The Cell Cycle and Turnover Times of the Small Intestinal Epithelia of the Pouchless Opossum, *Marmosa mitis*

The Didelphids (opossums) have several unique characteristics that make them potential experimental tools for developmental and cellular biologists. Developmentally the opossum is considered to be equivalent to an 8- to 10-week-old human embryo at birth and spends, therefore, its fetal life in the pouch[1]. In addition, the new-born opossum appears to be immunologically incompetent until about the eighth day of life[2,3]. The value of Didelphids for the cellular biologist results from simple karyotypes. The number of chromosomes in the diploid condition have been reported at either 14 or 22[4-8]. In addition, recent evidence suggests that chromosomes of *Didelphis* lymphocytes in vitro behave similar to higher mammals with respect to initiation of DNA synthesis and duplication[7,8]. In spite of these unique characteristics, research of the literature has failed to reveal any data upon the cell cycle and turnover times of renewing cell populations in opossums. Therefore, this study was undertaken in order to measure the average duration of the cell cycle and its phases as well as the turnover times in the small intestinal epithelia of the pouchless opossum, *Marmosa mitis*.

Materials and methods. Wild trapped adult *Marmosa mitis* from Barrenquilla (Colombia) were obtained from the Pet Farm, Miami (Florida). The animals were shipped from Miami to Los Angeles by air in compartmentalized crates to avoid fighting in transit. The opossums were quarantined and treated for helminth infestations[9]. The animals were individually housed, fed and placed on a light cycle as previously described[9,10].

A total of 32 wild trapped *Marmosa* were given a single s.c. injection of tritium thymidine (^{3}H Tdr), methyl labeled, Sp. Act. 17.4 C/m*M* at a concentration of 1 µc/g body weight. The animals were sacrificed in pairs by ether anesthesia from $^1/_2$ to 96 h following ^{3}H Tdr administration. Segments of the duodenum (0.5 cm below the pylorus), jejunum (mid small intestinal length) and ileum (0.5 cm above the cecum) were removed, fixed in Bouin's fluid, embedded in paraffin and processed for autoradiography as previously described[11]. The autoradiograms were scored for a) the percentage of labeled mitotic figures from $^1/_2$ to 14 h after thymidine injection, b) the percentage of labeled cryptal cells at 30 to 60 min after ^{3}H Tdr injection and c) the percentage of the villus height (total of 10 villi counted) that labeled progeny had migrated at 1, 10, 12, 24, 36 and 48 h. The measurements of the duration of G_2, D and S phases were made from constructed mitotic curves[11,12]. The duration of the cell cycle was estimated from the formula $T = S/I$[11,13]. G_1 was measured by subtracting the total of G_2, D and S from the duration of the cell cycle. Estimates of the turnover time of the 3 portions of the small intestine were made according to the method of LORAN and ALTHAUSEN[14].

Results. Kinetics of labeled mitotic figures. Curves representing the percentage of labeled mitotic figures in the duodenum, jejunum and ileum are given in Figure 1. The kinetics of labeled mitotic figures were similar in the 3 regions of the small intestine. Labeled mitoses first appeared at 30 min (duodenum) and 1 h (jejunum and ileum) after ^{3}H Tdr injection. The percentage of labeled mitotic figures rose rapidly thereafter and approached 100% by $2^1/_2$ h, remaining at this plateau until 8 h (duodenum and jejunum) and 10 h (ileum) following isotope injection. The labeling fell, thereafter, reaching lower values between 12–14 h.

Labeling index; cell cycle and its phases; turnover times. Data for the values obtained for the labeling index, the cell cycle and its phases as well as turnover times for the 3 regions of the small intestine are summarized in the Table. The rate of migration of labeled progeny is presented in Figure 2. Estimates for the minimum duration of G_2 were 0.5 h (duodenum) and 1 h (jejunum and ileum). Division time was approximated to be 2 h

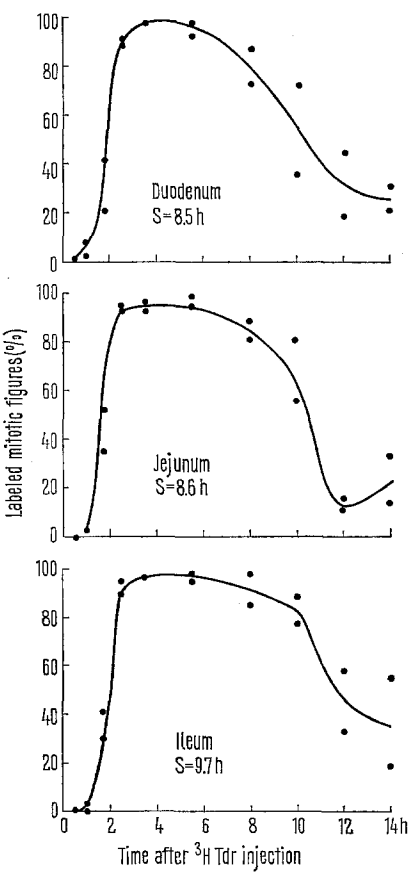

Fig. 1. Curves that demonstrate the percentage of labeled mitotic figures at $^1/_2$ to 14 h after ^{3}H Tdr injection in the duodenum, jejunum and ileum of the pouchless opossum, *Marmosa mitis*.

[1] M. BLOCK, Ergebn. Anat. EntwGesch. *37*, 237 (1964).
[2] E. S. LAPLANTE, R. G. BURRELL, A. L. WATNE and B. ZIMMERMANN, Surg. Forum *17*, 200 (1966).
[3] D. T. ROWLANDS, M. F. LAVIA and M. W. BLOCK, J. Immun. *93*, 157 (1964).
[4] J. D. BIGGERS, H. I. FRITZ, W. C. D. HARE and R. A. MCFEELEY, Science *148*, 1602 (1965).
[5] M. LEGATOR, C. JACOBSON, M. PERRY and D. DOLIMPO, Life Sci. *5*, 397 (1966).
[6] O. A. REIG, Experientia *24*, 185 (1968).
[7] L. K. SCHNEIDER and W. O. RIEKE, J. Cell Biol. *33*, 497 (1967).
[8] A. K. SINHA, Experientia *23*, 889 (1967).
[9] R. D. BARNES, in *Animal Models for Biomedical Research*, National Academy of Sciences *1594*, 88 (1968).
[10] J. D. THRASHER, Lab. Animal care. *19*, 67 (1969).
[11] J. D. THRASHER, in *Methods in Cell Physiology* (Ed. D. M. PRESCOTT; Academic Press, New York 1966), vol. 2, p. 323.
[12] H. QUASTLER and F. G. SHERMAN, Expl. Cell Res. *17*, 420 (1959).
[13] I. L. CAMERON and R. C. GREULICH, J. Cell Biol. *18*, 31 (1963).
[14] M. R. LORAN and T. L. ALTHAUSEN, J. biophys. biochem. Cytol. *7*, 667 (1961).

(duodenum) and 1.5 h (jejunum and ileum). The average duration of the S-phases, as measured from the 50% level on the mitotic curve, were 8.5, 8.6 and 9.7 h, respectively. The duration of the cell cycle was calculated to be 20, 28 and 37 h for each region of the small intestine. The difference in the duration of the cell cycle for the 3 portions of the small intestine was absorbed by variation in G_1: 9, 16.4 and 24.8 h for the duodenum, jejunum and ileum, respectively.

The rate of migration was very slow in all 3 portions of the small intestine (Figure 2). The labeled progeny migrated most rapidly in the duodenum reaching approximately 63% of the villus height by 48 h after ^{3}H Tdr injection. In contrast, the wave of labeled progeny reached only 58% (jejunum) and 33% (ileum) of the villus height by 48 h in the 2 lower portions of the small intestine. Preliminary values for the turnover times, as estimated from extrapolation of Figure 4, were 75, 82 and 120 h for the duodenum, jejunum and ileum, respectively.

Discussion. The estimates for the minimum duration of G_2 and D phases of the cell cycle in the duodenum, jejunum and ileum of *M. mitis* fall within the times reported for eutherian mammals[11,15–17] and chickens[18,19]. However, the values obtained for the average duration of the S-phase (8.5–9.7 h) are slightly longer than corresponding times (7–8 h) reported for the mouse[11–13,20] and rat[21] small intestine. The longer S-phase in *Marmosa* intestinal epithelial cells corroborate observations made on the S-phase duration of *Didelphis* lymphocytes (9 h) and the gastric epithelium (10 h) of young Virginia opossums[7,22]. Therefore, at the present time it appears that S-phases in excess of 8 h are a real phenomenon in marsupial cells both in vivo and in vitro. The longer S-phases observed in marsupial cells may be a function of either the lower body temperature (34 °C) commonly observed[23] or the low modal number of chromosomes[7,8]. In addition, the great amount of autosomal heterochromatization and the late replication of autosomes may also be important with regards to the longer S-phases[8]. However, more data is needed in order to determine the role that chromosome replication may have in this phenomenon as well as the variability in the marsupial cell cycle under various environmental conditions and in other tissues.

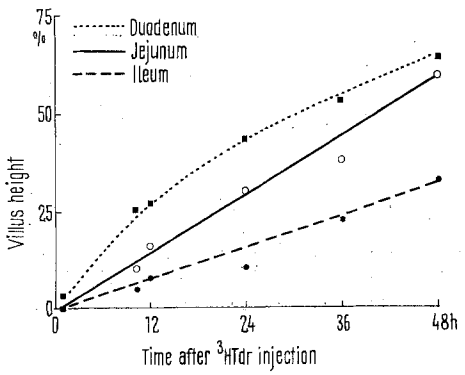

Fig. 2. A graph that shows the percentage of the villus height that labeled progeny have migrated at 1 to 48 h after injection of ^{3}H Tdr in the duodenum, jejunum and ileum of the pouchless opossum, *Marmosa mitis*.

Organ	Labeled Cells (%)	Time (h)					
		S	D	G_2	G_1	Cell Cycle	Turnover
Duodenum	42.3	8.5	2	0.5	9	20	75
Jejunum	31.1	8.6	1.5	1.0	16.4	28	82
Ileum	26.4	9.7	1.5	1.0	24.8	37	120

It is recognized that estimates of the cell cycle duration by the *S/I* method give values in excess of those obtained by the labeled mitotic method. However, such data are taken as representing a fair estimate of the cell cycle times[11–13]. Thus, it appears that the duration of the cell cycle in the duodenal epithelium of *Marmosa* falls within the range of estimates made on mice[11–13]. The values for the jejunum and ileum, on the other hand, are considerably longer than corresponding data published on rodents[13,21,24]. In spite of the longer times estimated for the cell cycle in *Marmosa*, the variability that exists in the cell cycle is absorbed by G_1.

The turnover times of the 3 regions of the small intestine (Table) also indicate that population kinetics of renewing cell populations in *Marmosa* are considerably slower than eutherian mammals. For comparison, the turnover of the mouse small intestinal epithelia occurs as follows: duodenum, 54 h[25]; jejunum, 50 h[26] and ileum, 38–44 h[27]. The values obtained for the turnover time in *Marmosa* small intestine are at least 21, 32 and 76 h longer for each respective region. Thus, not only are the cell cycle and S-phases extended, the turnover of the small intestinal epithelium is considerably slower. Explanation of this phenomenon must await further data on domestic generations produced in this laboratory.

In conclusion, the duration of the cell cycle and the S-phases of marsupial cells are on the average longer than corresponding times reported for eutherian mammals. However, inspite of these differences, it appears that marsupials can be used as a model to investigate the effects of radiation, carcinogens, drugs, etc., upon the cell cycle and turnover times of the small intestine. These studies become particularly interesting from the fact that the Genus *Marmosa* has a very simple karyotype and only seven pairs of chromosomes[6].

Zusammenfassung. Es wird erstmals der in vivo Zellzyklus von intestinalen Zellen des Marsupialiers *Marmosa mitis* beschrieben. Die bei diesem beutellosen Opossum beobachteten längeren Generationszeiten gehen gleichzeitig mit längeren Erneuerungszeiten der Epithelzellen in oberen Darmabschnitten überein.

J. D. THRASHER

Department of Anatomy, UCLA School of Medicine, Los Angeles (California 90024, USA), 9 February 1970.

[15] J. POST and J. HOFFMAN, Expl. Cell Res. *36*, 111 (1964).
[16] J. POST and J. HOFFMAN, Expl. Cell Res. *37*, 183 (1964).
[17] A. B. REISKIN and M. L. MENDELSOHN, Cancer Res. *24*, 1131 (1964).
[18] I. L. CAMERON, J. Cell Biol. *20*, 185 (1964).
[19] S. FUJITA, Expl. Cell Res. *28*, 52 (1962).
[20] J. D. THRASHER and R. C. GREULICH, J. expl. Zool. *159*, 39 (1965).
[21] A. B. CAIRNIE, L. F. LAMERTON and G. G. STEEL, Expl. Cell Res. *39*, 528 (1965).
[22] J. D. THRASHER, Expl. Cell Res. *57*, 441 (1969).
[23] G. B. WISLOCKI, Q. Rev. Biol. *8*, 385 (1933).
[24] S. LESHER, R. J. M. FRY and H. I. KOHN, Gerontologia *5*, 176 (1961).
[25] S. LESHER, R. J. M. FRY and H. I. KOHN, Lab. Invest. *10*, 291 (1961).
[26] R. J. M. FRY, S. LESHER and H. I. KOHN, Am. J. Physiol. *201*, 213 (1961).
[27] R. J. M. FRY, S. LESHER and H. I. KOHN, Lab. Invest. *11*, 289 (1962).