Scientific Basis of Gastroenterology

EDITED BY

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Preface

Our objective has been to provide a set of authoritative summaries of the basic knowledge of the principal areas of gastroenterology to give a scientific basis for clinical practice. We have tried to steer a middle path between detailed review articles with numerous references on the one hand and purely didactic texts on the other. A restricted number of references has been allotted to each contributor but these should permit tracing of the relevant literature.

We are grateful for the generous cooperation of all our contributors and our publishers.

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Contents

BASIC PRINCIPLES

1

Cell population kinetics in the mucosa of the gastrointestinal tract

The mucosal layer of the digestive tract is a dynamic entity in which the surface cells are continuously renewed. The cellular desquamation from the superficial epithelia and villi of the gastrointestinal mucosa results in a constant cell loss. A sustained proliferative activity of 'stem' cells compensates for this cellular shedding and maintains a histological steady state. When the cell loss increases, the proliferative activity of the stem cells must increase proportionally or the total number of cells in the mucosa decreases. Theoretically a negative imbalance between cell production and cell loss may result in mucosal atrophy, and perhaps in mucosal ulceration. Conversely, the prolonged survival of cells or excessive mitotic activity of stem cells is likely to result in hyperplasia or even of tumour formation.

Alterations of the mechanism regulating cell loss and cell production have been described recently in patients with disease of the digestive tract. Atrophic gastritis, haemorrhagic gastritis, stress ulcers, Zollinger–Ellison syndrome, idiopathic sprue, postradiation diarrhoea, rectocolitis and of course, all neoplastic lesions of the mucosa of the digestive tract are accompanied, and perhaps caused by abnormal cell kinetic patterns in the mucosal glands. Moreover, it has been suggested that more common diseases, such as peptic ulcer and gallbladder lithiasis also involve abnormalities of the mucosal cell kinetics. Interest in this topic has been stimulated by experimental data which have indicated the existence of factors which control cell proliferation and which can modify the mitotic activity, the life duration and the number of different types of cell in the digestive mucosa, since information of this sort is likely to open new therapeutic prospects in human pathology.

The understanding of deviations of cell kinetics of a tissue from normal is possible only if the normal proliferation characteristics of this tissue are known and if the physiological factors which control cell renewal can be defined. This is far from being the case in the human digestive tract since ethical considerations have prevented most studies in patients. Fortunately, experimental studies in animals have permitted considerable progress in this field during the last decade, while new techniques have been introduced which can be safely applied in clinical research. The recent progress in the methodology of kinetic studies as well as in the knowledge of the normal mitotic activity and of its variations in the digestive mucosa will be examined in view of their possible implications in human digestive pathology. An excellent review of the literature on this subject has recently been published by Eastwood (1977).

METHODS AND TECHNIQUES

Interpretation of histological sections after staining has been widely used to determine whether a mucosa contains many or few mitoses and whether the cells in a tissue are more or less numerous than normal. It is evident that results obtained by such methods cannot be submitted to statistical analysis and must be taken with caution. Whenever possible, quantitative data must be obtained in the study of cell populations.

The methods used for investigating the kinetics of cell populations distinguish between three different aspects of cell renewal:

1 The production of new cellular elements.
2 The total number of cells in the mucosa.
3 The loss of cells from the mucosa.

1 Methods for studying cell proliferation

The cell cycle is the interval between a mitosis and the completion of the subsequent mitosis in the daughter cells. In most mammalian cells, the cell cycle is subdivided into four successive stages: a pre-DNA-synthesis resting phase (G_1), a DNA-synthesis phase (S), a post-DNA-synthesis resting phase (G_2) and the mitosis phase (M). The mean total duration of this cycle is called the cell cycle time (Tc). Resting cells which do not participate in the cell cycling are called G_0 cells.

Mitotic and DNA-synthesis index

The mitotic index (MI) of a cell population is the percentage of cells in mitosis in a tissue, calculated by the formula $MI = \frac{Nm}{Nt} \times 100$, where Nm is the number of mitotic figures and Nt the total number of cells in the population, Mitotic figures may be counted in classical histological sections. Because the mitotic phase is relatively short (approximately 20–30 minutes) when compared to the length of the cell cycle in the gastrointestinal mucosa (Table 1.1), the number of mitotic figures, and thus the mitotic index in the digestive glands, is low. Therefore the precision of this type of measurement is low and results misleading in slowly proliferating populations like the gastric glandular epithelium.

The DNA synthesis phase in most stem cells of the gastrointestinal mucosa is 15–20 times longer than the mitotic phase and cells in the DNA synthesis phase are found 15–20 times more frequently than mitotic figures. Labelled precursors of DNA, such as ^{3}H-thymidine or ^{14}C-thymidine, are incorporated into nuclear DNA during the DNA synthesis phase so that it is possible to recognise and to count the cells by using autoradiography of histological sections after injection of these precursors, and to identify cells in the phase of DNA synthesis at the time of the radioactive injection. The DNA synthesis index, or labelling index (LI), is the percentage of labelled cells in a tissue shortly after injection of labelled thymidine and is calculated from the formula $LI = \frac{Ns}{Nt} \times 100$ where Ns represents the number of cells in the cell population

Table 1.1 Kinetic parameters in normal digestive glands

TISSUE			Species	LI	S phase (hours)	Cell cycle (hours)	Turnover (hours)
OXYNTIC MUCOSA							
Stem cells	Lipkin	(1965)	Man		10	99	
	Willems	(1977)	Man (fasted)	4%	9		223
	Castrup *et al.*	(1975)	Man	10%	7	100	
	Hart-Hansen *et al.*	(1975)	Man	12%			
Chief cells	Willems *et al.*	(1972)	Mouse	0·33%		2 months	
ANTRUM							
Stem cells	Kaku	(1966)	Hamster	30%			
	Willems *et al.*	(1974)	Dog	8%			
	Hart-Hansen *et al.*	(1975)	Man	14%			
Gastrin cells	Lehy *et al.*	(1976)	Mouse	0·25%			3 months
DUODENUM	Trasher	(1967)	Mouse	30–40%	7	15	45–80
JEJUNUM	Cairnie *et al.*	(1965)	Rat	35%	7	15	40
	Lipkin	(1965)	Man		11	24	100
	Tytgat *et al.*	(1975)	Man	38%	11	30	
ILEUM	Lipkin	(1965)	Man		7	15	72
COLON	Sawicki *et al.*	(1968)	Hamster	14%	7		48
	Lipkin *et al.*	(1965)	Man				72
RECTUM	Lipkin	(1965)	Man		11	40	80
	Willems *et al.*	(1970)	Dog	26%	8		29
	Bleiberg *et al.*	(1970)	Man	10%	9		86

under consideration. Owing to the higher number of labelled cells, precise determination of the DNA synthesis index is more easily made than are estimations of mitotic index.

Two main errors must be avoided when the rate of cell proliferation is evaluated by counting mitotic figures or DNA synthetising cells in the digestive mucosa. Firstly, it must be emphasised that an increase in mitotic index, even when adequately estimated, does not necessarily reflect an increased cell turnover since the absolute duration of the mitotic phase can be prolonged under certain circumstances; such as poisoning with colchicine. In this case, cells are blocked in the metaphase, the relative number of mitotic figures in the tissues increases and the high mitotic index is paradoxically the result of decreased cell turnover. In order to avoid this type of error, the duration of the mitotic phase may be determined using a stathmokinetic method with vincristine (Clarke, 1970), but the potential toxic effects of this mitotic poison prevents its use in man. The duration of the S phase may be modified by the experimental conditions and the determination of the DNA synthesis index alone cannot, therefore, provide a measure of the rate of cell proliferation. Combined estimations of DNA synthesis rate and of mitotic index are better because the probability that experimental conditions can lengthen both phases of the cell cycle without affecting G_1 or G_2 is low. Measuring both the labelling index and the length of the S phase (Ts) at the same time appears to be the ideal solution when a quantitative estimation of cell turnover is required.

The second error frequently committed when mitotic or labelling indices are measured involves the histological methods used in the determination of these indices. Cell division occurs in the deeper portions of the crypts and not in the superficial epithelia or in the villi of the digestive mucosa, so that if all the mucosal cells are used in calculating the percentage of mitoses or of labelled cells, cells which never divide will be included in the cell counts and the mitotic or labelling indices will be underestimated. A decrease in non-dividing cells, for instance through superficial desquamation, will increase the calculated proportion of mitoses and of labelled cells in the glands and will incorrectly indicate that proliferation has been stimulated, while if measurement of the percentage of cells in DNA synthesis or in mitosis is restricted to the zones in which labelled cells are seen in each gland section a number of cells in G_1 or G_0 phases will be overlooked and the indices will be overestimated. In order to prevent such errors, the proliferative compartment of the mucosa should be predetermined with accuracy and the cell counts should be restricted to this area in all the mucosae.

Several other errors may occur during the use of histo-autoradiography. The background values should be controlled. Excessive thickness of the histological sections may affect the resolution of autoradiograms. When tritium compounds are used the radioactivity of certain cells from paraffin sections cannot reach the emulsion layer, so that the paraffin sections must be as thin as possible, and using semi-thin Epon section may offer considerable advantages. The volume of a mitotic figure is larger than the volume of a normal mucleus, so that the probability of sectioning a mitotic spindle is relatively higher and mitotic counts are overestimated in histological sections, especially when the sections are thin.

An accurate crypt squash technique was developed in order to determine precise values of labelled and of mitotic figures in digestive glands (Kovacs and Potten, 1973) but the theoretical precision of the data is frequently counterbalanced by the technical difficulties of these methods. Practical failures of the technique and time-loss during preparation may, in fact, introduce much larger errors than those actually avoided.

Duration of the cell cycle phase

An increase in both labelling and mitotic index is highly suggestive of accelerated cell turnover but the absolute demonstration of the shortening of the cell cycle requires the measurement of other criteria. The length of the different phases of the cell cycle can be measured by the autoradiographic method of the labelled mitosis wave (Quastler and Sherman, 1959). This accurate technique involves the injection of triated thymidine and multiple sampling of the tissue under study. A few authors have measured kinetic indices in the human digestive glands in this way (Table 1.1) but for obvious reasons the number of subjects has been small and the individuals have had a limited life expectancy, so that the normality of the observed values has not been established.

Techniques using double labelling with ^{3}H-thymidine and ^{14}C-thymidine, or with two different doses of ^{3}H-thymidine (Galand and Chretien, 1969) have been described for measuring the length of the phase of DNA synthesis particularly for use *in vitro* in incubated mucosal specimens since this method may be applied without any risk to patients. When the durations of the S phase (Ts) and the DNA synthesis index (or labelling index – LI) are known, the turnover-time (Tot) can be calculated by the formula of Quastler and Sherman $\text{Tot} = \frac{\text{Ts}}{\text{LI}} \times 100$ if the tissue under study is in a steady state.

Labelled cells and mitosis in slowly proliferating cell populations are sometimes so infrequent that these methods cannot be used. Measurement of the time needed for doubling the labelled cell population after one or more injections of the precursor may provide approximate values of the cell cycle time under these circumstances. In view of the dangers of injection of radioactive materials, it seems probable that indices of cell cycles of cells such as endocrine cells of the gut or gastric peptic cells will remain unknown in the human unless new techniques are introduced.

The stathmokinetic method with vincristine has been used to study the length of the mitotic phase and hence, of the cell cycle in animal tissues. Since the cell nucleus doubles its DNA content during the S phase, micro-spectrofluorometry may be used to study the amount of DNA in the nuclei and to distinguish tetraploid G_2 cells. No reports are available concerning the G_2 cell populations in the intestinal mucosa.

Recently, bromodeoxyuridine has been used as a non-radiolabelled precursor of DNA for the analysis of cellular kinetics (Tice, Schneider and Rary, 1976). The bromodeoxyuridine which is incorporated *in vitro* into DNA can be stained with certain fluorescent compounds and autoradiography can therefore be avoided. Another new technique is available which is based on the fact that cellular DNA synthesis and nuclear immunoreactivity to antinucleoside anti-

bodies vary in parallel during the S phase. Immunoperoxidase-labelled anti-nucleoside antibodies may thus be used to stain electively the cell nuclei in DNA synthesis without any preliminary injection of the patient (Liebeskind *et al.*, 1977). These new techniques of investigation promise to reveal very useful information about human mucosal tissues.

Other parameters

The time required for a labelled cell to reach the surface epithelium, starting from the progenitor region of the digestive glands, is called the 'migration time'. It represents the rate of cell renewal in the glandular tubes and depends on the proliferative activity of the stem cells. This criterion is generally measured after *in vivo* injection of radioactively labelled compounds. The migration of labelled cells in human biopsy specimens after *in vitro* labelling has been used to study migration time in human alimentary mucosae (Browning and Trier, 1969). However, the renewal of well differentiated cells such as parietal cells, which have lost their capacity to divide, cannot be studied with this technique because these cells do not synthesise DNA and do not incorporate the precursors of DNA. ^{3}H-thymidine may be used as a cellular tracer for this type of cell, because these cells originate through continuous transformation of undifferentiated 'mother cells' which are able to divide and do incorporate the precursor within their nucleus. The rate at which the specialised cells are produced may then be studied by measuring the increasing percentage of radioactive nuclei in the specialised cell population under study during a period of time (Ragins, Wincze and Liu, 1968).

Biochemical methods are available which allow the determination of the rate at which ^{3}H-thymidine is incorporated into mucosal tissue. Such assays are less time-consuming than are histological observations, such as cell counts, but they are less precise. Mucosal homogenates include non-epithelial cells which eventually incorporate the precursor and scintillation counts cannot distinguish these cells. Moreover, normal superficial epithelium never incorporates precursors and therefore represents an uncontrolled 'cold' fraction, which variably alters the proportion of 'warm' DNA in the homogenate. Superficial desquamation therefore increases the relative radioactivity of the homogenate despite an unchanged DNA synthetic activity in the glands and will therefore give a false picture of proliferative stimulation. The biochemical methods are even more hazardous after *in vitro* incubation with ^{3}H-thymidine because, under these circumstances, distinction cannot be made between inadequate uptake in some cells and normal uptake in a small number of cells.

2 Total number of cells

Information about the absolute number of cellular elements in a given cell population is important from a physiological point of view, since quantitative changes in types of cell may result in modified exocrine or endocrine secretory capacity. On the other hand, changes in the total number of cells may indicate factors controlling proliferation, differentiation and life span of the cell types under study.

The term 'hyperplasia' refers to an increase in the absolute number of cells

within a defined cell population. Hyperplasia is frequently confused with 'hypertrophy' which is a rather ambiguous term and has been used to describe an increase in the thickness, the volume or the weight of a mucosa or of an organ. In fact, interstitial or intraglandular accumulation of mucins; proteins or bacterial products as well as swollen cytoplasm or nuclei; lymphocytic infiltration; storage of lymph or of capillary blood as well as increased uptake of labelled precursors of any kind have been included under the general heading of 'hypertrophy'. Any factor capable of inducing one of the above changes can be called 'trophic' and acquire the reputation of promoting growth in the tissue. Ambiguity concerning 'tissue growth' must therefore also be avoided in studies of the kinetics of cell populations since 'growth' indicates non-specific increase in size or in weight of the tissue, as well as specific multiplication of the cellular elements. Changes in numbers of cells is the only appropriate criterion for studies of the kinetics of cell populations.

There are some practical problems involved in estimating mucosal cellular masses. The existence of hyperplasia or of hypoplasia cannot be confirmed by observing small biopsy specimens. Two criteria must be measured in order to derive an estimate of the total numbers of different types of cell in a mucosa, such as the mean concentration of cells per unit volume of mucosa, or per unit of mucosal surface area, together with the total volume or surface of the mucosa (Card and Marks, 1960). Such methods have allowed the determination of the parietal cell, peptic cell and gastrin cell mass in the gastric mucosa in animals and man. In the intestine, the total epithelial population of one crypt can be counted by the crypt squash technique, or more roughly, in histological sections. Since a fair approximation of the total number of crypts can be obtained, the total number of cells in the bowel can be calculated (Hardings and Cairnie, 1975).

Because DNA content is theoretically similar in all the diploid cells of the same animal, biochemical determination of the DNA content of a tissue provides an estimate of the total number of cells in the tissue. However, non-epithelial cells from inflammatory infiltrates or connective tissue are included in these estimations and must be separately evaluated by microscopical examination. In addition, cells in the phase of DNA synthesis or in the G_2 phase contain more DNA than cells in other phases. Increased DNA synthetic activity or lengthening of the G_2 phase in a tissue could therefore increase the total amount of DNA without necessarily indicating changes in cell numbers.

3 Loss of cells from the mucosa

The rate of the decrease of mucosal radioactivity after uptake of ^{3}H-thymidine may be used to estimate the survival of cells in the mucosa (Ragins *et al.*, 1968). The fact that the life span of radiolabelled cells may be shortened because of the possible radiobiological effects of ^{3}H-thymidine must of course be kept in mind when interpreting these data.

A different method for quantifying the rate of loss of cells from a mucosal surface involves measurement of the appearance of DNA in gastric or intestinal washings (Pink, Croft and Creamer, 1970). However, contamination caused by diapedesis of leucocytes must be controlled by suitable cytological examination

when the loss of only epithelial cells is being studied. Information about the magnitude of exfoliation of cells from mucosal surfaces is essential for the understanding of many pathological conditions affecting the mucosae of the alimentary tract. Increased loss of cells from a mucosa may elicit a compensatory increase in cellular proliferation, so that if only criteria of cellular proliferation are studied, the origin of any observed abnormalities may not be detected. In all pathological conditions involving abnormal cellular kinetics, all three aspects of the kinetics of cell populations should therefore be studied: the rate of production of new cellular elements; the total number of cellular elements; and the rate at which these elements are destroyed. None of these aspects, when investigated alone can provide adequate information about the disease being studied.

CELL RENEWAL IN THE GASTRIC MUCOSA

1 Normal cell renewal in the stomach

The oxyntic glands of the gastric body contain at least five different types of epithelial cells – mucous cells of surface and pits, mucous neck cells, zymogen (chief or peptic) cells, parietal (acid-secreting) cells and undifferentiated neck cells (Rubin, Ross and Sleisinger, 1968). The immature neck cells are found in the lower part of the pits and in the neck of the oxyntic glands. It is probable that these cells represent multipotential elements from which the four other cell types can differentiate. Most of the newly produced cells undergo upward migration to the surface while differentiating into mucous cells. During their upward migration, the pit cells loose their ability to divide.

Parietal cells are unable to divide. Their continuous renewal is assured by differentiation from immature neck cells, followed by slow downward migration towards the deeper segments of the glands (Willems *et al.*, 1972). The time required for a new parietal cell to differentiate from its immature precursors is less than seven days in the mouse and less than 15 days in the rat. No information is yet available for man. The survival of labelled parietal cells has been observed to be at least three months in the mouse.

The origin of zymogen cells is less certain. A number of observations indicate that these cells may differentiate from immature cells under circumstances involving gastric mucosal damage. In the normal mucosa, however, zymogen cells retain their ability to divide and, in contrast with parietal cells, the renewal of this cell population is not linked with the cell division and maturation activity in the glandular neck cell area (Willems and Lehy, 1975), so that stimulation of proliferative activity in the progenitor cells of the oxyntic gland may result in hyperplasia of the parietal cell population but does not necessarily influence the number of zymogen cells in the same glands.

Two main types of epithelial cell are observed in the antral glands – mucous cells from surface and pits and mucous cells of the pyloric type. The proliferative activity occurs in the immature cells at the base of the antral crypt and is more intense than in the fundic glands (Table 1.1). The gastrin producing cells of the antrum of adult mice are also able to replicate through mitosis despite their high degree of differentiation (Lehy and Willems, 1976).