestinal function (Galjaard & Bootsma, 1969; Galjaard *et al.*, 1970, 1972a, b). Other parameters, such as the ultrastructural development of the epithelial cells, remained unaffected (de Both *et al.*, 1974). In the present paper it was shown that the life span of the epithelial cells remain unaffected by reduced villus length as a result of irradiation. This implies that the observed immaturity of the villus cells

(Galjaard *et al.*, 1972b) is to be related to an increased proliferative activity in the crypt, and not to any changes in the life span of the epithelial cells.

ACKNOWLEDGMENTS

The assistance of Dr A. W. M. van der Kamp, Mr W. J. Visser and Mr A. Hoogeveen is gratefully acknowledged. Part of this study has been carried out with a grant from the Netherlands Foundation in Medical Research.

REFERENCES

BOTH, N.J., DE, DONGEN, J.M. VAN, KEULEMANS, J., HOFWEGEN, B. VAN, VISSER, W.J. & GALJAARD, H. (1974) The influence of various cell-kinetic conditions on functional differentiation in the small intestine of the rat. a. Enzymes bound to subcellular organelles. *Develop. Biol.* **38**, 119.

BULLOUGH, W.S., LAURENCE, E.B., IVERSEN, O.H. & ELGJO, K. (1967) The vertebrate epidermal chalone. *Nature*, **214**, 578.

CAIRNIE, A.B., LAMERTON, L.F. & STEEL, C.G. (1965) Cell proliferation studies in the intestinal epithelium of the rat. I. Determination of the kinetic parameters. *Exptl Cell Res.* **39**, 528.

ELGJO, K., LAERUM, O.D. & EDGEHILL, W. (1972) Growth regulation in mouse epidermis. II. G_1-inhibitor present in the differentiating cell layer. *Virchows Arch., Abt. B, Zellpath.* **10**, 229.

FORTIN-MAGANA, R., HURWITZ, R., HERBST, J.J. & KRETCHMER, N. (1970) Intestinal enzymes: indicators of proliferation and differentiation in the jejunum. *Science*, **167**, 1627.

GALJAARD, H. & BOOTSMA, D. (1969) The regulation of cell proliferation and differentiation in intestinal epithelium. II. A quantitative histochemical and autoradiographic study after low doses of X-irradiation. *Exptl Cell Res.* **58**, 79.

GALJAARD, H., DUUREN, M. VAN & GIESEN, J. (1970) A quantitative histochemical study of intestinal mucosa after X-irradiation. *J. Histochem. Cytochem.* **18**, 291.

GALJAARD, H., MEER-FIEGGEN, W. VAN DER & GIESEN, J. (1972a) Feedback control by functional villus cells on cell proliferation and maturation in intestinal epithelium. *Exptl Cell Res.* **73**, 197.

GALJAARD, H., MEER-FIEGGEN, W. VAN DER & BOTH, N.J. DE (1972b) In: *Cell Differentiation* (Ed. by D. Viza and H. Harris), p. 322. Munksgaard, Copenhagen.

HANNA, I.R.A. (1967) Response of early erythroid precursors to bleeding. *Nature*, **214**, 355.

HARRISON, D.D. & WEBSTER, H.L. (1969) The preparation of isolated intestinal crypt cells. *Exptl Cell Res.* **55**, 257.

HENNINGS, H. & ELGJO, K. (1970) Epidermal regeneration after cellophane tape stripping of hairless mouse skin. *Cell Tissue Kinet.* **3**, 243.

IEMHOFF, W.G.J. & HÜLSMANN, W.C. (1971) Development of mitochondrial enzyme activities in rat small intestinal epithelium. *Eur. J. Biochem.* **23**, 429.

IVERSEN, O.H. (1969) In: *Homeostatic Regulators* (Ed. by G. E. W. Wolstenholme and J. Knight), p. 29. Churchill, London.

KIVILAAKSO, E. & RYTÖMAA, T. (1971) Erythrocytic chalone, a tissue-specific inhibitor of cell proliferation in the erythron. *Cell Tissue Kinet.* **4**, 1.

LAJTHA, L.G. & OLIVER, R. (1962) Cell population kinetics following different regimes of irradiation. *Brit. J. Radiol.* **35**, 131.

LEBLOND, C.P. & STEVENS, C.E. (1948) The constant renewal of the intestinal epithelium in the albino rat. *Anat. Rec.* **100**, 357.

LOEHRY, C.A. & CREAMER, B. (1969) Three-dimensional structure of the human small intestinal mucosa in health and disease. *Gut*, **10**, 6.

MEER-FIEGGEN, W. VAN DER (1973) Regulation of cell proliferation and differentiation in intestinal epithelium. Thesis, Erasmus University, Rotterdam.

NORDSTRÖM, C., DAHLQVIST, A. & JOSEFSSON, L. (1968) Quantitative determination of enzymes in different parts of the villi and crypts of rat small intestine; comparison of alkaline phosphatase, disaccharidases dipeptidases. *J. Histochem. Cytochem.* **15**, 713.

QUASTLER, H. (1956). The nature of intestinal radiation death. *Radiat. Res.* **4**, 303.

RYTÖMAA, T. & KIVINIEMI, K. (1968) Control of granulocyte production. I. Chalone and antichalone, two specific humoral regulators. *Cell Tissue Kinet.* **1**, 329.

WEBSTER, H.L. & HARRISON, D.D. (1969) Enzymic activities during the transformation of crypt to columnar intestinal cells. *Exptl Cell Res.* **56**, 245.

ZAJICEK, G., MICHAELI, Y. & WEINREB, M. (1972) Kinetics of the inner enamel epithelium in the adult rat incisor during accelerated eruption. *Cell Tissue Kinet.* **5**, 35

Cell Tissue Kinet. (1975) **8**, 441–453. Publication II

THE EFFECT OF CONTINUOUS IRRADIATION ON CELL PROLIFERATION AND MATURATION IN SMALL INTESTINAL EPITHELIUM

R. P. C. RIJKE, H. PLAISIER, A. T. HOOGEVEEN, L. F. LAMERTON* AND H. GALJAARD

*Department of Cell Biology and Genetics, Erasmus University Rotterdam, The Netherlands, and *Biophysics Department, Institute of Cancer Research, Royal Cancer Hospital, Sutton, Surrey*

(*Received* 2 *January* 1975; *revision received* 10 *March* 1975)

ABSTRACT

Autoradiographic studies and scintillation counting of crypt material after pulse labelling with ^{3}H-thymidine showed that during continuous irradiation with 290 rads/day a reduced proliferative activity is present in the crypts of rat small intestine after 1 day of irradiation and of normal activity during the remaining period (5 days) irradiation. After cessation of irradiation an increase in proliferative activity can be observed after 1 day of recovery. From the time (36–48 hr after starting of the irradiation) that the number of villus cells is reduced an expansion of the proliferation zone in the crypt was observed. Both effects last until 1 day of recovery after cessation of irradiation. The process of crypt cell maturation and of villus cell function has also been studied during and after continuous irradiation by microchemical enzyme analyses in isolated crypts and villi. It was found that the expansion of the proliferation zone in the crypt is accompanied by a decrease in activity of only those enzymes (i.e. non-specific esterases) which normally become active during crypt cell maturation. The activity of enzymes normally present mainly in the functional villus cells remained relatively unaffected by changes in crypt cell kinetics. A hypothesis of different regulation mechanisms of the proliferative activity in the intestinal crypt and a possible explanation of the different behaviour of various enzyme activities as a result of changes in crypt cell proliferation is discussed.

INTRODUCTION

For several cell renewing systems, i.e. skin, enamel epithelium, haemopoietic and lymphopoietic systems, evidence has been obtained for a feedback control of the proliferative activity in the precursor cell compartment (Bullough *et al.*, 1967; Elgjo, Laerum & Edgehill,

1972; Hanna, 1967; Hennings & Elgjo, 1970; Kiger *et al.*, 1972; Kivilaaksoo & Rytömaa, 1971; Rytömaa & Kiviniemi, 1968; Zajicek, Michaeli & Weinreb, 1972).

Comparative studies on the recovery of the small intestinal epithelium after a single dose of X-radiation in conventional and germ-free rats gave support to the hypothesis of a feedback control of crypt cell proliferation by the functional villus cells (Galjaard, van der Meer-Fieggen & Giesen, 1972a; van der Meer-Fieggen, 1973). In studies on the recovery from different radiation doses it was found that the percentage of labelled crypt cells varies inversely with the number of cells per villus column. It was also suggested that the villus cell population has to be reduced to a critical number (approximately 70% of control value) to initiate an expansion of the proliferating crypt cell compartment (Rijke, van der Meer-Fieggen & Galjaard, 1974).

In the study of homeostatic mechanisms it is often very useful to perturb the system by an agent which leads to a new steady state or near steady state of cell population. Continuous irradiation is one such agent and published data on the effects of continuous irradiation on intestinal epithelium are of considerable interest, contrasting with the data from single radiation exposures described above. In experiments on the effects of continuous irradiation on the intestinal epithelium of rats no increase in labelling and mitotic indices was observed during irradiation (Cairnie, 1967; Lamerton, 1966; Quastel, 1963; Quastler *et al.*, 1959; Wimber & Lamerton, 1963), although a reduction in total villus population occurs (Lamerton *et al.*, 1961; Quastel, 1963).

The present studies were designed to follow up these findings by investigating, during the course of continuous irradiation, the changes in, and possible relationships between, the number of villus cells per column, the proliferative activity in the crypt (as measured by the labelling index and by scintillation counting of crypt material after pulse labelling with ^{3}H-thymidine) and the localization of the proliferative zone in the crypt. The effect of changes in crypt cell proliferation on the process of cell maturation has also been investigated by microchemical enzyme analyses on isolated crypts and villi, during continuous irradiation and during a subsequent recovery period. The activity of the non-specific esterase was measured as a parameter for crypt cell maturation (de Both *et al.*, 1974; Galjaard, van Duuren & Giesen, 1970; van der Meer-Fieggen, 1973) and the activities of alkaline phosphatase, leucine aminopeptidase and neutral α-glucosidase were analysed as parameters for villus cell function (de Both *et al.*, 1974; Fortin-Magana *et al.*, 1970; Galjaard, van der Meer-Fieggen & de Both, 1972b; Nordström, Dahlqvist & Josefsson, 1968; Webster & Harrison, 1969).

MATERIALS AND METHODS

Male hybrid rats of the female August and male Marshall strains, weighing 100–120 g and about 7 weeks old, were used. Irradiations were performed in a Caesium-137 irradiation unit (Quastler *et al.*, 1959), with a dose of 290 rads/day. The animals were exposed to continuous irradiation for 5 days and subsequently allowed to recover for 7 days. After 1, 2, 3 and 5 days of irradiation, and after 1, 2, 3, 5 and 7 days of recovery following irradiation for 5 days, two animals were anaesthesized, 0·5 hr after being injected intraperitoneally with 100 μCi ^{3}H-TdR (Amersham, specific activity 29 Ci/mole), and segments of small intestine (approximately 8 cm from the pylorus) were removed. At operation days segments of small intestine were also taken under the same conditions from one or two unirradiated control animals, in total five rats. All rats were sacrificed at approximately 12.00 hours except for two rats

which had been allowed a recovery of 2 days after 5 days of irradiation, which were sacrificed at approximately 18.00 hours. Because differences between labelling indices at 12.00 hours and 18.00 hours have been found to be almost nil (Pilgrim, Err & Maurer, 1963; Sigdestad, Bauman & Lesher, 1969) the results of the kinetic studies of these rats sacrificed at the later time are included in the results. The enzymatic activities are not given since differences due to the daily rhythm of enzymatic activities are considerable (Hugon, Charnel & Lamendeau, 1973; Saito, 1972).

Autoradiography

Segments of small intestine from each animal were fixed in 4% formalin and paraffin sections (5 μm) were processed for dipping autoradiography using K2-Ilford emulsion. After HE-staining, the number of cells was determined in fifty longitudinally cut crypt columns and villus columns, together with the number of labelled cells along the crypt column. The positions of the labelled cells along the crypt column were also determined in order to observe changes in the pattern of distribution of proliferating cells in the crypt; the data were plotted as described earlier (Galjaard *et al.*, 1972a). The leading edge of labelled cells along the crypt column was also recorded for this purpose.

Scintillation counting

From each animal segments of small intestine were quickly frozen in liquid nitrogen and stored in solid carbon dioxide, for scintillation counting and determination of enzymatic activities. Cryostat sections (20 μm) were freeze-dried (4 hr at $-25°C$, $p < 0{\cdot}001$ mmHg), crypt fragments were dissected, weighed on a quartz balance and placed in a liquid scintillation vial (two vials for each animal), containing 0·5 ml Soluene* for solubilization. After 48 hr 15 ml scintillation mixture (5 g PPO† plus 0·5 g POPOP† per litre toluene) were added. The samples were counted in a liquid scintillation spectrometer (Packard) and the results were expressed as number of disintegrations per minute per unit dry weight of crypt.

Enzymatic assays

Cryostat sections (12 μm) were freeze-dried, as described above, for microchemical analyses; crypts and villi were dissected and the tissue fragments were weighed on a quartz balance (Galjaard *et al.*, 1970). The activity of the non-specific esterase was determined using naphthyl acetate as a substrate in a final volume of 350 μl (Galjaard *et al.*, 1970), and the activity of neutral α-glucosidase with 4-methylumbelliferyl-α-D-glucopyranoside as a substrate in a final volume of 520 μl (Galjaard *et al.*, 1972c). The alkaline phosphatase activity was determined according to Lowry *et al.* (1954), using di-Na-paranitrophenylphosphate as a substrate, Mg^{++}-ions added, in a final volume of 250 μl. The leucine aminopeptidase was determined according to the 'LAP test' of Boehringer, with L-leucine-p-nitroanalid as a substrate in a final volume of 50 μl, as described earlier (Galjaard *et al.*, 1970).

The enzyme activities were estimated for each animal in ten samples of crypt-material and ten samples of villus-material. The non-specific esterase served as a parameter for crypt cell maturation and the alkaline phosphatase, leucine aminopeptidase and neutral α-glucosidase as parameters for villus cell function, this distinction being based on earlier experiments (de Both *et al.*, 1974; Galjaard *et al.*, 1970, 1972b).

* Soluene (Packard).

† PPO: 2,5-diphyloxazol; POPOP: 2,2′-phenylen-bis-(5-phenyloxazol).

RESULTS

1. *Compartment sizes and proliferative activity*

At various time intervals during 5 days of continuous irradiation, with a dose of 290 rads/day, and a subsequent 7 days' period of recovery, radioautographs of small intestine were prepared after pulse labelling with tritiated thymidine. The following parameters were investigated: number of cells per villus column and crypt column, percentage of labelled cells per crypt column and the localization of labelled cells in the crypt. Additional information on the proliferative activity was obtained by scintillation counting of crypt material dissected from freeze-dried cryostat sections of small intestine.

(a) *Crypt and villus column counts.* The numbers of cells per longitudinally cut crypt column and villus column at various periods of continuous irradiation and during subsequent recovery are given in Table 1. During continuous irradiation the number of cells per crypt column is hardly reduced and attains normal values after 1 day of recovery. After 2 and 3 days of recovery an overshoot in the number of cells per crypt column is observed and subsequently the number of cells per crypt column decreases again to approach control values.

The villus length is reduced to approximately 70% of its normal length after 2 days of continuous irradiation and remains so until day 2 of recovery, at which time control values are attained again.

TABLE 1. Number of crypt cells and villus cells in small intestinal epithelium during and after continuous irradiation*

	No. of cells per column			
	Continuous irradiation		Recovery after irradiation	
	Crypt	Villus	Crypt	Villus
Unirradiated controls	32 ± 2	74 ± 8	33 ± 2	73 ± 8
	32 ± 2	68 ± 6	32 ± 3	73 ± 8
	32 ± 2	73 ± 7		
1 day	29 ± 2	75 ± 8	32 ± 2	58 ± 4
	26 ± 2	71 ± 8	32 ± 2	47 ± 4
2 days	30 ± 3	53 ± 4	40 ± 4	74 ± 7
	31 ± 3	48 ± 5	38 ± 3	71 ± 6
3 days	28 ± 2	50 ± 4	36 ± 3	70 ± 6
	31 ± 3	57 ± 6	39 ± 3	70 ± 7
5 days	30 ± 5	51 ± 5	33 ± 2	70 ± 6
	29 ± 2	53 ± 6	35 ± 3	78 ± 8
7 days			34 ± 3	73 ± 8
			34 ± 2	76 ± 6

* Each value represents the mean (±S.D.) of fifty longitudinally cut columns of crypts and villi in various tissue sections from one animal.

(b) *Proliferative activity.* As shown in Fig. 1(a) the percentage of labelled crypt cells, as determined by autoradiography, shows an initial decrease at day 1 of irradiation, but control values are maintained during the remaining period of irradiation. After 1 day of recovery from the irradiation the percentage of labelled crypt cells is increased, but approximately normal values are observed during the remaining period.

Quantitative data based on the dry weight of isolated crypt material were obtained by scintillation counting after pulse labelling with tritiated thymidine. Fig. 1(b) illustrates the number of disintegrations per minute per mg dry weight of crypt, which, as grain counts

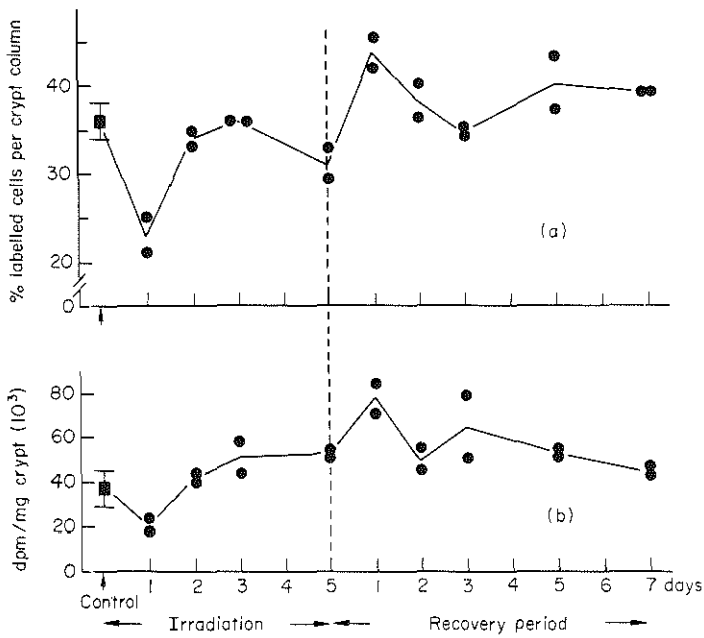

FIG. 1. Proliferative activity in small intestinal crypts during and after continuous irradiation (290 rads/day): (a) percentage of labelled cells per crypt column studied by autoradiography, (b) radioactivity in dpm/mg crypt, as determined by liquid scintillation counting. Each point represents the mean of determinations in one rat and control values represent the mean (±S.D.) of analyses in five animals.

revealed no significant differences, is a reflection of the number of cells synthesizing DNA per mg of crypt material. The results show that the radioactivity per unit weight of crypt is decreased after 1 day of irradiation, and is normal again during subsequent days of irradiation. After cessation of irradiation the cell production rises quickly (at day 1 of recovery) and subsequently decreases again to approach control values.

(c) *Localization of proliferative cell compartment in the crypt.* The distribution of labelled cells along the length of the crypt column, corrected for (minor) variations in crypt length, after various periods of irradiation is plotted in Fig. 2 and in Fig. 3 the same parameters are illustrated for the period of recovery after 5 days of irradiation.

Throughout the irradiation period there is a decrease in the percentage of labelled cells for those cell positions which are normally exclusively involved in proliferation (i.e. cell

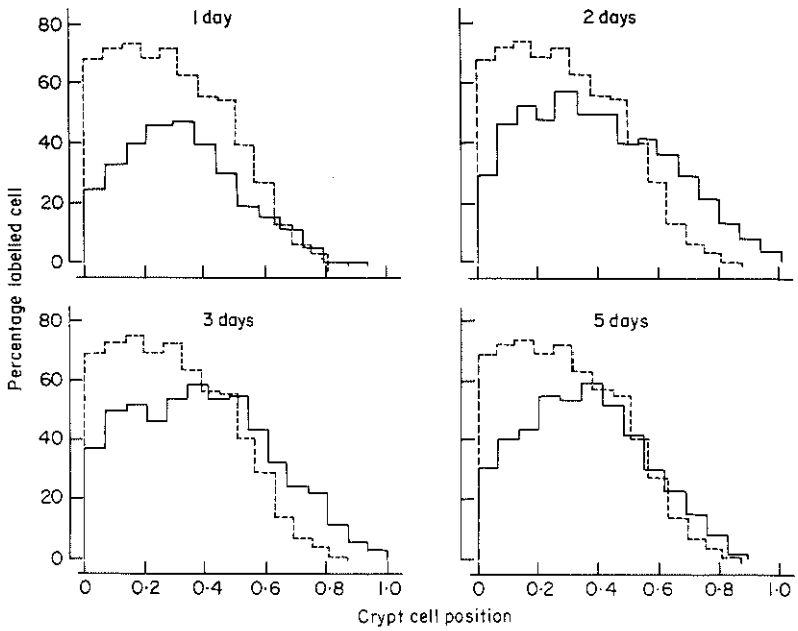

FIG. 2. Distribution of labelled cells in intestinal crypts during continuous irradiation (290 rads/day), expressed in percentage of labelled cells in each cell position along longitudinally cut crypt columns. Cell positions are given in fractional distances from bottom to top of the crypt. The dotted lines represent the distribution of control animals.

positions 1–16 from the bottom of the crypt). After irradiation for 2 and 3 days there is an expansion of the proliferation zone and more labelled cells are found in the upper half of the crypt. After 5 days of irradiation the distribution of proliferating cells in the crypt is approximately the same as that of the non-irradiated control. The results in Fig. 3 illustrate that the proliferation zone is expanded after the first day of recovery, and the distribution of proliferating cells in the crypt has already normalized after 2 days of recovery. During the whole period of recovery the percentages of labelled cells in the lower half of the crypt are similar to those in control animals.

Fig. 4 shows the relationship between the position of the leading edge of labelled cells along the crypt column, serving as a parameter for the localization of the proliferative cell compartment, and the number of cells per villus column. An expansion of the proliferation zone is observed after 2 and 3 days of irradiation and after 1 day of recovery, and the number of cells per villus column is reduced at these time intervals. After 5 days of irradiation the distribution of proliferating cells in the crypt is normalized while the number of villus cells per column is reduced. By day 2 of recovery both the number of villus cells and the size of the proliferative cell compartment have returned to normal values.

2. *Crypt cell differentiation during and after continuous irradiation*

As a parameter for crypt cell differentiation the activity of non-specific esterase has been used, whereas the activities of alkaline phosphatase, leucine aminopeptidase and neutral α-glucosidase, which are mainly developed in the villus cells, were considered to be indicators

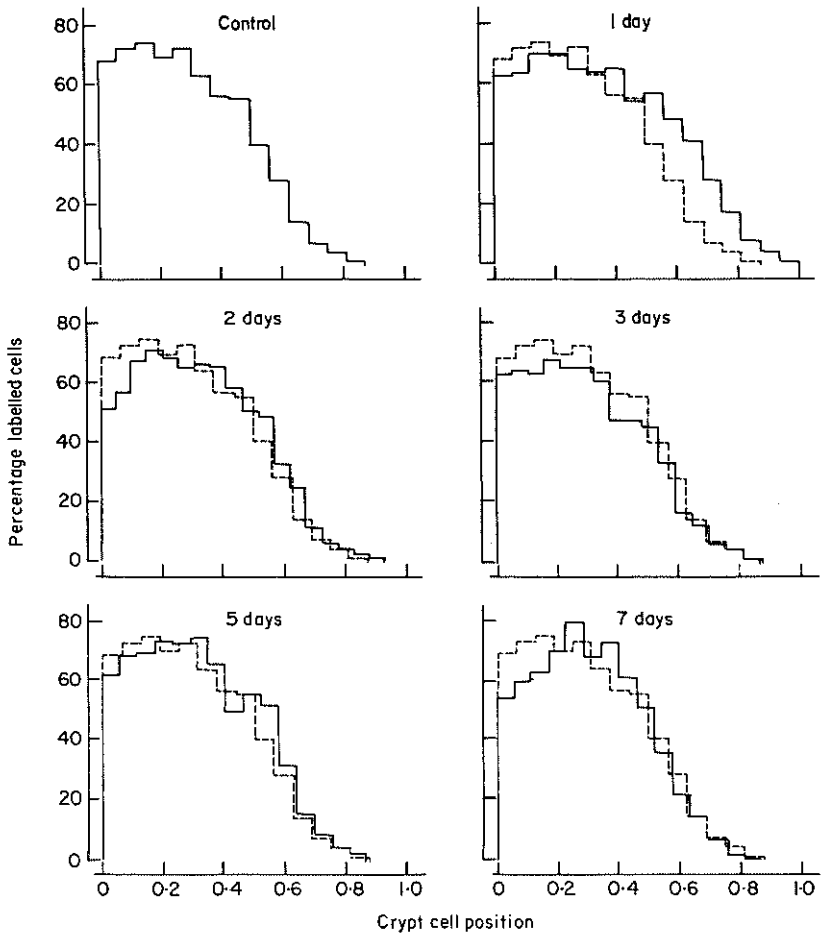

FIG. 3. Distribution of labelled cells along intestinal crypts during recovery after 5 days of continuous irradiation. Similar parameters as in Fig. 2.

for villus cell function. The results of the enzyme analyses in isolated crypts are illustrated in Fig. 5 and those for isolated villi in Fig. 6.

The activity of the non-specific esterase in the crypt decreases considerably between day 1 and 2 of irradiation and remains low during the whole period of irradiation, and during the initial part of the recovery period, returning to control values after 5 days of recovery. The reduced activity in the crypt is also reflected in the villus cells (Fig. 6), where a decreased esterase activity is observed during the irradiation and the subsequent recovery period. Immediately after cessation of irradiation there is an increase in esterase activity of the villus cells.

Figs. 5 and 6 also demonstrate the activities of alkaline phosphatase, leucine aminopeptidase and neutral α-glucosidase. In contrast to the non-specific esterase the activities of these enzymes are low in the crypt compared with the villus. For these enzymes the activities both in crypt cells and in villus cells remain relatively unaffected by changes in cell proliferation during the irradiation and the subsequent recovery period. Only after 1 day of irradiation

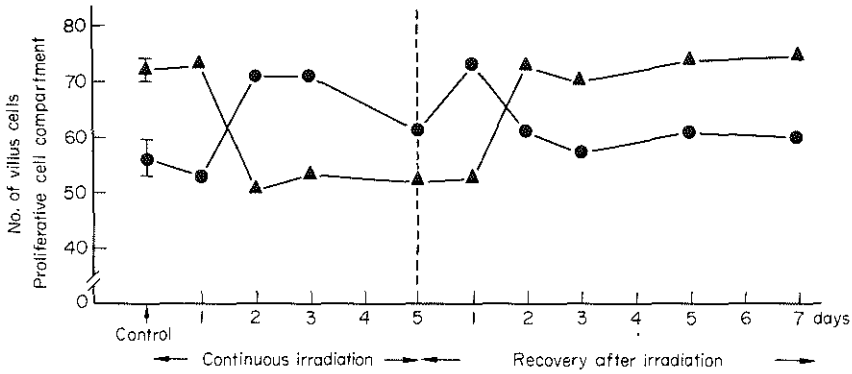

FIG. 4. Relationship between villus length (▲) and localization of proliferative cell compartment in the intestinal crypt (●) during and after continuous irradiation. The localization of the proliferative cell compartment is represented by the mean position of the highest labelled cell in fifty crypts per animal, expressed as percentage of crypt length, and villus length is expressed as number of cells per villus column. All points represent the mean of determinations in duplicate animals, except control values representing the mean (±S.D.) of five animals.

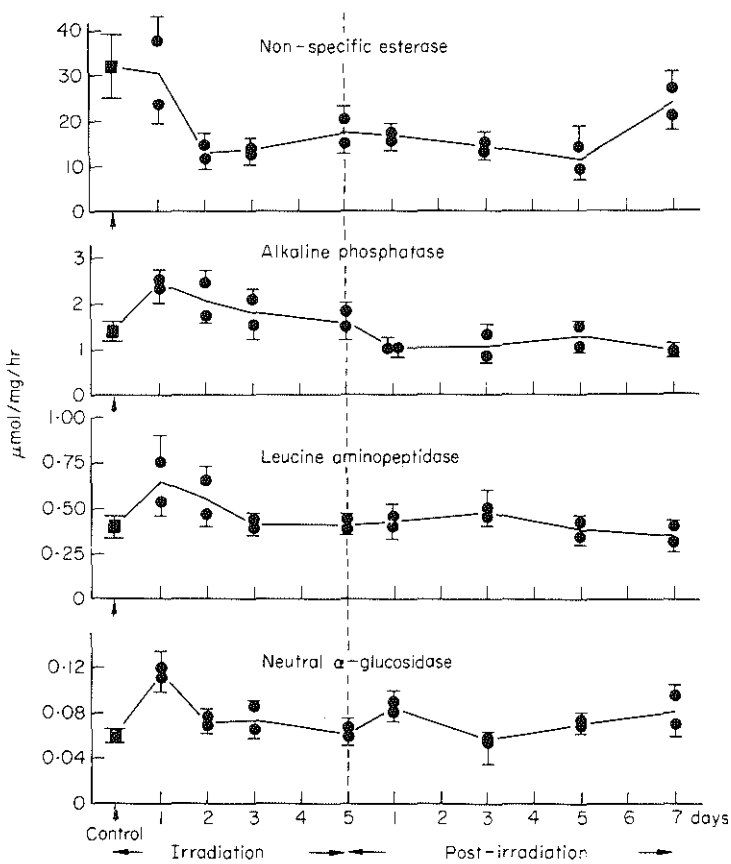

FIG. 5. Enzyme activities in small intestinal crypts during and after continuous irradiation. Each point represents the mean (±S.D.) of ten analyses from various tissue sections from one animal. Control values represent the mean (±S.D.) of enzyme analyses in five animals.

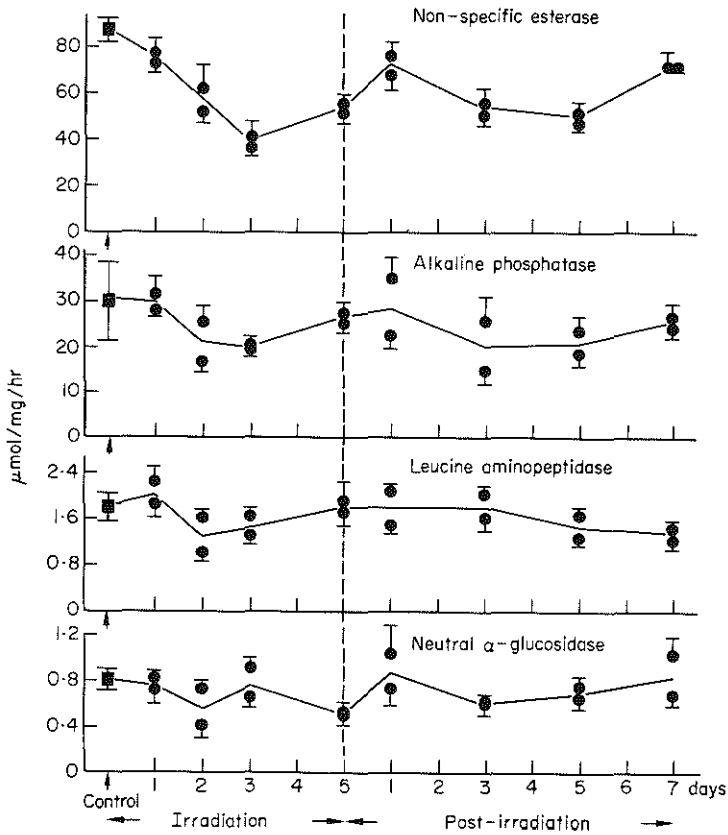

FIG. 6. Enzyme activities in small intestinal villi during and after continuous irradiation. Similar parameters as in Fig. 5.

increased activities in the crypt are observed for alkaline phosphatase, leucine aminopeptidase and neutral α-glucosidase (Fig. 5), which then gradually decrease to normal values.

DISCUSSION

There are various lines of evidence pointing to a feedback control of crypt cell proliferation in the epithelium of the small intestine. Comparative studies on the epithelium of rat small intestine in conventional and germfree rats led to the hypothesis of a feedback control of crypt cell proliferation by the functional villus cells (Galjaard *et al.*, 1972a; van der Meer-Fieggen, 1973). Recent studies on the recovery of rat intestinal epithelium after various doses of X-radiation revealed an inverse relationship between the number of cells per villus column and the percentage of labelled crypt cells (Rijke *et al.*, 1974). In the same studies it was suggested that the villus has to be reduced to a critical number to initiate an expansion of the proliferating cell compartment in the crypt (Rijke *et al.*, 1974). On the other hand, in his study of the existence of chemical factors in the small intestine affecting cell proliferation, Tutton (1973) found that a chemical factor inhibiting crypt cell proliferation could be isolated from crypt cells, but could detect no effect of an extract from villus cells. The timing of his

experiments would only allow the detection of factors which operated on the cell within a few hours of the start of mitosis, and presumably within the G_2 phase. From the work of Tutton nothing can therefore be said about feedback factors from crypt or villus operating on the earlier stages of the cell cycle.

The present experiments on the effect of continuous irradiation show that between 1 and 2 days of irradiation there is a slight decrease in the number of cells per crypt column while the number of cells per villus column remains unchanged (Table 1). The percentage of labelled cells per crypt column and the proliferative activity in the crypt as measured by scintillation counting are decreased and the distribution of proliferating cells in the crypt remains unchanged (Figs. 1 and 2). In the subsequent period of continuous irradiation the number of cells per villus column is reduced to approximately 70% of its normal cell number and at the same time the proliferative crypt cell compartment has expanded (Fig. 4). The percentage of labelled cells per crypt column returns to control values, as does the proliferative activity in the crypt determined by scintillation counting of crypt material. There is, however, an expansion of the proliferative cell compartment in the crypt after 2 and 3 days of irradiation, in spite of a normal percentage of labelled crypt cells. This apparent discrepancy is due to the decrease in labelling index of cells in the lower half of the crypt during irradiation (Fig. 2), possibly a consequence of a sterilizing action of the irradiation (Cairnie, 1967, 1969). During the whole period of continuous irradiation the number of cells per crypt column shows, in contrast to the number of cells per villus column, only a slight reduction. The results obtained are consistent with the hypothesis that an expansion of the proliferative cell compartment in the crypt is determined by changes in the villus cell population, though of course they do not prove a causal relationship.

It is also possible that the total crypt cell population influences the distribution of proliferating cells in the crypt since it was observed, using the squash technique, that the total crypt cell population is markedly reduced during continuous irradiation (Lamerton, 1963; Wimber & Lamerton, 1963). It was found, however, that after 1 day of irradiation there is a reduction of the crypt cell population and that the crypt cell population is already recovering at 1 day cessation of irradiation (Lamerton, 1963). These results do not appear to correspond with the data on the distribution of proliferating cells in the crypt, as illustrated in Fig. 4, but it has to be recognized that the column counts may not provide a good measure of the total cell population if the crypt (or villus) is changing in shape or if the cell density is changing. It is for this reason that conclusions based on column counts of crypt and villus have to be treated with a certain reserve.

On the first day of recovery after 5 days of continuous irradiation a distinct increase of the proliferative activity in the crypt occurs (Figs. 1a and 1b). The proliferative cell compartment remains expanded and the number of villus cells per column is still reduced, in contrast with the number of cells per crypt column, which has fully returned to control values. It would appear that it is a consequence of this increased proliferative activity that the villus is able to regain its normal length very quickly, and explains the observation that after 2 days of recovery the number of villus cells per column has already returned to control values. At the same time both the proliferative activity and the distribution of proliferative cells in the crypt return to control values. The number of crypt cells per column shows an overshoot at day 2 and 3 of recovery and subsequently decreases towards control values.

During the period of expansion of the proliferative cell compartment in the crypt, and hence reduction in the length of the non-proliferating maturation cell compartment in the

crypt, only the non-specific esterase shows a distinct decrease of activity in the crypt, which is in accordance with earlier observations after acute irradiation (Galjaard & Bootsma, 1969). The decreased esterase activity in the crypt is also reflected in a lower activity in the functional villus cells (Figs. 5 and 6), which has been shown not to result from changes in the life span of the epithelial cells (Rijke & Galjaard, 1973; Rijke *et al.*, 1974). Such an inverse relationship between cell proliferation and activity of non-specific esterase has also been reported to exist under other circumstances (Smejkalová & Smejkal, 1973). During the first days of recovery crypt cell maturation, as reflected by the activity of the non-specific esterase, is still markedly reduced. A relatively late recovery of esterase activity in the crypt has also been observed following acute irradiation (Galjaard & Bootsma, 1969; Galjaard *et al.*, 1972a), and may be an indication that the consequences of changes in cell proliferation last longer than the changes themselves.

After 1 day of irradiation there is an increase in activity of three enzymes involved in intestinal function, i.e. alkaline phosphatase, leucine aminopeptidase and α-glucosidase, and during subsequent days of irradiation there is a gradual decrease towards normal values (Fig. 5). However, changes in the distribution of proliferating cells in the crypt do not seem to influence the activities of those enzymes which are localized in the microvilli of the epithelial cell and the development of which mainly occurs when the epithelial cell enters the functional villus compartment (de Both *et al.*, 1974; Galjaard *et al.*, 1972b). Thus an expansion of the proliferative cell compartment does not interfere with the activity of the enzymes alkaline phosphatase, leucine aminopeptidase and neutral α-glucosidase.

Cairnie (1969) reported that the length of the $G_2 + \frac{1}{2}M$ part of the cell cycle time is increased after 1 day of continuous irradiation and subsequently gradually decreases to reach control values after 5 days of irradiation. It is interesting to note that the activities of alkaline phosphatase, leucine aminopeptidase and α-glucosidase in the crypt cells correspond approximately with Cairnie's data on the length of the $G_2 + \frac{1}{2}M$ part of the cell cycle time during and immediately after continuous irradiation. The activity of the non-specific esterase in the crypt follows a different pattern; it was not increased after 1 day of irradiation and it was decreased throughout the remaining period of irradiation and the first day of recovery after the irradiation. This suggests that the activity of the non-specific esterase in the crypt is not influenced by changes in the $G_2 + \frac{1}{2}M$ part of the cell cycle time.

Following acute or continuous irradiation an increase in the percentage of labelled crypt cells can be observed, which may be initiated by a reduction of the villus cell population or a reduction of the total crypt cell population. An increase in the labelling index following irradiation could be brought about by two different mechanisms, i.e. by an expansion of the proliferative cell compartment in the crypt and by a shortening of the cell cycle time. Recently Elgjo and his colleagues (Elgjo *et al.*, 1972; Elgjo & Edgehill, 1973) suggested the existence of two different 'chalones' for the epidermis, one being produced by the functional cells and arresting cells in the G_1-phase of the cell cycle in the proliferation compartment, and another produced by the proliferating cells supposed to arrest cells in the G_2-phase. The data presented herein are consistent with the view that the distribution of proliferating cells in the crypt is determined by changes in the villus cell population. Thus a reduction of the villus cell population may, for a number of cells migrating along the crypt, delay the decision to stop proliferating, so that the 'critical decision zone' (Cairnie, Lamerton & Steel, 1965) shifts upwards in the crypt. The chemical fraction from crypt cells, recently isolated by Tutton (1973), appears to inhibit cell proliferation by some hold-up of cells in the G_2-phase of the

cell cycle. It is possible that in small intestinal epithelium, in the same way as suggested for the epidermis by Elgjo, there are two different mechanisms to increase the proliferative activity in the crypt, regulated by different cell populations. The cell cycle time of proliferating cells in the crypt could be determined by the crypt cell population and the distribution of proliferating cells in the crypt by the villus cell population.

ACKNOWLEDGMENTS

The assistance of Dr A. W. M. van der Kamp is gratefully acknowledged. Part of this study has been carried out with a grant from the Netherlands Foundation in Medical Research.

REFERENCES

BOTH, N.J. DE, DONGEN, J.M. VAN, KEULEMANS, J., HOFWEGEN, B. VAN, VISSER, W.J. & GALJAARD, H. (1974) The influence of various cell-kinetic conditions on functional differentiation in the small intestine of the rat. a. Enzymes bound to subcellular organelles. *Devl Biol.* **38**, 119.

BULLOUGH, W.S., LAWRENCE, E.B., IVERSEN, O.H. & ELGJO, K. (1967) The vertebrate epidermal chalone. *Nature*, **214**, 578.

CAIRNIE, A.B. (1967) Cell proliferation studies in the intestinal epithelium of the rat: response to continuous irradiation. *Radiat. Res.* **32**, 240.

CAIRNIE, A.B. (1969) Further studies on the response of the small intestine to continuous irradiation. *Radiat. Res.* **38**, 82.

CAIRNIE, A.B., LAMERTON, L.F. & STEEL, G.G. (1965) Cell proliferation studies in the intestinal epithelium of the rat. *Exp. Cell Res.* **39**, 528.

ELGJO, K. & EDGEHILL, W. (1973) Epidermal growth inhibitors (chalones) in dermis and serum. *Virchows Arch., Abt. B, Zellpath.* **13**, 14.

ELGJO, K., LAERUM, O.D. & EDGEHILL, W. (1972) Growth regulation in mouse epidermis. II. G_1-inhibitor present in the differentiating cell layer. *Virchows Arch., Abt. B, Zellpath.* **10**, 229.

FORTIN-MAGANA, R., HORWITZ, R., HERBST, J.J. & KRETCHMER, N. (1970) Intestinal enzymes: indicators of proliferation and differentiation in the jejunum. *Science*, **167**, 1627.

GALJAARD, H. & BOOTSMA, D. (1969) The regulation of cell proliferation and differentiation in intestinal epithelium. II. A quantitative histochemical and autoradiographic study after low doses of X-irradiation. *Exp. Cell Res.* **58**, 79.

GALJAARD, H., DUUREN, M. VAN & GIESEN, J. (1970) A quantitative histochemical study of intestinal mucosa after X-irradiation. *J. Histochem. Cytochem.* **18**, 291.

GALJAARD, H., MEER-FIEGGEN, W. VAN DER & GIESEN, J. (1972a) Feedback control by functional villus cells on cell proliferation and maturation in intestinal epithelium. *Exp. Cell Res.* **73**, 197.

GALJAARD, H., MEER-FIEGGEN, W. VAN DER & BOTH, N.J. DE (1972b) In: *Cell Differentiation* (Ed. by D. Viza and H. Harris), p. 322. Munksgaard, Copenhagen.

GALJAARD, H., FERNANDES, J., JAHODOVA, M., KOSTER, J.F. & NIERMEIJER, M.F. (1972c) Prenatal diagnosis of genetic disease. *Bull. Europ. Soc. Hum. Genet.* Nov. 1972, 79.

HANNA, I.R.A. (1967) Response of early erythroid precursors to bleeding. *Nature*, **214**, 355.

HENNINGS, H. & ELGJO, K. (1970) Epidermal regeneration after cellophane stripping of hairless mouse skin. *Cell Tissue Kinet.* **3**, 243.

HUGON, J.S., CHARNEL, CL. & LAMENDEAU, D. (1973) Circadian rhythm of alkaline phosphatase in the Golgi-zone of mouse duodenal enterocytes. *Histochemie*, **35**, 263.

KIGER, N., FLORENTIN, I., GARCIA-GIRALT, E. & MATHÉ, G. (1972) Lymphocyte inhibitory factors (chalones) extracted from lymphoid organs. Extraction, partial purification and immunosuppressive properties. *Transpl. Proc.* **4**, 531.

KIVILAAKSOO, E. & RYTÖMAA, T. (1971) Erythrocytic chalone, a tissue-specific inhibitor of cell proliferation in the erythron. *Cell Tissue Kinet.* **4**, 1.

LAMERTON, L.F. (1963) Late effects of continuous irradiation. *Laval Med.* **34**, 156.

LAMERTON, L.F. (1966) Cell proliferation under continuous irradiation. *Radiat. Res.* **27**, 119.

LAMERTON, L.F., STEEL, G.G. & WIMBER, D.R. (1961) Sensitivity of mammalian tissues to continuous exposure. *Brookhaven Symp. Biol.* **14**, 158.

LOWRY, O.H., ROBERTS, N.R., WU, M.L., HIXON, W.S. & CRAWFORD, E.J. (1954) The quantitative histochemistry of the brain. I. Enzyme measurements. *J. biol. Chem.* **207**, 39.

MEER-FIEGGEN, W. VAN DER (1973) Regulation of cell proliferation and differentiation in intestinal epithelium. Thesis, Erasmus University Rotterdam.

NORDSTRÖM, C., DAHLQVIST, A. & JOSEFSSON, L. (1968) Quantitative determination of enzymes in different parts of the villi and crypts of rat small intestine; comparison of alkaline phosphatase, disaccharidases and depeptidases. *J. Histochem. Cytochem.* **15**, 713.

PILGRIM, C., ERR, W. & MAURER, W. (1963) Diurnal fluctuations in the numbers of DNA synthesizing nuclei in various mouse tissues. *Nature*, **189**, 863.

QUASTEL, M.R. (1963) Continuous irradiation of the rat at high dose rates: a study of jejunal damage, recovery and radioresistance using tritiated thymidine. *Radiat. Res.* **18**, 46.

QUASTLER, H., BENSTED, J.P.M., LAMERTON, L.F. & SIMPSON, S.M. (1959) Adaptation to continuous irradiation: observations on the rat intestine. *Brit. J. Radiol.* **32**, 501.

RIJKE, R.P.C. & GALJAARD, H. (1973) The effect of X-irradiation on the life span of epithelial cells in small intestine. (Abstract). *Int. J. Radiat. Biol.* **24**, 319.

RIJKE, R.P.C., MEER-FIEGGEN, W. VAN DER & GALJAARD, H. (1974) Effect of villus length on cell proliferation and migration in small intestinal epithium. *Cell Tissue Kinet.* **7**, 577.

RYTÖMAA, T. & KIVINIEMI, K. (1968) Control of granulocyte production. I. Chalone and antichalone, two specific humoral regulators. *Cell Tissue Kinet.* **1**, 329.

SAITO, M. (1972) Daily rhythmic changes in brush border enzymes of the small intestine and kidney in rat. *Biochim. biophys. Acta*, **286**, 212.

SIGDESTAD, C.P., BAUMAN, J. & LESHER, S.W. (1969) Diurnal fluctuations in the number of cells in mitosis and DNA synthesis in the jejunum of the mouse. *Exp. Cell Res.* **58**, 159.

SMEJKALOVÁ, E. & SMEJKAL, V. (1973) Cytochemical study of non-specific esterase, acid and alkaline phosphatase in the human thyroid neoplasma. *Acta Histochem.* **46**, 74.

TUTTON, P.J.M. (1973) Control of epithelial cell proliferation in the small intestinal crypt. *Cell Tissue Kinet.* **6**, 211.

WEBSTER, H.L. & HARRISON, D.D. (1969) Enzymic activities during the transformation of crypt to columnar intestinal cells. *Exp. Cell Res.* **56**, 245.

WIMBER, D.R. & LAMERTON, L.F. (1963) Cell population studies on the intestine of continuously irradiated rats. *Radiat. Res.* **18**, 137.

ZAJICEK, G., MICHAELI, I. & WEINREB, M.M. (1972) Kinetics of the inner enamel epithelium in the adult rat incisor during accelerated eruption. *Cell Tissue Kinet.* **5**, 35.

Gastroenterology Publication III

THE EFFECT OF ISCHEMIC VILLUS CELL DAMAGE ON CRYPT CELL PROLIFERATION IN THE SMALL INTESTINE

Evidence for a feedback control mechanism

R.P.C. Rijke, W.R. Hanson, Ph.D., H.M. Plaisier, and J.W. Osborne, Ph.D.

Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois, and Radiation Research Laboratory, College of Medicine, University of Iowa, Iowa City, Iowa

In recent years the hypothesis that the number of villus cells regulates crypt cell proliferation in the epithelium of the small intestine has been brought forward by a number of investigators. To test this hypothesis, the villus cell population was reduced by clamping the superior mesenteric artery and vein in rats for 1 hr and the effects on the intestinal epithelium were studied during the first 24 hr. It was shown that temporary interruption of the blood flow to the small intestine led to a marked decrease in the number of functional

Received January 5, 1976. Accepted May 24, 1976.
This study has been carried out with a grant from The Netherlands Foundation in Medical Research, and was partly supported by Grant AM-15758 from the National Institutes of Health.
The authors gratefully acknowledge Professor H. Galjaard and Professor L.F. Lamerton for the stimulating discussions, criticisms, and advice. The illustrations have been prepared by Mr. P. Hartwijk, Mr. J.G.H. Fengler, and Mr. T.M. van Os.

villus cells within 2 hr; preferentially, cells from the upper part of the villus were lost and the number of crypt cells was not affected. This reduction in the number of villus cells led to an increase in the percentage of labeled crypt cells after pulse labeling with (^{3}H)thymidine, and an expansion of the proliferative cell compartment in the crypt. After a peak of proliferative activity at 16 hr, the investigated crypt cell kinetic parameters approached control values after 24 hr, as did the number of villus cells. The enzyme activities of nonspecific esterase and neutral α-glucosidase showed marked decreases in isolated crypt and villus cell compartments as crypt cell proliferation increased. These data support the hypothesis of a feedback control of crypt cell proliferation by the functional villus cells, and confirm earlier data on the influence of changing cell kinetics on crypt cell maturation. Additional data which were obtained after creating temporary ischemia in part of the small intestine support the view that the feedback control of proliferation by the villus cells is a local control mechanism.

In recent years, the hypothesis of a feedback control of crypt cell proliferation by the functional villus cells in the small intestine has been put forward by a number of investigators (1-4).

Comparing the response of the small intestinal epithelium to X-irradiation in germ-free and normal rats (1), an inverse relation was found between the number of cells per villus column and the percentage of labeled cells per crypt column. The response of rat intestinal epithelium to

various doses of X-irradiation showed the same relationship (2). It was observed that at the time at which there was a reduced number of villus cells after X-irradiation, there was an expansion of the proliferative cell compartment in the crypt (1). In studies using continuous X-irradiation it was found that an expansion of the proliferative cell compartment in the crypt coincided with a decrease in the number of villus cells, even though the percentage of labeled cells per crypt column was normal (3).

X-irradiation as a tool to decrease the number of functional villus cells has the disadvantage of acting via changes in the crypt cell population, the response of which is subsequently studied. Procedures selectively affecting the functional cell compartment, which have already contributed in studies of other cell renewal systems (5-10), are needed to test the hypothesis of a feedback control of cell proliferation in the intestinal epithelium.

A considerable loss of villus cells in the rat small intestine was observed after clamping of the superior mesenteric artery for 1 hr (11). The fast recovery of the villus epithelium which subsequently occurred suggested that the crypt cell population was not damaged, or only to a minor degree. In recent studies on dogs, it was found that clamping of a mesenteric artery and its accompanying vein for 1 hr resulted in extensive desquamation from the villi, whereas the crypts remained relatively intact (12,13).

The purpose of the present study was to investigate the effect of a reduction in the number of villus cells, brought about by temporary ischemia, on the proliferative activity and the localization of the proliferative cell compartment in the crypt. In addition, the activities of nonspecific esterase and neutral α-glucosidase in isolated crypt and villus compartments were measured after temporary

ischemia. Earlier work has shown that these are parameters for intestinal cell maturation that change under altered crypt cell kinetics (3,14-18). Finally, in a number of animals temporary ischemia was created in approximately one-half of the small intestine and observations were made proximal to the damaged area to investigate whether the response is of a local or systemic character.

Materials and Methods

Clamping procedure. Male Glaxo-Wistar rats, weighing approximately 250 g, were subjected to pentobarbital anesthesia (40 mg per kg of body weight, intraperitoneally) after a fasting period of 24 hr and a midline laporotomy was performed.

The small intestine, from ligament of Treitz to cecum, was exteriorized and delivered on a saline-moistened sponge, and the superior mesenteric artery and vein were clamped using Diffenbacher serrefine clamps. After 1 hr the clamp was removed and the small intestine was returned to the peritoneal cavity. The wound was closed in two layers: muscle layer and peritoneum with 3-0 silk and the skin with wound clips. Control animals underwent the same procedure except for the clamping. The animals were returned to cages and were given 5% sucrose.

Tissue preparation. After 2, 8, 16, and 24 hr, segments of small intestine, approximately halfway between the ligament of Treitz and the cecum, were resected under ether anesthesia, 1 hr after the rats had been given intravenous injections of (^{3}H)thymidine, 1 µc per g of body weight (specific activity 15.2 c per mmole, Radiochemical Centre, Amersham, England). At each time interval tissues were removed from 4 animals which underwent clamping and 2 control animals. All animals were killed between 11:00 AM and

12:00 noon.

Autoradiography. Segments of small intestine were fixed in 10% formalin and paraffin sections (4 μ) were processed for dipping autoradiography using Eastman Kodak NTB emulsion. After hematoxylin and eosin staining the number of cells was counted in 20 longitudinally cut crypt columns and villus columns. Also, the number and distribution of labeled cells in the crypt were recorded. For each crypt column the position of the proliferative cell compartment was calculated as a fraction of the total number of cells between bottom and top of the crypt to correct for differences in crypt length (19).

Microchemical enzyme assays. From each animal segments of small intestine were quickly frozen in liquid nitrogen and stored at $-70^{\circ}C$. Cryostat sections (12 μ) were freeze-dried (4 hr at $-25^{\circ}C$, $P < 0.15$ N per m^2), crypts and villi were dissected, and the tissue fragments were weighed on a quartz balance (17). The activity of the nonspecific esterase was determined using naphthyl acetate as a substrate in a final volume of 350 μl (17). The activity of neutral α-glucosidase was determined using 4-methylumbelliferyl-α-D-glucopyranoside as a substrate in a final volume of 520 μl (15). For each animal the enzyme activities were estimated in 10 samples of crypt material and 10 samples of villus material.

Determination of site of villus cell loss. To determine whether preferential cell loss would occur from the upper part of the villus, a number of animals were killed at 24 hr after injection with (^3H)thymidine. One-half of these rats were subjected to clamping between 2 and 3 hr before being killed. After autoradiographic processing, the number of cells per villus column and the cell position of the leading edge of labeled cells along the villus column were determined from tissue sections.

Partial clamping. In a number of animals the mesenteric blood vessels of approximately 50% of the small intestine (30 to 80 cm distal to the ligament of Treitz) were clamped for 1 hr in a way similar to that described above. After 16 hr, segments of the nonclamped intestine were resected, immediately distal from the ligament of Treitz. In addition, segments of the clamped part, approximately halfway along the small intestine, were resected. One hour before being killed, the animals were injected with (^{3}H)thymidine. After autoradiographic processing the cell numbers, labeling index, and position of the leading edge of labeled cells in the crypt were determined in 20 longitudinally cut crypt and villus columns. The same procedure was followed for control animals except for the clamping.

Results

Proliferative activity in the crypt. At various time intervals after clamping of the superior mesenteric artery and vein, radioautographs of small intestine were prepared after pulse labeling with (^{3}H)thymidine (fig. 1).

The number of epithelial cells per longitudinally cut crypt column and villus column at various periods after clamping are presented in figure 2. The number of cells per crypt column, except for minor variations, was not influenced by the interruption of blood flow to the small intestine. The number of cells per villus column, however, showed a considerable decrease to about 50% of the control value at 2 hr after clamping. At 8 hr after clamping the number of cells per villus column was still markedly reduced, but increased subsequently to approach control values at 16 and 24 hr after clamping.

The villus cell position of the leading edge of labeled cells at 24 hr after pulse labeling with tritiated thymi-

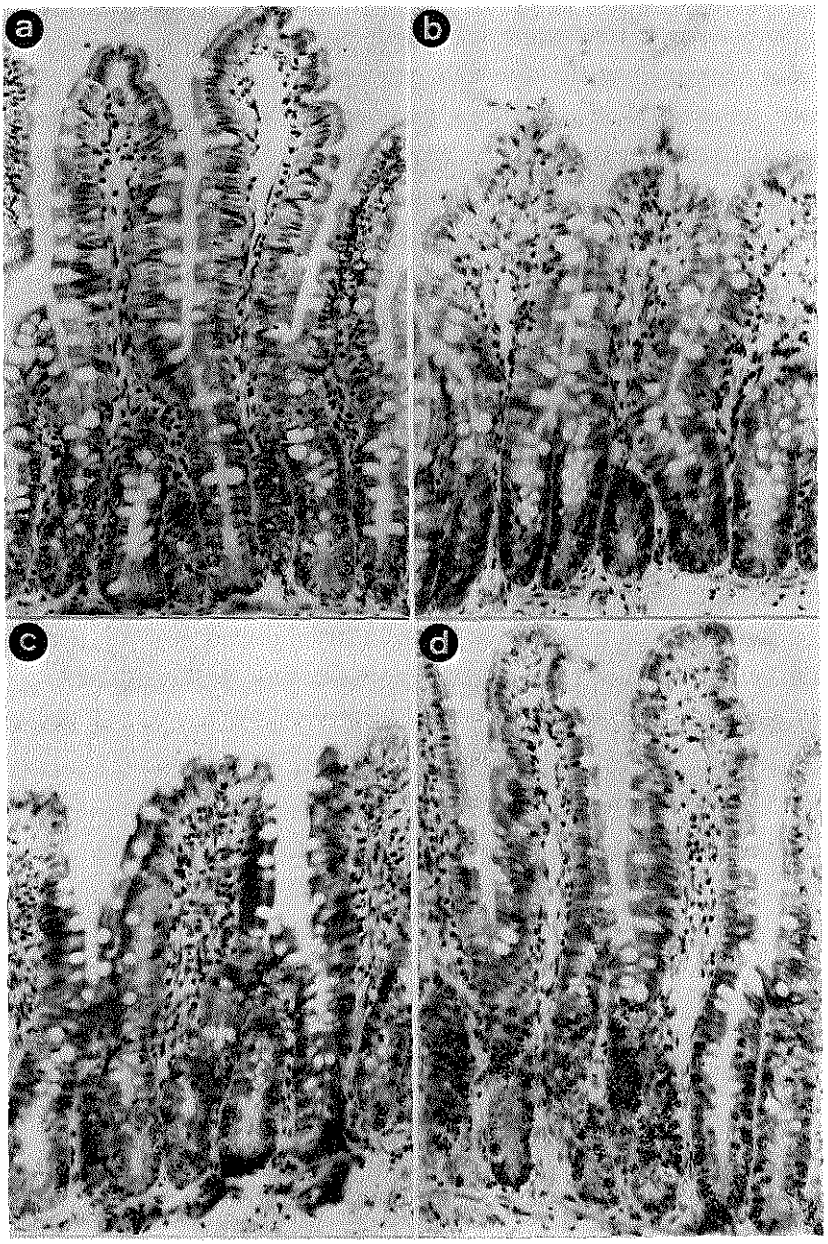

Fig. 1. Photomicrographs of the small intestinal epithelium of control rats (a), and at 2 (b), 8 (c),and 16 hr (d) after temporary ischemia. All photomicrographs were taken at the same magnification.

dine and the number of cells per villus column for animals that underwent clamping (2 to 3 hours before being killed) and for control animals are presented in figure 3. The number of cells per villus column showed a considerable decrease, comparable with the data in figure 2. However, there was no difference in cell position of the leading edge of labeled cells along the villus column at 24 hr after labeling (control: 16 $\pm$ 2; after clamping: 17 $\pm$ 1).

The percentage of labeled cells per crypt column, as illustrated in figure 4, already showed a significant increase above control values at 8 hr after clamping and reached a maximum value after 16 hr. At 24 hr the percentage of labeled crypt cells was markedly reduced again to approach control values.

The mean value for the highest cell position of labeled cells along the crypt column (leading edge), determined 1 hr after pulse labeling with (^{3}H)thymidine, is presented in table 1. A distinct expansion of the proliferative cell compartment in the crypt was observed at 8 and 16 hr after

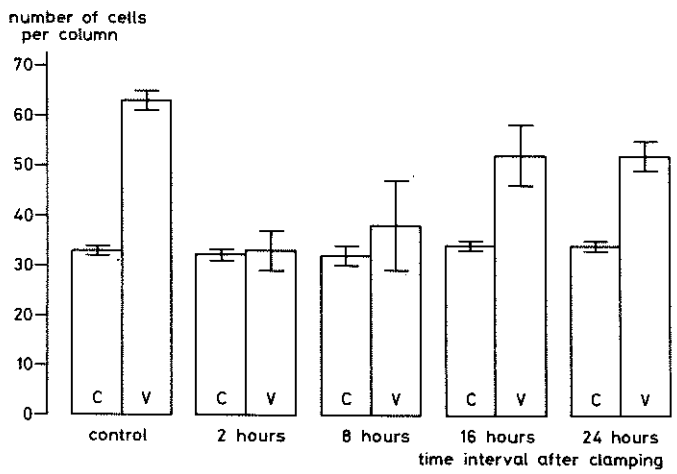

Fig. 2. Number of cells per crypt column (C) and villus column (V) after temporary ischemia. Each value represents the mean ($\pm$ SD) of 4 animals, except for the control which is the mean of 8 animals.

clamping of the superior mesenteric artery and vein, whereas the number of cells per crypt column was normal (fig. 2). After 24 hr the localization of the proliferative cell compartment resembled again the localization in control animals.

Next, the labeling index for each crypt cell position was determined to investigate the distribution of labeled cells along the crypt column. Figure 5 illustrates the percentage of labeled cells for each crypt cell position, expressed in fractional distance from the bottom of the crypt to the crypt mouth. Each crypt column was standardized in order to exclude a possible influence of the number of cells per crypt column. At 8 and 16 hr after clamping there is an expansion of the proliferative cell compartment in the crypt, the labeled cells occupying 70% of the crypt length compared with 50% in control animals. At 2 and 24 hr after clamping the distribution of labeled cells along the crypt column is almost identical to that in control animals. At all time intervals there is a slight increase in the percentages of labeled cells in those cell positions which, under normal circumstances, are completely involved in cell proliferation, i.e., the lower half of the crypt.

Table 1. Leading edge of labeled cells along the crypt column (1 hr after labeling with (^{3}H)thymidine) after temporary clamping of mesenteric blood vessels

Time interval after clamping	Highest labeled cell position+	SD	No. of animals
Control	0.53	0.04	8
2 hours	0.56	0.02	4
8 hours	0.67	0.08	4
16 hours	0.73	0.04	4
24 hours	0.61	0.02	4

+ Expressed as fraction of total number of cells from bottom to top of crypt.

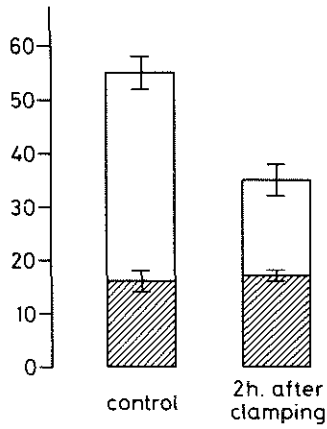

Fig. 3. Ordinate: villus cell position. Total length of columns represents the number of cells per villus column and length of shaded parts represents the leading edge of labeled cells on the villus column, 24 hr after labeling with (^{3}H)thymidine. Each value represents the mean (± SD) of 4 animals.

Enzyme activities in crypt and villus as parameters for cell maturation. The activities of nonspecific esterase and neutral α-glucosidase in crypts and villi, dissected from freeze-dried cryostat sections, after clamping of the superior mesenteric artery and vein, are illustrated in figures 6 and 7, respectively.

At 2 hr after clamping no change was observed in the ac-

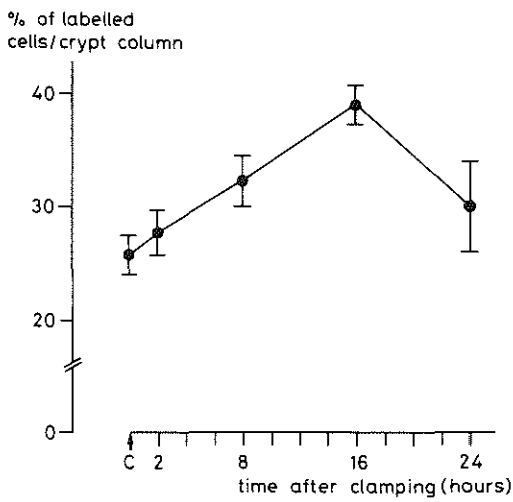

Fig. 4. Percentage of labeled cells per crypt column after temporary ischemia. Control values represent mean (± SD) of 8 animals, other values of 4 animals.

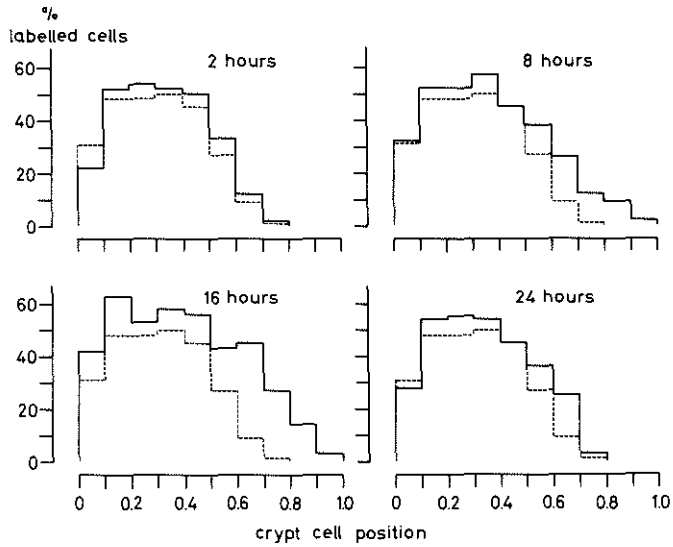

Fig. 5. Localization of the proliferative cell compartment in the crypt at various time intervals after temporary clamping of mesenteric blood vessels. Each distribution is based on data of 4 animals, control (dotted lines) on data of 8 animals.

tivity of nonspecific esterase in the crypt or villus compartment (fig. 6). The activity of nonspecific esterase in the crypt cells was decreased at 8 hr after clamping and remained low. The villus cells also showed a similar decrease in nonspecific esterase activity, but to a lesser degree.

The pattern of activity of neutral α-glucosidase (fig. 7) was approximately the same as that of nonspecific esterase. Again, a decrease in activity was observed from 8 hr on after clamping, although some increase in activity was noted at 24 hr.

Partial clamping. The blood vessels of approximately 50% of the middle and distal part of the small intestine were clamped for 1 hr. After 16 hr tissues were resected, 1 hr after pulse labeling with (^{3}H)thymidine, and radioautographs were prepared to investigate whether the immediate

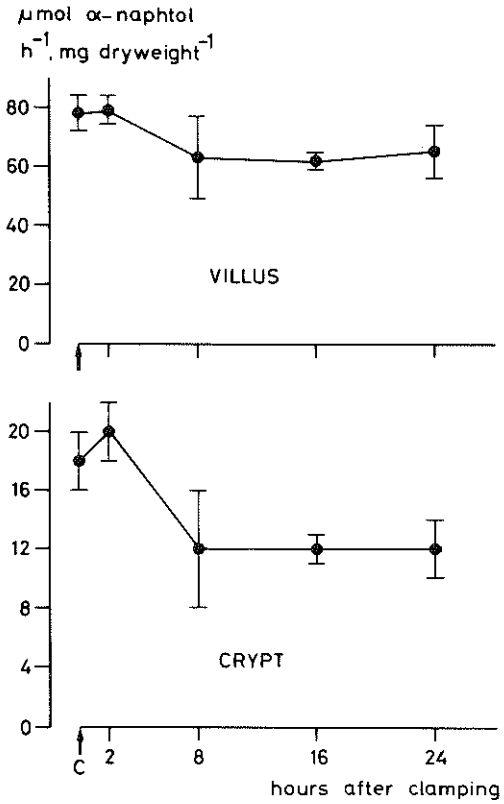

Fig. 6. Enzyme activity of nonspecific esterase in isolated crypts and villi at various time intervals after intestinal blood flow interruption. Each point represents the mean (± SD) of 4 animals, control of 8 animals.

response of the intestinal epithelium is of a local or systemic nature. The results, presented in table 2, show that in the ischemic intestinal epithelium in the midportion of the small intestine the villus length was reduced, the percentage of labeled crypt cells was increased, and the proliferative cell compartment, as judged from the leading edge of labeled cells along the crypt column, was expanded. These changes are identical to the response after clamping of the superior mesenteric artery and vein (figs. 2 and 4 and table 1). In the jejunal epithelium,

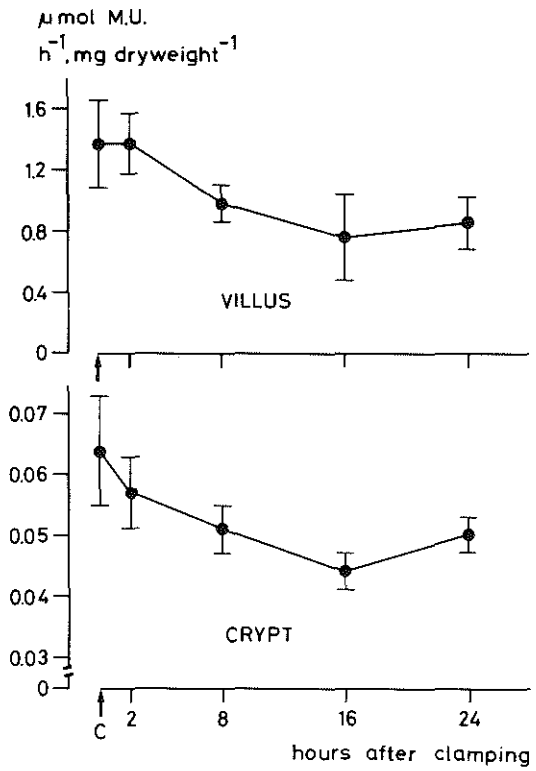

Fig. 7. Enzyme activity of neutral α-glucosidase in isolated crypts and villi at various time intervals after intestinal blood flow interruption. Each point represents the mean (± SD) of 4 animals, control of 8 animals.

taken immediately distal from the ligament of Treitz where the blood flow was not restricted, there were no differences in the determined parameters compared to control values (table 2).

Discussion

The intestinal epithelium lining the crypts and villi of the small intestine consists of a number of cell types which seem to originate from the same precursor cell and of which the columnar absorptive cell is the major compo-

Table 2. Cell kinetics in intestinal epithelium at different sites of small intestine 16 hr after temporary interruption of the blood flow to middle and lower part of small intestine+

Group	No. of cells per column crypt	No. of cells per column villus	% of labeled crypt cells	leading edge of labeled cells (fraction of crypt column)
3 cm distal from lig. of Treitz				
- control	33 ± 1	76 ± 2	28.6 ± 1.8	0.51 ± 0.01
- partial clamping	33 ± 1	73 ± 2	25.7 ± 2.5	0.52 ± 0.04
mid-small intestine				
- control	33 ± 1	64 ± 2	25.3 ± 1.7	0.53 ± 0.05
- partial clamping	32 ± 1	49 ± 2	38.2 ± 4.2	0.73 ± 0.04

+ Each value represents the mean (± SD) of 3 animals.

nent (20-24). The intestinal epithelium is constantly renewed through proliferation in the lower half of the crypt with an extremely short cell cycle time, followed by cell migration to the villus and extrusion from the villus top (25-29).

The steady state growth in the intestinal epithelium seems to be influenced by a number of factors (30), and may be perturbed by a variety of experimental procedures (16, 19,31-36). However, little is known about the way(s) in which cell proliferation is regulated in the intestinal epithelium (37).

Celiac disease in man presents a situation in which compensation for an increased cell loss from the villi seems to occur through increased proliferative activity in the crypts (38,39). In a number of experimental studies it was suggested that the number of functional villus cells is involved in the regulation of crypt cell proliferation (1-4). Using a single dose of X-irradiation to perturb the steady

state, an inverse relationship was found between the number of villus cells and the proliferative activity in the crypt (1,2). In a later study, using continuous irradiation, a reduction in the number of villus cells was accompanied by an expansion of the proliferative cell compartment in the crypt, despite the fact that the labeling index remained normal (3). Changes in crypt cell proliferation also affect the activity of a number of enzymes involved in intestinal function (1,3,14-18) and goblet cell differentiation (40) The development of the ultrastructure during crypt cell maturation remains relatively unaffected under altered crypt cell kinetics (14).

Interruption of the intestinal blood supply for a short period of time leads to a loss of villus cells, whereas the crypts seem to be relatively unaffected (11-13,41,42). The difference in sensitivity to ischemia between crypt and villus cells may be based on the specific pattern of mucosal oxygen supply (43,44). Therefore, temporary clamping of the intestinal blood vessels seemed to be a useful tool to test the hypothesis of a feedback control of proliferation by the villus cell population.

The present results demonstrate that temporary intestinal blood flow interruption led to a 50% decrease in the number of cells per villus column after 2 hr, whereas the number and proliferative activity of the crypt cells remained unaffected (figs. 1,2, and 4). It was also shown that villus cell loss occurred especially from the upper part of the villus (fig. 3). After clamping, the percentage of labeled cells per crypt column and the relative size of the proliferative cell compartment increased rapidly above control values, to reach a maximum after 16 hr (figs. 1,4, and 5, and table 1).

Cells, which normally leave the mitotic cell cycle halfway up the crypt (25), will now go through one more cell

cycle when the number of villus cells is reduced. A similar effect was also found during recovery after X-irradiation when the number of villus cells was decreased (1,2)

In the present study an expansion of the proliferative cell compartment in the crypt was found to occur within 8 hr after temporary ischemia (fig. 5 and table 1). After X-irradiation, a similar expansion of the proliferative cell compartment was observed; however, 48 hr elapsed (1), which correlates with the time interval needed before a considerable reduction of the number of villus cells occurs (2). Therefore, it is most likely that the number of villus cells, and not the number of crypt cells, is involved in the regulation of the relative size of the proliferative cell compartment in the crypt.

In the present experiments the activities of nonspecific esterase and neutral α-glucosidase were not changed at 2 hr after temporary intestinal ischemia (figs. 6 and 7). However, both enzyme activities, especially in the crypt cell compartment, are markedly reduced after 8 hr and do not recover within the first 24 hr. This decrease is probably caused by an increase in crypt cell proliferation, as in earlier studies an inverse relationship was observed between nonspecific esterase activity in the crypt and the size of the proliferative cell compartment (3,18). For the neutral α-glucosidase activity such a relationship has not been found, although it is influenced by changes in crypt cell proliferation (3,15,18). These findings, together with the ones presently reported, indicate that a reduction of the villus cell population affects crypt cell maturation, possibly as a result of changing cell kinetics.

Creating temporary ischemia in a large part of the small intestine was shown, in the present study, not to lead to changes in proliferative activity in the adjacent normal intestine (table 2). This suggests that the response of the

crypt cell population to a reduction of the villus cell population is not a generalized one, but of a local character.

The data seem to indicate that the villus cell population is involved in the regulation of crypt cell proliferation by influencing the size of the proliferative cell compartment, which also affects crypt cell maturation. In addition, data were obtained substantiating that this control mechanism is of a local character.

Such a mechanism has also been found in a number of tissues and organs (45). Control of proliferation may be mediated by the so-called chalones, and for epidermis this seems to be well established (7,45).

In rat intestinal epithelium a chalone-like fraction, which was found to hold up crypt cells in the G2-phase, has been isolated from crypt cells (46). Recently the existence of two chalone-like fractions affecting cells in the G1- and G2-phases of the cell cycle was described for the newt (47).

In addition to the possibility that one or more chalones may be isolated from the small intestinal epithelium, it seems important to investigate the way(s) in which these act during disturbances of the steady state growth of the intestinal epithelium. In addition, other factors, such as intercellular communication in the epithelial layer (48) and the role of subepithelial fibroblasts in the control of proliferation (30,45,49,50), should be studied in relation to tissue damage and tissue recovery.

References

1. Galjaard H, Meer-Fieggen W van der, Giesen J: Feedback control by functional villus cells on cell proliferation and maturation in intestinal epithelium. Exp Cell Res 73:197-207, 1972
2. Rijke RPC, Meer-Fieggen W van der, Galjaard H: Effect of villus length on cell proliferation and migration in small intestinal epithelium. Cell Tissue Kinet 7:577-586, 1974

3. Rijke RPC, Plaisier H, Hoogeveen AT, et al: The effect of continuous irradiation on cell proliferation and maturation in small intestinal epithelium. Cell Tissue Kinet 8:441-453, 1975
4. Sato F, Muramatsu S, Tsuchihashi S, et al: Radiation effects on cell populations in the intestinal epithelium of mice and its theory. Cell Tissue Kinet 5:227-235, 1972
5. Hanna IRA: Response of early erythroid precursors to bleeding. Nature 214:355-357, 1967
6. Hennings H, Elgjo K: Epidermal regeneration after cellophane tape stripping of hairless mouse skin. Cell Tissue Kinet 3:243-252, 1970
7. Iversen OH, Bhangoo KS, Hansen K: Control of epidermal cell renewal in the bat web. Virchows Arch (Zellpathol) 16:157-179, 1974
8. Pinkus H: Examination of the epidermis by the strip method of removing horny layers. J Invest Dermatol 16:383-386, 1951
9. Potten CS, Allen TD: The fine structure and cell kinetics of mouse epidermis after wounding. J Cell Sci 17:413-447, 1975
10. Zajicek G, Michaeli Y, Weinreb MM: Kinetics of the inner enamel epithelium in the adult rat incisor during accelerated eruption. Cell Tissue Kinet 5:35-39, 1972
11. Cameron GR, Khanna SD: Regeneration of the intestinal villi after extensive mucosal infarction. J Pathol Bacteriol 77:505-511, 1959
12. Robinson JWL, Mirkovitch V: The recovery of function and microcirculation in small intestinal loops following ischaemia. Gut 13:784-789, 1972
13. Robinson JWL, Haroud M, Mirkovitch V: The recovery of structure and function of the intestine following acute ischaemia. In Intestinal Adaptation. Edited by RH Dowling, EO Riecken. New York, Schattauer Verlag, 1974, p 139-147
14. Both NJ de, Dongen JM van, Hofwegen B van, et al: The influence of various cell kinetic conditions on functional differentiation in the small intestine of the rat. A study of enzymes bound to subcellular organelles. Dev Biol 38: 119-137, 1974
15. Both NJ de, Plaisier H: The influence of changing cell kinetics on functional differentiation in the small intestine of the rat. A study of enzymes involved in carbohydrate metabolism. J Histochem Cytochem 22:352-360, 1974
16. Galjaard H, Bootsma D: The regulation of cell proliferation and differentiation in intestinal epithelium. II. A quantitative histochemical and autoradiographic study after low doses of X-irradiation. Exp Cell Res 58:79-92, 1969
17. Galjaard H, Buys J, Duuren M van, et al: A quantitative histochemical study of intestinal mucosa after X-irradiation. J Histochem Cytochem 18:291-301, 1970
18. Galjaard H, Meer-Fieggen W van der, Both NJ de: Cell differentiation in gut epithelium. In Cell Differentiation. Edited by D Viza, H Harris. Copenhagen, Munksgaard, 1972, p 322-328
19. Cairnie AB: Cell proliferation studies in the intestinal epithelium of the rat: response to continuous irradiation. Radiat Res 32:240-264, 1967

20. Cheng H, Leblond CP: Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. I. Columnar cell. Am J Anat 141:461-480, 1974
21. Cheng H: Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. II. Mucous cells. Am J Anat 141:481-502, 1974
22. Cheng H, Leblond CP: Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. III. Entero-endocrine cells. Am J Anat 141:503-520, 1974
23. Cheng H: Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. IV. Paneth cells. Am J Anat 141:521-536, 1974
24. Cheng H, Leblond CP: Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. V. Unitarian theory of the origin of the four epithelial cell types. Am J Anat 141:537-562, 1974
25. Cairnie AB, Lamerton LF, Steel GG: Cell proliferation studies in the intestinal epithelium of the rat. I. Determination of the kinetic parameters. Exp Cell Res 39:528-538, 1965
26. Cairnie AB, Lamerton LF, Steel GG: Cell proliferation studies in the intestinal epithelium of the rat. II. Theoretical aspects. Exp Cell Res 39:539-553, 1965
27. Leblond CP, Messier B: Renewal of chief cells and goblet cells in the small intestine as shown by radioautography after injection of thymidine-H^3 into mice. Anat Rec 132:247-259, 1958
28. Quastler H, Sherman FG: Cell population kinetics in the intestinal epithelium of the mouse. Exp Cell Res 17:420-438, 1959
29. Schultze B, Haack V, Schmeer AC, et al: Autoradiographic investigation on the cell kinetics of crypt epithelia in the jejunum of the mouse. Cell Tissue Kinet 5:131-145, 1972
30. Sprinz H: Factors influencing intestinal cell renewal. A statement of principles. Cancer 28:71-74, 1971
31. Altmann GG: Influence of bile and pancreatic secretions on the size of the intestinal villi in the rat. Am J Anat 132:167-178, 1971
32. Clarke RM: Control of intestinal epithelial replacement: lack of evidence for a tissue-specific blood-borne factor. Cell Tissue Kinet 7:241-250, 1974
33. Dowling RH, Gleeson MH: Cell turnover following small bowel resection and by-pass. Digestion 8:176-190, 1973
34. Hanson WR, Osborne JW: Epithelial cell kinetics in the small intestine of the rat 60 days after resection of 70 per cent of the ileum and jejunum. Gastroenterology 60:1087-1097, 1971
35. Hanson WR: Intestinal compensation following intestinal resection in the rat. Ph.D. thesis. University of Iowa, 1975
36. Lesher S: Compensatory reactions in intestinal crypt cells after 300 roentgens of cobalt-60 gamma irradiation. Radiat Res 32:510-519, 1967
37. Galjaard H: Some unresolved questions relevant to intestinal adaptation. In Intestinal Adaptation. Edited by RH Dowling, EO Riecken. New York, Schattauer Verlag, 1974, p 263-268

38. Trier JS, Browning TH: Epithelial-cell renewal in cultured duodenal biopsies in celiac sprue. N Engl J Med 283:1245-1250, 1970
39. Wright N, Watson A, Morley A, et al: The measurement of cell production rates in the crypts of Lieberkuhn. An experimental and clinical study. Virchows Arch (Pathol Anat) 364:311-323, 1974
40. Dongen JM van, Kooyman J, Visser WJ: The influence of 400 R X-irradiation on the number and the localization of mature and immature goblet cells and Paneth cells in intestinal crypt and villus. Cell Tissue Kinet 9:65-75, 1976
41. Robinson JWL, Haroud M, Winistofer B, et al: Recovery of function and structure of dog ileum and colon following two hours' acute ischaemia. Eur J Clin Invest 4:443-452, 1974
42. Rottger P, Oran M: Histochemische und biochemische Untersuchungen zur Frage des Enzym-Gehaltes der Dunndarmschleimhaut nach temporarer Ischaemie. Verh Dtsch Ges Pathol 53:189-195, 1969
43. Kampp M, Lundgren O, Nilsson NJ: Extravascular shunting of oxygen in the small intestine of the cat. Acta Physiol Scand 72:396-403, 1968
44. Lundgren O: The circulation of the small bowel mucosa. Gut 15:1005-1013, 1974
45. Bullough WS: Mitotic control in adult mammalian tissues. Biol Rev 50:99-127, 1975
46. Tutton PJM: Control of epithelial cell proliferation in the small intestinal crypt. Cell Tissue Kinet 6:211-216, 1973
47. Brugal G, Pelmont J: Existence of two chalone-like substances in intestinal extract from the adult newt, inhibiting embryonic intestinal cell proliferation. Cell Tissue Kinet 8:171-187, 1975
48. Loewenstein WR: Membrane junctions in growth and differentiation. Fed Proc 32:60-64, 1973
49. Marsh MN, Trier JS: Morphology and cell proliferation of subepithelial fibroblasts in adult mouse jejunum. I. Structural features. Gastroenterology 67:622-635, 1974
50. Marsh MN, Trier JS: Morphology and cell proliferation of subepithelial fibroblasts in adult mouse jejunum. II. Radioautographic studies. Gastroenterology 67:636-645, 1974

Gastroenterology Publication IV

THE INFLUENCE OF EXPERIMENTAL BY-PASS ON CELLULAR KINETICS AND MATURATION OF SMALL INTESTINAL EPITHELIUM

R.P.C. Rijke, H.M. Plaisier, H. de Ruiter, M.D., Ph.D., and H. Galjaard, M.D., Ph.D.

Dept. of Cell Biology and Genetics, Erasmus University Rotterdam, The Netherlands

The effect of experimental by-pass on the intestinal epithelium was investigated from 2 to 360 days after creating a Thiry-Vella fistula. Within 7-14 days the number of cells per villus column and, to a lesser degree, the number of cells per crypt column decreased and subsequently these values remained constant up to one year. Autoradiography did not show any changes in the percentage of labelled cells per crypt column, after incorporation of (^{3}H)-thymidine. Also the relative size of proliferative cell compartment in the crypt, the number of crypts per unit length of small intestine and the crypt-villus ratio remained unchanged. Scintillation counting of isolated crypts from the Thiry-Vella fistulas showed that the number of cells per crypt was markedly reduced. Scanning electronmicroscopy revealed that the three-dimensional size of the villi was also reduced. The

Received July 23, 1976.
This study has been carried out with a grant from The Netherlands Foundation in Medical Research.
The authors gratefully acknowledge Dr. W.R. Hanson for his stimulating criticisms and advice, Mr. W.J. Visser and Mr. J. Bos for technical assistence, and Mr. N.H.C. Brons and Dr. W. van Ewijk for preparation of the scanning electron micrographs.

activities of non-specific esterases and neutral α-glucosidase remained unchanged in crypt cells and were above control values in villus cells. The development of these biochemical characteristics of the villus cells seems to be independent of the luminal contents. It was concluded that deprivation of food passage and gastric, pancreatic and liver secretions from a small intestinal segment primarily causes a reduction in the number of cells per crypt, which leads to a lower cell production. In turn, this is responsible for the reduction of the functional villus cell compartment. Arguments are presented for the hypothesis that crypt cell proliferation is regulated by at least two different control mechanisms.

The epithelium of the small intestine is known to be influenced by a wide variety of factors, but little is known about the way(s) in which the functional capacity and the continuous cell renewal is regulated (1,2). Intraluminal factors, like pancreatic secretions, food substances, bile and bile products have been shown to cause considerable changes in the small intestinal epithelium (3-15).

The functional capacity of the small intestine depends to a large extent on the number of functional cells on the villus and on the degree of maturation of these cells. The number of functional villus cells is determined by the cell production in the crypts and by the life span of the epithelial cells. A change in functional capacity of the small intestine may thus be caused by changes in many parameters, like the number of cells per crypt and villus, the number of crypts and villi per unit length of small intestine, the proliferative activity in the crypt and maturation of the epithelial cells.

The isolation of a small segment of jejunum into a permanent Thiry-Vella fistula, without affecting its nerve and blood supply, seems to be an attractive model to study the influence of luminal contents on the various parameters of the intestinal epithelium, as mentioned above.

Earlier reports about the by-pass of a segment of small intestine showed that the microscopic changes were relatively minor compared with the gross alterations in macroscopic appearance (10). These authors observed only slight changes in villus and crypt length in Thiry-Vella fistulas (10), whereas others reported a considerable decrease in the length of villus and crypt after experimental by-pass (6). Also a decrease in villus length has been reported to be accompanied by an increase in crypt length (15). However, in the various studies the surgical procedures and the size of the segment differed to a large extent. The proliferative activity was found to be decreased in by-passed small intestine (4,15), whereas the migration rate and the life span of the epithelial cells were not affected (10). Enzyme activities in by-passed small intestine were found to be unchanged (11,13,16).

In the present study an attempt was made to extend the observations of changes in the small intestinal epithelium following by-pass, and to clarify the underlying mechanisms. Cell kinetics and cell maturation in jejunal Thiry-Vella fistulas were studied from 2 to 360 days following by-pass. Also the three-dimensional size of crypt and villus, the number of crypts and villi per unit length of small intestine and the migration rate and life span of the epithelial cells were studied following experimental by-pass.

Materials and Methods

Male Glaxo-Wistar rats, weighing approximately 200 g at the time of surgery were used and all animals were sacrificed between 10.00 and 11.00 hours.

Surgical procedure and tissue sampling

Animals were subjected to pentobarbital anesthesia (40 mg/kg of body weight, i.p.), after a fasting period of 24 hours, and the abdomen was opened through a midline incision. The small intestine was divided at approximately 5 cm distal to the ligament of Treitz and again at 12-13 cm. The remaining small intestine was anastomosed, mucosa to mucosa, using 6/0 silk suture and the serosa was pulled over the anastomosis with a number of interrupted sutures. The ends of the dissected segment were temporarily closed, brought to the abdominal surface through separate stab wounds and fixed to the abdominal wall to create a permanent Thiry-Vella fistula. The midline incision was closed in two layers using interrupted 3/0 silk sutures. During the first two days following surgery, 5% sucrose was given to the rats, after which the animals resumed the standard pellet diet.

At intervals of 2,3,7,14,30,60,90,120,180,270 and 360 days after surgery, 2 or 3 animals were injected intraperitoneally with (^{3}H)-thymidine, 0.5 µCi per gram body weight (Amersham, specific activity 15.2 Ci/mmole). After 30 minutes the animals were lightly anesthesized with ether, their abdomens opened and segments were removed from the Thiry-Vella fistulas. At 2,120,180 and 360 days after surgery, control segments were removed from the small intestine in continuity at approximately 3 cm distal to the anastomosis. Also intestinal segments at 5-10 cm distal to the ligament of Treitz were removed from control animals of the same age as the animals with a Thiry-Vella fistula

which were sacrificed at 2,120,180 and 360 days after surgery.

Cellular kinetics

The small intestinal segments were fixed in 10% formalin and paraffin sections (4 µm) were processed for dipping autoradiography using K2 Ilford emulsion. After hematoxylin and eosin staining, the number of cells was determined in 20 longitudinally cut crypt and villus columns. Also the percentage of labelled cells per crypt column and their positions along the crypt column were recorded. The positions of the labelled cells were calculated for each crypt column as fractions of the total number of cells between bottom and top of the crypt to correct for possible influences of differences in crypt length (17). The leading edge of labelled cells along the crypt column was recorded as described earlier (18).

To determine the migration rate of the epithelial cells, a number of animals was injected with (^{3}H)-thymidine at about 4 months after surgery. At intervals of 12,24 and 36 hours, segments were removed from the Thiry-Vella fistula and from the small intestine in continuity at approximately 3 cm distal to the anastomosis. After fixation in 10% formalin, paraffin sections (4 µm) were processed for dipping autoradiography. After hematoxylin and eosin staining the position of the leading edge of labelled cells was determined along 20 longitudinally cut villus columns per tissue.

Three-dimensional parameters

A number of animals was injected intravenously with (^{3}H)-thymidine, 1 µCi per gram body weight, at approximately 4 months after surgery. One hour later segments were removed from the Thiry-Vella fistula and from the small intestine in continuity at approximately 3 cm distal to the

anastomosis.

The total proliferative activity per crypt was determined according to Hagemann et al. (19). From each tissue 150 crypts were isolated and transferred to 3 liquid scintillation vials (50 to each vial), containing 0.5 ml Soluene (Packard) for solubilization. After 48 hours 15 ml scintillation mixture (5 g PPO plus 0.5 g POPOP/l toluene) was added. The samples were counted in a liquid scintillation spectrometer (Packard) and the results were expressed as disintegrations per minute (dpm's) per crypt.

From the same animals, about 2 cm long segments were tied off at both ends after intraluminal injection of physiological saline at a hydrostatic pressure of 33 cm of water. A weight of 25 g was attached to one end, three segments with a length of exactly 4 mm were simultaneously resected and transferred to liquid scintillation vials (one for each segment). After drying in an oven at 60^{o}C for 24 hours, 1 ml Soluene was added to each vial and 48 hours later 15 ml scintillation mixture. The samples were counted in a liquid scintillation spectrometer, and the results were expressed as dpm's per cm of small intestine. By dividing these values by the number of dpm's per crypt for each tissue, the number of crypts per unit length of small intestine can be calculated.

The number of crypts per villus was determined according to Clarke (20) in intestinal segments, which were resected from Thiry-Vella fistulas and small intestine in continuity (appr. 3 cm distal to the anastomosis) at 4 months after surgery. The number of crypts and the number of villi per unit of serosal surface were determined under the microscope.

To study the three-dimensional shape of the villi, segments from Thiry-Vella fistulas and small intestines in continuity (appr. 5 cm distal to the anastomosis) were pre-

pared for scanning electron microscopy, 4 months after surgery. The tissues were fixed in Carnoy's solution at 4°C for 24 hours, dehydrated in graded series of ethanol, transferred to amylacetate and dried in a critical point drying apparatus. The tissues were mounted on specimen stages, covered with gold using a sputter coater and examined in a Cambridge MK II A scanning electron microscope.

Enzymatic assays

Intestinal segments from all animals and locations, mentioned under tissue sampling, were quickly frozen in liquid nitrogen and stored at -70°C. Cryostat sections (12 µm) were freeze-dried (4 hr at -25°C, $p < 0.15$ N/m^2), crypt and villus compartments were dissected under the microscope and the tissue fragments were weighed on a quartz balance (21). The activity of non-specific esterases was determined spectrophotometrically using naphthyl acetate as a substrate in a final volume of 350 µl (21). The activity of neutral α-glucosidase was measured fluorometrically using 4-methylumbelliferyl-α-D-glucopyranoside as a substrate at pH 6.5 in a final volume of 520 µl (16). For each tissue, the enzyme activities were determined in 10 samples of dissected crypts and 10 samples of dissected villi.

Results

Cell compartment sizes and proliferative activity

The number of epithelial cells per crypt column and per villus column at various time intervals after establishing a jejunal Thiry-Vella fistula is illustrated in fig. 1. The number of cells per villus column in the jejunum of control animals of various ages and in the jejunum in continuity of animals that underwent surgery does not show any differences. In the Thiry-Vella fistula the number of cells per villus column decreases within 1 week to about 80% of the control values and subsequently remains constant up to one year after surgery. The number of cells per crypt column only shows a slight decrease in the Thiry-Vella fistula to reach constant values of 29-30 cells per crypt column, com-

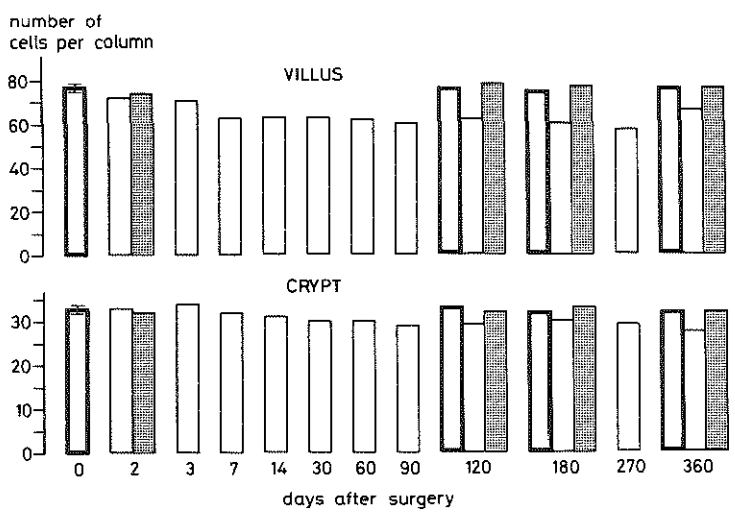

Fig. 1. Number of cells per crypt column and villus column at various time intervals after experimental by-pass.
☐ Thiry-Vella fistula, ▦ jejunum in continuity, ☐ jejunum of age controls.
Each value represents the mean of 2-3 animals, control values represent the mean (± SD) of 5 animals.

Table 1. Proliferative activity in jejunal crypts after experimental by-pass.[x]

days after surgery	percentage of labelled cells per crypt column			leading edge of labelled cells along crypt column[+]		
	T.V.-fistula	jejunum in continuity	jejunum of age controls	T.V.-fistula	jejunum in continuity	jejunum of age controls
control			28.0 ± 1.5			0.53 ± 0.02
2	27.3	29.1		0.60	0.62	
3	24.0			0.54		
7	29.3			0.55		
14	22.6			0.51		
30	24.5			0.49		
60	26.7			0.52		
90	28.0			0.51		
120	23.8	27.7	28.4	0.50	0.56	0.52
180	24.8	25.7	27.9	0.50	0.52	0.51
270	23.7			0.51		
360	25.5	28.8	26.2	0.52	0.53	0.53

[x] Each value represents the mean of 2-3 animals, control values represent the mean (± SD) of 5 animals.

[+] Expressed as fraction of number of cells from bottom to top of crypt.

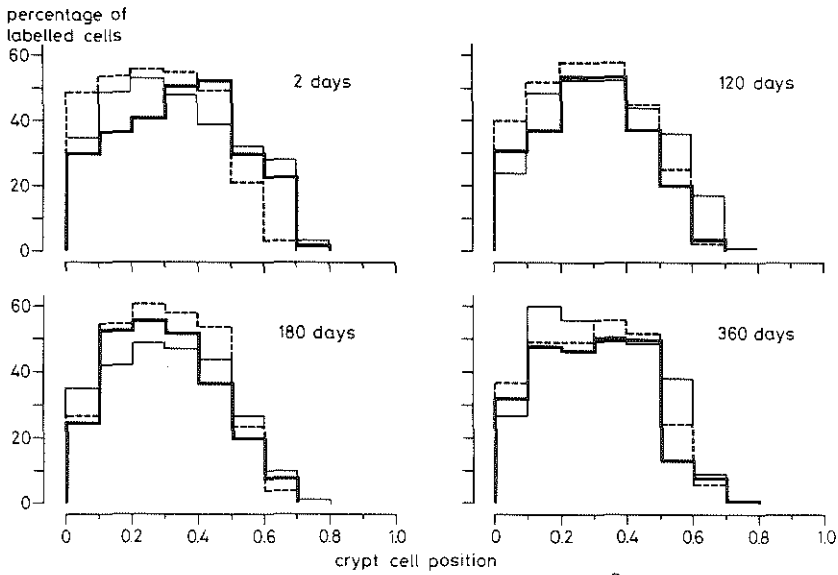

Fig. 2. Distribution of labelled cells after (^{3}H)-thymidine labelling in jejunal crypt columns at various time intervals following experimental by-pass.
——— Thiry-Vella fistula, ——— jejunum in continuity,
------- jejunum in age controls.
Each distribution is based on data from 2-3 animals.

pared to 32-33 cells per column in the jejunum of control animals and in the jejunum in continuity of operated rats.

The percentage of labelled cells per crypt column, half an hour after (^{3}H)-thymidine injection, was determined in the Thiry-Vella fistula and in the jejunum in continuity at various time intervals after surgery. The results are given in table 1, together with the values of the age controls. No consistent changes can be observed between the various controls and the Thiry-Vella fistulas. The relative size of the proliferative cell compartment in the crypt, estimated by determining the leading edge of labelled cells along the crypt column, is also given in table 1. In the first days following surgery there is a slight expansion of the proliferative cell compartment in the Thiry-Vella fistula as well as in the jejunum in continuity. At all other time in-

Table 2. Epithelial cell migration on villi after experimental by-pass.+

hours after ^{3}H-thymidine	Thiry-Vella fistula	jejunum in continuity
12	0.03	0.02
24	0.3	0.2
36	0.7	0.5

+ Values represent cell position of leading edge of labelled cells, expressed as fraction of number of cells per villus column. Each value is the mean of 2 animals.

tervals after surgery no differences are observed.

The distribution of labelled cells along the crypt column is illustrated for the various experimental groups in fig. 2. In all age controls the percentage of labelled cells at each cell position is approximately 50% in the proliferative cell compartment, which is localized in the lower half of the crypt. The distribution of labelled cells in the Thiry-Vella fistula and in the jejunum in continuity is about the same as in the age controls, except for a slight expansion of the proliferative cell compartment at 2 days after surgery.

Cell migration of epithelial cells

Animals with permanent Thiry-Vella fistulas were injected with (^{3}H)-thymidine and 12, 24 and 36 hours later, the position of the leading edge of labelled cells was determined (table 2). Cell migration along the upper half of the crypt

Table 3. Three-dimensional parameters of intestinal epithelium following experimental by-pass.+

	Thiry-Vella fistula	Jejunum in continuity
dpm/crypt	10.0 ± 3.1	15.0 ± 2.0
crypts/cm of intestine	29400 ± 6800	28700 ± 1600
crypt-villus ratio	9.2 ± 1.0	10.2 ± 1.0

+Each value represents the mean (± SD) of 3 animals.

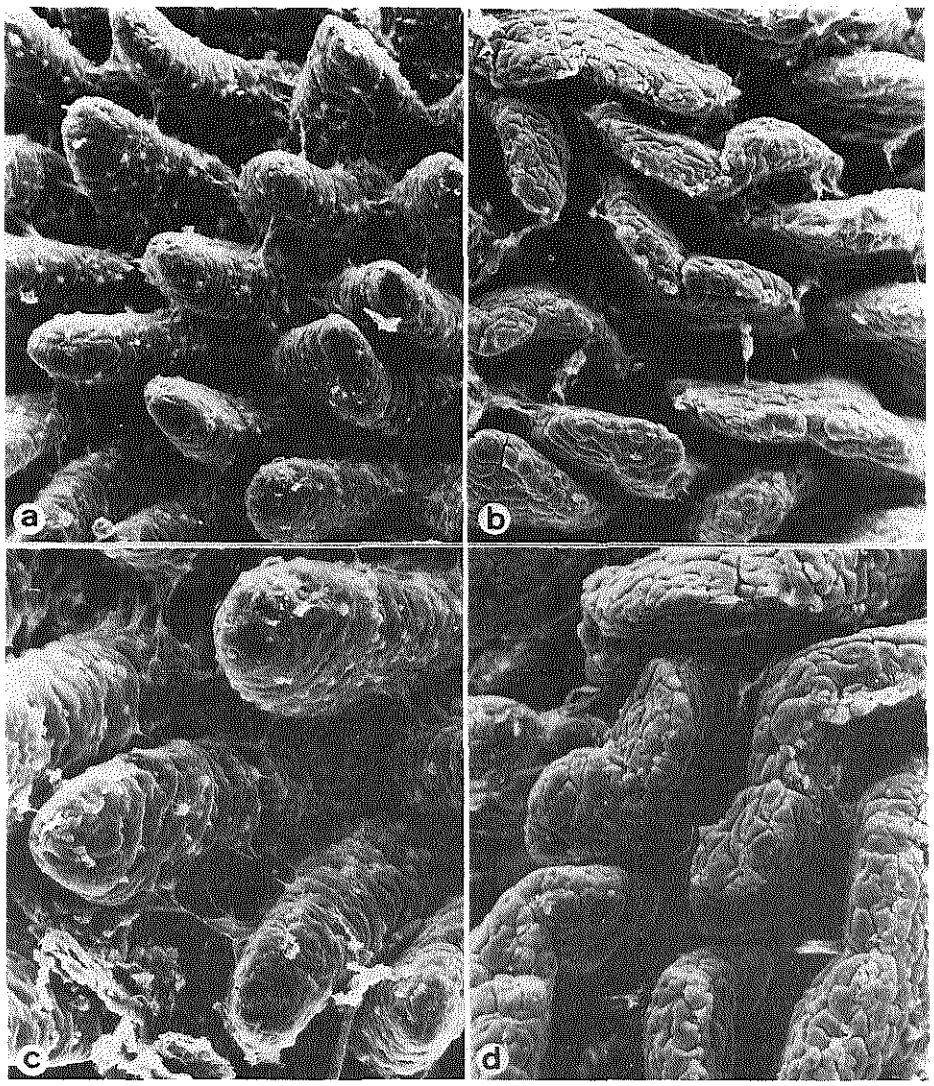

Fig. 3. Scanning electron micrographs of jejunal villi.
(a) Thiry-Vella fistula, and
(b) jejunum in continuity of the same rat;
magnification 122x
(c) Thiry-Vella fistula, and
(d) jejunum in continuity;
magnification 244x.

takes somewhat less than 12 hours in all experimental groups. During the subsequent migration along the villus the migration rate seems to be somewhat faster in the Thiry-Vella fistula than in the jejunum in continuity.

Three-dimensional parameters

The total radioactivity in whole crypts, determined as dpm's per crypt after pulse labelling with (^{3}H)-thymidine and crypt isolation, is considerably smaller in the Thiry-Vella fistula than in the jejunum in continuity (table 3), indicating a clear difference in crypt cell production. The number of crypts per cm length of small intestine and the number of crypts per villus in the Thiry-Vella fistula do not differ from those in the jejunum in continuity.

To observe any changes in the three-dimensional structure

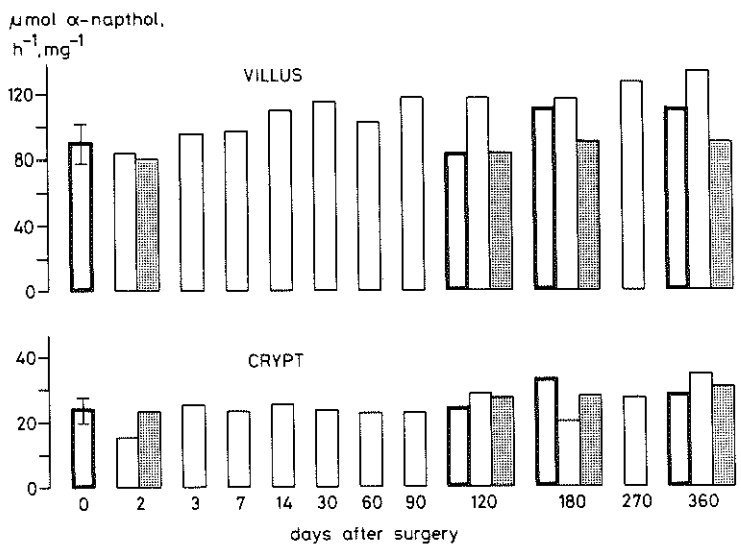

Fig. 4. Enzyme activity of non-specific esterases in isolated crypt and villus cell compartments at various time intervals following experimental by-pass.
☐ Thiry-Vella fistula, ▦ jejunum in continuity, ☐ jejunum in age controls.
Each value represents the mean of 2-3 animals, control values represent the mean (± SD) of 5 animals.

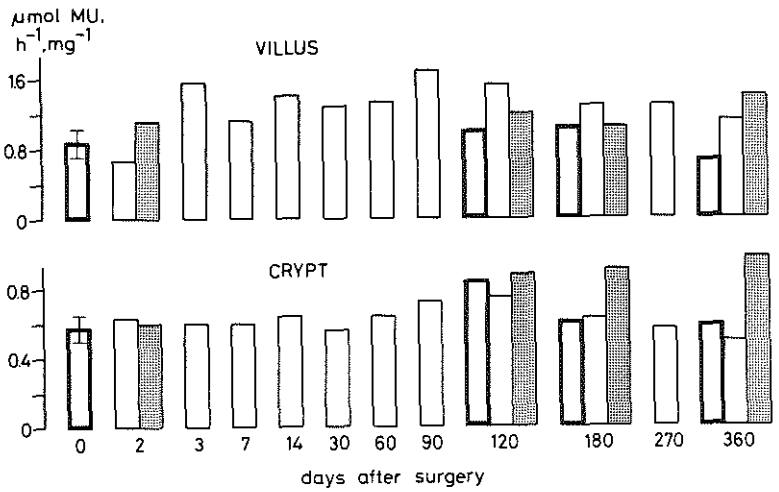

Fig. 5. Enzyme activity of neutral α-glucosidase in isolated crypt and villus cell compartments after experimental by-pass. For explanation of columns see legend to Fig. 4.

of the villi, small intestinal segments were investigated with a scanning electron microscope. As fig. 3 shows there is a large difference in villus shape between the fistula and the jejunum in continuity. The latter shows the normal appearance of leaf-shaped villi. The villi in the Thiry-Vella fistula are conical structures with broad bases and narrow tips.

Enzyme activities as parameters for cell maturation

The activity of non-specific esterases was measured in isolated crypt and villus compartments. The results at the various time intervals after establishment of a Thiry-Vella fistula, are illustrated in fig. 4. The enzyme activity in the crypt in Thiry-Vella fistulas is reduced at 2 days after surgery, and subsequently returns to control values. The esterase activity in the villus cell compartment increases with time in the Thiry-Vella fistula, and this also occurs in the controls, although to a lesser degree.

Neutral α-glucosidase was also assayed in isolated crypt and villus compartments in the various experimental groups,

and the activities are illustrated in fig. 5. The activity of neutral α-glucosidase in the crypt cell compartment following by-pass does not show any differences, as compared to the controls. In all three experimental groups the activities show considerable variations. The activities of neutral α-glucosidase in villus cells also show large variations. The enzyme activities in villus cells are mostly higher in the Thiry-Vella fistula than in the control groups.

Discussion

Among the factors influencing small intestinal morphology and function, luminal factors seem to be very important. Although the number of villus cells and crypt cells are known to adapt to various circumstances, the regulatory mechanisms of intestinal proliferation and maturation are unknown (1).

The number of crypt and villus cells may be increased by pancreatic secretions (3), or partial resection of the small intestine (5,22,23). In the latter case, the proliferative activity, as determined by the labelling index after (^{3}H)-thymidine, has been reported to be normal, while cell production per crypt increased (22,23). A decrease in cell numbers was observed following experimental by-pass (4,10). The total number of villi in the small intestine seems to be constant, as shown by experiments using starvation, irradiation and partial resection (20,24,25). The number of crypts, however, do change under some circumstances (20, 25).

The influence of luminal contents on the cellular kinetics and maturation of intestinal epithelium was studied by creating permanent Thiry-Vella fistulas of proximal jejunum.

The number of cells per villus column, and to a lesser degree the number of cells per crypt column, decreases in the by-passed jejunum within 7-14 days, and subsequently remains constant up to one year. No changes were observed in the percentage of labelled crypt cells and relative size of the proliferative cell compartment in the crypt. However, the total proliferative activity per crypt, as determined by scintillation counting of isolated crypts after labelling with (^{3}H)-thymidine, was found to be markedly reduced in by-passed jejunum. This decrease is largely due to a reduction in the total crypt cell population, as no changes were observed in the percentage of labelled cells per crypt column. A decrease in crypt cell production was also reported by Clarke (4), who determined the number of colchicin-arrested mitoses per crypt in by-passed intestine.

Using scanning electron microscopy the three-dimensional size of the villi was found to be markedly reduced in the Thiry-Vella fistula. Structural changes have also been reported by other investigators using dissection microscopy (10,15). The number of crypts per unit length of intestine remained unchanged, as did the crypt-villus ratio. From the data presented in table 3 it is clear that also the number of villi per unit length of intestine remains constant after experimental by-pass.

It seems that the macroscopic hypotrophy of a by-passed segment of small intestine, aside from changes in the muscle layer, results from a reduction in the size of villi and crypts, in length as well as in width. The reduction of the villus cell population is caused by a lowered cell production per crypt, due to a reduction in number of cells per crypt. Our experimental work also shows that luminal contents, like food substances and secretions from stomach, pancreas and liver, are not essential for maintaining the

structure of the epithelium of the small intestine. Adaptation to the new environment occurs within two weeks and subsequently the cell compartments remain constant up to one year after by-pass.

The migration rate of epithelial cells on the villus appears to be somewhat larger in the Thiry-Vella fistula than in control jejunum. This probably indicates some shortening of the life span of epithelial cells in by-passed jejunum, which also contributes to the decrease in the number of villus cells. Earlier studies on by-passed small intestine did not reveal any changes in cell migration rate, but migration of epithelial cells was followed during a shorter time period (10).

The observed changes in cellular kinetics do not lead to any consistent changes in crypt cell maturation, as reflected by unchanging activities of non-specific esterases and neutral α-glucosidase in isolated crypts. The esterase activity in the villus cell compartment is increased in by-passed jejunum, and this also seems to be true for neutral α-glucosidase, although the variations in activity of the latter enzyme are considerable. This increase in enzyme activities of the villus cells may be explained by the lack of pancreatic secretions as well as bile products, which have been shown to reduce the activity of a number of enzymes in small intestine (7,12,26,27).

The observed response of the small intestinal epithelium to experimental by-pass is quite different from the response to an experimental reduction of the villus cell population. In the latter case an increase in crypt cell production is observed which is primarily achieved by an expansion of the proliferative cell compartment in the crypt (18,28,29). This is accompanied by a considerable decrease in crypt cell maturation, as reflected in lower activities of a number of enzymes involved in intestinal function (16,

18,29-31).

Studies on the response to partial resection of the small intestine revealed that the crypt and villus cell populations increase in size, but no change in proliferative activity occurs (5,22,23). Also during lactation the number of cells per crypt increases without any changes in labelling index, number of villi and crypts, or life span of the epithelial cells (32).

It is most likely that crypt cell proliferation is regulated by at least two different mechanisms. Firstly, there is a feedback control of the relative size of the proliferative cell compartment in the crypt by the number of functional villus cells (18,29,30,33). Secondly, there is a mechanism which regulates crypt cell production via changes in the size of the crypt cell compartment. The question remains unanswered how a change in number of cells per crypt is brought about, although a shortening of the cell cycle may be involved in an enlargement of the crypt (22, 32).

References

1. Galjaard H: Some unresolved questions relevant to intestinal adaptation. In Intestinal Adaptation. Edited by RH Dowling, EO Riecken. New York, Schattauer Verlag, 1974, p 263-268
2. Sprinz H: Factors influencing intestinal cell renewal. Cancer 28:71-74, 1971
3. Altmann GG: Influence of bile and pancreatic secretions on the size of the intestinal villi in the rat. Am J Anat 132:167-178, 1971
4. Clarke RM: Control of intestinal epithelial replacement: lack of evidence for a tissue-specific blood-borne factor. Cell Tissue Kinet 7:241-250, 1974
5. Dowling RH, Gleeson MH: Cell turnover following small bowel resection and by-pass. Digestion 8:176-190, 1973
6. Altmann GG, Leblond CP: Factors influencing villus size in the small intestine of adult rats as revealed by transposition of intestinal segments. Am J Anat 127:15-36, 1970
7. Caspary WF, Winckler K, Lankisch PG, et al: Influence of exocrine and endocrine pancreatic function on intestinal brush border enzymatic activities. Gut 16:89-92, 1975

8. Cook RM, Powell PM, Moog F: Influence of biliary stasis on the activity and distribution of maltase, sucrase, alkaline phosphatase, and leucylnaphthylamidase in the small intestine of the mouse. Gastroenterology 64:411-420, 1973
9. Fry RJM, Staffeldt E: Effect of a diet containing deoxycholate on the intestinal mucosa of the mouse. Nature 203: 1396-1398, 1964
10. Gleeson MH, Cullen J, Dowling RH: Intestinal structure and function after small bowel by-pass in the rat. Clin Science 43:731-742, 1972
11. Gleeson MH, Dowling RH, Peters TJ: Biochemical changes in intestinal mucosa after experimental by-pass in the rat. Clin Science 43:743-757, 1972
12. Gracey M, Houghton M, Thomas J: Deoxycholate depresses small-intestinal enzyme activity. Gut 16:53-56, 1975
13. Hietanen E, Hänninen O: Effect of chyme on mucosal enzyme levels in small intestine of rat. Metabolism 21:991-1000, 1972
14. Johnson LR, Copeland EM, Dudrick SJ, et al: Structural and hormonal alterations in the gastrointestinal tract of parenterally fed rats. Gastroenterology 68:1177-1183, 1975
15. Keren DF, Elliott HL, Brown GD, et al: Atrophy of villi with hypertrophy and hyperplasia of Paneth cells in isolated (Thiry-Vella) ileal loops in rabbits. Light-microscopic studies. Gastroenterology 68:83-93, 1975
16. Both NJ de, Plaisier H: The influence of changing cell kinetics on functional differentiation in the small intestine of the rat. A study of enzymes involved in carbohydrate metabolism. J Histochem Cytochem 22:352-360, 1974
17. Cairnie AB: Cell proliferation studies in the intestinal epithelium of the rat: response to continuous irradiation. Radiat Res 32:240-264, 1967
18. Rijke RPC, Plaisier H, Hoogeveen AT, et al: The effect of continuous irradiation on cell proliferation and maturation in small intestinal epithelium. Cell Tissue Kinet 8:441-453, 1975
19. Hagemann RF, Sigdestad CP, Lesher S: A method for quantification of proliferative intestinal mucosal cells on a weight base: some values for C57B1/6. Cell Tissue Kinet 3:21-26, 1970
20. Clarke RM: The effect of growth and of fasting on the number of villi and crypts in the small intestine of the albino rat. J Anat 112:27-33, 1972
21. Galjaard H, Buys J, Duuren M van, et al: A quantitative histochemical study of intestinal mucosa after X-irradiation. J Histochem Cytochem 18:291-301, 1970
22. Hanson WR, Osborne JW: Epithelial cell kinetics in the small intestine of the rat 60 days after resection of 70 per cent of the ileum and jejunum. Gastroenterology 60:1087-1097, 1971
23. Hanson WR: Intestinal compensation following intestinal resection in the rat. Ph D thesis, University of Iowa, 1975
24. Forrester JM: The number of villi in rat's jejunum and ileum: effect of normal growth, partial enterectomy, and tube feeding. J Anat 111:283-291, 1972

25. Cairnie AB, Millen BH: Fission of crypts in the small intestine of the irradiated mouse. Cell Tissue Kinet 8:189-196, 1975
26. Alpers DH, Tedesco FJ: The possible role of pancreatic proteases in the turnover of intestinal brush border proteins. Biochim Biophys Acta 401:28-40, 1975
27. Arvanitakis C, Olsen WA: Intestinal mucosal disaccharidases in chronic pancreatitis. Dig Dis 19:417-421, 1974
28. Galjaard H, Meer-Fieggen W van der, Giesen J: Feedback control by functional villus cells on cell proliferation and maturation in intestinal epithelium. Exptl Cell Res 73:197-207, 1972
29. Rijke RPC, Hanson WR, Plaisier HM, et al: The effect of ischemic villus cell damage on crypt cell proliferation in the small intestine: evidence for a feedback control mechanism. Gastroenterology (accepted for publication)
30. Both NJ de, Dongen JM van, Hofwegen B van, et al: The influence of various cell kinetic conditions on functional differentiation in the small intestine of the rat. A study of enzymes bound to subcellular organelles. Developm Biol 38:119-137, 1974
31. Galjaard H, Meer-Fieggen W van der, Both NJ de: Cell differentiation in gut epithelium. In Cell Differentiation. Edited by D Viza, H Harris. Copenhagen, Munksgaard, 1972, p 322-328
32. Harding JD, Cairnie AB: Changes in intestinal cell kinetics in the small intestine of lactating mice. Cell Tissue Kinet 8:135-144, 1975
33. Rijke RPC, Meer-Fieggen W van der, Galjaard H: Effect of villus length on cell proliferation and migration in small intestinal epithelium. Cell Tissue Kinet 7:577-586, 1974

Gut Publication V

RECOVERY OF EPITHELIUM IN BY-PASSED JEJUNUM AFTER 700R X-IRRADIATION

R.P.C. Rijke, H.M. Plaisier, H. de Ruiter and H. Galjaard
Dept. of Cell Biology and Genetics,
Erasmus University Rotterdam, The Netherlands

SUMMARY Crypt cell proliferation in the small intestine is probably regulated via a feedback mechanism by the villus cell population. Such a mechanism also plays a role in the recovery of the intestinal epithelium after irradiation. The present study deals with the question whether the normal luminal contents are necessary for the feedback regulation to operate. Permanent jejunal Thiry-Vella fistulas were established in rats and 3-4 months later the animals were exposed to whole-body irradiation. Crypt cell proliferation and maturation were studied up to one week after irradiation. After irradiation a reduction in the crypt cell population was observed, which was followed by a recovery at 72 hours after irradiation. The number of villus cells decreased up to 48 hours and control values were found again between 96 and 168 hours after irradiation. From 48 hours after irradiation on an increase in crypt cell proliferation was observed, which was mainly due to an expansion of the proliferative cell compartment in the crypt. No differences were found in the pattern of crypt cell proliferation after irradiation between by-passed jejunum and jejunum in continuity. The same was true for the activities of non-specific esterases, neutral α-glucosidase and alkaline phosphatase in isolated crypt and villus compartments. Hence it seems that the absence of normal lumi-

nal contents does not affect the response of crypt cell proliferation to an experimental reduction of the villus cell population.

INTRODUCTION

A number of investigators have suggested a feedback control of crypt cell proliferation by the villus cell compartment in the small intestine (Galjaard et al, 1972; Rijke et al, 1974 and 1976a; Sato et al, 1972). This hypothesis is mainly supported by the inverse relationship between the number of villus cells and the proliferative activity in the crypt under a number of experimental circumstances. The reduction in the number of villus cells following irradiation or temporary intestinal ischaemia is accompanied by an expansion of the proliferative cell compartment in the crypt. During the subsequent recovery of the villus cell compartment, the proliferative cell compartment in the crypt decreases in size (Galjaard et al, 1972; Rijke et al, 1976a).

Since luminal, and possibly functional, factors are known to influence epithelial cell kinetics in small intestine (Altmann and Leblond, 1970; Dowling and Gleeson, 1973; Feldman et al, 1976; Rijke et al, 1976b), it seemed worthwhile to investigate whether experimental by-pass of a jejunal segment would interfere with the regulation of cell proliferation in that segment.

Therefore jejunal Thiry-Vella fistulas were established in rats. The animals were exposed to 700R whole-body irradiation at 3-4 months after surgery. The epithelial cell kinetics and maturation were subsequently studied from 24 to 168 hours after irradiation in the by-passed jejunum as well as in the jejunum in continuity.

MATERIALS AND METHODS

Male Glaxo-Wistar rats, weighing approximately 200 g at the time of surgery, were used and all animals were sacrificed between 10.00 and 11.00 hours.

Jejunal Thiry-Vella fistulas were constructed as described earlier (Rijke et al, 1976b). At 3-4 months after surgery, the animals were exposed to 700R whole-body X-irradiation from a Philips X-ray machine. At 24, 36, 48, 72, 96 and 168 hours after irradiation, intestinal segments were resected in two animals from the jejunum in continuity (approximately 3 cm distal to the anastomosis) and from the Thiry-Vella fistula (middle part). All animals had been injected intraperitoneally, together with five unirradiated animals, with ^{3}H-thymidine, 0.5 µCi per gram body weight (Amersham, specific activity 18.4 Ci/mmole) 30 minutes earlier.

The small intestinal segments were fixed in 10% formalin, and paraffin sections (4 µm) were processed for dipping autoradiography with Ilford K2 emulsion. After hematoxylin and eosin staining, the number of cells per crypt column and per villus column was determined in 20 longitudinally cut crypt and villus columns. The percentage of labelled cells per crypt column was determined and the positions of the labelled cells per crypt column was recorded. The positions of the labelled cells along the crypt column were calculated as fractions of the total number of cells between bottom and top of crypt to correct for the possible influence of differences in crypt length (Cairnie, 1967). Also the leading edge of labelled cells along the crypt column was determined as a parameter for the relative size of the proliferative cell compartment in the crypt (Rijke et al, 1975).

From all animals also segments of the jejunum in conti-

nuity and of the Thiry-Vella fistula were quickly frozen in liquid nitrogen and stored at -70°C. Cryostat sections (12 µm) were freeze-dried in vacuo (4 hours at -25°C, $p < 0.15$ N/m^2). Crypt and villus cell compartments were dissected under the microscope and the tissue fragments were weighed on a quartz fiber balance (Galjaard et al, 1970). The activity of non-specific esterases was determined using naphthyl acetate as a substrate in a final volume of 350 µl (Galjaard et al, 1970). The alkaline phosphatase activity was determined according to Lowry et al (1954), using di-Na-paranitrophenylphosphate as a substrate in a final volume of 250 µl. The activity of neutral α-glucosidase was determined with 4-methylumbelliferyl-α-D-glucopyranoside as a substrate in a final volume of 520 µl (de Both and Plaisier, 1974). For each tissue activities were determined in 10 samples of dissected crypts and 10 samples of dissected villi.

RESULTS

The number of epithelial cells per crypt column and per villus column, at the various time intervals after irradiation, is illustrated for by-passed jejunum and jejunum in continuity in Figure 1. In the unirradiated animals the number of cells per villus column is smaller in by-passed jejunum than in jejunum in continuity. Following 700R irradiation the number of cells per crypt column decreases during the first 24 hours, and has recovered again 72 hours after irradiation. No differences occur between by-passed jejunum and jejunum in continuity. The number of cells per villus column decreases during the first 48 hours and subsequently increases to approach control values between 96 and 168 hours after irradiation. The number of cells per villus column in by-passed jejunum remains smaller than in

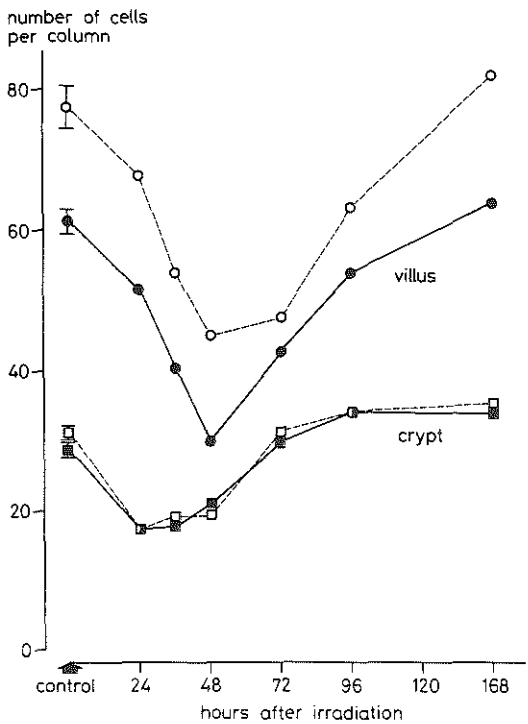

Fig. 1. Number of cells per crypt and per villus column at various time intervals after 700R X-irradiation in by-passed jejunum (closed symbols) and jejunum in continuity (open symbols). Control values represent the mean (± SD) of 5 animals, other values represent the mean of 2 animals.

jejunum in continuity throughout the recovery period.

The percentage of labelled cells per crypt column, determined half an hour after ^{3}H-thymidine labelling, is reduced during the first 36 hours following irradiation (Fig. 2). Subsequently the percentage of labelled crypt cells increases above control values at 48 and 72 hours, and decreases again to approach control values at 96 hours after irradiation. The leading edge of labelled cells along the crypt column, which is a parameter for the relative size of the proliferative cell compartment in the crypt, follows approximately the same pattern as the percentage of labelled

cells (Fig. 2). Throughout the recovery period after irradiation no differences are observed in the pattern of proliferative activity between by-passed jejunum and jejunum in continuity.

The distribution of labelled cells along the crypt column, which is the resultant of cell cycle parameters and size of the proliferative cell compartment in the crypt, is illustrated for jejunum in continuity in Figure 3 and for by-passed jejunum in Figure 4. These distributions show that a considerable expansion of the proliferative cell compartment occurs in the crypt, with labelled cells up to

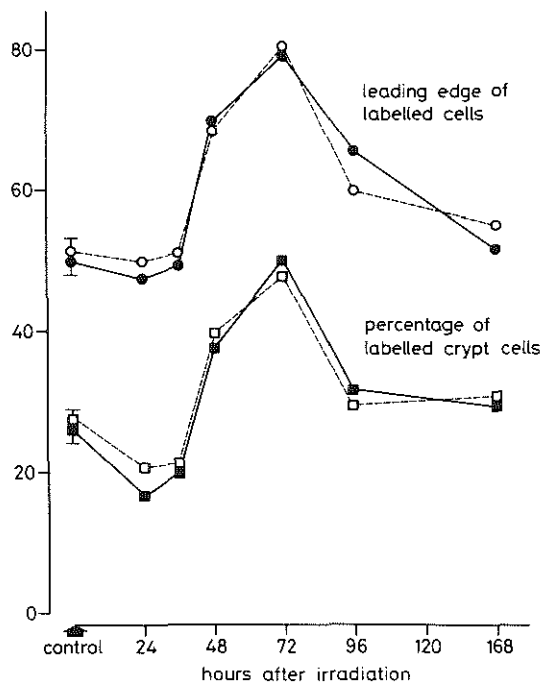

Fig. 2. Percentage of labelled crypt cells after irradiation in by-passed (■) and control jejunum (□), and relative size of the proliferative cell compartment in the crypt in by-passed (●) and control (○) jejunum, as estimated by the relative position of the leading edge of labelled cells along the crypt column (expressed as percentage of crypt cell column).

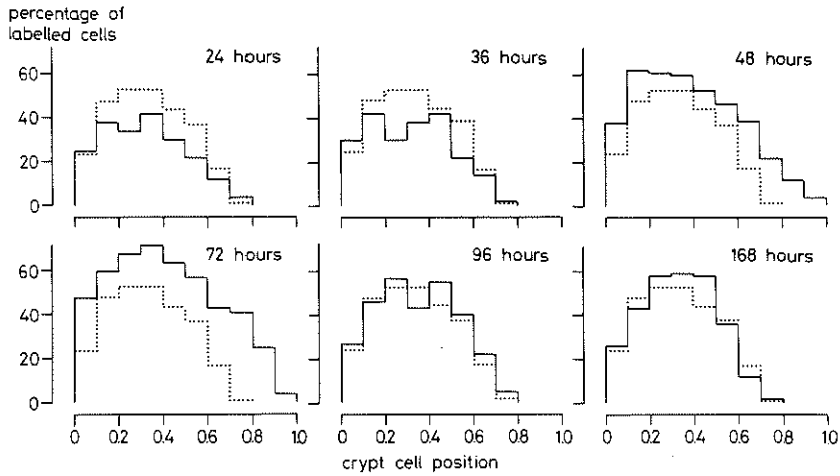

Fig. 3. Distribution of labelled cells along the crypt column in jejunum in continuity following irradiation. Dotted lines represent the distribution in unirradiated animals.

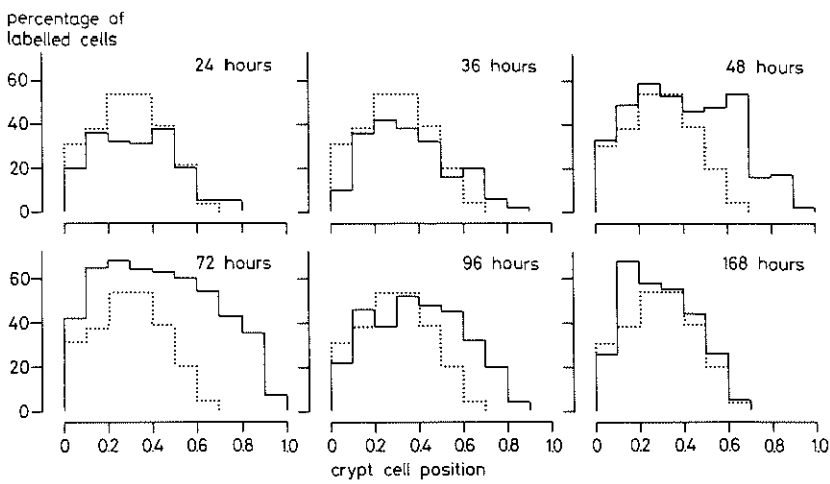

Fig. 4. Distribution of labelled cells along the crypt column in by-passed jejunum after irradiation. Dotted lines represent the distribution in unirradiated animals.

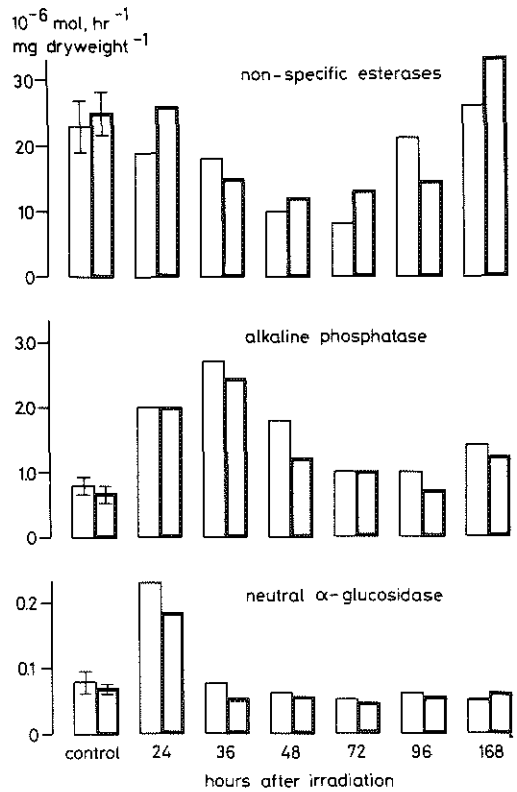

Fig. 5. Crypt cell enzyme activities in by-passed (▣), and control (□) jejunum at various time intervals after irradiation. Control values represent the mean (± SD) of 5 animals, other values the mean of 2 animals.

the mouth of the crypt, in jejunum in continuity at 48 and 72 hours after irradiation. The same is true for by-passed jejunum at 48, 72 and, to a lesser degree, 96 hours after irradiation. During the subsequent period the relative size of the proliferative cell compartment in the crypt becomes normal again. The relative length of the S-phase of the proliferative cells, as reflected by the percentage of labelled cells per crypt cell position, does not change very much during recovery after irradiation.

The crypt cell activities of non-specific esterases, alkaline phosphatase and neutral α-glucosidase following

irradiation are illustrated in Figure 5. The activity of non-specific esterases decreases from 36-48 hours after irradiation, increases after 72 hours and is normal again at 168 hours after irradiation. Alkaline phosphatase activity in crypt cells is increased during the first 48 hours after irradiation and is subsequently within control values. Neutral α-glucosidase activity shows control values during the recovery period except for an increase at 24 hours. No differences in enzyme activities are observed between jejunum in continuity and by-passed jejunum.

The villus cell activities for jejunum in continuity and by-passed jejunum are shown in Figure 6. The esterase activity in the villus cell compartment following irradiation

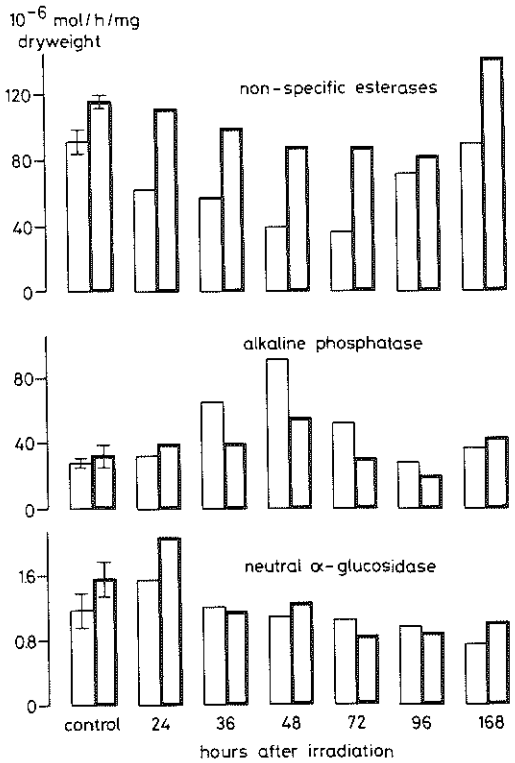

Fig. 6. Villus cell enzyme activities in by-passed (▣), and control (□) jejunum after irradiation.

shows a pattern comparable with the activity in the crypt cell compartment. However, the esterase activity in villus cells, in unirradiated animals as well as during recovery from irradiation, is larger in by-passed jejunum than in jejunum in continuity. The alkaline phosphatase and neutral α-glucosidase activities in the villus cell compartment do not show any considerable changes, except for an increase in alkaline phosphatase activity after 36-48 hours. No consistent differences in villus cell activities of alkaline phosphatase and neutral α-glucosidase exist between jejunum in continuity and by-passed jejunum.

DISCUSSION

The response of crypt cell proliferation to irradiation and the accompanying changes in crypt cell differentiation have been presented in detail elsewhere (de Both et al, 1974; de Both and Plaisier, 1974; van Dongen et al, 1976a and b; Galjaard et al, 1972; Rijke et al, 1974). Also the arguments for a feedback control of crypt cell proliferation by the villus cell population in the small intestine have been discussed previously (Rijke et al, 1976a).

The present data show that the response of crypt and villus cell populations to irradiation in by-passed jejunum and jejunum in continuity is identical. Also no differences were observed in the pattern of proliferative activity. Although esterase activity in the villus cell compartment is larger in by-passed jejunum than in jejunum in continuity, the pattern of activity of this enzyme in crypt and villus cell compartment after irradiation is the same. The latter is also true for neutral α-glucosidase and alkaline phosphatase. These findings indicate that changes in enzyme activities following irradiation are related to changes in cell kinetics (de Both et al, 1974; de Both and Plaisier,

1974), and not to luminal or functional changes.

The present data also indicate that luminal and/or functional factors do not affect the response of the crypt cell compartment to an experimental reduction of the villus cell compartment. The response of the by-passed intestinal epithelium to the irradiation could also be attributed to a systemic response of the intestinal epithelium. However, it was recently found by studying the recovery of parabiotic pairs, of which one rat was irradiated, that no systemic factors influence crypt cell proliferation under these circumstances (van Dongen, unpublished). Furthermore, indications for a local character of the feedback control of crypt cell proliferation were obtained by studying crypt cell proliferation in different parts of the small intestine after a reduction of the villus cell compartment was induced by temporary ischaemia in only part of the small intestine (Rijke et al, 1976a).

Summarizing the available data, it is most likely that the number of functional villus cells regulates crypt proliferation by controlling the relative size of the proliferative cell compartment in the crypt. This feedback mechanism is of a local character and is not influenced by luminal or functional factors.

ACKNOWLEDGMENTS

This study was supported by a grant from The Netherlands Foundation in Medical Research.

The authors wish to thank Mr. W.J. Visser and Mr. J. Bos for their technical assistance.

REFERENCES

Altmann, G.G., and Leblond, C.P. (1970). Factors influencing villus size in the small intestine of adult rats as revealed by transposition of intestinal segments. American journal of Anatomy **127**, 15-36.

Both, N.J. de, Dongen, J.M. van, Hofwegen, B. van, Keulemans, J., Visser, W.J., and Galjaard, H. (1974). The influence of various cell kinetic conditions on functional differentiation in the small intestine of the rat. A study of enzymes bound to subcellular organelles. Developmental Biology **38**, 119-137.

Both, N.J. de, and Plaisier, H. (1974). The influence of changing cell kinetics on functional differentiation in the small intestine of the rat. A study of enzymes involved in carbohydrate metabolism. Journal of Histochemistry and Cytochemistry **22**, 352-360.

Cairnie, A.B. (1967). Cell proliferation studies in the intestinal epithelium of the rat: response to continuous irradiation. Radiation Research **32**, 240-264.

Dongen, J.M. van, Kooyman, J., Visser, W.J., Holt, S.J., and Galjaard, H. (1976a). The effect of increased crypt cell proliferation on the activity and subcellular localization of esterases and alkaline phosphatase in rat small intestine. Histochemical journal (accepted for publication).

Dongen, J.M. van, Visser, W.J., Daems, W.Th., and Galjaard, H. (1976b). The relation between cell proliferation, differentiation and the ultrastructural development in rat intestinal epithelium. Cell and Tissue Research (accepted for publication).

Dowling, R.H., and Gleeson, M.H. (1973). Cell turnover following small bowel resection and by-pass. Digestion **8**, 176-190.

Feldman, E.J., Dowling, R.H., McNaughton, J., Peters, T.J. (1976). Effects of oral versus intravenous nutrition on intestinal adaptation after small bowel resection in the dog. Gastroenterology **70**, 712-719.

Galjaard, H., Buys, J., Duuren, M. van, and Giessen, J. (1970). A quantitative histochemical study of intestinal mucosa after X-irradiation. Journal of Histochemistry and Cytochemistry **18**, 291-301.

Galjaard, H., Meer-Fieggen, W. van der, and Giesen, J. (1972). Feedback control by functional villus cells on cell proliferation and maturation in intestinal epithelium. Experimental Cell Research **73**, 197-207.

Lowry, O.H., Roberts, N.R., Wu, M.-L., Hixon, W.S., and Crawford, E.J. (1954). The quantitative histochemistry of the brain. II. Enzyme measurements. Journal of Biological Chemistry **207**, 19-37.

Rijke, R.P.C., Hanson, W.R., Plaisier, H.M., and Osborne, J.W. (1976a). The effect of ischaemic villus cell damage on crypt cell proliferation in the small intestine: evidence for a feedback control mechanism. Gastroenterology (accepted for publication).

Rijke, R.P.C., Meer-Fieggen, W. van der, and Galjaard, H. (1974). Effect of villus length on cell proliferation and migration in small intestinal epithelium. Cell and Tissue Kinetics **7**, 577-586.

Rijke, R.P.C., Plaisier, H., Hoogeveen, A.T., Lamerton, L.F., and Galjaard, H. (1975). The effect of continuous irradiation on cell proliferation and maturation in small intestinal epithelium. Cell and Tissue Kinetics 8, 441-453.

Rijke, R.P.C., Plaisier, H.M., Ruiter, H. de, and Galjaard, H. (1976b). The influence of experimental by-pass on cellular kinetics and maturation of small intestinal epithelium. Gastroenterology (submitted for publication).

Sato, F., Muramatsu, S., Tsuchihashi, S., Shiragai, A., Hiraoka, T., Inada, T., Kawashima, K., Matsuzawa, H., Nakamura, W., Trucco, E., and Sacher, G.A. (1972). Radiation effects on cell populations in the intestinal epithelium of mice and its theory. Cell and Tissue Kinetics 5, 277-235.

Cell Tissue Kinet. Publication VI

THE EFFECT OF TRANSPOSITION TO JEJUNUM ON EPITHELIAL CELL KINETICS IN AN ILEAL SEGMENT

R.P.C. Rijke, W.R. Hanson*, and H.M. Plaisier

Department of Cell Biology and Genetics,
Erasmus University Rotterdam, The Netherlands

ABSTRACT

Epithelial cell kinetics and maturation were investigated after transposition of an ileal segment to proximal jejunum. At 7 days after surgery the number of cells per villus column in the transposed ileum increased to reach jejunal values after 14-30 days. This increase was accompanied by a simultaneous increase in the number of cells per crypt column up to 130% of values in jejunum and remaining ileum. The percentage of labelled crypt cells, after labelling with ^{3}H-thymidine, and the relative size of the proliferative cell compartment in the crypt in transposed ileum did not differ from values in control ileum. The total proliferative activity per crypt, as determined by scintillation counting of isolated crypts after ^{3}H-thymidine labelling, increased twofold from 7 days after surgery on. Cell migration studies revealed that the increase in number of villus cells in transposed ileum is not caused by a change in life span of the epithelial cells. Cell maturation, as studied by determining the enzyme ac-

*Present address: Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois, U.S.A.

tivities of non-specific esterases and neutral α-glucosidase in dissected crypt and villus compartments, did not increase in transposed ileum. It appears that the adaptation of a transposed ileal segment consists of an increase in the number of villus cells, which is brought about by an increase in crypt size, while labelling index and relative size of the proliferative crypt cell compartment do not change.

INTRODUCTION

The epithelium of the small intestine has a great potential for adaptation to various circumstances (Dowling & Riecken, 1974). Among the many factors influencing epithelial cell kinetics and maturation, luminal factors seem to be especially important (Altmann & Leblond, 1970; Altmann, 1971; Dowling & Gleeson, 1973; Feldman et al., 1976). However, little is known about the way(s) in which the small intestinal epithelium adapts to various circumstances.

An attractive model to study the way(s) in which the intestinal epithelium adapts to altered circumstances, is to create a change of environment for a small segment of the small intestine, without affecting its blood and nerve supply. This segment may be studied after various time intervals while the remainder of the small intestine may serve as control tissue. After transposition of an ileal segment to proximal jejunum, Altmann and Leblond (1970) found that the number of cells per villus in the transposed ileal segment increased up to jejunal values. More recently, Altmann (1974) postulated that this increase in number of villus cells is due to a change in life span of the epithelial cells. In these studies, however, epithelial cell proliferation and maturation were not studied.

To study the way in which the increase in number of villus cells takes place in ileal epithelium after transposition to jejunum, epithelial cell kinetics were studied from 2 to 30 days after transposition of an ileal segment to proximal jejunum. Also the enzyme activities of non-specific esterases and neutral α-glucosidase, which are normally lower in ileum than in jejunum (Harrison & Webster, 1971), were determined in crypt and villus cell compartments to observe a possible adaptation in the transposed ileal segment.

MATERIALS AND METHODS

Surgical procedure

Male Glaxo-Wistar rats, weighing approximately 250 g at the time of surgery were used. Animals were subjected to pentobarbital anesthesia (40 mg/kg of body weight, i.p.), after a fasting period of 12 hours, and a midline laparotomy was performed. The small intestine, from ligament of Treitz to cecum, was exteriorized and delivered upon a saline moistened sponge. The small intestine was kept moist at all times with warm physiological saline. An ileal segment, supplied by blood vessels of one mesenteric arcade, was isolated by dividing the small intestine at approximately 4 cm and again at approximately 12 cm from the cecum. The continuity of the ileum was re-established by end-to-end anastomosis. The mesenterium of the isolated segment was incised to allow mobilization while retaining vascularization and innervation. The jejunum was divided at approximately 3 cm from the ligament of Treitz and the ileal segment was inserted by end-to-end anastomosis. The anastomoses were done by means of a continuous suture using 6/0 silk. The midline incision was closed in two layers: the muscle layer with 3/0 silk sutures and the skin with wound

clips. During the first two days following surgery, 5% sucrose was given to the animals, after which they resumed the standard pellet diet.

Tissue sampling

At intervals of 2,3,4,7,14 and 30 days after surgery, 3 animals were injected intravenously with ^{3}H-thymidine, 1 µCi per gram body weight (spec. act. 17 Ci/mmole) between 10.00 and 11.00 hours. After 1 hour the animals were anesthesized with ether, and their abdomens were opened. Segments were removed from the middle part of the transposed ileal segment, from the jejunum at 3 cm proximal to the transposed segment, and from the ileum at 3 cm proximal to the ileal anastomosis. Also 5 control animals were injected with ^{3}H-thymidine, and 1 hour later segments were removed from the corresponding parts of jejunum and ileum.

Cell kinetic studies

After fixation of the intestinal tissues in 10% formalin, paraffin sections (3 µm) were processed for dipping autoradiography with Kodak NTB-2 emulsion. After hematoxylin and eosin staining, the number of cells per column was determined for each tissue in 20 longitudinally cut crypt and villus columns. Also the percentage of labelled cells per crypt column was determined. The leading edge of labelled cells along the crypt column was recorded as a parameter for the relative size of the proliferative cell compartment in the crypt, as described earlier (Rijke et al., 1975).

The total proliferative activity per crypt was determined as described by Hagemann, Sigdestad and Lesher (1970). From each tissue 200 crypts were isolated and transferred to 4 liquid scintillation vials (50 to each vial), containing 0.5 ml Soluene (Packard) for solubilization. After 48 hours

15 ml of scintillation mixture (5 g PPO* plus 0.5 g POPOP** per litre of toluene) was added. The samples were counted in a liquid scintillation spectrometer (Packard), and the results were expressed as disintegrations per minute per crypt.

To determine the life span of the epithelial cells, a number of animals was injected with ^{3}H-thymidine at 30 days after surgery. At 12 and 36 hours after injection of ^{3}H-thymidine, segments were removed from jejunum, ileum, and transposed ileum. After autoradiographic processing the position of the leading edge of labelled cells was determined along 20 longitudinally cut villus columns for each tissue.

Enzyme assays

Segments from jejunum, ileum and transposed ileum were resected in rats at 2-30 days after surgery, together with jejunal and ileal segments in control animals. The tissues were quickly frozen in liquid nitrogen and stored at -70^{o}C. Cryostat sections (12 µm) were freeze-dried in vacuo, crypt and villus compartments were dissected, and the tissue fragments were weighed on a quartz-fibre balance (Galjaard et al., 1970). The activity of non-specific esterases was determined spectrophotometrically using naphthyl acetate as a substrate in a final volume of 350 µl (Galjaard et al., 1970). The activity of neutral α-glucosidase was measured fluorometrically with 4-methylumbelliferyl-α-D-glucopyranoside as a substrate at pH 6.5 in a final volume of 520 µl (de Both & Plaisier, 1974). For each tissue enzyme activities were determined in 5 samples of dissected crypts and 5 samples of dissected villi.

*PPO: 2,5-diphyloxazol; **POPOP: 2,2'-phenylen-bis-(5-phenyloxazol).

RESULTS

Epithelial cell kinetics

At various time intervals (2-30 days) after transposition of an ileal segment to proximal jejunum, radioautographs of jejunum, ileum, and transposed ileum were were prepared 1 hour after pulse labelling with ^{3}H-thymidine.

The cell kinetic data of the jejunum are given in Table 1. During the first days after surgery, an increase in proliferative activity is observed, together with an increase in the number of cells per crypt column. There is an expansion of the proliferative cell compartment in the crypt, as reflected by the leading edge of labelled cells along the crypt column. All parameters are normal again from 7-14 days after surgery on. To discriminate between this general response of the entire small intestine, which was also found during the first days after partial resection of the intestine (Hanson, 1975) and after experimental

Table 1. Cell kinetics in jejunal epithelium* after transposition** of an ileal segment to proximal jejunum.

time after surgery	No. of cells per column		% labelled crypt cells	leading edge of labelled cells***
	crypt	villus		
Control	32 ± 1	73 ± 2	23.8 ± 1.3	0.48 ± 0.02
2 days	37 ± 1	71 ± 2	29.1 ± 0.9	0.68 ± 0.03
3 days	36 ± 1	73 ± 5	27.4 ± 0.5	0.62 ± 0.05
4 days	35 ± 3	74 ± 3	26.7 ± 0.3	0.59 ± 0.08
7 days	34 ± 1	82 ± 4	26.2 ± 1.5	0.52 ± 0.01
14 days	33 ± 1	76 ± 1	25.5 ± 2.4	0.51 ± 0.01
30 days	32 ± 1	75 ± 1	23.7 ± 0.2	0.50 ± 0.02

*At 3 cm proximal to the transposed segment
**Control values represent the mean (± SD) of 5 animals, other values the mean (± SD) of 3 animals.
***The leading edge of labelled cells along the crypt column is expressed as fraction of the number of cells from bottom to top of crypt.

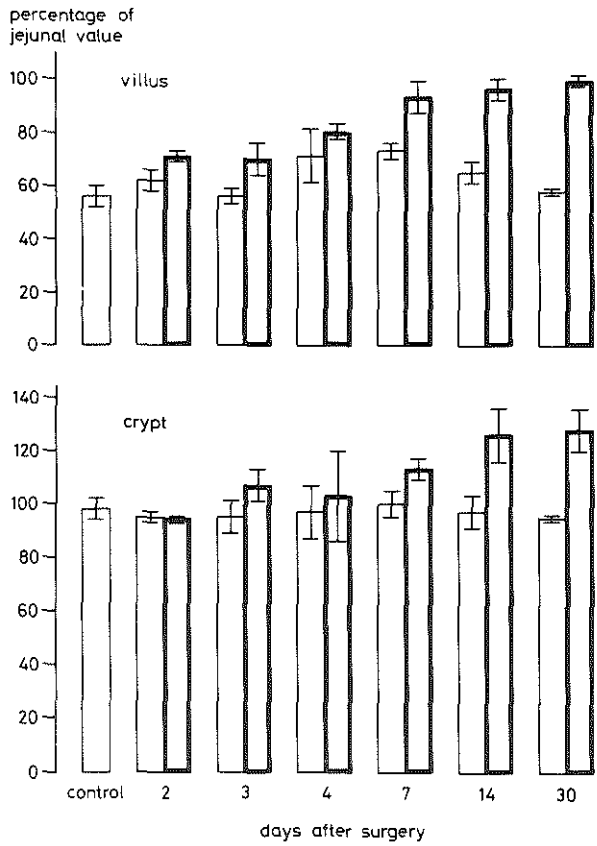

Fig. 1. The number of cells per crypt column and villus column at various time intervals after transposition of an ileal segment to proximal jejunum. ☐ remaining ileum, ☐ transposed ileum.
Each value is the mean (± SD) of 3 animals, control value is the mean (± SD) of 5 animals.

by-pass of an intestinal segment (Rijke et al., 1976b), and a possibly specific response of the transposed ileum, the data of transposed ileum and remaining ileum were calculated as percentages of jejunal values of the same rat (see Figs. 1 and 2).

The number of epithelial cells per crypt column and per villus column in transposed ileum and remaining ileum at the various time intervals after surgery is illustrated in

Fig. 1. The number of cells per villus column in the ileum of control animals is approximately half of that in jejunum. In the remaining ileum after surgery the number of cells per villus column varies between 55 and 75% of jejunal values. The number of cells per villus column in the transposed ileum increases above that in the remaining ileum from day 7 on, and is the same as that in jejunum at day 30 after surgery. The number of cells per crypt column in the ileum of control animals and the remaining ileum of operated animals is the same as in the jejunum. The number of cells per crypt column in the transposed ileum increases above that in jejunum and ileum from day 7 after surgery on to remain markedly enlarged.

Thus it seems that a specific response occurs in an ileal segment after transposition to proximal jejunum. This response consists of an increase in the number of cells per crypt column and per villus column from day 7 after surgery on.

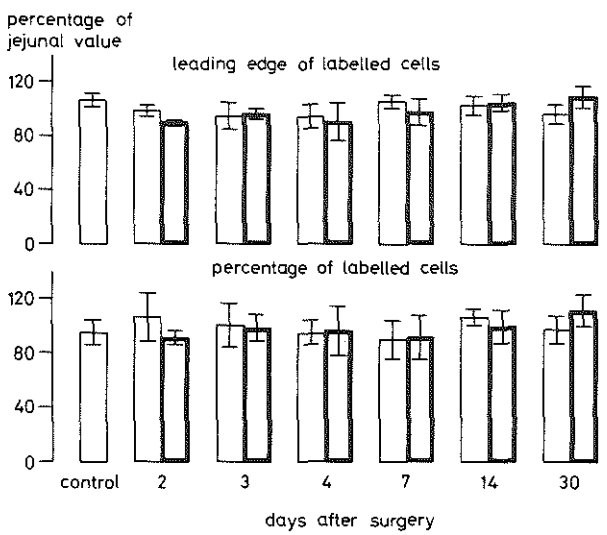

Fig. 2. Proliferative activity in intestinal crypts after transposition of an ileal segment to jejunum. ☐ remaining ileum, ☐ transposed ileum.

Table 2. Total proliferative activity* per crypt in jejunum, ileum, and transposed ileum at various time intervals after surgery.

time after surgery	jejunum	transposed ileum	remaining ileum
Control	7.0 ± 2.5		10.0 ± 4.5
2 days	17.5 ± 4.5	17.5 ± 1.0	17.0 ± 2.5
3 days	8.0 ± 2.0	10.0 ± 2.5	10.5 ± 1.5
4 days	9.0 ± 2.5	10.0 ± 0.5	8.0 ± 6.0
7 days	13.5 ± 1.0	24.0 ± 4.5	14.5 ± 2.0
14 days	9.0 ± 2.0	16.5 ± 2.0	10.5 ± 1.5
30 days	10.5 ± 1.0	20.0 ± 4.5	9.0 ± 2.0

*Expressed as d.p.m.'s per crypt after pulse labelling with ^{3}H-thymidine.

The percentage of labelled cells per crypt column, 1 hour after labelling with ^{3}H-thymidine, is illustrated for transposed ileum and remaining ileum in Fig. 2. At all time intervals the percentage of labelled cells per crypt column in the remaining ileum as well as in the transposed ileum is about the same as that in the jejunum.

The relative size of the proliferative cell compartment, estimated by determining the leading edge of labelled cells along the crypt column 1 hour after ^{3}H-thymidine labelling, is also illustrated in Fig. 2. The leading edge of labelled cells in transposed ileum and remaining ileum is approximately the same as that in jejunum at all time intervals after surgery.

The total proliferative activity per crypt in transposed ileum, ileum and jejunum was determined by scintillation counting of isolated crypts after ^{3}H-thymidine labelling, and expressed as number of disintegrations per minute per crypt (Table 2). At 2 days after surgery there is an increase in proliferative activity in all three parts of the small intestine of operated animals. In the following days the values for jejunum and ileum are within control values

Table 3. Epithelial cell migration on villi 30 days after transposition of an ileal segment to proximal jejunum.*

hours after ^{3}H-TdR	jejunum	transposed ileum	remaining ileum
12	5 ± 3	4 ± 2	3 ± 1
36	60 ± 10	60 ± 20	70 ± 10

*Values represent the position of the leading edge of labelled cells along the villus column, expressed as percentages of number of cells per villus column. Each value is the mean (± SD) of 3 animals.

again. At 7 days after surgery the proliferative activity per crypt increases in the transposed ileum above the values in jejunum and remaining ileum, to remain high.

It appears that the observed increase in number of crypt cells and villus cells in transposed ileum is not accompanied by changes in the percentage of labelled crypt cells or in the relative size of the proliferative cell compartment in the crypt. Comparing the data for the percentage of labelled crypt cells (Fig. 2) and the number of d.p.m.'s per crypt (Table 2), it is clear that the total number of cells per crypt in transposed ileum increases even more than the number of cells per crypt column.

Life span of the epithelial cells

An estimate of the life span of the epithelial cells was obtained by following the migration of labelled cells along the villus between 12 and 36 hours after labelling with ^{3}H-thymidine. From the results of this study (Table 3), which was performed at 30 days after surgery, it is clear that no difference in life span exists between epithelial cells in transposed ileum and those in remaining ileum, that may account for the observed difference in number of cells per villus column (Fig. 1).

Table 4. Enzyme activities of non-specific esterases and neutral α-glucosidase in crypt and villus compartments of jejunal epithelium after transposition of an ileal segment to jejunum.*

time after surgery	non-specific esterases		neutral α-glucosidase	
	crypt	villus	crypt	villus
Control	18 ± 3	67 ± 7	0.065 ± 0.013	1.0 ± 0.4
2 days	9 ± 1	64 ± 21	0.049 ± 0.002	0.8 ± 0.2
3 days	12 ± 2	59 ± 12	0.053 ± 0.003	0.8 ± 0.2
4 days	18 ± 2	78 ± 4	0.048 ± 0.004	0.6 ± 0.2
7 days	28 ± 10	89 ± 10	0.053 ± 0.002	0.8 ± 0.1
14 days	21 ± 2	73 ± 16	0.057 ± 0.001	0.7 ± 0.1
30 days	24 ± 3	74 ± 5	0.068 ± 0.007	0.9 ± 0.2

*Enzyme activities are expressed as 10^{-6} mol substrate split per hour per mg dryweight. Control values represent the mean (± SD) of 5 animals, other values the mean (± SD) of 3 animals.

Cell maturation of epithelial cells

Epithelial cell maturation was studied after transposition of an ileal segment to proximal jejunum by determining the enzyme activities of non-specific esterases and neutral

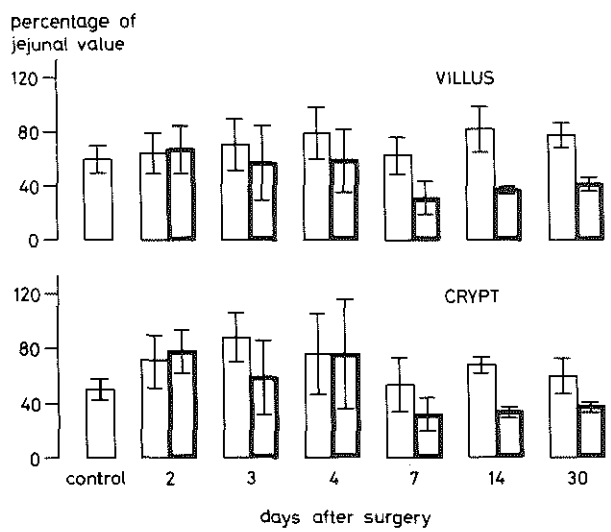

Fig. 3. Enzyme activity of non-specific esterases in dissected crypt and villus cell compartments after transposition of an ileal segment to jejunum. □ remaining ileum, ▣ transposed ileum.

α-glucosidase in dissected crypt and villus cell compartments.

From the enzyme activities in the jejunal epithelium, given in Table 4, it appears that the surgery and its consequences influence crypt and villus cell enzyme activities. The enzyme activities are reduced during the first days after surgery, at which time the proliferative activity is increased (Table 1), and subsequently return to control values. To determine whether specific changes in enzyme activities occur in the transposed ileum, the enzyme activities of the transposed ileum and the remaining ileum are given as percentages of activities in the jejunum of the same rat (Figs. 3 and 4).

The activity of non-specific esterases in the villus cell compartment of transposed ileum and remaining ileum is 60-80% of the activity in jejunum (Fig. 3) during the first days after surgery. From 7 days after surgery on the

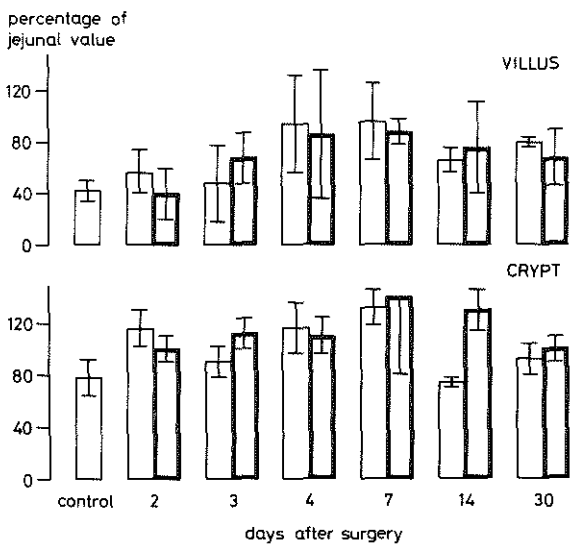

Fig. 4. Enzyme activity of neutral α-glucosidase in crypt and villus cell compartments after transposition of an ileal segment to jejunum. ☐ remaining ileum, ☐ transposed ileum.

esterase activity in the transposed ileum is reduced, as compared to the activity in the remaining ileum. The same is true for the esterase activity in the crypt cell compartment. The activity of neutral α-glucosidase in transposed ileum and remaining ileum, as illustrated in Fig. 4, increases somewhat above control values in the crypt and villus cell compartment. However, no consistent differences are observed between the transposed ileum and the remaining ileum.

From the enzyme studies, it appears that the epithelium of an ileal segment, which is transposed to the jejunum, does not adapt by an increase in enzyme activities: the enzyme activities remain below jejunal levels. The activity of non-specific esterases in transposed ileum is even reduced compared to the values for the remaining ileum.

DISCUSSION

The mechanisms underlying the potential of the small intestinal epithelium for adaptation are still largely unknown. The activity per cell of a number of enzymes involved in intestinal function, like disaccharidases, dipeptidases, alkaline phosphatase, and non-specific esterases, do not change, or only slightly, following procedures like partial resection of the intestine or experimental by-pass, which are known to affect intestinal function (Weser & Hernandez, 1971; Gleeson, Dowling & Peters, 1972; Hietanen & Hännine, 1972; Dowling & Gleeson, 1973; Menge et al., 1975; Rijke et al., 1976b). This indicates that changes in the number of functional villus cells are responsible for the functional adaptation of the intestinal epithelium to various circumstances. As the number of villi in the small intestine is remarkably stable under various experimental procedures (Clarke, 1972; Forrester, 1972; Cairnie &

Millen, 1975; Rijke et al., 1976b), the number of cells per villus appears to be primarily responsible for intestinal adaptation.

During lactation and after partial resection of the small intestine an increase in the number of villus cells and crypt cells was found throughout the small intestine, whereas no change was observed in the percentage of labelled crypt cells after ^{3}H-thymidine labelling (Hanson & Osborne, 1971; Hanson, 1975; Harding & Cairnie, 1975). During starvation a decrease in the number of villus cells and crypt cells was found throughout the small intestine, while the mitotic index remained normal or showed only a slight decrease (Stevens Hooper & Blair, 1958; Altmann, 1972).

A local adaptation of the small intestine may be observed after experimental by-pass or transposition of an intestinal segment. A decrease in the number of villus cells and crypt cells was found in a by-passed jejunal fistula, whereas the percentage of labelled crypt cells after ^{3}H-thymidine labelling remained unchanged (Rijke et al., 1976b). After transposition of an ileal segment to proximal jejunum, an increase in the number of villus cells was observed in the transposed segment (Altmann & Leblond, 1970). In the latter study, however, it was not investigated how the increase in the number of villus cells is brought about.

In the present study, epithelial cell kinetics and maturation were investigated from 2 to 30 days after transposition of an ileal segment to proximal jejunum. The results clearly demonstrate that the number of cells per villus column increases in transposed ileum up to jejunal values, and that the number of cells per crypt column markedly increases above that in jejunum and remaining ileum. This adaptation takes 14-30 days, which is a remarkably long

time considering the fast turnover of the intestinal epithelium. No changes were observed in the percentage of labelled cells per crypt column and the relative size of the proliferative cell compartment in the crypt. The total proliferative activity per crypt increases almost two-fold in transposed ileum at 7 days after surgery, indicating a large increase in the total number of cells per crypt. The results from the cell migration study reveal that the increase in number of villus cells in transposed ileum is not caused by a change in life span of the epithelial cells. The enzyme activities of non-specific esterases and neutral α-glucosidase are lower in ileal epithelium than in jejunal epithelium (see Figs. 3 and 4). However, no increase in enzyme activities was observed in ileal epithelium after transposition to proximal jejunum. The activity of non-specific esterases in crypt and villus cell compartments is even reduced in transposed ileum from 7 days after surgery on.

These findings lead to the conclusion that the adaptation of an ileal segment after transposition to proximal jejunum consists of an increase in the number of cells per villus. This increase is caused by an increase in the number of cells per crypt, which leads to a larger cell production per crypt, as no changes in the percentage of labelled crypt cells after ^{3}H-thymidine labelling or in the relative size of the proliferative crypt cell compartment occur.

Also following partial resection of the small intestine and during lactation an increase in crypt cell production is achieved by an enlargement of the crypt without changes in the relative size of the proliferative cell compartment (Hanson, 1975; Harding & Cairnie, 1975; McDermott & Roudnew, 1976). Furthermore it was observed that after partial resection of the intestine and during lactation some shortening of the cell cycle time occurs in the prolifera-

tive crypt cells (Hanson & Osborne, 1971; Harding & Cairnie, 1975; McDermott & Roudnew, 1976). Some shortening of the cell cycle time in all cells of the proliferative cell compartment could possibly lead to an increase in the total number of cells per crypt. However, it seems more likely that the cell cycle time of the proliferative cells in the bottom of the crypt, which is considerably longer than that of the remaining crypt cells (Cairnie, 1965; Wright, Morley & Appleton, 1972), is shortened under these circumstances, as this would lead more easily to an enlargement of the crypt.

An increase in crypt cell production was also found after an experimental reduction of the number of villus cells (Galjaard, van der Meer-Fieggen & Giesen, 1972; Rijke et al., 1976a). In these studies, however, the increase in crypt cell production was achieved by a considerable increase in the relative size of the proliferative cell compartment in the crypt, and the increase in crypt cell production occurred within 24 hours after reduction of the villus cell population (Galjaard et al., 1972; Rijke et al., 1976a). Following partial resection of the intestine or transposition of an ileal segment to jejunum an increase in crypt cell production is found only from 7 days after surgery on (Hanson, 1975; see Table 2).

It seems that cell proliferation in the intestinal epithelium is regulated by at least two separate control mechanisms. After reduction of the villus cell population an immediate increase in crypt cell production is achieved by an enlargement of the relative size of the proliferative cell compartment in the crypt, while an increase in crypt cell production during lactation, after partial resection of the intestine, or after transposition of an ileal segment to jejunum is brought about by an increase in the number of cells per crypt without changes in the relative size of the

proliferative crypt cell compartment. Furthermore, the latter mechanism takes a relatively long time to enlarge crypt cell production.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Prof. H. Galjaard and Prof. J.W. Osborne for their stimulating criticisms and advices. Mr. W.J. Visser is acknowledged for technical assistence, and Mr. P. Hartwijk and Mr. J.G.H. Fengler for preparation of the illustrations.

This study has been carried out with a grant from The Netherlands Foundation in Medical Research.

REFERENCES

Altmann, G.G. & Leblond, C.P.(1970)Factors influencing villus size in the small intestine of adult rats as revealed by transposition of intestinal segments. Am.J.Anat.**127**,15.

Altmann, G.G.(1971)Influence of bile and pancreatic secretions on the size of the intestinal villi in the rat. Am.J.Anat.**132**,167.

Altmann, G.G.(1972)Influence of starvation and refeeding on mucosal size and epithelial cell renewal in the rat small intestine. Am.J.Anat.**133**,391.

Altmann, G.G.(1974)Demonstration of a morphological control mechanism in the small intestine. In: Intestinal Adaptation (Ed. by R.H. Dowling and E.O. Riecken), p. 75. Schattauer Verlag, New York.

Both, N.J. de & Plaisier, H.M.(1974)The influence of changing cell kinetics on functional differentiation in the small intestine of the rat. A study of enzymes involved in carbohydrate metabolism. J.Histochem.Cytochem.**22**,352.

Cairnie, A.B.(1965)Cell proliferation studies in the intestinal epithelium of the rat. I. Determination of the kinetic parameters. Exptl.Cell Res.**39**,528.

Cairnie, A.B. & Millen, B.H.(1975)Fission of crypts in the small intestine of the irradiated mouse. Cell Tissue Kinet.**8**,189.

Clarke, R.M.(1972)The effect of growth and of fasting on the number of villi and crypts in the small intestine of the albino rat. J.Anat.**112**,27.

Dowling, R.H. & Gleeson, M.H.(1973)Cell turnover following small bowel resection and by-pass. Digestion **8**,176

Dowling, R.H. & Riecken, E.O.(eds.)(1974)Intestinal Adaptation. Schattauer Verlag, New York.

Feldman, E.J., Dowling, R.H., McNaughton, J. & Peters, T.J.(1976) Effects of oral versus intravenous nutrition on intestinal adaptation after small bowel resection in the dog. Gastroenterology 70,712.

Forrester, J.M.(1972)The number of villi in rat's jejunum and ileum: effect of normal growth, partial enterectomy, and tube feeding. J.Anat. 111,283.

Galjaard, H., Buys, J., Duuren, M. van & Giesen, J.(1970)A quantitative histochemical study of intestinal mucosa after X-irradiation. J.Histochem.Cytochem.18,291.

Galjaard, H., Meer-Fieggen, W. van der & Giesen, J.(1972)Feedback control by functional villus cells on cell proliferation and maturation in intestinal epithelium. Exptl.Cell Res.73,197.

Gleeson, M.H., Dowling, R.H. & Peters, T.J.(1972)Biochemical changes in intestinal mucosa after experimental small bowel by-pass in the rat. Clin.Sci.43,743.

Hagemann, R.F., Sigdestad, C.P. & Lesher, S.(1970)A method for quantification of proliferative intestinal mucosal cells on a weight base: some values for C57B1/6. Cell Tissue Kinet.3,21.

Hanson, W.R. & Osborne, J.W.(1971)Epithelial cell kinetics in the small intestine of the rat 60 days after resection of 70 per cent of the ileum and jejunum. Gastroenterology 60,1087.

Hanson, W.R.(1975)Intestinal compensation following intestinal resection in the rat. Ph.D. Thesis, University of Iowa.

Harding, J.D. & Cairnie, A.B.(1975)Changes in intestinal cell kinetics in the small intestine of lactating mice. Cell Tissue Kinet.8, 135.

Harrison, D.D. & Webster, H.L.(1971)Proximal to distal variations in enzymes of the rat intestine. Biochim.Biophys.Acta 244,432.

Hietanen, E. & Hännine, O.(1972)Effect of chyme on mucosal enzyme levels in small intestine of the rat. Metabolism 21,991.

McDermott, F.T. & Roudnew, B.(1976)Ileal crypt cell population kinetics after 40% small bowel resection. Autoradiographic studies in the rat. Gastroenterology 70,707.

Menge, H., Gräfe, M., Lorenz-Meyer, H. & Riecken, E.O.(1975)The influence of food intake on the development of structural and functional adaptation following ileal resection in the rat. Gut 16,468.

Rijke, R.P.C., Plaisier, H.M., Hoogeveen, A.T., Lamerton, L.F. & Galjaard, H.(1975)The effect of continuous irradiation on cell proliferation and maturation in small intestinal epithelium. Cell Tissue Kinet.8,441.

Rijke, R.P.C., Hanson, W.R., Plaisier, H.M. & Osborne, J.W.(1976a)The effect of ischemic villus cell damage on crypt cell proliferation in the small intestine: evidence for a feedback control mechanism. Gastroenterology (accepted for publication).

Rijke, R.P.C., Plaisier, H.M., Ruiter, H. de & Galjaard, H.(1976b)The influence of experimental by-pass on cellular kinetics and maturation of small intestinal epithelium. Gastroenterology (submitted for publication).

Stevens Hooper, C. & Blair, M.(1958)The effect of starvation on epithelial renewal in the rat duodenum. Exptl.Cell Res.14,175.
Weser, E. & Hernandez, M.H.(1971)Studies of small bowel adaptation after intestinal resection in the rat. Gastroenterology 60,69.
Wright, N., Morley, A. & Appleton, D.(1972)Variation in the duration of mitosis in the crypts of Lieberkuhn of the rat: a cytokinetic study using vincristine. Cell Tissue Kinet.5,351.