JCI The Journal of Clinical Investigation

CELL POPULATION KINETICS IN THE COLON OF THE MOUSE

Martin Lipkin, Henry Quastler

J Clin Invest. 1962;41(1):141-146. https://doi.org/10.1172/JCI104456.

Research Article

Find the latest version:

https://jci.me/104456/pdf

Journal of Clinical Investigation
Vol. 41, No. 1, 1962

CELL POPULATION KINETICS IN THE COLON OF THE MOUSE *

By MARTIN LIPKIN AND HENRY QUASTLER

(From the Department of Medicine, Cornell University Medical College, Second (Cornell) Medical Division, Bellevue Hospital, New York; and Biology Department, Brookhaven National Laboratories, Upton, N. Y.)

(Submitted for publication July 15, 1961; accepted September 14, 1961)

In recent years interest in the cell population kinetics of gastrointestinal mucosa has been stimulated by several factors. One is the realization that the turnover rates of mucosal cell populations are among the most rapid in the body (1). Another is the possibility that abnormal histologic appearance of mucosal epithelial cells in certain disease states (for example ulcerative colitis, peptic ulcer disease) in humans may be related to impaired differentiation or proliferation of these elements (2–4).

The micro-radioautographic methods of measuring cell proliferation and migration that are now available should prove useful in the analysis of the renewal of human mucosal tissues (1, 5). These techniques have been used in several studies to measure epithelial cell regeneration in animals, including studies of population kinetics in the small intestine of mice (6, 7). In the work reported here, similar methods were used to measure rates of epithelial cell proliferation and migration in mouse colon. Estimates were made from the data of the duration of the proliferative cycle and its various portions, and of the speed of migration of the epithelial cells.

MATERIAL AND METHODS

Thirty-five adult C57 Brown male mice were used. The animals were labeled by intraperitoneal injection of tritiated thymidine (0.7 μc per g of animal, specific activity 360 cpm, dissolved in 1 ml saline); 25 were sacrificed between 0.5 and 40 hours and 10 were killed 1 week after injection. Specimens of ascending and sigmoid colon were removed, fixed in neutral formol-saline, embedded in paraffin, and sectioned at 5 μ. Slides were dipped into NTB (Kodak) liquid emulsion, exposed for 2 to 4 weeks, developed, and stained with basic fuchsin. A representative radioautograph is shown in Figure 1.

The following measurements were made. The position of labeled epithelial cells in the colonic crypts were recorded at 1 and 24 hours after labeling[1]; counts were made of labeled mitoses at various intervals after labeling[2]; and the persistence of labeled cells at long intervals after injection was studied.

* This work was aided by grants from The John A. Hartford Foundation, Inc., the National Institute of Arthritis and Metabolic Diseases (Grant A-3165), G. D. Searle and Co., and The Upjohn Co. Part of the work was done at Brookhaven National Laboratories under the auspices of the U. S. Atomic Energy Commission.

[1] Tritiated thymidine is incorporated specifically into DNA in process of formation at the time of labeling. The subsequently decaying tritium atoms emit beta rays, the range of which in tissue is about 1 μ. Therefore, the photographic grains produced by the beta rays are closely apposed to the structures originally labeled. In all mammalian and plant tissues so far studied, DNA synthesis is restricted to a portion of the interphase called the S phase, and only cells in S phase at the time the label is administered appear labeled (see Figure 1).

[2] DNA synthesis partitions the interphase into 3 portions, or *phases*. The phase between the termination of DNA synthesis and the commencement of mitosis is called the G_2 phase, or "postsynthetic premitotic gap"; according to how mitoses are scored, this phase will include more or less of the prophase. The phase between termination of mitosis and commencement of DNA synthesis is called the G_1 phase, or "presynthetic postmitotic gap"; it may include some of the telophase. The method of labeled mitoses is particularly useful in determining the duration of the G_2 and S phases. This method has been described in detail elsewhere (6). Briefly, the principle is as follows. Cells in mitosis at the time of sacrifice are synchronous within a narrow period of time, particularly if only meta- and anaphases are scored as "mitoses." Cells labeled at the time of injection must have been in S phase during the short time when the label was available. Therefore, restricting the observation to cells in mitosis is equivalent to looking backward into the time preceding sacrifice, through a "window" which is approximately 0.5 to 1 hour wide at the beginning (corresponding to the length of time during which label is available, plus the duration of mitosis) and widens subsequently owing to variations in speed of proliferation. Separating mitotic figures into those labeled and unlabeled is equivalent to establishing what fraction of the cells contained in the time "window" were in S phase at the time the label was available. For short intervals between labeling and sacrifice, cells in mitosis at sacrifice will have been in the phase immediately preceding mitosis at the time of la-

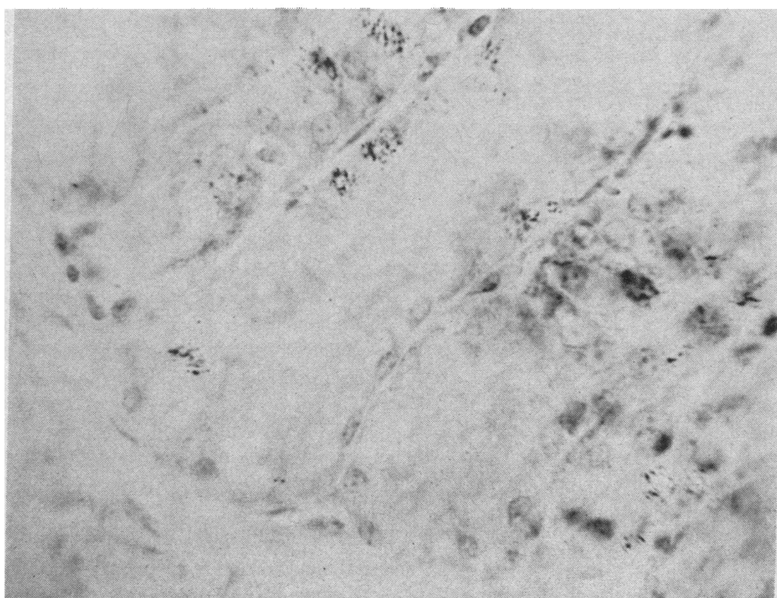

FIG. 1. LABELING OF NEWLY SYNTHESIZED DNA IN THE COLON. The micro-radioautograph shows the lower portions of several crypts in the colon of a mouse, 1 hour after injection of tritiated thymidine. Feulgen stain; × 1100.

The anatomical unit used in studying the distribution of labeled cells is the *crypt cell column,* or a column of cells situated in one optical plane and extending from the bottom to the mouth of the crypt. The cell at the bottom of the crypt occupies "position 1," its nearest neighbor in the direction of the mouth "position 2," and so on. In scoring labeled cells it is important to keep in mind the effects of self-absorption. A nucleus not actually cut by the plane of section could be scored as negative even if labeled. The incidence of false negatives was minimized by restricting the scoring to cell columns that were in the same optical plane as other definitely labeled cells; if this precaution is taken thick sections can be used as well as thin ones. Since the optical plane scanned comprises several cell columns in adjacent crypts, the restriction gave no bias against cell columns that actually contained no labeled cells.

beling (the G_2 phase) and, since cells do not take up thymidine into DNA during this phase, the mitoses will be unlabeled. For longer intervals, cells in mitosis at sacrifice will have been in the S phase at labeling and, therefore, will be labeled. If the fraction of mitoses labeled is plotted against the interval between labeling and sacrifice (e.g., Figure 2), the S phase will appear as a wave of labeled mitoses recording the time distribution of cells emanating from the preceding S phase. For still longer intervals, cells in mitosis at sacrifice will have been in one of the nonlabeling phases preceding the S phase—namely (counting backward from the S phase), G_1, mitosis, and G_2. The trough following the large wave of labeled mitoses contains cells in G_1, mitosis, and G_2, and is followed by a second wave corresponding to the S phase twice removed from the mitosis at sacrifice, and so on. The duration of the S phase can be estimated from the wave of labeled mitoses by measuring the distance (in time) between the midpoints of the ascending and descending limbs of the wave.

RESULTS AND ANALYSIS OF DATA

Count of labeled mitoses. Figure 2 shows the fraction of mitoses labeled at various time intervals after injection of thymidine. Each circle represents the count in one mouse. The numbers of mitoses counted at each point range from 22 to 212, with a mean of 65.4. The vertical bars in Figure 2 measure the limits of a 95 per cent confidence interval according to the table by Mainland and Herrera (1956). Almost no mitoses are labeled if a mouse is killed within 1 hour after labeling. Therefore, this is the minimum duration of the postsynthetic, premitotic "gap," or G_2 phase. The fraction of mitoses labeled rises rapidly to about 0.93. Since the fraction of labeled mitoses is low at 1 hour and maximal beyond 3 hours, the midpoint of the rise must lie between 1 and 2.5 hours; this is approximately the mean duration of G_2 plus half the mitosis time. The midpoint of the subsequent decline of the wave can be similarly

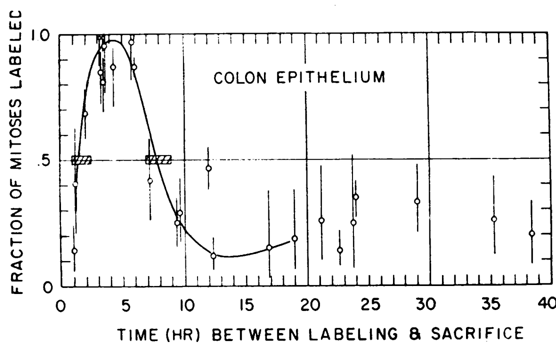

FIG. 2. FRACTION OF MITOSES LABELED VS TIME BETWEEN LABELING AND SACRIFICE, IN THE COLON EPITHELIUM OF THE MOUSE. Each circle represents the count in one mouse. The vertical bars show the limits of a 95% confidence interval according to the table by Mainland and Herrera (1956). The line is fitted by eye, and the hatched areas indicate the limits between which the curve is thought to cross the 0.5 line.

bracketed. Since the fraction of labeled mitoses is still near maximal at 6, and definitely low at 9.5 hours, it must lie between 7 and 9 hours. The mean duration of the portion of the interphase, during which DNA is synthesized (the "S phase"), is estimated [2] as the distance between the midpoints of rise and fall of the wave of labeled mitoses. The lowest estimate is the time interval between 2.5 and 7 hours, or 4.5 hours; the highest, the interval between 1 and 9 hours, or 8 hours. The true value is likely to be near the midportion of the interval so bracketed. Greater precision could be obtained by using more animals, but this was not deemed necessary at this time, for the value estimated agrees with those found in other rapidly growing mouse tissues (6, 8, 9). The fraction of labeled mitoses is lowest between 11 and 20 hours after injection. This trough, appearing at the end of the wave of labeled mitoses and containing in order, G_1, mitosis, and G_2 phases, can be used to estimate the total generation time. Since G_2 is very short, the portion of the trough corresponding to the mitoses in the preceding cycle should be near the far end, and the total generation time can be estimated to be about 16 hours. Subtraction of about 8 hours (for S, G_2, and mitosis) gives an estimated duration for the G_1 phase of 8 hours. A second wave of labeled mitoses, corresponding to the S phase once removed from that preceding the mitosis observed, is very poorly expressed. This has been found in several other cell populations. The peak of the wave of labeled mitoses does not quite reach 100 per cent. This could be due to false negatives or to variations in the duration of the G_2 and S phases sufficiently large that there is no period of time when there are not at least a few unlabeled cells contributing unlabeled mitoses. Detailed analysis of a similar situation in the small bowel (6) led to the conclusion that the difference between the peak of the wave and 100 per cent was due to false negatives in that case.

For intervals of 1 day and more, the fraction of mitoses labeled is around 0.25. If variations in generation time abolished within 24 hours the synchronicity of cells in phase at the time of sacrifice, then the average fraction of mitoses labeled should equal the ratio of duration of S phase to total generation time, provided the proliferating population is homogeneous. The S phase lasts between 4.5 and 8 hours; for a generation time of about 16 hours the ratio should be between 0.28 and 0.50, which is slightly higher than the value found. This

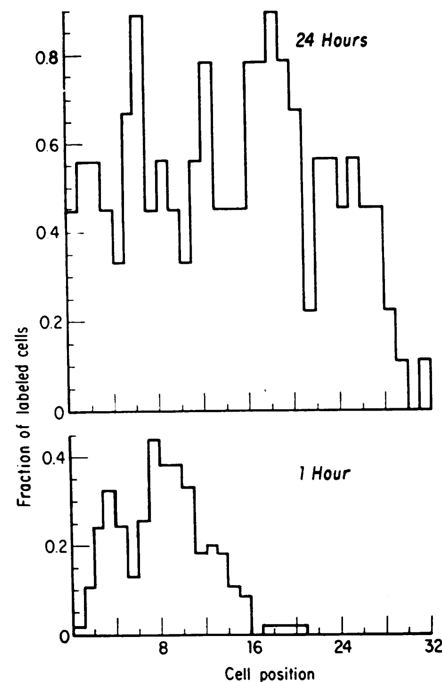

FIG. 3. LABELING INDEX VS POSITION IN THE CRYPTS OF THE COLON. The position number "1" is that of the cell at the bottom of the crypt, position "2" that of the next cell toward the lumen, and so on; 24 hours after labeling most of the cells found labeled after 1 hour have divided, and some have divided twice.

phenomenon has also been found in other cell populations. It may be due to false negatives, or that labeled cells divide less easily than do unlabeled ones, or that unlabeled cells are fed into the cell production compartment.

Labeled cells 1 hour after injection of thymidine. The number of cells in 45 crypt cell columns ranged from 13 to 28 (mean, 20.3) in the colons of the C57 Brown mice studied. Figure 3 shows the occurrence of labeled cells 1 hour after injection. The highest number of labeled cells was in the middle portion of the crypt; the cells near the bottom show less, and the peripheral third very much less label. This agrees with the distribution reported by Messier and Leblond (1). On sections that do not go through the bottom of the crypt the lowest cells often seem to be labeled. We verified on serial sections that the bottom cells are indeed infrequently labeled. The average number of labeled cells per crypt column was 3.9, or 19 per cent. In a homogeneous population of proliferating cells, the fraction of cells labeled should equal the ratio of DNA synthesis time to generation time. According to the data on labeled mitoses, this ratio should be between 0.28 and 0.50; the ratio found in the middle of the crypt is about 0.35.

Labeled cells 24 hours after injection of thymidine. As seen in Figure 3, the mean number of labeled cells has increased, and the labeled cells are distributed more evenly throughout the crypt. The average number of labeled cells per crypt column is 16, a fourfold increase. In some instances labeled cells were seen at the surface, which indicates that some labeled cells might have been lost into the lumen at this period. Hence, each cell originally labeled has given rise to 4 or more labeled cells 1 day later. According to the analysis of the data shown in Figure 2, cells originally labeled should start dividing about 1 hour after injection, and all should have divided by about 12 hours. By that time the number of labeled cells will be doubled if there are no abortive mitoses. Divisions of unlabeled cells will leave the number of labeled cells per crypt unchanged as long as no labeled cells are lost. A new wave of increase in the number of labeled cells should follow the first one by about one generation time and lead to a quadrupling of the number of labeled cells. If all cells proliferated at the same speed, the time needed for quadrupling of labeled cells would be the length of two generation times minus the duration of one G_1 period. According to estimates made on the basis of the fraction of labeled mitoses, this is about 24 hours, in agreement with the measured number of labeled cells 24 hours after labeling.

The change in distribution of labeled cells from 1 to 24 hours after labeling is compatible with the assumption that cells move in a coherent sheet toward the lumen (the zone of labeled cells may also expand toward the bottom of the crypt, into "positions 1 and 2," but this would affect only a few cells). The boundary between labeled and unlabeled cells on the lumen side is in "position 14" 1 hour after labeling; in addition, a few scattered labeled cells are nearer the mouth of the crypt. The comparable point at 24 hours is "position 30." This means that the 14 cells contained within the area originally labeled have produced 16 new cells. It is estimated that 12 of these are descended from the 4 labeled cells originally present, leaving only about 4 cells as products of the nonlabeled proliferating cells in the crypt. Since the labeled cells are thought to have divided at the beginning and again near the end of the 24-hour period following, the unlabeled cells might have divided, on the average, only once toward the middle of this period. It may thus be estimated that there are about 4 proliferating cells in G phase per crypt column, in addition to the 4 found in S phase. Then, about 6 of the 14 cells between the leading edge and the bottom of the crypt either are not proliferating or grow at a speed much slower than the others.

Labeled cells 1 week after injection of thymidine. Each time a labeled cell divides the label is reduced by about one-half. With a mean generation time of 16 hours each labeled cell should divide about 10 times during 1 week after labeling, giving rise to 1,000 cells, if cell loss were not present. If there were no somatic crossing over, then less than 10 per cent of these should contain a labeled chromosome, and the average grain count of these cells should be about 1 per cent of the grain count immediately after labeling. With frequent crossover, all descendants would be labeled, but the grain count of each labeled cell would be reduced to 0.1 per cent. In either case the label would be indistinguishable from background, with the exposure times used in this study. Actually, 1 week

after injection of thymidine, 7.2 per cent of the cells in the colon are still distinctly labeled, and a small percentage is still heavily labeled, in agreement with findings of Messier and Leblond (1). Some of the labeled cells seem to have divided only once or twice, some possibly not at all. These cells are found in any position between the bottom of the crypt and the surface.

DISCUSSION

All findings reported are compatible with a generation time of about 16 hours, divided into an S phase lasting about 6.5 hours, a G_2 phase, which together with mitosis, lasts about 1.5 hours, and a G_1 phase of about 8 hours. Variation in duration must exist for all periods observed but must be most pronounced for the duration of G_1. These numbers have a precision of about 20 per cent; other values might, and probably will, be found in different strains and species and under different conditions. Roughly similar values have been found in all rapidly growing tissues of the mouse.

In some renewal tissues the locus of proliferation is fairly sharply separated from the location of mature cells. This is not the case in the colon, where the zone of proliferation blends into that occupied by mature cells, and where a fair fraction of mature cells is mixed in even at the place of highest proliferation.

In the crypts of the small intestine of the mouse, all proliferating cells seem to divide at about the same speed. In the colon the cells near the bottom of all or some crypts proliferate at a much slower rate than those in the middle third. They may represent a different population, a possibility raised by Messier and Leblond (1) and supported by the finding of Wattenberg (10) that these cells can be cytochemically differentiated. Alternatively, the cells at the origin of a column of proliferating cells may divide less often than the others, a situation found by Kember in the cartilage plate (11), and well known in situations where the least mature cells are morphologically identifiable (spermiogonia A, possibly stem cells of the blood cell lines), and postulated by Osgood (12) for all "type A" cells.

It should be said that the distinction between the alternatives could well be a difficult one. In the small bowel the cells at the bottom of the crypts (the Paneth cells) are readily identifiable with routine microscopic methods. Even in this case it is not yet certain whether they are a separate population or are derived from the principal cells in the crypt.

The persistence of labeled cells in the crypts of the colon cannot be explained unequivocally at this time. The persisting cells do not follow the pattern of proliferation kinetics described for the majority of the cells. A question that must be considered is whether these cells are exceptional members of the general population, which (for some time, at least) do not participate in the general pattern of division and migration, or whether they are members of a separate population. They are not argentaffine cells as demonstrated by the diazo stains and the Gibbs and Fontana methods (13); they could be migrating descendants of the slowly growing cells at the bottom of the crypts. The answer to the problem may depend on a detailed study of the kinetics of proliferation of the cells at the very bottom of the crypts.

SUMMARY

Interest in cell population kinetics of gastrointestinal mucosa has been stimulated by the possibility that impaired proliferation and differentiation of cellular elements may be present in certain disease states. To aid in standardizing methods of measurement of these functions, measurements were made of epithelial cell proliferation and migration in normal mouse colon after injection of H^3-thymidine and micro-radioautography. Estimates were made of the duration of the proliferative cycle and its various portions, and the rate of migration of the epithelial cells.

The findings revealed for the majority of epithelial cells a generation time of 16 hours. The duration of the DNA synthesis (S phase) was about 6.5 hours. After incorporation of thymidine and DNA synthesis, the majority of epithelial cells went rapidly through G_2 and mitosis phases, the total durations of which lasted about 1.5 hours. The duration of the G_1 phase was estimated to be about 8 hours.

The zone of rapid epithelial cell proliferation was located in the middle third of the colonic crypts, and rapidly proliferating cells were blended with mature cells, in contrast to jejunum where

zones of proliferation and differentiation are more sharply localized. Although the majority of cells underwent mitosis shortly after labeling, some epithelial cells did not follow the pattern of proliferation kinetics described for the majority of cells. One week after labeling, some of these cells appeared to have divided only once and some not at all. The origin of these cells cannot be precisely described at this time.

ACKNOWLEDGMENT

The technical assistance of Mrs. Eileen Kairys is gratefully acknowledged.

REFERENCES

1. Messier, B., and Leblond, C. P. Cell proliferation and migration as revealed by radioautography after injection of thymidine-H^3 into male rats and mice. Amer. J. Anat. 1960, **106**, 247.
2. Quastler, H. The nature of intestinal radiation death. Radiat. Res. 1956, **4**, 303.
3. Kirsner, J. B. Drug-induced peptic ulcer. Ann. intern. Med. 1957, **47**, 666.
4. Brown, H. O., Levine, M. L., and Lipkin, M. Effect of starvation on regeneration of intestinal mucosa. Clin. Res. 1961, **9**, 150.
5. Hughes, W. L., Bond, V. P., Brechner, G., Cronkite, E. P., Painter, R. B., Quastler, H., and Sherman, F. G. Cellular proliferation in the mouse as revealed by autoradiography with tritiated thymidine. Proc. nat. Acad. Sci. (Wash.) 1958, **44**, 476.
6. Quastler, H., and Sherman, F. G. Cell population kinetics in the intestinal epithelium of the mouse. Exp. Cell Res. 1959, **17**, 420.
7. Leblond, C. P., and 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. 1958, **132**, 247.
8. Mendelsohn, M. L., Dohan, F. C., Jr., and Moore, H. A., Jr. Autoradiographic analysis of cell proliferation in spontaneous breast cancer of C3H mouse. I. Typical cell cycle and timing of DNA synthesis. J. nat. Cancer Inst. 1960, **25**, 485.
9. Cattaneo, S. M., Quastler, H., and Sherman, F. G. The proliferative cycle in the growing hair follicle of the mouse. Nature (Lond.). In press.
10. Wattenberg, L. W. A histochemical study of five oxidative enzymes in carcinoma of the large intestine in man. Amer. J. Path. 1959, **35**, 113.
11. Kember, N. F. Cell division in endochondral ossification. A study of cell proliferation in rat bones by the method of tritiated thymidine autoradiography. J. Bone Jt Surg. 1960, **42B**, 824.
12. Osgood, E. E. Number and distribution of human hemic cells. Blood 1954, **9**, 1141.
13. Pearse, A. G. E. Histochemistry, Theoretical and Applied, 2nd ed. London, Churchill, 1960, pp. 919–925.