Cellular Kinetics of Gastrointestinal Mucosa, with Special Reference to Gut Endocrine Cells

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Summary. After ^3H -thymidine autoradiography was introduced to the study of cellular kinetics, the epithelial cells of the gastrointestinal mucosa have been examined extensively with regard to their kinetic behavior. After reviewing the knowledge on the kinetics of different epithelial cells in the stomach and small and large intestine, this article concentrates on the origin, differentiation and turnover of gut endocrine cells which have been under much controversy.

Autoradiographic and other investigations of EC cells support the view that these cells are not neuroectodermal in origin, but differentiate from an immature type of gut epithelial cells. We investigated the kinetics of the antral G cells in the hamster, G cells and S cells in the rat duodenum by the combined utilization of ^3H -thymidine autoradiography with immunohistochemistry, and confirmed that these cells are derived from the same precursor cells as the other mucosal cells of the gastrointestinal tract. The half-life of the antral G cells was estimated at 10-15 days, while the turnover time of the G cells and S cells in the duodenum were calculated at 3-4 days and 2 days, respectively. These data also support the belief that gut endocrine cells originate from the endoderm.

Histopathological alterations such as antral gastritis and intestinal metaplasia, as well as vagal denervation, were shown to influence the population of the antral G cells in man and the hamster. This supports the view that intrinsic and extrinsic stimuli might alter the kinetics of gut endocrine cells.

Study of cell renewal of the gut mucosa has a long history. Before the second half of the nineteen-fifties, however, most investigations were qualitative or remained at the level of a hypothesis. No dynamic experimental means of revealing the kinetic aspect of a cell had yet been found.

In 1958, autoradiography by ^3H -thymidine was introduced to the study of cellular kinetics, and caused rapid progress in research in the field of the gastrointestinal tract. Thymidine is a component of DNA, and, when given to experimental animals, thymidine labelled with ^3H is taken specifically into cells synthesizing DNA. As β -ray from ^3H in the nucleus is detectable with photosensitive emulsion, the cells with mitotic activity are able to be identified. ^3H -thymidine, once taken into a cell, remains as a stable DNA component as long as the nuclear DNA is not resolved and the daughter cells can also be identified as labelled cells, though ^3H -thymidine is diluted by cell divisions. Application of ^3H -thymidine autoradiography made it possible to pursue various kinds of cells of gut mucosa as labelled cells. To date, a great deal of detailed

data and new knowledge concerned with surface epithelium and various kinds of glandular cells in almost all sections of the gastrointestinal tract have been accumulated. In contrast, the kinetics of gut endocrine cells had not been studied until recently.

The cells today known as gut endocrine elements have been recognized as "basal-granulated cells" scattered throughout the gastrointestinal mucosa since the early days of HEIDENHEIN (1870) and KULTSCHITZKY (1897). Recent striking progress in ultrastructural study and immunocytochemistry made the specific identification of gut endocrine cells possible among them. At present, more than fifteen different gut endocrine cells have been identified and classified ultrastructurally. Nevertheless, to the present, the main interest of most studies on gut endocrine cells has lain in areas such as their ultrastructure, their distribution in normal or pathological states, and the physiology or pathophysiology of gut hormones contained in the cells.

These cells had been traditionally considered to be a static cell group with no division and no renewal (PATZELT, 1936). PEARSE (1966, 1968, 1969), on the other hand, found a common property between the gut endocrine cells and other endocrine cells, i.e., the ability of their amine-precursor-uptake and decarboxylation (APUD), and postulated that gut endocrine cells are derived from the neural crest (PEARSE, 1969; PEARSE and POLAK, 1971). The cells with APUD activity are widely distributed in the pineal body, hypophysis, pancreas, thyroid gland and carotid body. PEARSE (1977) categorized these cells including basal-granulated cells in the gut as the APUD series. This concept was broadly supported not only because of its proposal of a comprehensive interpretation of the endocrine cells in a whole body and its new position to the basal-granulated cells, but also from its original viewpoint on histogenesis. Nevertheless, there is much controversy over the actual origin of the gut endocrine cells (CHENG and LEBLOND, 1974c; ANDREW et al., 1982). In such a historical context, investigating the detailed kinetics of each type of the gut endocrine cells is also expected to cast more light on its enigmatic origin.

In this paper, the cellular kinetics of gut endocrine cells which have interested the authors for a few years will be mainly reviewed. The kinetics of these cells, however, should not be discussed separately from that of other mucosal cells. Accordingly, discussion on the data by ^3H -thymidine autoradiography of the surface epithelium and various exocrine cells in each part of the gastrointestinal tract will be included.

CELLULAR KINETICS OF GASTROINTESTINAL MUCOSA

1. The stomach

Human gastric mucosa consists of the cardiac, fundic and pyloric gland area. Both the cardiac and pyloric glands are simple mucus-secreting structures with indistinguishable similarities in morphological and chemical nature. The mucosal surface is lined with a layer of columnar surface epithelium. The fundic gland is basically a simple tubular gland, and consists of generative cells (proliferative cells) localized from the bottom of the gastric pit to the isthmus and four kinds of cells localized below the isthmus, i.e., parietal cells (oxyntic cell), chief cells (zymogenic cell), mucous neck cells (immature chief cell) and endocrine cells. On the other hand, the pyloric gland is a simple alveolar gland with branching, and consists of three kinds of cells, i.e., generative cells, mucoid cells and endocrine cells.

In this chapter, the cellular kinetics of the gastric mucosal cells except endocrine cells (described later) will be discussed.

The surface epithelium

Mitotic figures are frequently encountered in the epithelial cells immediately above the isthmus of the fundic glands. BIZZOZERO (1888, 1893) found transitional forms to each cell on the way from the bottom of the gastric pit to the mucosal surface, and concluded that the surface epithelium is renewed actively by the epithelial cells at this generative cell zone.

After ^3H -thymidine autoradiography was introduced to the study of cellular kinetics in 1958, QUASTLER and SHERMAN (1959), LEBLOND and MESSIER (1958), MESSIER and LEBLOND (1960), HUNT and HUNT (1962), LIPKIN et al. (1963) and KAKU et al. (1962) successively reported on the cellular kinetics of the stomach or intestine. By this series

Fig. 1. Autoradiograph of the fundic mucosa of the hamster by flash labeling of ^3H -thymidine. Arrows indicate labelled cells.

of studies, the distribution of generative cells in the gastric mucosa was clearly shown and the kinetics of the surface epithelium was clarified. As ^3H -thymidine is taken specifically into the cells synthesizing DNA, the number and location of the proliferating cells can be clearly identified by autoradiography after a single administration of ^3H -thymidine (flash labelling method, Fig. 1). Further, application of ^3H -thymidine autoradiography enabled the investigation of the kinetic parameters, i.e., DNA synthesizing time and cell cycle time, and the cell migration patterns.

MESSIER and LEBLOND (1960) traced labelled cells at regular intervals after ^3H -thymidine administration (pulse labelling method), and confirmed that most of the cells produced at the generative cell zone migrated to the mucosal surface and were transformed into surface epithelium. The life span of the surface epithelium, from the reports of many investigators (MESSIER and LEBLOND, 1960; HUNT and HUNT, 1962; HATTORI, 1975; HATTORI and FUJITA, 1976a), amounts to 3-4 days in the fundic, pyloric and cardiac glands, with little species difference.

On the other hand, KAKU (1966) and HATTORI and FUJITA (1976b) estimated the cell cycle time and DNA synthesizing time of a generative cell in the rat and hamster at approximately 30 hrs and 7 hrs, respectively. LIPKIN et al. (1963) reported the cell cycle time and DNA synthesizing time in generative cells of the human stomach as about 40 and 10 hrs, respectively. Thus, the generative cells at the isthmus repeat mitosis at 30 hr intervals in the hamster and rat, and 40 hr intervals in humans. These continuous cell divisions lead the immature surface epithelium after the final mitosis, to migrate towards the mucosal surface, while the intracellular organelles develop and secreting granules begin to form (HATTORI and FUJITA, 1976a). The cell migration from the gastric pit to mucosal surface is successive, just like the movement of an escalator.

The fundic gland

The kinetics of the glandular cells is considerably different from that of the surface epithelium. Before the latter half of the 1960's no kinetic analysis of the fundic gland had been reported. Although the surface epithelium is renewed at relatively short intervals, the cell migration to the lower part of the glandular portion is extremely slow and complex.

WILLEMS et al. (1972) proposed that chief cells are renewed by self-replication, judging from the observation that there were always DNA synthesizing cells, scanty as they were, among the chief cells and mucous neck cells. Contrary to this, from the investigation using the cumulative and pulse labelling method of ^3H -thymidine, HATTORI (1974) and HATTORI and FUJITA (1976a) insisted on a cell supply to the chief cell coming from the generative cell zone at the isthmus. They suggested that, although a few chief cells have the ability for self-replication, most of them are differentiated from the mucous neck cells in about 50 days. Furthermore, simulating a stochastic migration model, they estimated the life span of both parietal and chief cells at around 200 days. Thus, when mucous neck cells are regarded as immature and intermediate cells as chief cells, this model well explains the characteristic distribution pattern of mucous neck cells located at the upper part of the fundic gland and chief cells at the lower part. They also inferred that the difference in the distribution between parietal cells and chief cells is based on the difference in their migration speed downwards from the generative cell zone. This migration pattern, different from the pipe line system of the intestinal epithelium (first produced, first lost), is attended with the phenomenon of "passing and being passed" cell migration, and has been named the "stochastic flow system."

Fig. 2. Autoradiograph of the pyloric mucosa of the hamster by flash labelling of ^3H -thymidine. Arrows indicate labelled cells.

The pyloric gland

The main component cell of the pyloric gland is a large mucus-secreting cell. Kinetic studies of the pyloric gland have been extremely scarce. One of the reasons for this is that the pyloric gland was long regarded as a simple mucous gland and received less attention. Nowadays, however, it has been shown that the pyloric gland contains several kinds of gut endocrine cells and plays as important a role as the fundic gland in the gastric function.

HATTORI and FUJITA (1976b) labelled the pyloric gland cells of a hamster with ^3H -thymidine by a cumulative and pulse labelling method, and investigated the process by which the labelled cells were lost. They reported that the renewal of the pyloric gland cells of the hamster had a life span of around 14 days via the pipe line system. Concurrently they inferred, from an analysis of the decreasing pattern of the labelling index, that there must be another cell group different in cell kinetics from the pyloric gland cells with the life span of 14 days. This cell group has been clarified by the identification of the endocrine cells and the recent analysis of their kinetics.

2. The small intestine

As previously stated, it has been known since BIZZAZERO (1888, 1893) that the epithelium of the gastrointestinal mucosa is in the process of constant renewal. Figure 3 shows a ^3H -thymidine autoradiograph of a rat intestinal crypt after a single injection. The labelled cells, as seen in Figure 3, are distributed mainly in the lower part of the crypt, and it is clear that only at this portion (generative cell zone) are the cells constantly

Fig. 3. Autoradiograph of the upper jejunum of the rat by flash labelling of ^3H -thymidine. Arrows indicate labelled cells.

renewed. All the component cells, e.g., columnar cells, goblet cells or Paneth cells, are supplied from this zone.

The frequency of mitosis in the generative cell zone is particularly higher at the bottom of crypts. The labelling indices calculated in sections sliced parallel to the surface from the bottom of a crypt result as shown in Figure 4. The low labelling indices at the cell location numbers 10 to 30 are due to the intermixture of villus cells without mitotic activity, and relatively low labelling indices at the bottom may be due to mixed pre-Paneth cells.

The cell cycle time of a generative cell in the small intestine has been, so far, found to be around 14 hrs in the mouse or rat (QUASTLER and SHERMAN, 1959; KAKU, 1962; CAIRNIE et al., 1965; HAGEMANN et al., 1970; SCHULTZE et al., 1972; KOVACS and POTTEN,

Fig. 4. Correlation between labelling index (LI) and cell position in the crypt. (Reproduced by permission from S. FUJITA, *Nihon Ishikai Zasshi* 67, 1972).

Fig. 5. Velocity of cell migration in the crypt. (Reproduced by permission from S. FUJITA, *Nihon Ishikai Zasshi* 67, 1972).

1973), from which the migration speed of a cell at a certain site in a crypt can be inferred as in Figure 5. As shown in Figure 5, the deeper a cell is located in a crypt, the slower it moves. For instance, it takes 120 hrs for a cell at the base of a crypt to reach its opening, while a cell at the location number 20 can emerge from the crypt in around 15 hrs. Such migration in the crypt is basically an upwards perpendicular progression, but a movement in a right-angled direction, shifting horizontally in a crypt, may occur in a few cases.

Under normal conditions, both villus cells and generative cells are constant in number, and preserve the dynamic equilibrium. In addition, the generative cell zone is always located at the lower part of a crypt. This dynamic equilibrium and peculiar distribution can not be explained from the ambivalent mitosis of generative cells.

Fig. 6. Diagram of the p, q model. (Reproduced by permission from S. FUJITA, *Nihon Ishikai Zasshi* 67, 1972). p: probability to remain as a generative cell. q: probability to differentiate to a villus cell.

Figure 6 is a model explaining the difference in distribution between generative cells and villus cells, that is, according to the location of the generative cells, the probabilities whether a daughter cell remains as a generative cell or differentiates to a villus cell are fixed. The point of intersection of these two probabilities p and q is called the "critical zone of differentiation."

The columnar cell

In the small intestine the mucosa contains columnar cells, goblet cells, Paneth cells and endocrine cells, and yet the columnar cells form a large majority and play a decisive role in cell renewal. The columnar cells produced in the crypts overflow out of the opening of the crypts and migrate smoothly to the villus tips (LEBLOND and STEVENS, 1948; LEBLOND and MESSIER, 1958; QUASTLER and SHERMAN, 1959; MESSIER and LEBLOND, 1960; KAKU, 1962; CAIRNIE et al., 1965; CHENG and LEBLOND, 1974a). This migration can be considered an escalator-like movement by its way of sliding on the basement membrane. Furthermore, by tracing the labelled cells with ^3H -thymidine, it can be seen that newly labelled villus cells climb up the villi and push up the non-labelled villus cells. The border between labelled and non-labelled cells in this process is apparently clear, and there seems to be neither passing nor being passed procedures among the villi. This process called the "turn over by pipe line system" and the motive power of this type of cell migration is thought to be based on cell proliferation in the crypts. In the process of the cell's migration to the villus tips, the cells move temporally in a slow stream with a big horizontal vector at the transitional zone, and it is believed that several crypts can supply cells to one villus. This may mean that, in the transitional zone, cells from the right and left sides are mixed with each other. Once reaching a villus, a cell goes up by the pipe line system and falls into the intestinal lumen from the extrusion zone on the villus tip (BERTALANFFY and NAGI, 1961).

According to reports up to the present, the time transpiring for the replacement of

the villus cells is 1–2 days, and the turnover time of columnar cells is estimated at 3–4 days.

The goblet cell

Goblet cells are ordinarily found in the intestinal mucosa. Although a few goblet cells are capable of mitosis (CAIRNIE, 1970; SHIMAMOTO, 1970), a majority is produced from generative cells in crypts. Labelled goblet cells appear in the villus 20–24 hrs later than labelled columnar cells after ^3H -thymidine administration (MERZEL and LEBLOND, 1969; CAIRNIE, 1970). Both cells, however, migrate along the villi at the same speed. This suggests that the differentiation of goblet cells occurs at a deeper position in the crypts, corresponding to the above-mentioned time of 20 hrs, than that of columnar cells (CHENG and LEBLOND, 1974a; CHENG, 1974a). Specifically, the cells determined to differentiate to goblet cells stay as pre-goblet cells for a while, and then appear in the villi as mature goblet cells.

The Paneth cell

Paneth cells are usually located at the bottom of the crypts and, unlike the other cell types, do not show any upward migration. To date, it has been shown that the Paneth cell has no self-replicating ability (THROUGHTON and TRIER, 1969; CHENG et al., 1969; CAIRNIE, 1970; CHENG, 1974b), and seems to be derived from crypt-base columnar cells and degenerate in situ (CHENG et al., 1969; CHENG, 1974b). The turnover time of the population is thought to be much longer than that of goblet cells (CHENG et al., 1969; CAIRNIE, 1970; CHENG, 1974 a, b).

Brunner's gland

NIKI et al. (1980) reported the kinetic behavior of Brunner's gland from an analysis of ^3H -thymidine autoradiography stating that, basically, the cells of Brunner's gland are not supplied from the generative cell zone in the crypts but produced in situ corresponding to the cell loss by the proliferation of mature glandular cells at need and at random. They estimated a half life time of approximately 40 days.

3. The large intestine

The mucosa of the large intestine consists of columnar cells, goblet cells and endocrine cells, and are also renewed very rapidly. The generative cells are located in the lower half of a crypt (Fig. 7). The cell cycle time of the generative cells measured by the fraction mitosis curve has been estimated at 23 hrs in the mouse (CHANG and NADLER, 1975) and human (LIPKIN et al., 1963). In addition SUNTER et al. (1979) reported a varied cell cycle time according to different sites of the rat colon, namely, 25 hrs in the cecum, 35 hrs in the ascending colon, 42 hrs in the transverse colon and 58 hrs in the descending colon.

The fashion of cell renewal in the large intestine is more or less similar to that in the small intestine but the renewal rate or turnover time of each component cell is quite longer than in the small intestine. CHANG and LEBLOND (1971) identified three types of cell lines in the mouse large intestine, i.e., vacuolated-columnar, mucus (goblet) and argentaffin cell lines. In the series of their studies (CHANG and LEBLOND, 1971; CHANG and NADLER, 1975; TSUBOUCHI and LEBLOND, 1979), it was described that vacuolated-columnar cells and mucus cells were found to be capable of mitosis in the mid or lower crypts, to migrate upwards and be lost from the mucosal surface within a life span of about 4.6 days.

Fig. 7. Autoradiograph of the colonic mucosa of the hamster by flash labelling of ^3H -thymidine. Arrows indicate labelled cells.

CELLULAR KINETICS OF GUT ENDOCRINE CELLS

1. The enterochromaffin cell (EC cell)

The kinetics of the EC cell has been investigated mostly in gut endocrine cells, because the combined use of ^3H -thymidine autoradiography with more conventional techniques such as diazo-reaction was not difficult, whereas the specific identification of other gut endocrine cells was nearly impossible until the introduction of immunohistochemistry.

So far, only one report by HATTORI *et al.* (1977) on the kinetics of EC cells in pyloric gland is available. They found no labelled diazo positive EC cells in the pyloric gland of the hamster after flash labelling of ^3H -thymidine, and observed that cumulative

labelling for 10 and 20 days caused EC cells to be labelled at 31% and 63% respectively; labelled EC cells initially appeared at the isthmus near the generative cell zone and then with time in the lower part of the glands after the cessation of ^3H -thymidine administration. They subsequently postulated that EC cells in the pyloric glands of the hamster are produced from precursor cells at the isthmus (=generative cell) and then migrate downwards. As the regression pattern in the labelling index showed an exponential decreasing curve with time, they inferred that the cell loss conformed to the "random loss system," and estimated the half life of the EC cell population at around 15 days on the average.

The kinetics of EC cells in the small intestine has been studied in the mouse and hamster (ODARTCHENKO et al., 1970; FERREIRA and LEBLOND, 1971; CHANG and LEBLOND, 1974; NIKI, 1980). These investigators employed diazo-reaction or electron microscopy for the identification of the cells, in combination with ^3H -thymidine autoradiography. On the basis of the first report by DESCHNER and LIPKIN (1966) by the combination of diazo-reaction with ^3H -thymidine autoradiography, ODARTCHENKO et al. (1970) investigated EC cells in the mouse duodenum using the same procedures. Recognizing the labelling in diazo-positive cells in the duodenal crypts, the frequency of the labelling being high in the medium and weak diazo-positive cells, they concluded that the proliferation of EC cells in the duodenum occurs mainly in less differentiated cells and possibly in a group of precursors that are hardly recognizable as belonging to the EC cell series. At the same time, they concluded the replacement time of EC cells in villi to be 72 hrs, due to the fact that EC cells were labelled in 33% in 24 hrs by ^3H -thymidine multi-injections, and that the migration speed of EC cells is slower than that of other epithelial cells. In succession, FERREIRA and LEBLOND (1971), and CHENG and LEBLOND (1974b) gave a full picture of the kinetics by making a detailed and comprehensive study of "entero-endocrine cells" in the mouse small intestine, using iron hematoxylin staining and electron microscopy together with ^3H -thymidine autoradiography. In a series of reports they described that a self-proliferative ability was not seen in entero-endocrine cells including EC cells but only in a small number of premature "young entero-endocrine cells" at the crypt-base. By analyzing the ^3H -thymidine autoradiography, they concluded that EC cells in the small intestine are derived from immature precursor cells at the crypt-base, lose their proliferative ability with the advance of maturation and move towards the villus tip to fall into the intestinal lumen. The maturation time and turnover time were estimated as about 2 and 4 days respectively. From these results, the observation by ODARTCHENKO et al. (1970) that labelled EC cells appeared later than other labelled epithelial cells suggests that the differentiation to EC cells occurs at a deeper position than the other epithelial cells. Incidentally, whether or not the EC cell is judged proliferative by light microscopy depends on not only its stage of maturation but the sensitivity of the staining method for EC cells as well. We have reported on an immunohistochemical demonstration of EC cells using anti-serotonin serum (INOKUCHI et al., 1982) and recently found that this method is much more sensitive in selectively detecting the EC cells than conventional techniques such as diazo-reaction (unpublished data). Actually, some EC cells could be shown to have mitotic activities via immunohistochemical demonstration.

The kinetics of EC cells in the large intestine was reported earliest of all the endocrine cells in the gastrointestinal tract (DESCHNER and LIPKIN, 1966). Its details were, however, not clearly explained until recently. Observing mitosis and labelling after the administration of ^3H -thymidine among diazo-positive cells, DESCHNER et al. (1966) reported the proliferative activity of EC cells in the human rectum, and estimated the

replacement time of the EC cell population as 35–100 days from the rate of increase in the labelled cells. On the other hand, CHANG and LEBLOND (1971) and TSUBOUCHI and LEBLOND (1979) investigated EC cells in the descending colon of the mouse and found no proliferative activity in those cells. Analyzing autoradiography by continuous infusion of ^3H -thymidine in their consecutive studies, they concluded that EC cells are derived from immature precursor cells, migrate more slowly than other cell lines and are probably lost from the mucosal surface. They estimated the turnover time of EC cells in the mouse colon as 23.3 days.

By the kinetic studies, the EC cell population has been proven not to be static but to be constantly renewed. It has also been shown that EC cells are produced from the immature precursor cells and their kinetic behaviors are quite different among the pyloric gland, small intestine and large intestine. Such differences in their migration pattern and life span in the different sites of the gut is thought to be based on the site-specific mucosal architecture and the difference in the cell cycle time among them.

2. The gastrin cell (G cell)

MCGUIGAN (1968) first identified G cells by an immunofluorescence method for gastrin. G cells are distributed mainly in the pyloric gland but they are also found in the duodenal mucosa in a considerable number.

LEHY and WILLEMS (1975), combining ^3H -thymidine autoradiography with an immunohistochemical demonstration of gastrin, observed a number of mitotic figures in mouse antral G cells after colchicine administration; labelled gastrin cells comprised 0.45–0.48% by a single injection of ^3H -thymidine. Consequently they concluded that the G cell population is not static but continuously renewed. Furthermore, by analyzing autoradiographs after a single and a series of six injections of ^3H -thymidine (LEHY

Fig. 8. Labelling index of G cells during and after a series of 5 injections of ^3H -thymidine. (Reprinted by permission of the publisher from the article by S. FUJIMOTO et al., *Gastroenterology* 79: 785–791, 1980. Copyright 1980 by the American Gastroenterological Association).

and WILLEMS, 1976), they postulated that a large majority of G cells originates from the replication of other gastrin cells, as the rate of increase in labelling index during the initial 1.5 days was approximately equal to the theoretical increase by mitotic replications, and estimated the turnover time as 2–4 months. They left the origin of antral G cells as an unsolved matter, saying that they could not prove the origin to be either immature pregonitor cells or the neural crest. We, therefore, investigated in detail and gave a full picture of the kinetics of antral G cells in the hamster by flash and cumulative labelling methods which consisted of series of five and forty-one injections of ^3H -thymidine at 6 hrs intervals (FUJIMOTO et al., 1980a). We observed the labelling index of 0.3% in G cells by flash labelling, which confirmed the proliferative ability in some antral G cells, as LEHY and WILLEMS (1976) previously described. The increase curve of the labelling index after five injections of ^3H -thymidine revealed the completion of the differentiation of G cells within 5 days (Fig. 8). The increase of labelled G cells, however, was much higher than the theoretical increase by self-replication. Thus, the renewal of antral G cells was ascribed to some process other than self-proliferation. The pattern of regression of the labelling index (Fig. 9) and time course of the spatial distribution of labelled G cells (Fig. 10) after cumulative labeling for 10 days indicated that G cells migrate downwards from the isthmus with time and are lost randomly ("random loss system") with a half life of 10–15 days. From these results it was concluded that a large majority of antral G cells originates from the immature precursor cells (generative cells) at the isthmus of pyloric glands and show downward migration, while a small number of G cells undergo mitosis.

G cells in the duodenal mucosa are distributed in both the villi and the crypts, but usually more numerous in the latter. In our recent observation, only a small number of G cells in the duodenal crypts of the rat were labelled, while most were non-labelled after a single injection of ^3H -thymidine; one third of the G cells, especially those in the villi, were labelled on the second day after five injections at 6 hr intervals. This indicated that most G cells in the rat duodenum were produced from the immature precursor cells in the crypts and their functional differentiation was completed within 3 days. Almost all were replaced by non-labelled cells within 3–4 days, which indicated

Fig. 9. Labelling index of G cells during and after a series of 41 injections of ^3H -thymidine. (Reprinted by permission of the publisher from the article by S. FUJIMOTO et al., *Gastroenterology* 79: 785–791, 1980. Copyright 1980 by the American Gastroenterological Association).

Fig. 10. Spatial distribution of labelled G cells on various days. (Reprinted by permission of the publisher from the article by S. FUJIMOTO et al., *Gastroenterology* 79: 785–791, 1980. Copyright 1980 by the American Gastroenterological Association). **A:** 5th day of cumulative labelling. **B:** 10th day of cumulative labelling. **C:** 10th day of pulse labelling. **D:** 20th day of pulse labelling. **E:** 50th day of pulse labelling.

that the G cells after maturation migrated upwards and were lost within 3–4 days. From this preliminary study we inferred the renewal pattern of G cells in the duodenum as shown in Figure 11.

Thus, G cells in two different sites, i.e., pyloric and duodenal mucosa, show different kinetic behaviors. The kinetics of antral G cells is quite similar to that of antral EC cells, while the kinetics of duodenal G cells is similar to that of EC cells in the small intestine. The cellular kinetics of them, consequently, depends on where they are rather than what they are.

3. The S cell

In recent years we have also studied the kinetics of secretin cells (S cells) in rat duodenum. According to all existing reports, opinion is divided over the spatial distribution of S cells in the duodenum. Some investigators reported that S cells were more numerous in the crypts (BUSSOLATI et al., 1971) or at the transitional zone (POLAK et al., 1971a, b; CHEY and ESCOFFERY, 1976), while others found them mainly in the villi (LARSSON et al., 1977). In our observation of the rat, S cells were distributed from the transitional zone to the villus tip and more numerous in the upper two thirds of the villi. This result agrees with the observation of KRAWITT et al. (1966) who reported that the bioactivity of secretin was concentrated in the transitional zone and villi.

In our preliminary study by ^3H -thymidine autoradiography, the S cell showed no proliferative ability, and over 60% of the S cells in the villi were labelled on the second day after five injections of ^3H -thymidine at 6 hr intervals. All labelled cells were then replaced by non-labelled cells within 2 days. From these results, we calculated the renewal pattern of S cells as shown in Figure 12. It is thought that the reason why no S cell was found in the crypt of the duodenum is because its functional differentiation does not occur till the cell reaches the villi.

4. Other endocrine cells in the gut mucosa

Although more than fifteen kinds of gut endocrine cells have been identified, we have

Fig. 11. Diagram of G cell kinetics in the rat duodenum. The functional differentiation of G cells is completed in a crypt within 3 days after the proliferation from immature precursor cells. After the maturation, the cells migrate towards the villus tip and are lost with life span of 3-4 days.

Fig. 12. Diagram of S cell kinetics in the rat duodenum. The maturation of S cells occurs during the migration along a villus within 3 days. The mature S cells are lost with life span of 2 days.

very little knowledge about the kinetics of those cells except for EC, G and S cells, thus far. As far as the small intestine is concerned, however, almost all cells throughout the crypts and villi can be labelled by consecutive administrations of ^3H -thymidine, and there is no definite cell free from the labelling. It seems reasonable to conclude that all mucosal cells of the small intestine are derived from the generative cells in crypts and renewed continuously.

FACTORS INFLUENCING THE CELLULAR DYNAMICS OF GUT ENDOCRINE CELLS

At present we must admit that the factors which might influence the cellular dynamics of gut endocrine cells mostly remain unknown subjects. So far, hyperplasia of antral gastrin cells with chronic antral stimulation (LEHY et al., 1975) and in several pathological states (CREUTZFELDT et al., 1971; POLAK et al., 1971c, d; PEARSE et al., 1972) have been described, so it seems that some intrinsic or extrinsic factors could influence the kinetics of gut endocrine cells, such as the antral G cells.

Although we now understand that the G cell population is continuously renewed, it is not known what is responsible for the hyperplastic state among different possible mechanisms such as an increased renewal rate, a shortening of differentiation time or a prolonged life span of antral G cells, or their combination. In this chapter our observations, which might give some clues to those questions, are discussed.

The G cell population in histopathological alterations

STOCKBRUGGER *et al.* (1977) described histopathological alterations such as a reduced G cell population in human antral gastritis. Lately, we confirmed their observation and, in addition, found no G cells in the area occupied by intestinal metaplasia (FUJIMOTO *et al.*, 1980b).

Antral gastritis is characterized by inflammatory cell infiltration and a decrease in glandular cells. Therefore, the reduced G cell population is thought to be a decrease proportional with the other components. By contrast, the finding that no G cell exists in the intestinalized glands indicates that an intestinalized gland is incapable of producing G cells.

RUBIN (1969), on the other hand, described the proliferation of endocrine cells in atrophic fundic mucosa in patients with pernicious anemia characterized by the disappearance of normal fundic glands and replacement with pseudopyloric glands or intestinal metaplasia. Lately, CREUTZFELDT *et al.* (1971) and POLAK *et al.* (1971c, 1973) found G cells in the fundic gland area in patients with pernicious anemia. As basically no G cell exists in the fundic gland area, the origin of these G cells is apparently mysterious.

Recently we found G cells in the regenerated pseudopyloric glands after mucosal injury, and observed the transformation of a normal fundic gland into a pseudopyloric gland containing G cells along the fundopyloric border (Fig. 13) in a resected human stomach. It is certain that this alteration in glandular architecture comes from the characteristic change in immature precursor cells at the generative cell zone, i.e., precursor cells producing fundic gland components such as chief cells and parietal cells which seem to have been transformed to those producing other cell types such as mucoid cells and G cells. This seems to be a possible explanation for the origin of G cells in the fundic gland area, because a pseudopyloric change appears to be inevitable in atrophic gastritis in patients with pernicious anemia.

Fig. 13. Transformation of the fundic gland into the pseudopyloric gland containing G cells along the fundopyloric border in human gastric mucosa. A pseudopyloric change starts at the neck portion of the fundic gland, then progresses downwards.

G cell hyperplasia after vagotomy

In man and the rat, hypergastrinemia (BECKER *et al.*, 1973a, b; SCHRUMPF *et al.*, 1974; DELINCE *et al.*, 1978; HAKANSON *et al.*, 1974; ALUMETS *et al.*, 1980), increase in antral gastrin content (BECKER *et al.*, 1973b; HUGHES

and HERNANDEZ, 1976) and G cell hyperplasia (DELINCE et al., 1978; ALUMETS et al., 1980) have been described after vagotomy. These observations were obtained at least 3 weeks after vagotomy. In ^3H -thymidine autoradiography of the antral portion of hamsters by the flash labelling method during the 7 days after vagotomy, we observed a remarkable expansion of the generative cell zone in the pyloric glands at 24 hrs after vagotomy (Fig. 14), and also an increased labelling index of G cells as high as 7–8 times that of the controls. The labelling index increased more or less transiently and gradually diminished, settling at slightly high values compared to the controls (unpublished data).

These findings indicate that not only G cells but other mucosal cells in the pyloric glands were affected and responded to the denervation at such early stages as 24 hrs

Fig. 14. Autoradiograph of the pyloric mucosa of the hamster. **a.** Control, **b.** 24 hrs after vagotomy; note the expansion of the generative cell zone.

after vagotomy. As far as G cells were concerned, it was obvious that G cells capable of mitosis increased significantly in number during the 24 hrs after vagotomy. However, it is unclear whether such an increase in the G cell population with mitotic ability is due to rapid functional differentiation of the cell prior to loss of mitotic activity, its preserved mitotic ability after maturation, or its recovered mitotic ability at its full maturity. The life span of G cells after vagotomy is also unknown.

Many factors other than histopathological alterations and denervation may also influence the kinetics of gut endocrine cells, for example the interaction of various kinds of hormones including gut hormones, or the administration of drugs, etc. These issues thus far remain unsolved.

In summary, gut endocrine cells come from the same origin as other cell types of endoderm and never represent a special cell group in mucosal cell dynamics. A generative cell in each part of the gut mucosa is multipotential in its differentiation and maintains the site-specific mucosal architecture of the gastrointestinal tract. However, many questions, i.e., what sort of stimuli might determine their differentiation into endocrine cells under normal or pathological conditions, how the alteration of this cellular kinetics might be induced by the stimuli and whether or not it might involve other types of cells, remain to be solved in future studies.

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